Bioactive Food as Dietary Interventions for

The Aging Population

Edited by Ronald Ross Watson and Victor R. Preedy

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ACKNOWLEDGMENTS FOR BIOACTIVE FOODS IN CHRONIC DISEASE STATES

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Mature and aging animals and people have physiological systems that function quite distinctly from the young, growing ones. Increased tissue oxidants and decreased dietary antioxidant compounds result and accentuate some of these changes. As humans age their reduced physical activity and food consumption accentuate changes associated with aging. Lower incomes substantially reduce the ability to maintain health and reduce oxidants via adequate consumption of fruits and vegetables. Many chronic diseases are found in higher frequency in the aged and increase nutritional stresses in adults. The association with dietary inadequacies or sufficiency may be important by increasing longevity and prolonging health. Treatment of chronic disease states in the aging adult represent major health care and economic liabilities, which may be mitigated by herbs, foods, and dietary supplements discussed in this book. The aging adult offers a number of challenges including determining which bioactive foods and their extracts will promote health and how they affect cell structure and function. Cells in older adults have altered nutritional needs, biochemical activities, and protein turnover. The major objective of this book is to review in detail how foods and herbs affect aging cellular systems and chronic diseases. The dramatically increasing numbers of older people require a detailed study and directed research to optimize nutrition and use of health promoting foods and herbs.

The book has 34 chapters and leads with three chapters dealing with an overview of antioxidant supplementation in health of the aged, and mechanisms of action including changing epigenetics and longevity genes. Micronutrient, vitamin, and mineral supplements play key roles in restoring levels and health. The expert scientists provide five reviews covering vitamins, selenium, minerals, and zinc on a range of health problems, including bone structure, and age related disorders. The experts in two chapters evaluate the molecular mechanisms of diet and bone structure, as well as providing an overview of nutrition bone health. Similarly, other small non-nutritional molecules, taurine and creatine, have activity without being nutrients as defined in two chapters. Major impacts of dietary supplements beyond nutrients occur in bone and skeletal health or disease. In four chapters, soy, soy proteins, and isoflavones, as well as other plant products are reviewed relative to bone and lifespan and muscle mass retention. Macronutrients and special diets have been shown to be helpful for seniors. Ayurvedic medicinal plants are anti-aging drugs, while energy intake and the Mediterranean lifestyle and diet are active supporting methods to sustain seniors' health. Bioactive foods' actions on cancer in seniors are carefully described and documented. In three reviews the role of diet and social inequalities

affect older adults' health, including in economically developing countries. The amount of calories and exercise affect heart and overall muscle function positively.

Key components of the book are expert reviews on possible mechanisms of action of dietary materials in older adults. There are three chapters looking at antioxidants and aging, the brain and diet, and preventing the epidemic of mental health to help define the actions of nutrients. Finally seven chapters describe various specific herbs and their components with well documented activities. These include modulation of autoimmune diseases in the elderly, sirtuin 1 and nutrition in the aged, and the beneficial effects of fruits on health. In addition, legumes show genome maintenance for optimal health, while Asian medicinal remedies for alleviating aging are defined. Ginseng and nutritional hormetins affect aging and cognition, while bioactive prairie plants' actions are documented.

Such reviews help define the overall goal of providing the current, scientific appraisal of the efficacy and mechanisms of action of key foods, nutrients, herbs, and dietary supplements in preventing or treating a major factor in chronic diseases in older adults. There is compelling evidence that oxidative stress is implicated in its pathophysiology. Increased free radical formation and reduced antioxidant defenses contribute to increased oxidative stress. Importantly, diets rich in antioxidants in human dietary studies reduce the incidence, suggestive of potential protective roles of antioxidant nutrients. This book investigates the role of foods, herbs, and novel extracts in moderating the pathology promoting and preventing the aging process and its risk for other chronic diseases.

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Antioxidant Supplementation in Health Promotion and Modulation of Aging: An Overview

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1. OXYGEN AND OXIDATIVE STRESS

Antioxidants have become a necessity as a consequence of adaptation to life under aerobic conditions. Oxygen (or dioxygen, triplet O_2) is strictly necessary for our energetic metabolism and evolution has found a way to increase its concentration in aqueous environments, and to transport it into our internal fluids by means of hemoglobin and other heme-containing proteins. Oxygen is needed for its oxidizing property, that is, oxidizing food (carbohydrates, lipids, and some amino acids) and using the released electrons to reduce NAD^+ and oxidized flavins to NADH, $FMNH_2$, and $FADH_2$. These, in turn, are used in the mitochondria to produce adenosine triphosphate (ATP), again exploiting the oxidizing ability of O_2 (that will be converted to H_2O) as the driving force for the overall reaction. Incidentally, the oxidizing activity of oxygen (or its derivatives) sometimes goes out of control and results in so-called oxidative stress, which can lead to biological damage if not balanced by antioxidant defenses. Indeed, *oxidative stress* can be defined as the imbalance between generation of oxidating oxygen derivatives and antioxidant defenses, while the *oxidative stress status* (OSS) is a measure of such an imbalance (Halliwell and Gutteridge, 1999).

Reduction of oxygen to water using NADH in the inner membrane of the mitochondria is a spontaneous yet highly controlled process, occurring through a cascade of redox reactions called the electron transport chain, as exemplified in Figure 1.1. Such a sophisticated molecular machine is, however, not perfect and can leak electrons throughout the chain. Particularly, complexes I and III have been identified as the weak rings of the chain, due to the involvement of the intermediate semiquinone radical CoQH•, which can react with molecular oxygen to form superoxide radical anion ($O_2^{•-}$), one of the most abundant reactive oxygen species (ROS) in biological systems (Finkel and Holbrook, 2000). Approximately 10–15% of the total oxygen intake is consumed in uncatalyzed chemical oxidation or by a variety of oxygenases and oxidases and

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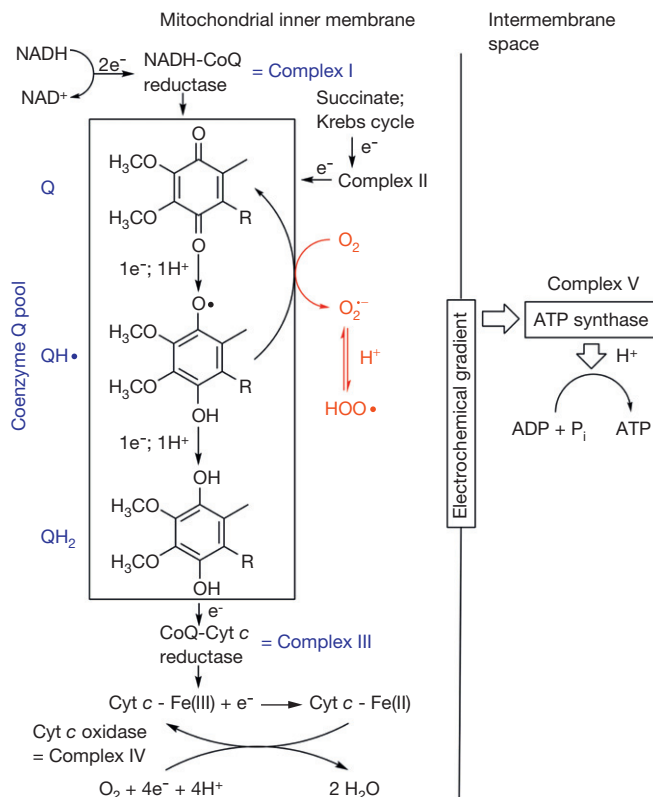
Figure 1.1 The mitochondrial electron-transport chain starts with the transfer of electrons from NADH to coenzyme Q (ubiquinone, CoQ) to form the reduced hydroquinone (CoQH$_2$): although the overall reaction involves the transfer of two electrons, the intermediate one-electron reduced form, ubisemiquinone radical (CoQH•), is also present. Reduction of CoQ by NADH is controlled by NADH: coenzyme Q reductase called complex I. CoQ also accepts electrons from reduced flavoproteins generated by the Krebs cycle (complex II, including succinate dehydrogenase) and oxidation of fatty acids. CoQH$_2$ in turn passes the electrons to coenzyme Q:cytochrome c reductase or complex III. Cytochromes are heme-proteins, and the electrons are used, one at a time, to reduce Fe^{III} to Fe^{II}: Cyt-Fe^{3+} + e$^-$ → Cyt-Fe^{2+}. The reaction then goes backward (to regenerate Cyt-Fe^{3+}) in the next step, when the electron is passed over to the multienzyme cytochrome c oxidase (complex IV) that uses e electrons to reduce one molecule of O$_2$ to two molecules of H$_2$O. The movement of electrons through the cascade also induces a migration of H$^+$ into the intermembrane space, and the energy associated to this electrochemical gradient is stored by ATP synthase (complex V) into ATP molecules. During the chain, electrons are transferred from CoQH• to O$_2$ to form superoxide radicals.

not used for energetic metabolisms (in the mitochondria). This is a very relevant source of ROS, particularly the P450 superfamily of monooxygenase.

Cytochrome P450 enzymes are involved in the oxidation of several compounds, in–cluding xenobiotics, and their expression is induced by the xenobiotics themselves, such as ethanol. Similar to cytochrome c, their operation, represented in Figure 1.2, is not
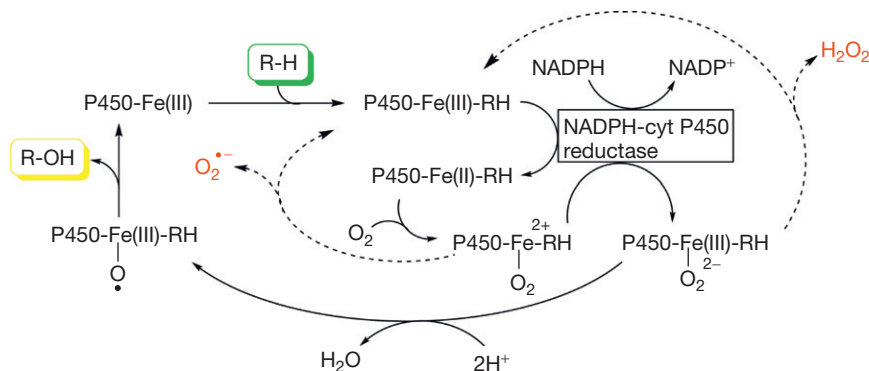
Figure 1.2 Hydroxylation of an organic substrate RH by P450 oxygenases. ROS production occurs as a side event.

error–proof and can lead to the formation of superoxide and hydrogen peroxide (H_2O_2). Hydrogen peroxide and superoxide radicals are also formed by several cytosolic oxidases, whose primary task appears to be indeed the formation of such species, which serve as both chemotactic factors and chemical messengers in a multitude of redox–sensitive regulatory processes within the cell.

As part of the inflammatory process, organic peroxides (ROOR) and hydroperoxides (ROOH) are formed in the arachidonic acid cascade. A very relevant source of oxidative stress comes from Fenton–type chemistry (Eq. 1.1), which occurs spontaneously to hydrogen peroxide and organic hydroperoxides in the presence of transition metal ions such as Fe^{2+} and Cu^+ in solution, and leads to the formation of hydroxyl (HO•) and alkoxyl (RO•) radicals. Ionizing radiations or photochemical reactions in skin exposed to sunlight can be an additional source of reactive species.

$$Fe^{2+} + H_2O_2(\text{or ROOH}) \rightarrow Fe^{3+} + HO^- + HO^{\bullet}(\text{or } HO^- + RO^{\bullet}) \qquad (1.1)$$

1.1 ROS, Reactive Nitrogen Species (RNS), and Free Radicals Involved in Oxidative Stress

Radicals have an unpaired electron in their outer (valence) electronic shell, which normally makes them highly unstable and reactive. They might be free radicals, that is, neutral, without a counterion, or radical ions (anion or cation). They may or may not be oxidizing species; this depends on their redox potential, on the reactivity of other molecules in the surroundings, and on the environment. ROS comprise both radical and molecular oxygen metabolites involved in oxidative damage to biomolecules, particularly superoxide radical ($O_2\bullet^-/HOO\bullet$), peroxyl radicals (ROO•), hydroxyl radicals (HO•), alkoxyl radicals (RO•), hydrogen peroxide (H_2O_2), alkyl hydroperoxides (ROOH), organic peroxides (ROOR), and hypochlorite (ClO^-). In addition to

ROS, other compounds involved in cellular redox homeostasis and signaling are the so-called reactive nitrogen species (RNS). These include nitric oxide or nitrogen monoxide (NO•), nitrogen dioxide (NO_2•), peroxynitrite ($ONOO^-$), alkyl peroxynitrite (ROONO), and nitroxyl anion (NO^-), among others, all originating from NO•, which in turn is mainly produced by nitric oxide synthase enzymes, being predominantly a chemical messenger rather than a harmful species under physiologic conditions.

$$O_2{}^{\bullet-} + H^+ \rightleftharpoons HOO^{\bullet} \tag{1.2}$$

The superoxide radical anion ($O_2{}^{\bullet-}$) is the prevailing form of superoxide in water at neutral pH (Eq. 1.2). It is a relatively persistent radical species, with limited reactivity toward biomolecules and a modest oxidizing character. Indeed, the standard redox potential of the redox couple $O_2/O_2{}^{\bullet-}$ (−0.3 V vs. SHE at pH 7.0) suggests that it can, instead, reduce free and most chelated Fe^{3+} (e.g., Fe^{3+}-citrate, Fe^{3+}-ADP or Fe^{3+}-cytochrome *c*) to the ferrous (Fe^{2+}) species. Its modest reactivity is paradoxically the main reason for its importance in oxidative stress, as it allows this species to diffuse at relatively long distance from the site of origin and act as a chemical messenger, influencing a multitude of redox-regulated processes. Conversely, its neutral form (HOO•), which may predominate at lower pH ($pK_a = 4.8$) or locally, in the proximity of a carboxylic group (COOH, e.g., in proteins), possesses a far higher reactivity and oxidizing ability, similar to peroxyl radicals.

At the opposite end of the reactivity scale, among the radical species found in biological systems, HO• and RO• radicals have largely unselective behavior, being able to attack almost any biomolecule found in the proximity of their site of generation. These species, formed predominantly by Fenton-type chemistry (Eq. 1.2), by radiolysis of water, or by photolysis of peroxides and hydroperoxides, commonly react by H–atom abstraction from a CH moiety (e.g., from lipids) or by addition to CC double bonds. The resulting carbon-centered radicals, under aerobic conditions, will react at near-diffusion controlled rates with oxygen to form a peroxyl radical, the main protagonis of oxidative stress. Peroxyl radicals are electron-poor highly reactive species that, more often, attack biomolecules by H–atom abstraction from OH, SH, and CH functions. Unlike HO•, they are quite selective and attack only specific molecular sites.

1.2 Oxidative Damage to Biomolecules

1.2.1 *Lipid peroxidation*

The main ROS-related damage to lipids is lipid peroxidation, a radical-chain reaction mediated by peroxyl radicals. Several radical species including HO•, HOO•, RO•, and ROO• can act as initiating species by attacking unsaturated fatty acid residues and abstracting a hydrogen atom in the allylic (or *bis*-allylic) position to yield the corresponding alkyl (C-centered) radical, which will rapidly add molecular oxygen to form a lipid-peroxyl radical. This, in turn, will abstract a hydrogen atom from another lipid molecule to produce the corresponding hydroperoxide and another lipid-peroxyl radical. Hence,
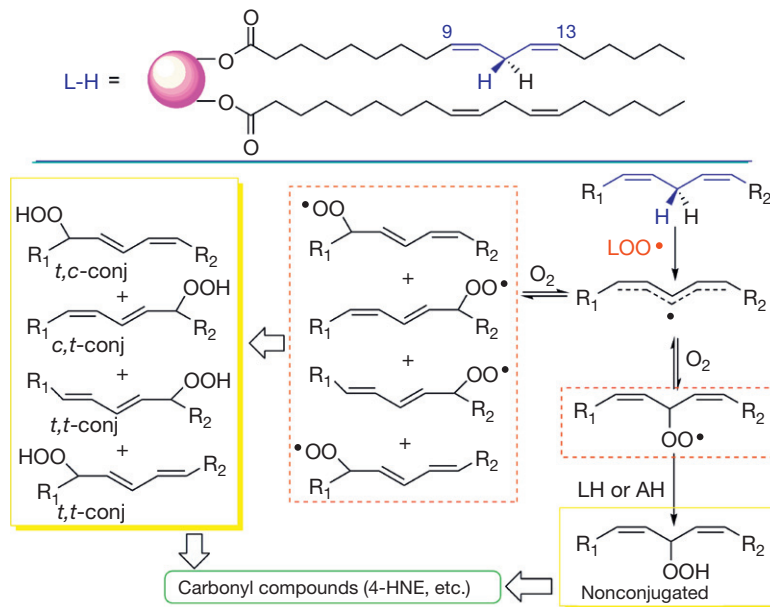
Figure 1.3 Formation of hydroperoxides during lipid peroxidation.

peroxyl radicals are formed cyclically, propagating the oxidative chain, and leading to the progressive oxidation of the substrate (the lipid) until two peroxyl radicals quench each other in a termination step, or an antioxidant stops the radical chain. In this process, a variety of isomeric hydroperoxides are formed (Figure 1.3), these, in turn, can react with metal ions to originate new radical species, or they can decompose (spontaneously or enzymatically) to form reactive carbonyl compounds (such as 4–hydroxynonenal) that, being potent electrophiles, will attack proteins and DNA, expanding the biological damage.

1.2.2 Oxidative damage to proteins

RNS such as peroxynitrite can attack aromatic amino acids such as tyrosine and phenyl-alanine in proteins causing nitration. Hydroxyl and alkoxyl radicals attack several amino acids yielding a multitude of by-products. Peroxyl radicals might attack the alpha CH position of any amino acid and/or the backbone of some side chains to form, in the presence of oxygen, aminoacyl–peroxyl radicals that will propagate the oxidative chain, similarly to what happens in lipid peroxidation, resulting in the formation of aminoacyl-hydroperoxides. These, in turn, might be decomposed by metal ions to generate hydroxyl radicals that will produce further chemical attack. Cysteine SH function is particularly sensitive to oxidation, and forms thiyl radicals (S•) that recombine to disulfides SS. Methionine is also easily oxidized to the corresponding sulfoxide and sulfone. Depending on the original role of the protein, these modifications might produce structural or functional alteration within the cell. Particularly, enzyme activity will be

compromised in case the modified amino acids are close to the enzyme active site or have a role in its catalytic activity. Similarly, receptors might be functionally altered by attack on the amino acids involved at the binding site. However, due to protein unfolding, even modification of aminoacyl residues far from the active/binding site might result in significant biological damage.

1.2.3 Oxidative damage to DNA

Purine and pyrimidine bases are quite resistant to attack by several ROS (e.g., ROO•, ROOH, $O_2^{\bullet-}$, and H_2O_2); however, they easily react with HO• radicals to yield a number of chemically modified bases (Figure 1.4), arising predominantly by addition of HO• in 4, 5, or 8 position in the purine ring, and in 5 or 6 position in the pyrimidine ring. Hydroxyl or peroxyl radicals can also attack the sugar moiety, producing sugar peroxyl radicals that will undergo subsequent reactions, including dehydration and CC bond
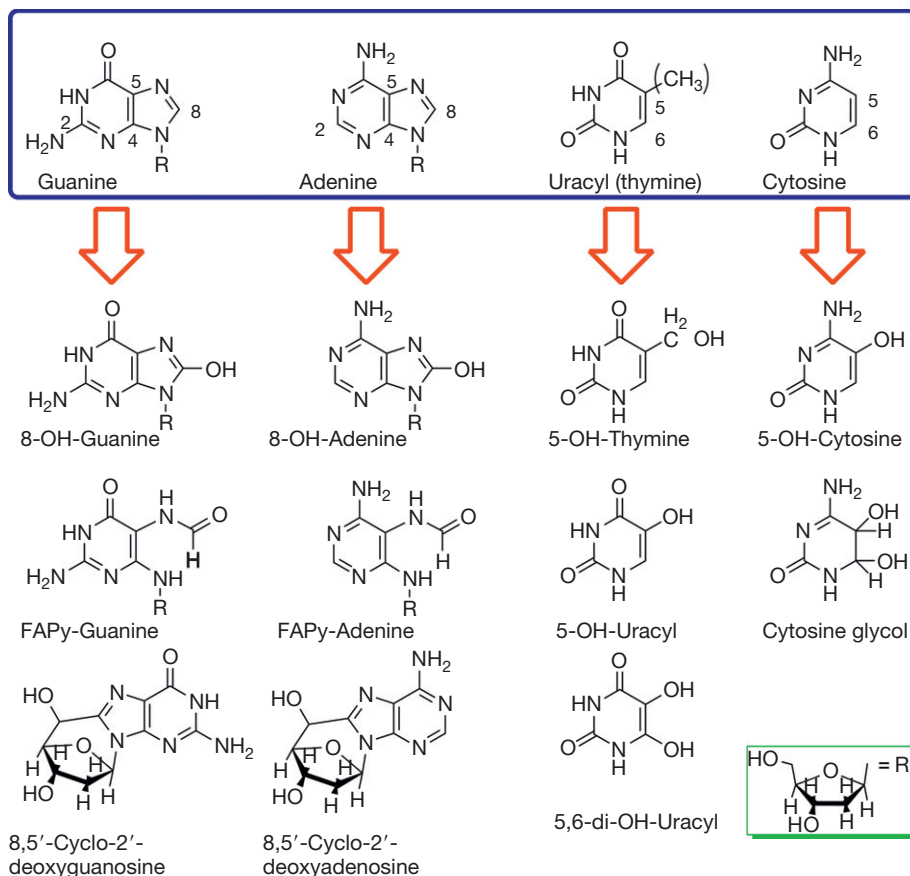
Figure 1.4 Some chemically modified DNA/RNA bases formed by reaction with ROS.

cleavage, to yield a variety of carbonyl products. In some cases, this could result in single- or double-strand cleavage. Nuclear proteins that normally protect DNA from radical attack can also be attacked by radicals and the resulting protein–derived radicals can cross-link to sugar-derived radicals, producing DNA–protein cross-links. The consequence of radical DNA damage largely depends on the efficiency of DNA repair systems, but clearly can lead to cell death, or mutations and cancer.

2. ANTIOXIDANT DEFENSES

2.1 Classification of Antioxidants

Antioxidants share the common task of lowering the concentration of oxygen-centered radicals, particularly peroxyl radicals, which are the chain-carrying species of lipid peroxidation and autoxidation of organic substrates. Due to its importance, lipid peroxidation is chosen as the model reaction to classify and evaluate antioxidants. *Direct antioxidants* are those small molecules or enzymes capable of impairing lipid peroxidation. Based on their mechanism of interference, direct antioxidants can be further classified as preventive or chain-breaking antioxidants, as illustrated in Scheme 1.1.

2.1.1 Preventive antioxidants

Preventive antioxidants are unable to stop the chain reaction carried on by peroxyl radicals; however, they impair lipid peroxidation by preventing the initiation event. This can be obtained in different ways.

UV filters, like melanin in human skin, prevent the photochemical decomposition of peroxides or that of other moderately light-stable biomolecules.

Metal deactivators chelate redox-reactive metal ions, particularly copper and iron, keeping them out of the solution or in a less reactive form, thereby preventing Fenton-type chemistry and the generation of HO• and RO• radicals. Ceruloplasmin and transferrin are examples of biological antioxidants of this kind, while curcumin and phytic acid are dietary examples of such antioxidants.

Peroxide decomposers act by decomposing hydrogen peroxide, hydroperoxides, or superoxide radicals via non-radical paths, that is, preventing their decomposition in radical species that would initiate the chain reaction. Catalase, glutathione peroxidase, superoxide dismutase (SOD), and several small molecules to large macromolecules containing sulfur or selenium in the reduced state can act with this mechanism.

2.1.2 Chain-breaking antioxidants

Chain-breaking antioxidants are arguably the most important and effective antioxidants as they inhibit or retard the autoxidation by quenching chain-carrying peroxyl radicals, that is, they interfere with the propagation event. Phenols like α-tocopherol (vit. E), or dietary flavonoids, as well as ubiquinol ($CoQH_2$) and ascorbic acid (vit. C), belong
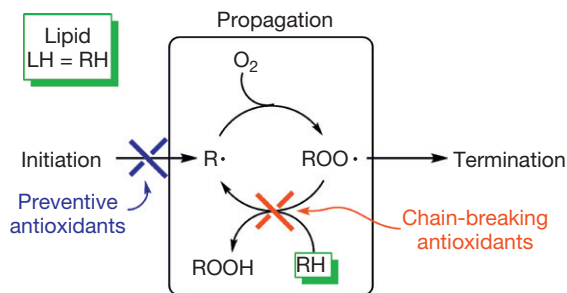
Scheme 1.1 Lipid peroxidation and antioxidants.

to this class. In order to inhibit chain propagation, these antioxidants react with peroxyl radicals much faster than they would react with the lipid molecule, to yield a stabilized phenoxyl (or ascorbyl) radical that is unable to propagate the chain reaction, being sufficiently unreactive and long-lived to wait for a second peroxyl radical. Therefore, one molecule of antioxidant is normally capable of quenching two peroxyl radicals. The main reactions involved are illustrated by Eqns. (1.3) and (1.4) for α-tocopherol.
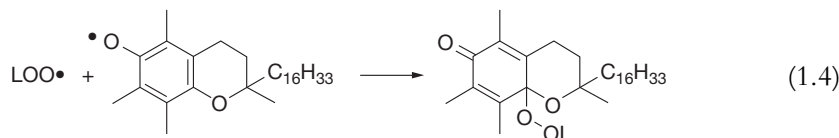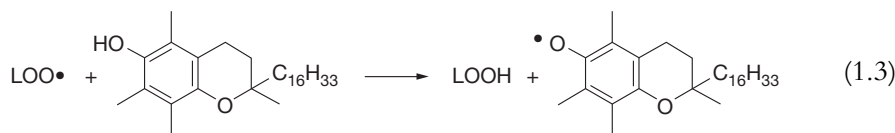
The reaction of chain-breaking antioxidants with peroxyl radicals occurs by formal hydrogen atom transfer from the reactive moiety (e.g., the phenolic OH in phenolic antioxidants) to ROO•. Therefore, their antioxidant activity depends on the dissociation enthalpy of the reactive OH bond: the lower the enthalpy, the higher is the antioxidant activity. The presence of electron-donating groups or unsaturated carbon chains in conjugated positions in the phenolic ring weakens the OH bond. In flavonoids, the catechol ring is a privileged structural feature (Eq. 1.5) and the actual active portion of the molecule, particularly in the case where the unsaturated system is extended as in flavonols or in cinnamic acids (see Figure 1.6).
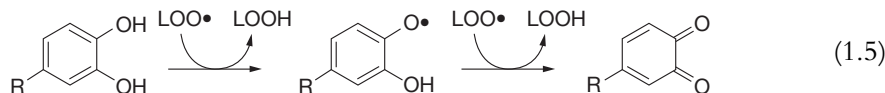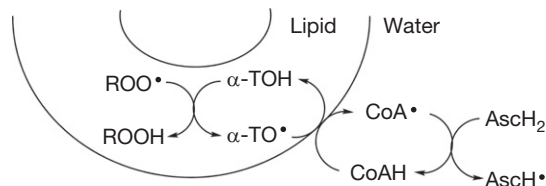
Figure 1.5 Synergic interplay of α-tocopherol (α-TOH), ascorbic acid (AscH$_2$), and dietary antioxidants (CoAH) in the protection of lipid membranes.

One important feature of chain-breaking antioxidants is that, when used in combination or in a mixture, they may display synergistic behavior, that is, they may have antioxidant activity significantly higher than that expected from the sum of individual contributions. This is due to recycling of the main or most active antioxidant by the other coantioxidant (s), in a similar fashion to the well-known behavior of vitamins E and C in biological systems (Amorati et al., 2003). The process is illustrated in Figure 1.5. Indeed, water-soluble dietary antioxidants such as flavonoids could act in synergy with α-tocopherol in the protection of lipid membranes and low-density lipoprotein (LDL).

2.1.3 Indirect antioxidants
Indirect antioxidants do not possess any appreciable antioxidant behavior in model solutions, that is, they are unable to efficiently quench peroxyl radicals, or their reaction with ROO• does not stop or retard the oxidative chain. Nonetheless, they decrease the oxidative stress in biologic systems and increase the resistance to oxidative insult by enhancing the antioxidant defenses. They can have different specific mechanisms, but they will ultimately increase the expression of the physiological antioxidant defenses, most often by inducing antioxidant enzymes, repair systems, or phase II detoxifying enzymes. Isothiocyanates (ITCs), derived from myrosinase hydrolysis of plant secondary metabolite glucosinolates (GLs), are the most notable example of this kind of dietary antioxidants, although it has been shown that many flavonoids could act in this way, as well as being chain-breaking antioxidants. Biliverdin reductases, quinone reductases, and glutathione reductases are examples of indirect physiological antioxidants because they increase the pool of active antioxidants. The main physiological antioxidants are summarized in Table 1.1.

2.2 Dietary Antioxidants
2.2.1 Structure and sources of dietary antioxidants
Most dietary antioxidants found in fruits and fresh vegetables are phenolics. Simple phenolic acids (e.g., gallic or cinnamic derivatives) can be found either as such or as acylating moieties connected to flavonoids. These, in turn, are polyphenolic compounds comprising a 15-carbon core, the aglycone, often glycosilated with one to several glycoside units

Table 1.1 Main Physiological Antioxidants and Their Role

Antioxidant	Mechanism	Reaction/function
Transferrin	Preventive	Binds Fe ions keeping them in unreactive form
Ceruloplasmine	Preventive	Binds Cu ions keeping them in unreactive form
Glutathione peroxidases (GPx)	Preventive	Reduce H_2O_2 and ROOH to H_2O and ROH at the expense of glutathione (GSH), which is oxidized to the GS-SG form
Superoxide dismutases (Mn-SOD and Cu, Zn-SOD)	Preventive	Dismutate superoxide radical to hydrogen peroxide and oxygen $(2O_2^{\bullet-} + 2\,H^+ \rightarrow H_2O_2 + O_2)$
Catalases (CAT)	Preventive	Dismutate H_2O_2 to H_2O and O_2 $(2H_2O_2 \rightarrow H_2O + O_2)$
Catalase peroxidases (KatGs)	Preventive	Dismutate H_2O_2 to H_2O and O_2 $(2H_2O_2 \rightarrow H_2O + O_2)$
NAD(P)H:quinone oxidoreductases (NQOR)	Preventive; indirect	Reduce oxidized coenzyme Q to the reduced form QH_2, preventing the formation of superoxide and increasing the pool of active antioxidants
Glutathione reductases (GR)	Indirect	Reduce oxidized glutathione GS-SG to the active form GSH
Thioredoxin reductases (TR)	Indirect	Reduce oxidized thioredoxin TS-ST to the active form TS-H
Biliverdin reductase	Indirect	Reduces biliverdin to bilirubin
Heme-oxygenases (HO-1 and HO-2)	Preventive; indirect	Convert prooxidant heme to biliverdin, precursor of antioxidant bilirubin
Bilirubin	Chain-breaking	Quenches ROO• radicals and other ROS
Thioredoxin	Chain-breaking; preventive	Quenches ROO• radicals and other ROS; modulates NF-κB and AP-1 signaling; inhibits ASK-1
Glutathione	Chain-breaking; preventive	Quenches ROO• radicals to ROOH and reduces H_2O_2 and ROOH to H_2O and ROH
Vitamin E	Chain-breaking	Quenches ROO• radicals to ROOH and quenches other ROS
Vitamin C	Chain-breaking	Quenches ROO• radicals to ROOH and quenches other ROS; regenerates vitamin E
Coenzyme Q	Chain-breaking	Quenches ROO• radicals to ROOH and quenches other ROS; regenerates vitamin E

(Crozier et al., 2009). Flavonoids are classified according to the aglycone structure, and the main structures of dietary interest are illustrated in Figure 1.6 and listed in Table 1.2. Nonphenolic dietary antioxidants comprise ascorbic acid, ITCs (Valgimigli and Iori, 2009), and sulfenic acids (McGrath et al., 2010).
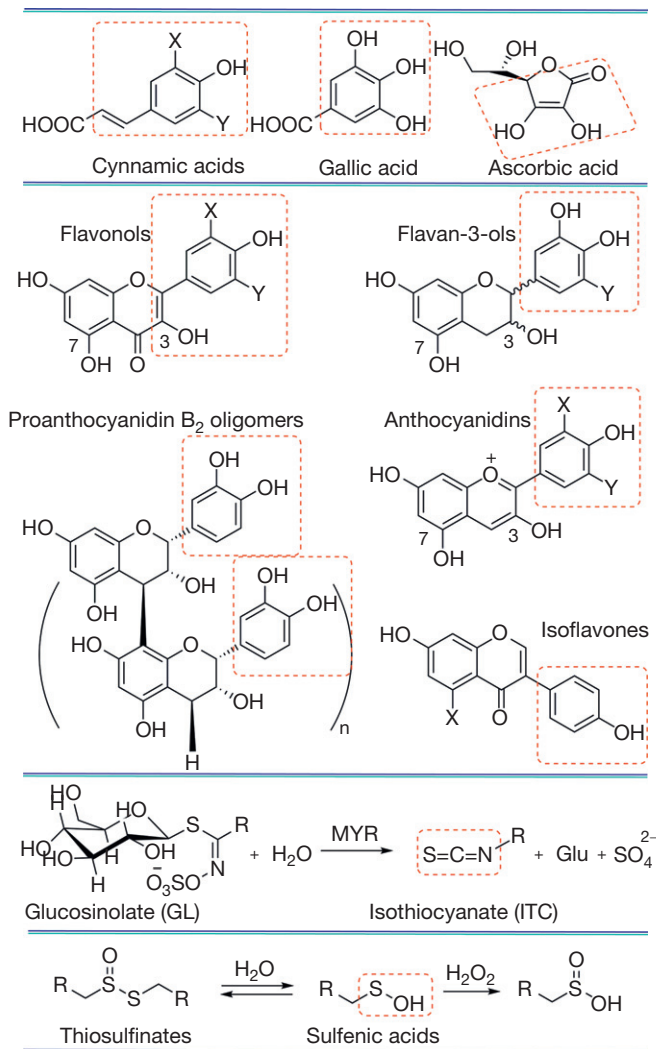
Figure 1.6 General structures of main classes of antioxidants. From top to bottom: organic acids, flavonoids, isothiocyanates, and sulfenic acids. The active moiety is squared.

2.2.2 Bioavailability of dietary antioxidants

Absorption of flavonoids occurs predominantly in the small intestine, typically by passive diffusion following hydrolysis of the aglycone in the brush border of intestinal cells, operated by broad substrate hydrolases. Active transport by sodium–dependent glucose transporters, due to the presence of the glycoside residues, has also been suggested. In this less relevant case, hydrolysis occurs later by cytosolic β-glucosidases. Prior to passage into systemic circulation, aglycones undergo metabolism, forming sulfates, glucuronides,

Table 1.2 Main Dietary Antioxidants, Sources, and Mechanisms (for Structures Refer to Figure 1.6)

Class	General structure	Examples	Main dietary sources	Antioxidant mechanism
Vitamin E	See Eq. (1.3)	α-Tocopherol, α-tocotrienol	Wheat germ, barley, nuts, grains	Chain-breaking (lipid-soluble)
Vitamin C	See Figure 1.6	Ascorbic acid	Fruits (citrus, berries), *Rosa canina* L., *Brassicaceae*	Chain-breaking (water-soluble)
Cinnamic acids	See Figure 1.6	Ferulic acid ($XOCH_3$; YH); Caffeic (XOH; YH)	*Brassicaceae*, berries, green tea, cocoa, coffee	Chain-breaking; indirect: ↑Nrf2 (ARE)
Gallic acid	See Figure 1.6	Gallic acid and gallyl glycosides	Green tea	Chain-breaking; indirect: ↑Nrf2 (ARE)
Flavan-3-ols	See Figure 1.6	(−)-Epicatechin-glycosides (+)-Catechin-glycosides Epigallocatechin gallate	Green tea, cocoa, coffee, red wine, French beans	Chain-breaking; indirect:↓NF-κB, ↓AP-1, ↑MAPK (ERK, JNK, p38)
Flavonols	Aglycones: Quercetin (XOH; YH); Kaempferol (XYH); ($XOCH_3$; YH); Myricetin (XYOH)	Quercetin-3-O-glycosides; Kaempferol-3-O-glycosides; Isorhamentin-3-O-glycosides; Myricetin-3-O-glycosides	Green tea, *Brassicaceae*, tomato, onions, fruits (peaches, apples)	Chain-breaking; indirect: ↓AP-1, ↑MAPK (ERK, JNK, p38); ↑Nrf2 (ARE)
Flavanones	Like flavan-3-ol without OH in 3	Naringenin-7-O-glycosides; hesperidin-7-O-glycosides	Citrus fruits (orange, grapefruit, lemon, lime)	Chain-breaking; indirect: ↑Nrf2 (ARE)
Anthocyanins	Mono-, di-, up to penta-glycosides or acylated glycosides of aglycones: pelargonidin (XYH), cyanidin (XOH; YH),	Malvidin-3,5-di-O-glucoside; Malvidin-3-O-(6″-O-acetyl) glucoside; Cyanidin-3-O-diglucoside-7-O-glucoside; Cyanidin-3,7-	Fruits (berries, grape, plums, apples, pears, cherries), red onion, red radishes, red cabbage,	Chain-breaking; indirect: ↑Nrf2 (ARE)

Continued

Table 1.2 Main Dietary Antioxidants, Sources, and Mechanisms (for Structures Refer to Figure 1.6)—cont'd

Class	General structure	Examples	Main dietary sources	Antioxidant mechanism
	delfinidin (XYOH), peonidin (XOCH$_3$; YH), malvidin (XYOCH$_3$), etc.	di-O-glucoside; Pelargonidin-3,7-di-O-glucoside; cyanidin-3,4′-di-O-glucoside; cyanidin-3-O-glucoside	eggplant, legume peel	
Proanthocyanidins	Dimers ($n=1$), trimers ($n=2$), oligomers (up to $n=50$) of flavan-3-ols or anthocyanins	Proanthocyanidin B2 dimer, trimer, tetramer, pentamer; prodelphinidins	Black tea, maritime pine, fruits (apples, berries), oak (wine barrels)	Chain-breaking; indirect: ↑Nrf2 (ARE)
Isoflavones	See Figure 1.6	Genistein (XOH); daidzein (XH)	Legumes (soy)	Chain-breaking; indirect: ↑Nrf2 (ARE), estrogen-like
Tannins	Polymers of phenolic acids (e.g., gallic) and sugars, or of other polyphenols	Tannic acid	Grape peel and red wine, black tea, woods	Preventive (metal-chelating); chain-breaking
Curcumin	1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	Curcumin	Zingiberaceae	Preventive (metal-chelating); chain-breaking; indirect: ↓NF-κB, ↓AP-1, ↑Nrf2 (ARE)
Phytates	Inositol hexakisphosphate	Phytic acid	Cereal bran	Preventive (metal-chelating)
Isothiocyanates	See Figure 1.6	Erucin, sulforaphane, sulforaphene	Released from corresponding GL contained in Brassicaceae (e.g., Eruca, Raphanus, Brassica genera)	Indirect: ↑Nrf2 (ARE); preventive (peroxides decomposers)

Continued

Table 1.2 Main Dietary Antioxidants, Sources, and Mechanisms (for Structures Refer to Figure 1.6)—cont'd

Class	General structure	Examples	Main dietary sources	Antioxidant mechanism
Thiosulfinates	See Figure 1.6	Allicin (RCH_2CH)	*Alliaceae* (e.g., garlic, onions, shallots, leeks)	Release of sulfenic acids that are chain–breaking and preventive, and indirect antioxidants

and/or methylated metabolites. In some cases, metabolites are subjected to phase II enzymes and/or might undergo enterohepatic recycling. Excretion occurs with urine within 24 h and no evidence for accumulation/storage is so far available.

Flavonols, in the form of *O*-glucuronides or *O*-sulfates, are retrieved in plasma in less than 1 h with a half–life of 2–5 h, and only about 5% of the intake is found in urine. The majority is converted into phenolic acids by intestinal flora in the colon (Figure 1.7), followed by their absorption (corresponding to about 20% of the flavonol intake). The bioavailability of intact aglycone largely depends on the glycosylation pattern. Flavan–3–ols are the only exception for which intact glycosilated compounds are found in the bloodstream following ingestion; however, their levels are modest, and most of the compounds are found as glucuronides or sulfates. Acylation with gallic acid increases the bioavailability, which can exceed 50% of the intake. Phenolic acids, in general, have very large bioavailability, with about 40% recovery in urine. Anthocyanins have more modest bioavailability for the intact aglycone, but they are mostly converted into phenolic acids before absorption. Bioavailability of proanthocyanidins depends on the molecular size, and they have been detected only up to pentamer size in the blood following intake of very large doses. Peak plasma levels for dietary flavonoids or their metabolites, following a fruit/tea–rich meal may span from the low nM range up to ≈ 1 μM depending on the compound and the dietary source (Crozier et al., 2009).

Allicin is readily absorbed through the intestine and partly decomposes in the stomach to yield sulfenic acids, disulfides, and other volatile lipophilic derivatives. Allicin is rapidly metabolized and/or spontaneously decomposed to a variety of metabolites in liver, blood, and other tissues, so studies failed to detect it in the blood even after abundant ingestion of garlic. Metabolites are excreted with breath, sweat, and urine.

Human tissues contain no significant enzymatic activity to convert inactive GLs into bioactive ITCs, and only GLs are contained in fresh vegetables. Hence, the bioavailability of dietary ITCs largely relies on myrosinase activity available in the vegetable source: if myrosinase has been inactivated (e.g., by cooking), intestinal microbial metabolism of GLs can still contribute. Following passive absorption, ITCs are conjugated with thiols
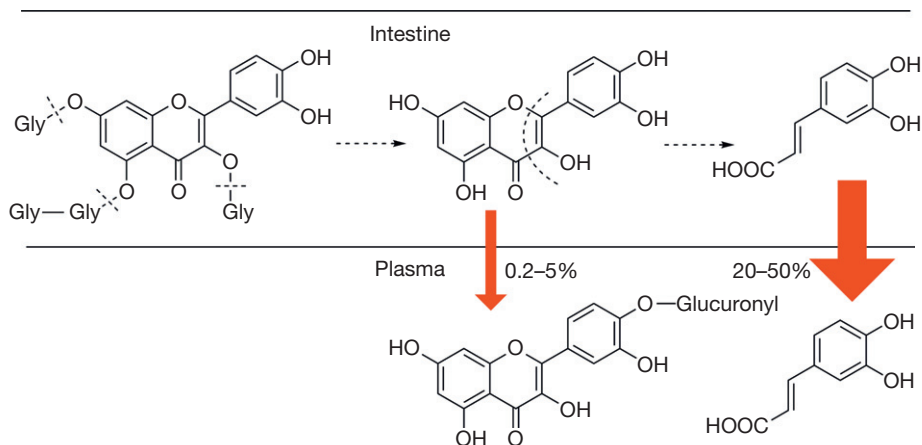
Figure 1.7 Intestinal uptake and metabolism of flavonoids.

such as glutathione (GSH) and protein SH residues in plasma, and only less than 1% remains in the unconjugated form. ITCs are metabolized principally by the mercapturic acid pathway, and excreted in urine as *N*-acetylcysteine–ITC conjugates. Human volunteers treated with a single dose of broccoli extract containing GLs (∼200 mmol, mainly glucoraphanin) and intact myrosinase activity had plasma peak levels of the corresponding ITCs at about 1–2 μM after 1.25 h with a half-life of 1.8 h. Urinary excretion of ITCs' metabolites corresponded to about 55–61% of GL intake in 8 h. In comparison, dietary intake of broccoli or other GL-rich vegetables can result in urinary excretion of ITCs' metabolites ranging from 1–8% of GL content for cooked vegetables to 17–77% for uncooked vegetables. The large variability depends particularly on the individual intestinal flora (Valgimigli and Iori, 2009)

3. OXIDATIVE STRESS AND AGING

Among the many theories on the nature of aging, the so-called free radical theory suggests that ROS and RNS produced in both metabolic processes and as a consequence of exogenous (environmental) insults, being capable of attacking and altering macromolecules such as nucleic acids, proteins, lipids, and complex carbohydrates within the cell (or cell membrane), will progressively cause a loss of functionality in the entire biological system, constituting the baseline of aging (Finkel and Holbrook, 2000). According to this view, the role of radicals and ROS in aging and longevity would be quite aspecific. However, the progressive discovery of the role of ROS in cell signaling has brought a deeper mechanistic understanding of the interplay among cellular redox balance, adaptation, senescence, and apoptosis. Indeed, the expression of over 100 proteins has been found to vary in response to redox stimuli (Finkel and Holbrook, 2000).

In some cases, such as in the induction of certain heat-shock proteins and in the activation of the ERK signaling pathway, the efficiency of such a response is attenuated with aging. As heat-shock proteins and ERK activation exert prosurvival signals in response to oxidative stress, their age-related attenuation has been interpreted as a mechanistic link between oxidative stress and aging. This is a very complex and important area of research that certainly deserves further efforts.

Mitochondrial membrane lipids and proteins are highly susceptible to oxidative damage. Furthermore, mitochondrial DNA is more sensitive to ROS than nuclear DNA because it is not protected by histone proteins. Hence, the mitochondrial function will progressively decline with exposure to ROS. The efficiency of the electron-transport chain progressively declines with aging and the generation of ROS is proportionally increased, establishing a vicious circle of oxidative stress and energetic decline. This lends support to the free radical theory of aging. On the other hand, it has also brought up the implicit expectation that oxidative stress increases with aging. Unlike a number of investigations on animal models, where single specific bio-indicators of oxidative stress were found to increase with aging, in a recent study on humans comparing a large group (188) of middle-aged healthy human subjects from France and the United Kingdom with a population (199) of older subjects from France and Italy, the effect of aging on OSS was assessed by the determination of TBARs, plasma thiols (SH), totalGSH, and the ferric reducing ability of plasma assay – all nonspecific indicators of the redox balance. Surprisingly, the results showed that oxidative stress was lower in older than in younger subjects (Andriollo-Sanchez et al., 2005). Clearly, the rate of metabolic activity is a major determinant of oxidative stress, as most of the ROS production comes as a side event of the mitochondrial production of ATP, and metabolic decline results in the decrease of oxidative stress despite the lower efficiency of the electron-transport chain. Apparently, this suggests that antioxidant supplementation in the elders may not be as useful and beneficial as in the young, where it will serve the purpose of counteracting the higher ROS generation associated with higher metabolic activity.

4. DIETARY ANTIOXIDANTS IN HEALTH PROMOTION AND CHRONIC DISEASE

It has been estimated that, *in vitro*, at least 1% of the total oxygen consumed in the mitochondria is transformed into $O_2^{\bullet-}$: if the same ratio is maintained *in vivo* for an adult of 70 kg of body weight, it corresponds to a production of superoxide of about 1.7 kg year^{-1}. Even in the case that ROS production *in vivo* is lower, this estimate suggests that our cells are unavoidably and continuously subjected to a massive attack from radicals and other oxygen metabolites, and their ability to survive and maintain functionality is totally dependent on the availability and effectiveness of antioxidant defenses and repair systems. It is not surprising that dietary antioxidants have attracted the attention as aids for general health promotion and modulation of aging. This idea has received support from evidence for oxidative stress involvement in the

pathophysiology of several chronic diseases, from cancer to neurologic and cardiovascular disorders, although the actual causal interplay between oxidative stress and pathology remains to be clarified in most cases.

A number of studies *in vitro* and in the animal model have so far documented that specific antioxidant therapy or supplementation with dietary antioxidants is beneficial for chronic diseases, for example, in inflammatory colitis, type-2 diabetes, Alzheimer's and Parkinson's diseases, and mutation and cancer development. In some cases, it can dramatically extend the lifespan (Melov et al., 2000). These studies have been stimulated by robust epidemiological evidence that a higher dietary intake of antioxidants and/or increased consumption of fruit and fresh vegetables are associated with a lower incidence of several nontransmissible diseases, particularly cardiovascular disease, cancer, diabetes, and neurological decline (Knekt et al., 2002). Similar evidence was obtained in cohort/observational studies where the dietary intake of specific antioxidant vitamins/provitamins, namely, β-carotene, retinol (vit. A), α-tocopherol (vit. E), and ascorbic acid (vit. C), was found to correlate with a lower incidence of cancer (stomach, breast, prostate, and lungs), arteriosclerosis, coronary heart disease (CHD), stroke, hypertension, cataracts, and macular degeneration (Fairfield and Fletcher, 2002). However, controlled clinical trials, trying to assess the benefits of diet supplementation with such specific vitamins in primary or secondary prevention, have generally produced disappointing results. Most controlled trials showed no beneficial properties of supplementation with α-tocopherol, ascorbic acid, or β-carotene on hypertension, mortality from cardiovascular diseases, and cataracts (Huang et al., 2006). Concerning cancer, no benefits have been recognized for α-tocopherol or ascorbic acid, while β-carotene was found to increase mortality from lung cancer in smokers and asbestos workers, and no beneficial activity was recorded in other cases (Myung et al., 2010). Only supplementation with selenium and with the combination of β-carotene, α-tocopherol, ascorbic acid, selenium, and zinc (LINXIAN and SUVIMAX studies) was found to moderately decrease cancer incidence and mortality (Bardia et al., 2008; Huang et al., 2006).

Interestingly, with the exception of β-carotene, several trials conducted over the years have not evidenced any significant toxicity associated with long-term dietary supplementation with the investigated vitamins/minerals, which is noteworthy on its own, considering that doses as high as 40-fold the recommended daily allowance have been used in some cases. Nevertheless, some investigators have discouraged supplementation with such vitamins/minerals and extended the warning to all antioxidants, due to the lack of strong clinical evidence for their beneficial role.

Despite the value of such controlled clinical trials, some design limitations should not be overlooked (Paolini et al., 2003).

4.1 Selection of Antioxidants

As illustrated, fruits, vegetables, and antioxidant–rich food contain an extraordinary variety of different antioxidants with different structures, bioavailabilities, and mechanisms.

α-Tocopherol and vit. C often accounts for less than 1% of the total antioxidant content. β-Carotene, on the other hand, has been shown to have no significant antioxidant activity in model systems and to be pro-oxidant and procarcinogenic *in vitro* (Paolini et al., 1999). The beneficial role of antioxidant-rich food is therefore not represented by high-dose supplementation of such single compounds. Intake of β-carotene might have been an arbitrary and misleading marker for antioxidant intake in observational studies, where flavonoid or ITC intake would also have been found to negatively correlate with the incidence of chronic diseases. Indeed, observational studies where the dietary intake of flavonoids was evaluated showed strong correlation with lower incidence and mortality associated with CHD, cerebrovascular disease, asthma, lung cancer, and prostate cancer (Knekt et al., 2002). However, no clinical trial has investigated flavonoid supplementation.

4.2 Combination of Antioxidants

The majority of clinical trials have assessed supplementation with single molecules, at variance with healthy food composition, comprising dozens to hundreds of antioxidant molecules. Antioxidants act in a synergistic manner to prevent/counteract damage by ROS. Highly lipid-soluble α-tocopherol, confined in the lipid core of LDL or biomembranes, is unable to provide protection from attack occurring in the aqueous compartments; indeed, when used *in vitro* to protect LDL in the absence of adequate amounts of vit. C or coantioxidants, it is known to cause so-called tocopherol-mediated peroxidation, in place of protection (Bowry et al., 1992). Apparently, the physicians' wisdom to recommend a diet as varied as possible has not driven the design of most trials. Even those involving a combination of vitamins/minerals have dealt with a limited number of molecules, which have been combined without consideration of their mechanism or possible synergism. It is well known that tocopherol gives no synergism with β-carotene – the most investigated combination in trials – while it gives complete synergism with catechols such as flavonoids or phenolic acids, a combination that would be found in standardized vegetable extract(s). The combination of chain-breaking (e.g., flavonoids) and indirect (e.g., ITCs) antioxidants would also appear to be very promising (Valgimigli and Iori, 2009).

GLOSSARY

Chain-breaking antioxidants Compounds or systems capable of impairing lipid peroxidation by quenching chain-carrying peroxyl radicals and interrupting chain propagation.
Chain reaction A chemical reaction sustained by a reactive intermediate, which is produced cyclically for several cycles, during the reaction. It is divided into initiation, propagation, and termination steps.
Indirect antioxidants Compounds or systems unable to impair lipid peroxidation in model solutions, but capable of enhancing the overall antioxidant defenses in a living system.
Preventive antioxidants Compounds or systems capable of impairing lipid peroxidation by preventing the formation of the initiating radical species.

Radical species A chemical species bearing an unpaired electron in the outer (valence) atomic or molecular electronic shell.

Synergic coantioxidants A mixture of antioxidants whose performance in the inhibition of lipid peroxidation is significantly higher than that expected from the sum of the individual contribution from mixture components.

Tocopherol–mediated peroxidation The pro-oxidant activity shown by α-tocopherol in the absence of synergic coantioxidants during lipid peroxidation in low-density lipoproteins.

REFERENCES

Amorati, R., Ferroni, F., Pedulli, G.F., Valgimigli, L., 2003. Modeling the co-antioxidant behavior of monofunctional phenols. Applications to some relevant compounds. Journal of Organic Chemistry 68, 9654–9658.

Andriollo-Sanchez, M., Hininger-Favier, I., Meunier, N., et al., 2005. Age-related oxidative stress and antioxidant parameters in middle-aged and older European subjects: the ZENITH study. European Journal of Clinical Nutrition 59, S58–S62.

Bardia, A., Tleyjeh, I.M., Cerhan, J.R., et al., 2008. Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis. Mayo Clinic Proceedings 83, 23–24.

Bowry, V.W., Ingold, K.U., Stocker, R., 1992. Vitamin E in human low-density lipoprotein. When and how this antioxidant becomes a pro-oxidant. Biochemical Journal 288, 341–344.

Crozier, A., Jaganath, I.B., Clifford, M.N., 2009. Dietary phenolics: chemistry, bioavailability and effects on health. Natural Product Reports 26, 1001–1043.

Fairfield, K.M., Fletcher, R.H., 2002. Vitamins for chronic disease prevention in adults. Scientific review. Journal of the American Medical Association 287, 3116–3126.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Halliwell, B.A., Gutteridge, M.C., 1999. Free radicals in Biology and Medicine, third ed. Oxford University Press, New York.

Huang, H.-Y., Caballero, B., Chang, S., et al., 2006. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a national institutes of health. Annals of Internal Medicine 145, 372–385.

Knekt, P., Kumpulainen, J., Järvinen, R., et al., 2002. Flavonoid intake and risk of chronic diseases. American Journal of Clinical Nutrition 76, 560–568.

McGrath, A.J., Garrett, G.E., Valgimigli, L., Pratt, D.A., 2010. The redox chemistry of sulfenic acids. Journal of the American Chemical Society 132, 16759–16761.

Melov, S., Ravenscroft, J., Malik, S., et al., 2000. Extension of life-span with superoxide dismutase/catalase mimetics. Science 289, 1567.

Myung, S.-K., Kim, Y., Ju, W., Choi, H.J., Bae, W.K., 2010. Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials. Annals of Oncology 21, 166–179.

Paolini, M., Cantelli-Forti, G., Perocco, P., Pedulli, G.F., Abdel-Rahman, S.Z., Legator, M.S., 1999. Co-carcinogenic effect of beta-carotene. Nature 398, 760–761.

Paolini, M., Sapone, A., Canistro, D., Chieco, P., Valgimigli, L., 2003. Antioxidant vitamins for the prevention of cardiovascular disease. Lancet 362, 920.

Valgimigli, L., Iori, R., 2009. Antioxidant and pro-oxidant capacities of ITCs. Environmental and Molecular Mutagenesis 50, 222–237.

FURTHER READING

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.D., Gluud, C., 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. Systematic review and meta-analysis. Journal of the American Medical Association 297, 843–857.

Bowry, V.W., Ingold, K.U., 1999. The unexpected role of vitamin E (α-tocopherol) in the peroxidation of human low-density lipoprotein. Accounts of Chemical Research 32, 27–34.

Burton, G.W., Ingold, K.U., 1984. Beta-carotene: an unusual type of lipid antioxidant. Science 224, 569–573.

Caruso, C., Lio, D., Cavallone, L., Franceschi, C., 2004. Aging, longevity, inflammation, and cancer. Annals of the New York Academy of Science 1028, 1–13.

Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Journal of Clinical Nutrition 78 (Suppl.), 517S–520S.

Valgimigli, L., Pratt, D.A., 2011. Antioxidants in chemistry and biology. In: Chatgilialoglu, C., Studer, A. (Eds.), Encyclopedia of Radicals in Chemistry, Biology and Materials. John Wiley & Sons Ltd., Chichester.

CHAPTER 2

Dietary Effects on Epigenetics with Aging

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ABBREVIATIONS

Acetyl–CoA Acetyl–coenzyme A
DMG Dimethylglycine
EGCG Epigallocatechin 3–gallate
HAT Histone acetyltransferase
HDAC Histone deacetylase
HDACI Histone deacetylase inhibitor
HMT Histone methyltransferase
SAH *S*-adenosylhomocysteine
SAM *S*-adenosylmethionine
SMM *S*-methylmethionine

1. INTRODUCTION

Probably most of us wonder what to eat and many of us wonder why. How much benefit is there in eating broccoli or apples instead of donuts or ice cream? Although a typical human life span is 70–80 years, a few people (currently less than 100 living worldwide) will live to 110 years and only a handful have ever reached 120 years of age (Young and Coles, 2011). These longest-lived people probably have good genetics, in fact, really good genetics – at least for long life. We will have to do something extraordinary with our foods if we hope to use them to extend our longevity to the lengths of these oldest old or beyond.

The dietary advice given to most Americans by the media, television in particular, is mainly for nutritionally imbalanced foods heavily emphasizing calories and causing deficiencies in several vitamins and minerals (Bell et al., 2009; Mink et al., 2010). One study found that advertisements for fruits and vegetables and nutrition-related public service announcements were each less than 2% of the total food advertisements (Bell et al., 2009). Deficiencies in micronutrients in some widely used foods may adversely affect epigenetics via methyl metabolism (Cooney, 2006) and may have a range of adverse effects including compromising mitochondrial function (Ames, 2010). These effects at the molecular and cellular levels can also affect our health.

Focused research is needed to address real issues in aging. Many studies are two steps back and one step forward. For example, we will not stop aging by stopping tobacco smoking. Studies to understand the health effects of tobacco are unlikely to help us understand aging. Stopping smoking only gets us back to normalcy and a normal life span (versus a shorter than normal life span for most smokers). The same can be said for other things that we did not evolve doing that currently shorten our lives such as relying on refined foods and soft drinks for a big part of our diets.

Instead, we need to know what things we can do in positive directions and well beyond normalcy. One of these is to choose foods that are high in vitamins, minerals, and other select micronutrients with known health benefits and then prioritizing foods rich in these nutrients but low in calories. Knowing how much of a nutrient we get from 100 kcal of a food is much more useful than knowing how much we get from 100 g or 100 ml of food. Except for those of us backpackers exploring the wilderness on foot, most of us probably aim for a certain daily caloric intake more than we aim to limit our daily weight or volume of food.

Here, the focus is on epigenetics, which is a collection of mechanisms by which we control our gene expression. Genetics gets us our genes but epigenetics determines how much and how often we use our genes. Thus, both genetics and epigenetics are important for our long-term health. A big difference is that we have at least some control over our epigenetics, but essentially no control over our genetics. Although we can imagine scenarios where we use foods, supplements, and drugs to maintain healthy epigenetics into old age, getting to this stage will require much more research (Cooney, 2010). For now, we have some ideas and some broad guidelines that should keep us moving in the right direction.

2. EPIGENETICS

Even though each of us has basically one genome, we produce hundreds, maybe thousands, of cell types and these make up our many organs and tissues – eyes, skin, liver, brain, etc. Our genomes produce these many cell types by expressing some genes but not others to give rise to each cell type. Many of the genes expressed in our liver are not expressed in our skin or brains, and vice versa.

The differences in gene expression between our different cell types are established in large part by molecular tags that are placed on the DNA itself or on the proteins that help package DNA, the histones. These tags include methyl groups on DNA and histones and acetyl groups on histones and are collectively called epigenetic controls. These epigenetic controls can be changed and often are when one cell type becomes another, as occurs in development or when pluripotent stem cells differentiate to make specific cell types. These and other epigenetic tags on DNA, histones, and other chromosomal proteins establish tissue-specific patterns of gene expression and can maintain these patterns over

many years or even many decades. Thus, cells can remember patterns of gene expression and their cell type (e.g., liver cells, epithelial cells) over many cell generations or in individual long-lived cells (e.g., neurons, lymphocytes). However, epigenetics drifts and degrades during aging and age-related diseases and this usually leads to the aberrant silencing of genes (Cooney, 2008, 2010).

Many of our genes contain CpG sequences, which are methylation targets of enzymes called DNA methyltransferases. They use *S*-adenosylmethionine (SAM) as their methyl donor and this SAM comes from our metabolism, as discussed later. DNA methylation almost always causes gene silencing. Numerous proteins that maintain silencing bind to methylated DNA. These include enzymes that tag or untag histones such as some histone methyltransferases (HMTs) and histone deacetylases (HDACs). Histone methylation by HMTs also requires SAM as its methyl donor. See Kouzarides (2007), Mato et al. (2008), Wallace et al. (2010), and Cooney (2006, 2008, 2010) for discussions of epigenetics and of SAM.

Methylation of some sites on histones promotes gene silencing, whereas methylation of other histone sites promotes gene activation (Kouzarides, 2007). Histone-methylated sites that promote gene activity recruit histone acetyltransferases (HATs) that acetylate histones using acetyl-coenzyme A (acetyl-CoA) as acetate donor. Acetyl-CoA comes from metabolism, as discussed later. Histone acetylation nearly always promotes gene activity. Histone acetylation is reversed (histones are untagged) and gene silencing promoted by HDACs of numerous classes and specificities. See Kouzarides (2007), Wellen et al. (2009), Wallace et al. (2010), and Cooney (2008, 2010) for discussions of epigenetics and of acetyl-CoA. Several studies show that gene activity can often be changed by manipulating either DNA methylation or histone acetylation (Champagne and Curley, 2009; Peleg et al., 2010).

3. SAM AND METHYL METABOLISM

SAM is produced by methyl metabolism, as shown in Figure 2.1 (Cooney, 2006; Mato et al., 2008). We need adequate methyl metabolism to maintain histone and DNA methylation. We need this not only to produce enough SAM but also to control levels of *S*-adenosylhomocysteine (SAH) because SAH inhibits most methyltransferase reactions. Methyl metabolism recycles SAH back to other useful components including SAM (Figure 2.1; Cooney, 2006; Mato et al., 2008). Histone methylation is involved in both the activation and the silencing of genes, so it is important for epigenetic control to maintain adequate SAM levels and SAM/SAH ratios to help assure adequate histone methylation. Presumably, epigenetic regulation will suffer without adequate histone methylation because some positive and negative signals will be lost and gene expression could drift. DNA methylation and gene silencing also require adequate SAM levels and adequate SAM/SAH ratios (Cooney, 2006).
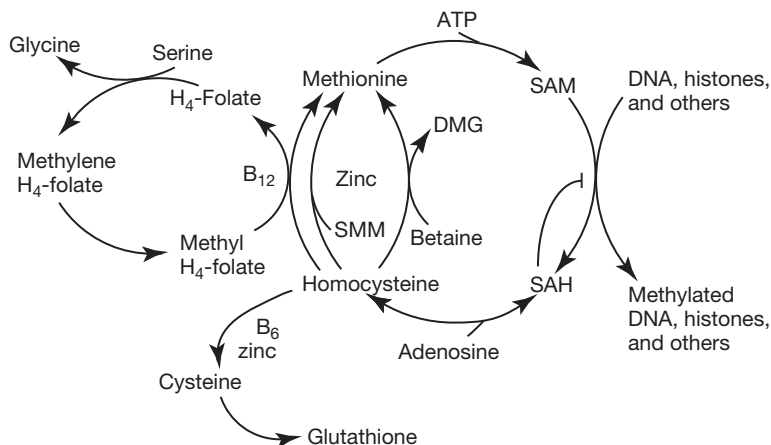
Figure 2.1 Methyl metabolism. SAM is the methyl donor for enzymatic methylation of histones, DNA, and many other molecules. The methylase reaction product SAH is an inhibitor of many methylation reactions that use SAM. Production of SAM and recycling of SAH occur through methyl metabolism as shown and require betaine and/or SMM and/or methylfolate as methyl donor. Several components in these cycles are essential nutrients or provide alternative metabolic pathways to make SAM. *Adapted from Cooney, C.A., 2009. Nutrients, epigenetics, and embryonic development. In: Choi, S.W., Friso, S. (Eds.), Nutrients and Epigenetics. CRC Press, Boca Raton, FL, pp. 155–174.*

Diet can provide large, even therapeutic amounts of nutrients important for methyl metabolism and for many nutrients this can be done without supplements (Cooney, 2006). One way to design such diets is to measure foods by calories rather than weight. By doing this, we can understand the benefits and energetic costs of choosing various foods. Foods that are micronutrient–dense but low in calories can help us achieve adequate or better levels of micronutrients while managing caloric intake. For example, 100 kilocalories (kilocalories are commonly called calories in nutrition) of raw spinach contain over 800 μg of folate, over 80 mg of choline, and over 400 mg of betaine (spinach item number 11457, refer to the USDA website). These levels of folate and betaine are substantial and are similar to amounts taken as supplements. In addition, spinach has substantial amounts of several other nutrients including lutein, zeaxanthin, vitamin K (phylloquinone), potassium, and beta carotene. S-Methylmethionine (SMM), a food component that clearly contributes to methyl metabolism (Figure 2.1) and presumably to SAM levels, is not yet in the major databases such as those of the USDA (Augspurger et al., 2005).

It is probably impossible to work effectively with methyl metabolism, DNA methylation, and histone methylation without also working with the sometimes congruous and sometimes countervailing effects of acetyl metabolism, histone acetylation, and the acetylation of other nuclear proteins including transcription factors.

4. ACETYL-COA AND ENERGY METABOLISM

Acetyl–CoA is a central metabolite produced from the metabolism of glucose, fats, and many other molecules (Figure 2.2; Cooney, 2008, 2010; Wallace et al., 2010). Acetyl–CoA is also a building block for fatty acids, cholesterol, and many other molecules. Acetyl–CoA from glucose or fats can be 'burned' for energy via the citric acid metabolic cycle and oxidative phosphorylation, ultimately giving off carbon dioxide and water. An abundance of acetyl–CoA usually indicates abundant energy because it can be 'burned' to make ATP, and it can be made into energy–rich stores in the form of fatty acids. Acetyl-CoA is also the acetyl donor for the acetylation of many proteins including histones and transcription factors. Protein acetylation acts as a tag or switch to change the enzyme activity or the binding affinity of the proteins that are acetylated. It is not surprising that histone acetylation acts to promote gene expression because abundant energy provides opportunity for growth, which in turn requires gene expression.

Acetyl–CoA may seem to be ubiquitous because of its role in so many processes. However, in some aged cells and most cancer cells, some of the machinery that makes and uses acetyl–CoA is dysfunctional or broken. In particular, mitochondria are responsible for the production of acetyl-CoA from glucose via pyruvate and for its 'burning' in the citric acid cycle and in oxidative phosphorylation. In senescence and cancer, mitochondria often fail in these and other tasks (Ames, 2010; Cooney, 2008, 2010; Wallace et al., 2010). Further, many aged cells and cancer cells rely on glycolysis to make small
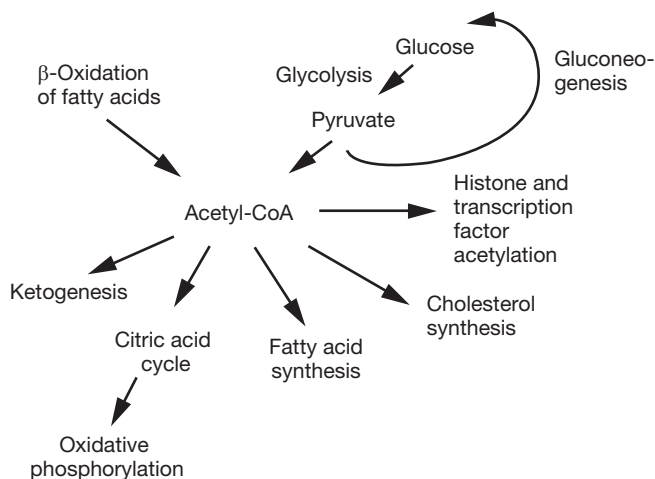
Figure 2.2 Acetate metabolism. Acetyl-CoA is the acetyl donor for enzymatic acetylation of histones, transcription factors, and many other molecules. Fats and glucose can be 'burned' for energy via acetyl-CoA, the citric acid cycle, and oxidative phosphorylation. However, when the mitochondria are dysfunctional, cells can rely on glycolysis and avoid production of acetyl-CoA from glucose. *Adapted from Cooney C.A., 2010. Drugs and supplements that may slow aging of the epigenome. Drug Discovery Today: Therapeutic Strategies 7, 57–64.*

amounts of energy from large amounts of glucose ultimately converting glucose (via py-ruvate), not into acetyl–CoA, but into lactate (which cells excrete; Figure 2.2). This pro-cess by which cancer cells use large amounts of glucose to make energy and excrete lactate is called the Warburg effect after Otto Warburg who discovered this effect in the 1920s (Semenza, 2008; Warburg, 1956). Despite using enormous amounts of glucose for their growth, cancer cells probably produce relatively little acetyl–CoA.

It is hypothesized that these metabolic effects in aging and cancer (such as the Warburg effect) leave little acetyl–CoA available for the acetylation of histones, transcrip-tion factors, and some other proteins. This in turn causes gene expression to gradually diminish and many genes to be gradually silenced (Figure 2.3; Cooney, 2008, 2010). This insidious process would promote senescence and cancer because it would silence genes needed for normal cell function. This could extend to neurons and other cells that would otherwise form memories except that their epigenetic machinery is now compromised. In mice, epigenetic silencing contributes to dementia (Kilgore et al., 2010; Peleg et al., 2010) and metabolism may contribute to this silencing. As discussed later, some foods, food compositions, and calorie levels may help prevent or reverse these processes by inhibiting HDACs and by promoting the availability of acetyl-CoA for histone and transcription factor acetylation.
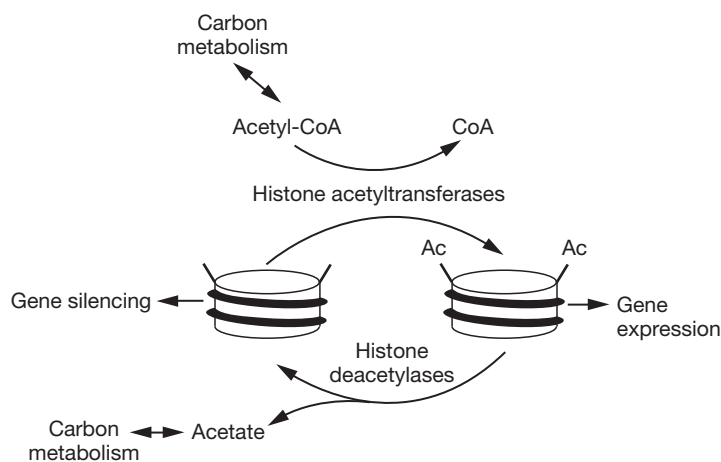
Figure 2.3 Acetyl-CoA levels are an indicator of a cell's metabolic and energy state and can affect histone acetylation and gene expression. Acetyl-CoA and histone acetyltransferases add acetyl groups while histone deacetylases remove acetyl groups. The balance of these affects net acetylation levels on histones. In normal young cells, these and related processes are balanced to allow for normal gene expression. When acetyl-CoA is low, or acetylase and deacetylase activities are out of balance, or under the effects of HDACIs, histone acetylation levels and gene expression levels can change. *Reproduced from Cooney, C.A., 2010. Drugs and supplements that may slow aging of the epigenome. Drug Discovery Today: Therapeutic Strategies 7, 57–64, with permission from Elsevier.*

5. AGE-RELATED DISEASE AND AGING

Epigenetics breaks down and becomes dysfunctional in age-related disease and with aging in general (Cooney, 2008, 2010). There is a global loss of DNA methylation where the bulk methylation level on DNA declines and yet on specific genes, there are huge methylation increases causing gene silencing. In aging, and especially in cancer, there are more examples of specific gene hypermethylation than hypomethylation. As discussed in examples given later, this occurs in many tissues including those where cancers are rare (e.g., neurons and vascular tissue) as well as in those where cancers are common (e.g., colon, lung, breast, and prostate). Histone methylation and acetylation also change in aging and cancer.

With age, there is gradual DNA hypermethylation of multiple genes in normal human prostate (Kwabi-Addo et al., 2007). These are some of the same genes that are hypermethylated in prostate cancer and these changes in aging prostate may predispose men to prostate cancer. Jean-Pierre Issa's group, the same group that discovered these changes in prostate, found DNA hypermethylation of genes in cardiovascular tissue related to age and atherosclerosis (Post et al., 1999). Studies of breast cancer show a loss of histone methylation and acetylation in cancer versus normal breast tissue (Elsheikh et al., 2009). In general, there seems to be a loss of normal epigenetic control in aging and cancer.

While epigenetics has long been called 'cell memory' because it helps cells remember their types and functions, more recent studies indicate that what we more commonly call memory, that is, our ability to recall people, places, and events, is also dependent on epigenetics. At least in adult rodents, the gene expression needed for memory is regulated by epigenetics. Andre Fischer and coworkers studied mice at 3, 8, and 16 months of age in various learning tests (Peleg et al., 2010). Old mice (16 months of age) did poorly in some of these tests compared to younger mice (3 and 8 months of age). In young mice, learning was associated with increased histone acetylation and it changed the expression of thousands of genes in a part of the brain called the hippocampus. By contrast, similarly experienced 16-month-old mice showed no significant changes in histone acetylation and only six genes were differentially expressed. Treatment of old mice with histone deacetylase inhibitors (HDACIs), namely a drug called suberoylanilide hydroxamic acid, or a component of foods and digestion called sodium butyrate, increased histone acetylation and improved learning. In another mouse memory study using a model of Alzheimer's disease, HDACIs significantly improved memory in mice suffering from memory loss and the mice maintained their new memories for at least 2 weeks (Kilgore et al., 2010). These studies show that at least some memory deficits with age can be reversed in mice.

6. FOODS, METABOLISM, AND EPIGENETICS

In early development, such as during pregnancy and the perinatal period, many studies have shown that diet and metabolism can change epigenetics and DNA methylation

(Cooney, 2009). In adults, manipulation of diet and methyl metabolism to affect aging or cancer through DNA methylation has mainly been used to induce cancer in experimental methyl deficiency studies (Mato et al., 2008). Changing methyl metabolism to treat cancer has been tested but has met with limited success. Methyl–deficient diets, high homocysteine, or high SAH can cause cancer, vascular disease, and dementia in animals and humans and thus make poor choices for treating gene hypermethylation with aging and cancer (see Cooney, 2010; Mato et al., 2008). As discussed earlier, histone methylation regulates both gene activation and gene silencing depending on the site-specific methylation and, thus, methyl-deficient diets, high homocysteine, or high SAH would probably dysregulate epigenetics. Instead, the aim is to maintain methyl sufficiency and preserve methylation of histones and global DNA methylation. It is not known why many genes become silenced with age and cancer while at the same time DNA genome-wide becomes hypomethylated. There may be multiple influences at work with multiple aspects of metabolism pulling in different directions (Cooney, 2008, 2010).

Even though epigenetic modifications including DNA methylation and histone acetylation sometimes change over short periods of minutes or days, it appears that there is less epigenetic plasticity with age and that memory formations and other responses that occur readily in young animals occur more slowly or not at all in older animals (Cooney, 2010). Some combinations of drugs, nutraceuticals, and foods might keep epigenetics plastic and help maintain memory, prevent cancer, and postpone aging.

We have a good idea about foods and supplements to maintain methyl metabolism and some ideas, though less well developed, about maintaining appropriate acetyl-CoA levels and energy metabolism. Variations in methyl metabolism and epigenetics have been studied extensively, whereas few data are available on variations in acetyl-CoA and energy metabolism, and their effects on epigenetics. Nevertheless, some possibilities and some prime areas for research can be discussed.

7. FOODS, SUPPLEMENTS, AND METHYL METABOLISM

Most of the various dietary components of methyl metabolism can be obtained by most people through a well-designed diet (Cooney, 2006). Expressed in terms of nutrients per calorie of food, high amounts of food folate can be had through leafy vegetables as in the earlier example of spinach. Many beans are also rich in folate although higher in calories than leafy vegetables. Betaine is found in many foods, but in high amounts in just a few including spinach, wheat, shrimp, and beets. Zinc is very abundant in oysters and available in significant amounts in meats and some other foods (food composition data available from the USDA website). Vitamin B12 is found in almost 'everything that moves' and in healthy young people, it is readily absorbed in digestion with the help of intrinsic factor. People with compromised digestion or absorption need B12 supplements or injections. Only moderate amounts of methionine (found in most proteins) are recommended for adults because high levels lower longevity in rodents (Miller et al., 2005) and can also raise homocysteine levels.

Without a carefully designed, consistently followed diet, moderate supplementation with folate, B12, zinc, and betaine provides good dietary insurance. A good functional indicator of methylation status is a blood plasma (or serum) homocysteine test and a level of 7 μM or lower is desirable. Combined tests of blood methylmalonic acid and homocysteine are good functional indicators of B12 adequacy or deficiency. See McCully (2007) for a discussion of homocysteine, vitamin B12, folate, and health.

8. FOODS, SUPPLEMENTS, AND ACETYL METABOLISM

The availability of acetyl–CoA is dependent on many factors including energy metabolism, the health of mitochondria, and many competing biochemical reactions. Therefore, we do not have simple recommendations to get adequate amounts of some nutrients to maintain acetyl–CoA at appropriate levels. The following are considerations for adequate acetyl–CoA and are as much basic and clinical research directions as they are ideas for everyday foods and supplements.

9. CARBOHYDRATES VERSUS FATS

The burning of fat must proceed through acetyl–CoA whereas many cells, especially aged and cancer cells, can derive energy from glucose (carbohydrate) without making acetyl–CoA (Cooney, 2008, 2010; Wallace et al., 2010). Therefore, relying on fats for energy and using an otherwise low glycemic index diet (to keep blood glucose levels low) is one approach. Another approach to keep blood glucose low is calorie restriction (e.g., a 1500 cal per day diet for many people). However, there are few data on how dietary fats versus carbohydrates or calorie restriction affect epigenetics. It is known though that both of these approaches affect many aspects of metabolism in many tissues, so the potential is there (Cooney, 2008; Wallace et al., 2010). Calorie restriction and low blood glucose levels have other health benefits even if the effects on epigenetics or the ultimate effect on human life span have not yet been determined.

10. MITOCHONDRIAL HEALTH

Mitochondrial function decays with age (Ames, 2010). Two metabolites of mitochondria have been shown to restore mitochondrial function in old rats. They are R–alpha lipoic acid (ALA) and acetyl-L-carnitine (ALCAR) (Ames, 2010). Although little is known about the effects of ALA and ALCAR on epigenetics, it is likely that supplementation with them will affect acetyl–CoA production and metabolism. At least one study shows that ALCAR can donate acetyl groups to make acetyl–CoA for nuclear protein acetylation (Madiraju et al., 2009).

Conversion of pyruvate (from glucose) to acetyl–CoA is performed by the pyruvate dehydrogenase complex in the mitochondria (Bonnet et al., 2007). An essential part of

this complex uses ALA bound as a cofactor to the enzyme dihydrolipoamide acetyl transferase. This critical juncture can determine the direction of glucose metabolism for energy – whether pyruvate is converted to lactate and on to gluconeogenesis (in the liver) or whether pyruvate is converted to acetyl–CoA where it can follow many paths, one of which is energy generation via the citric acid cycle (Figure 2.2; Bonnet et al., 2007; Cooney, 2008). Although 'burning' acetyl–CoA via the citric acid cycle may seem antithetical to the purpose of making acetyl–CoA available for histone and other protein acetylation, without this 'burning' function, many cells may make minimal acetyl–CoA while generating their energy from glycolysis instead (Figure 2.2; Cooney, 2008).

The essential nutrient pantothenate (vitamin B_5) is used as a building block to form HSCoA, which is part of acetyl-CoA. HSCoA is also a widely used cofactor for the intracellular transfer of fatty acids. Lit–Hung Leung has proposed that pantothenate is rate–limiting for the metabolism of fats for energy during fasting or restricted calorie intake (Leung, 1997). Thus, pantothenate is a candidate nutrient that could affect epi-genetics by facilitating the production of acetyl-CoA. Pantothenate is widely available in foods and obtaining at least recommended dietary allowance levels (10 mg for adults) is highly advisable. Optimal dietary levels for particular purposes are not known but could be much higher (Leung, 1997).

Pyrroloquinoline quinine is a nutrient (also available as a supplement) that can stim-ulate mitochondrial biogenesis in mice as reported by Robert Rucker and his colleagues (Rucker et al., 2009). More work is needed to know its ability to restore mitochondria in old mice and its effects on epigenetics.

Recent work by Craig Thompson and coworkers (Wellen et al., 2009) showed that acetyl-CoA derived from citrate (through the action of the enzyme adenosine triphos-phate citrate lyase) contributed acetyl groups to histones and other nuclear proteins. Thus, maintaining an active citric acid cycle (a mitochondrial function) and consuming foods rich in citrate and related organic acids may be important for maintaining gene activity and avoiding gene silencing.

11. ADDITIONAL NUTRITIONAL FACTORS IN EPIGENETICS

There are naturally occurring HDACIs in foods such as broccoli (sulforaphane) and garlic (allyl mercaptan and allicin). Both of these have activity against human cancer cells in the laboratory and cancers in mice and both, especially sulforaphane, are in clinical trials against cancer in humans (Meeran et al., 2010; Nian et al., 2009). Roderick Dashwood and coworkers at the Linus Pauling Institute have shown that broccoli sprouts can increase histone acetylation in the white blood cells of young healthy people (Nian et al., 2009). These HDACIs from food may play an everyday role in preventing adverse epigenetic change. This could extend beyond cancer to slowing or reversing dementia or slowing age-related decline in general.

In addition to producing acetyl–CoA it may be important to help direct acetyl–CoA away from some functions such as the synthesis of fats so that more will be available to acetylate histones. Green tea contains a fatty acid synthase inhibitor called epigallocatechin 3-gallate (EGCG), which is toxic to cancer cells grown *in vitro* and shows some activity against cancer in animal studies (Puig et al., 2009). EGCG is in clinical trials against cancer and Alzheimer's disease in humans. Preventing acetyl–CoA from going toward the synthesis of fats may make more acetyl–CoA available for maintaining active gene expression (Cooney, 2010).

12. CONCLUSIONS AND FUTURE DIRECTIONS

Combinations of diets and supplements that make more intracellular acetyl–CoA available may help slow or reverse age-related gene silencing. Such combinations may help prevent cancer, dementia, and other age-related diseases. Foods such as broccoli and garlic that contain HDACIs may also help prevent cancer, dementia, and other age-related diseases involving epigenetics. In clinical nutrition studies, measures of actual levels of white blood cell histone acetylation, gene hypermethylation, red blood cell folate, blood SAM, etc. are needed to determine if foods and supplements consumed are absorbed and active in the body and if they are affecting epigenetics. In the future, these and overall metabolic analyses may be available as part of regular physical exams to assess our health and provide us guidance for adjusting our diets. We can imagine a future where we use foods, supplements, and drugs to predictably maintain and improve our health, and one day, even extend our life spans. To get there, much more research is needed on the roles of foods and specific nutrients in mitochondrial function, acetyl–CoA availability, gene silencing, maintenance of epigenetic normalcy, and prevention of senescence.

REFERENCES

Ames, B.N., 2010. Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. Journal of Nucleic Acids 14.

Augspurger, N.R., Scherer, C.S., Garrow, T.A., Baker, D.H., 2005. Dietary *S*-methylmethionine, a component of foods, has choline-sparing activity in chickens. The Journal of Nutrition 135, 1712–1717.

Bell, R.A., Cassady, D., Culp, J., Alcalay, R., 2009. Frequency and types of foods advertised on Saturday morning and weekday afternoon English- and Spanish-language American television programs. Journal of Nutrition Education and Behavior 41, 406–413.

Bonnet, S., Archer, S.L., Allalunis-Turner, J., et al., 2007. A mitochondria-K$^+$ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11, 37–51.

Champagne, F.A., Curley, J.P., 2009. Epigenetic mechanisms mediating the long-term effects of maternal care on development. Neuroscience and Biobehavioral Reviews 33, 593–600.

Cooney, C.A., 2006. Maternal nutrition: nutrients and control of expression. In: Kaput, J., Rodriguez, R.L. (Eds.), Nutrigenomics: Concepts and Technologies. John Wiley and Sons, Hoboken, NJ, pp. 219–254.

Cooney, C.A., 2008. Cancer and aging. The epigenetic connection. In: Tollefssbol, T. (Ed.), Cancer Epigenetics. CRC Press, New York, pp. 303–316.

Cooney, C., 2009. Nutrients, epigenetics, and embryonic development. In: Choi, S., Friso, S. (Eds.), Nutrients and Epigenetics. CRC Press, Boca Raton, FL, pp. 155–174.

Cooney, C.A., 2010. Drugs and supplements that may slow aging of the epigenome. Drug Discovery Today: Therapeutic Strategies 7, 57–64.

Elsheikh, S.E., Green, A.R., Rakha, E.A., et al., 2009. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. Cancer Research 69, 3802–3809.

Kilgore, M., Miller, C.A., Fass, D.M., et al., 2010. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology 35, 870–880.

Kouzarides, T., 2007. Chromatin modifications and their function. Cell 128, 693–705.

Kwabi-Addo, B., Chung, W., Shen, L., et al., 2007. Age-related DNA methylation changes in normal human prostate tissues. Clinical Cancer Research 13, 3796–3802.

Leung, L.H., 1997. A stone that kills two birds: how pantothenic acid unveils the mysteries of acne vulgaris and obesity. Journal of Orthomolecular Medicine 12, 99–114.

Madiraju, P., Pande, S.V., Prentki, M., Madiraju, S.R., 2009. Mitochondrial acetylcarnitine provides acetyl groups for nuclear histone acetylation. Epigenetics 4, 399–403.

Mato, J.M., Martínez-Chantar, M.L., Lu, S.C., 2008. Methionine metabolism and liver disease. Annual Review of Nutrition 28, 273–293.

McCully, K.S., 2007. Homocysteine, vitamins, and vascular disease prevention. The American Journal of Clinical Nutrition 86, 1563S–1568S.

Meeran, S.M., Patel, S.N., Tollefsbol, T.O., 2010. Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. PLoS One 5, e11457.

Miller, R.A., Buehner, G., Chang, Y., et al., 2005. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell 4, 119–125.

Mink, M., Evans, A., Moore, C.G., Calderon, K.S., Deger, S., 2010. Nutritional imbalance endorsed by televised food advertisements. Journal of the American Dietetic Association 110, 904–910.

Nian, H., Delage, B., Ho, E., Dashwood, R.H., 2009. Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulfides: studies with sulforaphane and garlic organosulfur compounds. Environmental and Molecular Mutagenesis 50, 213–221.

Peleg, S., Sananbenesi, F., Zovoilis, A., et al., 2010. Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 328, 753–756.

Post, W.S., Goldschmidt-Clermont, P.J., Wilhide, C.C., et al., 1999. Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. Cardiovascular Research 43, 985–991.

Puig, T., Turrado, C., Benhamú, B., et al., 2009. Novel inhibitors of fatty acid synthase with anticancer activity. Clinical Cancer Research 15, 7608–7615.

Rucker, R., Chowanadisai, W., Nakano, M., 2009. Potential physiological importance of pyrroloquinoline quinone. Alternative Medicine Review 14, 268–277.

Semenza, G.L., 2008. Tumor metabolism: cancer cells give and take lactate. The Journal of Clinical Investigation 118, 3835–3837.

Wallace, D.C., Fan, W., Procaccio, V., 2010. Mitochondrial energetics and therapeutics. Annual Review of Pathology 5, 297–348.

Warburg, O., 1956. On respiratory impairment in cancer cells. Science 124, 269–270.

Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., et al., 2009. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324, 1076–1080.

Young, R.D., Coles, L.S., 2011. Validated worldwide supercentenarians, living and recently deceased. Rejuvenation Research 14, 99–104.

RELEVANT WEBSITES

http://www.clinicaltrials.gov – ClinicalTrials.gov: A Service of the U.S. National Institutes of Health.
http://www.grg.org – Gerontology Research Group.
http://www.nal.usda.gov – USDA, Agriculture Research Service, National Agricultural Library.
http://www.supercentenarian-research-foundation.org – Supercentenarian Research Foundation.

Bioactive Foods in Aging: The Role in Cancer Prevention and Treatment

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1. THE BURDEN OF CANCER

Cancer is a leading cause of death worldwide. In 2004, over 7.4 million people died of cancer, accounting for more than 13% of all deaths worldwide (WHO, 2010). In 2010, it is estimated that there will be over 1.5 million new cancer cases in the United States alone. Estimates for 2010 also project that close to 570 000 Americans will die from cancer, which translates into greater than 1500 cancer deaths per day (ACS, 2010). It is also projected that in 2020, 15 million new cancer cases will be reported and 12 million cancer patients will die worldwide (Bray and Møller, 2005). However, despite numerous outreach, education, and research efforts, cancer mortality rates continue to increase.

Notwithstanding its high mortality rate, cancer is largely a manageable condition if diagnosed and treated early. The World Health Organization estimates that up to one-third of all cancers could be cured if detected early and treated adequately (WHO, 2010). Furthermore, cancer is often a preventable condition. Recent estimates state that between 30 and 95% of all cancer cases could be prevented by modifying diet, lifestyle, and behavioral habits alone (ACS, 2010; Anand et al., 2008; WHO, 2010). Cancer, as has been astutely observed, is a 'preventable disease that requires major lifestyle changes' (Anand et al., 2008).

Such changes include modifying dietary and lifestyle habits. Tobacco use is the single largest preventable cause of cancer in the world, yet millions use tobacco regularly; among the total projected cancer deaths in the USA 2010, 171 000 (34%) are expected to be caused by tobacco use. Evidence also suggests that close to one-third of all projected cancer deaths in 2010 will be caused by overweight or obesity, physical inactivity, or poor nutrition (ACS, 2010; WHO, 2010). These conditions can be especially detrimental because prolonged inflammation in obese patients is thought to be critical for tumor initiation and progression (Schmid-Schonbein, 2006). Such conditions can be prevented through limiting consumption of energy-dense foods, avoiding sugary drinks and alcohol, limiting the frequent intake of red meats, and increasing consumption of foods of plant origin (AICR, 2007).

Many cancers caused by infectious agents can be prevented through the use of vaccines and antimicrobials, as well as lifestyle changes. Many types of skin cancers can also be prevented by avoiding excessive exposure to UV radiation. This can be accomplished by the implementation of sunscreen, the proper use of hats and clothing, and by limiting the use of indoor tanning equipment. The American Cancer Society also recommends regular screenings for many different types of cancer and reports that at least half of all new cancer cases could be prevented if these conditions were detected early while still in their precancerous states (ACS, 2010). It is clear that for serious improvements in cancer prevention to occur, significant lifestyle and behavioral changes must be made both in the United States and in the world at large.

2. BIOACTIVE FOODS

2.1 What Are Bioactive Foods?

As mentioned, one of the most important ways to decrease risk of cancer development and progression is by modifying diet, which is best done through limiting intake of energy-dense foods, reducing excessive salt intake, limiting alcohol consumption, and replacing these with foods that contain high levels of critical nutrients, specifically foods of plant origin, including fruits, vegetables, legumes, whole grains, nuts, and oils. Such foods provide essential vitamin and minerals, while also providing healthy lipids, sugars, and protein. 'Bioactive' foods are those which contain high levels of bioactive compounds, such as antioxidants, antithrombotics, and anti–inflammatory substances. These compounds protect the body from inflammation, accumulation of low-density lipoprotein (LDL) cholesterol, and oxidative stress, thereby helping to prevent the development of cancer, as well as other conditions such as heart disease and other cardiovascular diseases (Kris Etherton et al., 2002).

2.2 Examples of Bioactive Foods

Among the most widely studied bioactive compounds are lycopene, flavonoids, phytoestrogens, acetogenins, organosulfurs, resveratrol, L–ascorbic acid, and tocopherols. These are found in a vast array of fruits and vegetables and are important components in promoting a healthy metabolism and providing antioxidant, antitumor, and antithrombotic activities. These compounds will each be discussed below.

Lycopene is a carotenoid antioxidant found in tomatoes, gac, and pink grapefruit, which inhibits tumor cell growth and is thought to prevent prostate cancer. Many types of cereals, nuts, oils, fruits, vegetables, and red wine contain phenols of many varieties, including flavonoids. Phytoestrogens may be beneficial in reducing the risk of cardiovascular disease and are found in flaxseed oil, vegetables, grains, and soy (Kris Etherton et al., 2002). Acetogenins are polyketides that have demonstrated anti–inflammatory, antitumor, and

antioxidant activities and are found in many types of fruits, including *Annona cherimola*, guanabana, and other magnoliales (Chang et al., 1998; Chen et al., 1999; Gupta–Elera et al., 2011).

Organosulfur compounds, found in garlic and onions, have been shown to possess both cardioprotective and anticarcinogenic properties (Kris Etherton et al., 2002; Stan et al., 2008). Resveratrol has recently become a well-known potent antioxidant and is found in red grapes, blueberries, cranberries, and other types of *Vaccinium* berries (Rimando et al., 2004). It is also known for its antithrombotic, anti-inflammatory, antic-arcinogenic, and lifespan elongation effects, as well as its positive role in protection against insulin resistance (Baur et al., 2006; Hung et al., 2010; Kris Etherton et al., 2002; Lagouge et al., 2006).

L-Ascorbic acid (vitamin C) is an important essential nutrient found in a wide variety of fruit, vegetable, herb, and animal sources, including citrus fruits, berries, spices, leafy greens, cabbage, tomatoes, and potatoes. Known for its role in producing mature colla-gen and its usefulness in fighting the common cold, vitamin C was also linked to cancer treatment by Dr. Linus Pauling, who observed improved outcomes in cancer patients that were given very high doses of the vitamin as a supplement to their treatments. Since then, vitamin C has been the focus of many studies involving supplementation, cancer prevention, and epidemiology (Block, 1991; Doyle et al., 2006; Packer and Colman, 1999).

Tocopherols and tocotrienols together make up the vitamin E class of compounds. Vitamin E is found in many types of raw oils, nuts, rice bran oil, barley, and leafy green vegetables, with α-tocopherol having the highest bioavailability (Brigelius-Flohe and Traber, 1999). Vitamin E is an important bioactive food component and is well known for its protection against the harmful effects of UV light, its anti-inflammatory properties, its role in reducing the risk of prostate cancer, and its ability to inhibit cognitive decline (Aggarwal and Shishodia, 2006; Emerit et al., 2004; Masaki, 2010; Packer and Colman, 1999; Reiter, 1995).

3. THE PROCESSES OF AGING

3.1 Senescence

The English word *senescence* comes from the latin *sĕnex*, meaning old or aged, and refers to the general biological processes that occur in an organism, following maturation, that eventually lead to the functional decline and death of the organism. These processes in-clude decreased efficiency, loss of function, and decreased immune function.

These phenomena have been extensively researched at the cellular level as well as in vertebrates. On the vertebrate level, aging has been described in terms of decreasing levels of collagen, elastin, and bone mineral content, prostate pathologies, and neurode-generative diseases. On the cellular level, aging is the result of telomere shortening,

accumulation of waste, genetic mutations, and general cellular wear and tear, all of which ultimately culminate in either apoptosis or necrosis (Bianchi–Frias et al., 2010; Freeman, 2010; Hung et al., 2010; Pena Ferreira et al., 2010).

One of the first inquiries into the processes involved in cellular aging was performed in the early 1960s by Leonard Hayflick. In his experiments, Hayflick demonstrated that a population of normal human fetal cells, when grown in culture, normally divides between 40 and 60 times before entering the senescence phase. In this phase, telomeres shorten, cell division eventually ends, and apoptosis results (Hayflick, 1965). This observed limit of cell division came to be known as the 'Hayflick limit.' This limit of cell divisions was further investigated in terms of oxidative stress and aging, as incubation with α-tocopherol resulted in an extended lifespan of healthy human cells (WI-38 cells) to over 100 divisions (Packer and Smith, 1974). These results support the free-radical theory of aging.

3.2 Free-Radical Theory of Aging

In his investigations regarding the 'biological clock' of life, Denham Harman first proposed a free-radical theory of aging in 1956 (Harman, 1956). This theory of aging places oxidative stress as the main mechanism by which cells senesce, given that metabolic rate is linked to oxygen consumption. Although the original theory focused on the effects of superoxide, it has grown to include investigating the effects of all types of free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Harman later expanded his theory to address mitochondrial production of ROS (Harman, 1972).

Much of the research involving bioactive foods centers around the free-radical theory of aging and how the antioxidant compounds in these bioactive foods act to quench the radicals that damage cellular components and processes. In order to understand these interactions more fully, it is important to understand free-radical chemistry.

4. FREE RADICALS, AGING, AND CANCER

4.1 What Are Free Radicals?

Radicals are unpaired valence electrons found in various types of biological and chemical molecules. These compounds can either have one extra electron (giving them a slight negative charge) or be one electron deficient (giving them a slight positive charge). Either way, most compounds that have unpaired radicals are highly chemically reactive.

There are two main classes of free-radical compounds: ROS and RNS. The more common of the two groups is ROS, which are derivatives of O_2, with superoxide radical ($O_2^{\bullet-}$) being the most common. Other examples of ROS include hydrogen peroxide (H_2O_2), alkoxyl/peroxyl radical ($RO^{\bullet}/ROO^{\bullet}$), and peroxynitrite (ONOOH/ $ONOO^-$) (Liu et al., 2008). Recently, ROS have been linked to a variety of chronic

diseases, including Alzheimer's disease, Parkinson's disease, and cancer (Garrett et al., 2010; Markesbery, 1997). ROS have also been linked to p53 function (Liu et al., 2008), diabetes (Baynes and Thorpe, 1999; Singh et al., 2009), neurodegenerative diseases (Emerit et al., 2004), and cognitive decline (Pratico et al., 2002; Reiter, 1995).

RNS are species of free-radical compounds derived from nitric oxide ($NO^{\bullet}$), and include peroxynitrite ($ONOO^{-}$), nitrogen dioxide ($^{\bullet}NO_2$), and dinitrogen trioxide (N_2O_3). These and other RNS, similar to ROS, have been shown to cause damage to lipids, amino acids, nucleic acids, and other small molecules (O'Donnell et al., 1999).

In a recent review, the signaling, cytotoxic, and pathogenic characteristics of nitric oxide and peroxynitrite were discussed in detail (Pacher et al., 2007). Nitric oxide is an important signaling molecule because of its unique chemical properties, which include its rapid cellular diffusion (its diffusion coefficient in water is slightly higher than oxygen and carbon dioxide), and its propensity to quickly produce oxygen radicals. Peroxynitrite is another well-known RNS formed by the reaction of hydrogen peroxide and nitric oxide. Although peroxynitrite itself does not contain free radicals, it is a powerful oxidant, and its decomposition in the phagosome results in the formation of H_2O_2 and NO_2^{-} (Pacher et al., 2007).

Similar to ROS, not all RNS are detrimental, as both are important components of the innate immune system. A study performed by Iovine et al. demonstrated that murine macrophages significantly upregulated RNS production following exposure to *Campylobacter jejuni* and were much more effective at eliminating it when compared to mutant macrophages unable to produce RNS (Iovine et al., 2008). An earlier study by Neu et al. also demonstrated that prolonged inhibition of nitric oxide synthesis in human umbilical vein epithelial cells supports neutrophil adhesion, although it also leads to an increase in intracellular oxidative stress in those cells (Neu et al., 1994).

4.2 Intracellular Oxidative Stress

The amount of oxidative stress formed within the cell is highly dependent on the rate at which $O_2^{\bullet-}$ and H_2O_2 are produced (Imlay, 2003; Messner, 2002). During normal metabolic processes, baseline levels of free radicals are produced as by-products of several chemical reactions, including mitochondrial aerobic metabolism, but potential damage caused by these stresses is constantly prevented by constitutive antioxidant mechanisms (Sastre, 2003).

As mentioned, both RNS and ROS are produced as part of the innate immune system to attack and kill pathogens. These ROS and RNS are released nonspecifically, however, which can be harmful for surrounding tissues as chemical damage may result (Rice-Evans and Gopinathan, 1995). For this reason, the consequences of oxidative stress have recently been investigated with regard to inflammation, disease, and cancer development (Schmid-Schonbein, 2006).

5. CANCER

5.1 Cancer Epidemiology

Recent estimates state that as many as 90–95% of cancer cases are attributed to lifestyle factors, while the remaining 5–10% are due to genetics (Anand et al., 2008). As previously mentioned, the main lifestyle-related risk factors are alcohol consumption, tobacco use, diet, obesity, infectious agents (as in the case with several viruses like human papilloma-virus), radiation, and environmental pollutants (e.g., asbestos or benzene exposure in the workplace). From these data, cancer is a largely preventable condition if certain lifestyle behaviors are managed properly.

Among men in the United States, prostate cancer is the most common cancer with an estimated 217 730 new cases in 2010, accounting for 28% of all new cancers among men. Lung cancer and colorectal cancer follow, accounting for 15 and 9% of all new cases, respectively. Among women, breast cancer is most common, with an estimated 207 090 new cases in 2010 (28% of all new cases). Similar to cancers in men, lung cancer and colorectal cancer are the next most prevalent, accounting for 14 and 10% of all new cases in women, respectively (Jemal et al., 2010).

Currently, cancer accounts for nearly one in four deaths in the United States. Five-year relative survival rates from cancer have increased in recent years, up from 50% between 1975 and 1977 to 68% between 1999 and 2005. While these rates vary greatly among cancer types, progression upon diagnosis, and demographic factors, the improvements seen in survival rates may be attributed largely to more effective diagnoses, earlier diagnoses, and more effective treatments once cancer is diagnosed. The present chapter focuses on the role of bioactive foods in cancer prevention and treatment; for a more exhaustive review of cancer epidemiology data and figures, the reader is referred to literature which cover these topics in greater detail (ACS, 2010; AICR, 2007; Anand et al., 2008; Bray and Møller, 2005; Jemal et al., 2010; Schmid–Schonbein, 2006).

6. BIOACTIVE FOODS IN CANCER TREATMENT

6.1 Premise of Antioxidant Therapy

The principal treatments used for combating cancer today include surgery, radiation, and chemotherapy. Surgery is required when the cancer exists as a solid tumor mass that can be physically removed. Radiation is used to damage and/or kill cancer cells using a beam of high-energy particles. Both of these are local treatments because they affect only the site of treatment, where chemotherapeutic agents are systemic treatments because they can potentially affect many cells in the body.

Most chemotherapeutics developed for cancer are antineoplastic agents, which inhibit the proliferation of rapidly dividing cells. While these agents target cancer cells, they may also affect several other tissues in the body that rely on constant cell turnover to

function properly, including blood cells and cells of the digestive tract. Consequently, chemotherapy can have undesirable side effects such as mucositis and myelosuppression, leading to immunosuppression due to low white blood cell count. These side effects can lead to susceptibility to opportunistic infections, loss of appetite, and weight loss, all of which increase the difficulty of treatment and decrease the chance of recovery (Doyle et al., 2006). Such symptoms underscore the need for correct balance in the diet, which is vital to sustaining health during cancer treatment.

One of the leading causes of complications associated with cancer treatments is malnutrition (Doyle et al., 2006; Gupta et al., 2009). Studies have shown that chemotherapy may cause a decrease in the amount of nutrients absorbed from food. One study showed that rectal carcinoma patients treated with a combination of chemotherapy and radiation showed a decrease in serum levels of α-tocopherol (Dvořák et al., 2009). A second study demonstrated that plasma concentrations of the carotenoids lutein, α-carotene, and β-carotene are decreased in patients being treated for head and neck squamous cell carcinomas when compared to healthy controls (Sakhi et al., 2010).

6.2 Bioactive Food Components in Research

Bioactive foods may have the potential to decrease both the initiation and progression of cancer due to their rich antioxidant and nutrient contents, as previously discussed, and evidence is growing that may help define the role of bioactive foods in the prevention of cancer development and progression. Multiple studies have found correlations between plasma levels of bioactive food components (i.e., carotenoids, tocopherols, etc.) and the likelihood of survival of patients undergoing chemotherapy (Garg et al., 2009; McCann et al., 2009; Sakhi et al., 2010). Research has also shown a positive correlation between the amount of plant material consumed and a patient's chance of recovery. Two studies in particular followed the dietary intake of women with breast cancer as they went through treatment. These studies found an increased chance of survival in those women who had a high intake of plant-based foods and/or supplements containing bioactive food components when compared to those who consumed diets containing less of these components (McCann et al., 2009; Pierce et al., 2002).

6.2.1 Resveratrol

Resveratrol is a stilbene polyphenol that has recently garnered much attention for its antiinflammatory, antitumorigenic, and antioxidant properties (Alarcón de la Lastra and Villegas, 2005; Ndiaye et al., 2011). Found in high concentrations in *Vitis* and *Vaccinium* fruits, studies showing that resveratrol extends lifespan in *S. cerevisiae*, *C. elegans*, *Drosophila*, and murine models (through SIR2 activation) have given support to reports that regular consumption of red wine may increase longevity in humans (Alarcón de la Lastra and Villegas, 2005; Bass et al., 2007; Miller et al., 2010). The results from these studies may not be so easily applied to human models, however, due to resveratrol's low potency and

poor bioavailability after metabolism (Hsieh et al., 2010). Further research is needed to more fully elucidate the extent to which resveratrol may affect longevity and aging in humans.

In addition to its potential health-promoting effects, resveratrol may directly influence the development and progression of cancer. A recent report demonstrated that resveratrol-induced apoptosis in colorectal cancer cells is mediated by adaptive response gene ATF3 *in vitro*, supporting the idea that ATF3 may play an antitumorigenic role in colorectal tumorigenesis (Whitlock et al., 2011). Another recent report demonstrated the ability of resveratrol and resveratrol analogs to elicit blocks in the cell cycle of cultured human prostate cells, as well as the resulting increase in p53 and p21, providing further evidence of resveratrol's antitumorigenic abilities (Hsieh et al., 2010).

6.2.2 Carotenoids

Carotenoids, particularly lycopene, are known to be powerful antioxidants linked to oxidation-preventing mechanisms. In one case-control study, carotenoid plasma levels were measured to compare 118 non-Hispanic Caucasian men suffering from nonmetastatic prostate cancer with 52 healthy men in southeast Texas. Results showed that the risk for men with high levels of α-carotene, *trans*-β-carotene, β-cryptoxanthin, lutein and zeaxanthin in their plasma was less than half that of those with low levels of these compounds. No correlation was found between carotenoid plasma levels and the stage of aggressive disease in these patients. This study suggests that high plasma levels of carotenoids may help reduce prostate cancer development, but not its progression (Chang et al., 2005).

6.2.3 Vitamin D

Another well-known bioactive food component is vitamin D. Although vitamin D deficiency is mainly associated with bone-related diseases, interest has risen in vitamin D deficiency as a risk factor for different cancer types, mainly colon, breast, ovarian, and prostate cancers. A case-control study concluded that patients with plasma levels of 25(OH)D (the main form of circulating vitamin D and main marker for vitamin D deficiency) below $30 \, ng \, ml^{-1}$ had about twice the risk of developing colon cancer (Feskanich et al., 2004), while another revealed a doubling of colon cancer incidence for patients with less than $20 \, ng \, ml^{-1}$ of vitamin D (Tangrea et al., 1997). The association of 25(OH)D levels in different stages of colon cancer was investigated, and results suggested that vitamin D metabolites may have protective effects in all the stages of colon carcinogenesis (Braun et al., 1995).

Low levels of vitamin D have also been associated with breast cancer risk. Studies suggested that women in the lowest quartile of serum 25(OH)D had a five times greater risk of breast cancer than those in the highest quartile (Janowsky et al., 1999), while other case

studies suggested that low 25(OH)D plasma levels were associated with a more rapid progression of metastatic breast cancer (Brenner et al., 1998).

In a study including 19 000 men with prostate cancer, those with 25(OH)D levels below 16 ng ml^{-1} had a 70% higher incidence rate of prostate cancer than those with higher levels of vitamin D, and the incidence of prostate cancer for younger men was 3.5 times higher if their levels were below 16 ng ml^{-1} (Ahonen et al., 2000). These studies suggest that vitamin D levels have significant effects on the initiation of colon and prostate cancer and in the progression of metastatic breast cancer.

6.2.4 Vitamins A, E, and C

Additional studies on vitamins A, C, and E have shown inverse correlations between the presence of these vitamins and cancer incidence. In a study investigating the correlation of vitamins A, C, and E in 5454 colon cancer patients, results showed that the inverse correlation between vitamin intake and colon cancer incidence was statistically significant (Park et al., 2010). Also, a case-control study that examined the association of antioxidant vitamins A, C, and E and β-carotene for 144 cervical cancer patients in South Korea showed that total intakes of vitamins A, C, and E and β-carotene were inversely correlated with cervical cancer risk (Kim et al., 2010).

Vitamin E is a fat-soluble antioxidant known to quench oxygen radical species formed during fat oxidation, while vitamin A (retinol) is known for its role in vision and the production of retinoic acid. Both of these vitamins are well-known components in bioactive foods. A study involving 26 patients with gastroesophageal cancer examined vitamin A and E plasma levels compared to the plasma levels of healthy individuals in Eastern Anatolia. Contrastingly, these results showed that the difference in the plasma levels of vitamins A and E between healthy and cancer patients was not statistically significant (Yilmaz et al., 2010).

In contrast to vitamin E, vitamin C is a water-soluble vitamin that acts as a powerful antioxidant and is highly concentrated in citrus fruits. To better understand the role of citrus fruit in cancer risk, 955 patients with oral and pharyngeal cancer, 395 with esophageal, 999 with stomach, 3634 with large bowel, 527 with laryngeal, 2900 with breast, 454 with endometrial, 1031 with ovarian, 1294 with prostate, and 767 with renal cell cancer were studied in Switzerland and Italy. Results showed that there was a statistically significant inverse correlation between citrus fruit consumption and the risk of cancer for the digestive tract and the larynx (Foschi et al., 2010).

Several additional studies have also demonstrated that the overall nutrient state of an individual is positively correlated with the probability of both surviving cancer treatment and experiencing remission (Doyle et al., 2006; Garg et al., 2009; Gupta et al., 2009). Obesity, which can be prevented through modifying diet and activity, has been linked to increased probability of recurrence and incidence of secondary cancers in cancer patients. Indeed, studies have shown the importance of alternative feeding routes to help

sustain nutrition during treatment (Bower and Martin, 2009; Doyle et al., 2006; Garg et al., 2009).

7. CONCLUSION

It is becoming increasingly evident that lifestyle is a critical component of the prevention of many diseases, including cancer. As discussed, evidence of the direct correlation between healthy lifestyle habits and the low incidence of cancer is ever increasing. As the breadth of scientific research continues to grow, additional light will be shed on the importance of these behaviors in the prevention of cancer and other types of chronic diseases. The consumption of bioactive foods plays an important role in these lifestyle habits.

It is also becoming evident that patients with cancer who consume high amounts of bioactive foods during treatment have a higher chance of survival. Additional research is needed to elucidate the precise mechanisms of this interesting interaction. While some have speculated that the consumption of these foods may help boost the immune system, stemming off secondary infections, others have suggested that such a diet may help maintain proper nutritional levels, making the patient stronger overall. Whatever the case may be, the evidence is clear: eating a diet rich in bioactive foods helps prevent cancer and maintains more favorable health conditions through cancer treatment, thereby reducing the risk of secondary cancers and secondary infections (Doyle et al., 2006).

As additional research discoveries are made connecting healthy behavioral habits and cancer prevention, the onus of responsibility shifts toward the proper dissemination of this knowledge through educational outreach. Researchers, educators, government agencies, and nongovernmental organizations alike have the increasing responsibility of providing accurate, useful information to people of the world so that the formidable burden of cancer may be lessened for each successive generation.

REFERENCES

ACS, 2010. Cancer Facts and Figures 2010. American Cancer Society, Atlanta.

Aggarwal, B., Shishodia, S., 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology 71 (10), 1397–1421.

Ahonen, M.H., Tenkanen, L., et al., 2000. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes & Control 11 (9), 847–852.

AICR, 2007. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. World Cancer Research Fund/American Institute for Cancer Research, Washington, DC.

Alarcón de la Lastra, C., Villegas, I., 2005. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. Molecular Nutrition & Food Research 49 (5), 405–430.

Anand, P., Kunnumakara, A.B., et al., 2008. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research 25 (9), 2097–2116.

Bass, T., Weinkove, D., et al., 2007. Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*. Mechanisms of Ageing and Development 128 (10), 546–552.

Baur, J.A., Pearson, K.J., et al., 2006. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444 (7117), 337–342.

Baynes, J.W., Thorpe, S.R., 1999. Role of oxidative stress in diabetic complications. Diabetes 48 (1), 1–9.

Bianchi-Frias, D., Vakar-Lopez, F., et al., 2010. The effects of aging on the molecular and cellular composition of the prostate microenvironment. PLoS One 5 (9), e12501.

Block, G., 1991. Vitamin C and cancer prevention: the epidemiologic evidence. American Journal of Clinical Nutrition 53, 270–282.

Bower, M.R., Martin, R.C.G., 2009. Nutritional management during neoadjuvant therapy for esophageal cancer. Journal of Surgical Oncology 100 (1), 82–87.

Braun, M.M., Helzlsour, K.J., et al., 1995. Colon cancer and serum vitamin D metabolite levels 10–17 years prior to diagnosis. American Journal of Epidemiology 142 (6), 608–611.

Bray, F., Møller, B., 2005. Predicting the future burden of cancer. Nature Reviews. Cancer 6 (1), 63–74.

Brenner, B.M., Russell, N., et al., 1998. The effect of dietary vitamin D3 on the intracellular calcium gradient in mammalian colonic crypts. Cancer Letters 127 (1–2), 43–53.

Brigelius-Flohe, R., Traber, M.G., 1999. Vitamin E: function and metabolism. Federation of American Societies for Experimental Biology 13 (10), 1145–1155.

Chang, F.R., Chen, J.L., et al., 1998. Acetogenins from seeds of Annona reticulata. Phytochemistry 47 (6), 1057–1061.

Chang, S., Erdman, J.W., et al., 2005. Relationship between plasma carotenoids and prostate cancer. Nutrition and Cancer 53 (2), 127–134.

Chen, C.Y., Chang, F.R., et al., 1999. Aromin-A, an *Annonaceous acetogenin* from *Annona cherimola*. Phytochemistry 51, 429–433.

Doyle, C., Kushi, L.H., et al., 2006. Nutrition and physical activity during and after cancer treatment: an American Cancer Society Guide for informed choices. CA: A Cancer Journal for Clinicians 56 (6), 323–353.

Dvořák, J., Melichar, B., et al., 2009. Intestinal permeability, vitamin A absorption, alpha-tocopherol, and neopterin in patients with rectal carcinoma treated with chemoradiation. Medical Oncology 27 (3), 690–696.

Emerit, J., Edeas, M., et al., 2004. Neurodegenerative diseases and oxidative stress. Biomedecine & Pharmacotherapy 58 (1), 39–46.

Feskanich, D., Ma, J., et al., 2004. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiology, Biomarkers & Prevention 13 (9), 1502–1508.

Foschi, R., Pelucchi, C., et al., 2010. Citrus fruit and cancer risk in a network of case-control studies. Cancer Causes & Control 21 (2), 237–241.

Freeman, R., 2010. Skeletal implications of reproductive aging. Seminars in Reproductive Aging 28 (5), 422–425.

Garg, S., Yoo, J., et al., 2009. Nutritional support for head and neck cancer patients receiving radiotherapy: a systematic review. Supportive Care in Cancer 18 (6), 667–677.

Garrett, A.R., Murray, B.K., et al., 2010. Measuring antioxidant capacity using the ORAC and TOSC assays. Methods in Molecular Biology 594, 251–262.

Gupta, D., Lis, C.G., et al., 2009. Impact of improved nutritional status on survival in ovarian cancer. Supportive Care in Cancer 18 (3), 373–381.

Gupta-Elera, G., Garrett, A.R., et al., 2011. The antioxidant properties of the cherimoya (*Annona cherimola*) fruit. Food Research International 44 (7), 2205–2209.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology 11 (3), 298–300.

Harman, D., 1972. A biologic clock: the mitochondria? Journal of American Geriatrics Society 20 (4), 145–147.

Hayflick, L., 1965. The limited in vitro lifetime of human diploid cell strains. Experimental Cell Research 37 (3), 614–636.

Hsieh, T.C., Huang, Y.C., et al., 2010. Control of prostate cell growth, DNA damage and repair and gene expression by resveratrol analogues, in vitro. Carcinogenesis 32 (1), 93–101.

Hung, C.W., Chen, Y.C., et al., 2010. Ageing and neurodegenerative diseases. Ageing Research Reviews 9, (supplement) S36–S46.

Imlay, J.A., 2003. Pathways of oxidative damage. Annual Review of Microbiology 57, 395–418.

Iovine, N.M., Pursnani, S., et al., 2008. Reactive nitrogen species contribute to innate host defense against *Campylobacter jejuni*. Infection and Immunity 76 (3), 986–993.

Janowsky, E.C., Lester, G.E., et al., 1999. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. Public Health Nutrition 2 (3), 283–291.

Jemal, A., Siegel, R., et al., 2010. Cancer statistics 2010. CA: A Cancer Journal for Clinicians 60, 277–300.

Kim, J., Kim, M.K., et al., 2010. Intakes of vitamin A, C, and E, and beta-carotene are associated with risk of cervical cancer: a case-control study in Korea. Nutrition and Cancer 62 (2), 181–189.

Kris Etherton, P.M., Hecker, K.D., et al., 2002. Bioactive compounds in foods and their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113 (9B), 71S–88S.

Lagouge, M., Argmann, C., et al., 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 alpha. Cell 127, 1109–1122.

Liu, B., Chen, Y., et al., 2008. ROS and p53: a versatile partnership. Free Radical Biology & Medicine 44 (8), 1529–1535.

Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Radical Biology & Medicine 23 (1), 134–147.

Masaki, H., 2010. Role of antioxidants in the skin: anti-aging effects. Journal of Dermatological Science 58 (2), 85–90.

McCann, S.E., Thompson, L.U., et al., 2009. Dietary lignan intakes in relation to survival among women with breast cancer: the Western New York Exposures and Breast Cancer (WEB) Study. Breast Cancer Research and Treatment 122 (1), 229–235.

Messner, K.R., 2002. Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. Journal of Biological Chemistry 277 (45), 42563–42571.

Miller, R.A., Harrison, D.E., et al., 2010. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 66A (2), 191–201.

Ndiaye, M., Philippe, C., et al., 2011. The grape antioxidant resveratrol for skin disorders: promise, prospects, and challenges. Archives of Biochemistry and Biophysics 508 (2), 164–170.

Neu, X., Smith, C.W., et al., 1994. Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion to neutrophils. Circulation Research 74, 1133–1140.

O'Donnell, V.B., Eiserich, J.P., et al., 1999. Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion. Chemical Research in Toxicology 12 (1), 83–92.

Pacher, P., Beckman, J.S., et al., 2007. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews 87 (1), 315–424.

Packer, L., Colman, C., 1999. The Antioxidant Miracle. John Wiley & Sons, Inc., New York.

Packer, L., Smith, J.R., 1974. Extension of the lifespan of cultured normal human diploid cells by vitamin E. Proceedings of the National Academy of Sciences of the United States of America 71 (12), 4763–4767.

Park, Y., Spiegelman, D., et al., 2010. Intakes of vitamins A, C, and E and use of multiple vitamin supplements and risk of colon cancer: a pooled analysis of prospective cohort studies. Cancer Causes & Control 21 (11), 1745–1757.

Pena Ferreira, M.R., Costa, P.C., et al., 2010. Efficacy of anti-wrinkle products in skin surface appearance: a comparative study using non-invasive methods. Skin Research and Technology 16 (4), 444–449.

Pierce, J.P., Faerber, S., et al., 2002. A randomized trial of the effect of a plant-based dietary pattern on additional breast cancer events and survival: the Women's Healthy Eating and Living (WHEL) Study. Controlled Clinical Trials 23, 728–756.

Pratico, D., Clark, C.M., et al., 2002. Increase of brain oxidative stress in mild cognitive impairment. Archives of Neurology 59 (9), 972–976.

Reiter, R.J., 1995. Oxidative processes and antioxidative defense mechanisms in the aging brain. Federation of American Societies for Experimental Biology 9 (7), 526–533.

Rice-Evans, C.A., Gopinathan, V., 1995. Oxygen toxicity, free radicals and antioxidants in human disease: biochemical implications in atherosclerosis and the problems of premature neonates. Essays in Biochemistry 29, 39–63.

Rimando, A.M., Kalt, W., et al., 2004. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. Journal of Agricultural and Food Chemistry 52, 4713–4719.

Sakhi, A.K., Bohn, S.K., et al., 2010. Postradiotherapy plasma lutein, α-carotene, and β-carotene are positively associated with survival in patients with head and neck squamous cell carcinoma. Nutrition and Cancer 62 (3), 322–328.

Sastre, J., 2003. The role of mitochondrial oxidative stress in aging. Free Radical Biology & Medicine 35 (1), 1–8.

Schmid-Schonbein, G.W., 2006. Analysis of inflammation. Annual Review of Biomedical Engineering 8, 93–151.

Singh, P.P., Mahadi, F., et al., 2009. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2. Indian Journal of Clinical Biochemistry 24 (4), 324–342.

Stan, S.D., Kar, S., et al., 2008. Bioactive food components and cancer risk reduction. Journal of Cellular Biochemistry 104 (1), 339–356.

Tangrea, J., Helzlsour, K., et al., 1997. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes & Control 8 (4), 615–625.

Whitlock, N.C., Bahn, J.H., et al., 2011. Resveratrol-induced apoptosis is mediated by early growth response-1, Kruppel-like factor 4, and activating transcription factor 3. Cancer Prevention Research 4 (1), 116–127.

WHO, 2010. 10 Facts about Cancer. Retrieved 2 November 2010, from http://www.who.int/features/factfiles/cancer/en/index.html.

Yilmaz, O., Dursun, H., et al., 2010. Plasma vitamin A and E levels in patients with gastroesophageal cancer in Eastern Anatolia. Turkish Journal of Medical Sciences 40 (1), 115–119.

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Vitamins and Older Adults

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1. INTRODUCTION

In 2011, the baby boomers, those born between 1946 and 1964, began turning 65 years of age, reflecting a significant shift in the American population often referred to as the 'graying of America' (Harvard Kennedy School, 2006). Advancements in technology and improvements in healthcare, socioeconomic status, and health behaviors have led to a notable increase in longevity not only in the United States but also worldwide. In the United States, the fastest growing segment of the population is people over the age of 85 years old. Compared to younger adults, older adults comprise the segment of the population that is the most diverse and, therefore, at the greatest risk for undernutrition. Aging is associated with a number of physiological and psychosocial changes and increased needs for medical care, which place older adults at an increased risk for nutritional challenges. Eighty-seven percent of older adults have hypertension, diabetes, dyslipidemia, or a combination of these conditions (Center for Disease Control and Prevention, CDC, 2011) – all of which are associated with diet and lifestyle habits.

A number of factors associated with increasing age can affect nutritional status as illustrated in Table 4.1. Poor oral health in addition to changes in taste and smell affect the ability to detect and identify food flavors; this steadily declines with aging with the estimates that 85% of adults over 80 years of age have major olfactory impairments (Gripe et al., 1995). Individuals of 65–70 years of age may experience 'anorexia of aging.' Isolation, lower socioeconomic status, dysgeusia, dysphagia, gastroparesis, depression, and poor oral health are common reasons cited (Hays, 2006). Alterations in digestion-related hormone secretion and responsiveness, alterations in food intake-regulated regulatory mechanisms, and elevations in serum cytokine levels are additional factors.

The presence of chronic disease is also a key factor that influences the nutritional status of older Americans, as reportedly 85% of these individuals have one or more illnesses that impact absorption, transportation, metabolism, and excretion of nutrients (Drewnowski and Shultz, 2001). Therapeutic diets, such as low sodium, low cholesterol, low fat, and so on, are prescribed for the most common chronic diseases including heart disease, type 2 diabetes, and hypertension. Furthermore, the presence of disease may increase or decrease energy and/or nutrient needs. Hospitalization has been associated with accelerating age-associated

Table 4.1 Factors Associated with Nutrition Risk in Older Adults

Anorexia of aging
Alterations in oral health
  Dysphagia
  Poor dentition
  Ill fitting dentures
  Gingivitis
  Xerostomia
Bereavement
Chronic disease
Cognition
  Confusion
  Forgetfulness
Decreased taste and smell
Dementia
Depression
Economic status
Fatigue
Feeding dependency
Functional status
Impaired gastric motility
Isolation/lack of socialization
Lack of cooking skills
Lack of transportation
Malabsorption
Polypharmacy
Prescribed therapeutic diets

physiological changes and the promotion of malnutrition in adults over 65 years of age (Merk, 2010a). Malnutrition is also common in older adult populations residing in long-term care facilities, where reportedly 45–85% of older residents are undernourished (Merk, 2010a).

Given the numerous factors that can promote declines in nutritional status, nutrition screening and assessment are critical when working with older adults. Common micro-nutrient deficiencies reported in this population include vitamins A, C, E, and D and poor intake of calcium, magnesium, and zinc (Fletcher and Fairfield, 2002). This article will provide an overview of key vitamins and highlight the potential deficiencies that may occur in this population.

2. VITAMINS

2.1 Vitamin A

Vitamin A is a family comprised of a subgroup of compounds known as retinoids. Retinal, retinol, and retinoic acid are the three preformed compounds that exhibit metabolic

activity. Several plant-derived carotenoids also possess vitamin A activity and are included in the vitamin A family. Of the over 500 identified carotenoids, < 10% can be transformed by the body into the active form of vitamin A. Of the family of carotenoids, β-carotene can be most efficiently converted into retinol based on the body's vitamin A needs. α-carotene and β-cryptoxanthin can also be converted but not as efficiently. Lutein, lycopene, and zeaxanthin, while important carotenoids for a variety of health reasons, do not possess any vitamin A activity. The amount of vitamin A that results from conversion of these carotenoids depends on absorption, which ranges from 5% to 50% (Grune et al., 2010).

Vitamin A is required for a number of physiological functions including vision, immune function, normal reproduction, and osteogenesis. Deficiencies can arise because of malabsorption, poor dietary intake, and/or impaired transport (e.g., abetalipoproteinemia, liver disease, and malnutrition). The most significant adverse outcome associated with vitamin A deficiency is blindness, which is more common in developing countries. Follicular hyperkeratosis can also occur because of vitamin A deficiency.

Vitamin A deficiency is fairly common in older adults with about 25% affected because of inadequate dietary intake (Sebastian et al., 2007). Alternatively, 5–9% were found to exceed the upper limits for vitamin A primarily from food and use of dietary supplements. Given the concern regarding consumption of large doses of vitamin A and an increased incidence of hip fractures in postmenopausal women consuming over $3000\ \mu g\ day^{-1}$ of retinol, evaluation of vitamin A should be routinely assessed in older adults (Fletcher and Fairfield, 2002; Melhus et al., 1998; Promislow et al., 2002). It appears that the increase in fracture risk may be related to the use of dietary supplements containing large amounts [greater than recommended daily allowance (RDA)] of preformed vitamin A. Long-term ingestion of excess vitamin A can also lead to liver damage. Vitamin A toxicity is generally manifested by changes in skin and mucus membranes such as dry lips, nasal mucosa, and eyes; peeling skin, hair loss, nail fragility, irritability, fatigue, and hepatomegaly; abnormal liver function tests are later signs (Merck, 2010b). Table 4.2 summarizes the Institute of Medicine (IOM) recommendations for vitamin A intake.

2.2 B Vitamins

The B vitamins, thiamine, riboflavin, folate, niacin, vitamin B_6, and vitamin B_{12}, are a diverse group of compounds that are associated with numerous physiological functions in the body. While deficiencies of riboflavin, niacin, and pyridoxine are not common in older adults, thiamine, vitamin B_{12}, and folate levels can be more variable. Adequate thiamine levels can be affected by diuretics, alcohol consumption, and dialysis. Use of diuretics, such as furosemide, can promote marginal thiamine deficiency secondary to increased thiamine excretion (Zenuk et al., 2003). This is particularly of concern in individuals with congestive heart failure where loop diuretics are commonly prescribed for disease management.

Table 4.2 Institute of Medicine Recommended Dietary Intake Recommendations (DRI) for Selected Vitamins and Minerals [Recommended Daily Allowance (RDA) or Adequate Intake (AI)]

Micronutrients	Gender	
	Males	Females
Vitamins		
Vitamin A (RDA)		
Ages 51–70	900 µg day^{-1}	700 µg day^{-1}
Age >70	900 µg day^{-1}	700 µg day^{-1}
Vitamin C[a] (RDA)		
Ages 51–70	90 mg day^{-1}	75 mg day^{-1}
Age >70	90 mg day^{-1}	75 mg day^{-1}
Vitamin D (AI)		
Ages 51–70	600 IU day^{-1}	600 IU day^{-1}
Age >70	800 IU day^{-1}	800 IU day^{-1}
Vitamin E (RDA)		
Ages 51–70	15 mg day^{-1}	15 mg day^{-1}
Age >70	15 mg day^{-1}	15 mg day^{-1}
Vitamin K (AI)		
Ages 51–70	120 µg day^{-1}	90 µg day^{-1}
Age >70	120 µg day^{-1}	90 µg day^{-1}
Folate (RDA)		
Ages 51–70	400 µg day^{-1}	400 µg day^{-1}
Age >70	400 µg day^{-1}	400 µg day^{-1}
Vitamin B$_{12}$ (RDA)		
Ages 51–70	2.4 µg day^{-1}	2.4 µg day^{-1}
Age >70	2.4 µg day^{-1}	2.4 µg day^{-1}
Calcium (AI)		
Ages 51–70	1200 mg day^{-1}	1200 mg day^{-1}
Age >70	1200 mg day^{-1}	1200 mg day^{-1}
Magnesium (RDA)		
Ages 51–70	420 mg day^{-1}	320 mg day^{-1}
Age >70	420 mg day^{-1}	320 mg day^{-1}
Zinc (RDA)		
Ages 51–70	11 mg day^{-1}	8 mg day^{-1}
Age >70	11 mg day^{-1}	8 mg day^{-1}
Iron (RDA)		
Ages 51–70	8 mg day^{-1}	8 mg day^{-1}
Age >70	8 mg day^{-1}	8 mg day^{-1}
Selenium (RDA)		
Ages 51–70		
Age >70		

[a] Recommended intake for smokers is 35 mg day^{-1} greater than for nonsmoker for both genders.

Deficiencies of B vitamins, specifically of vitamins B_{12}, B_6, and folate, are associated with cognitive decline and neurologic dysfunction (Selhub et al., 2010). Whether supplementation with these nutrients or prevention of these deficiencies will prevent declines in cognitive function is unclear. This is reviewed in greater detail under the discussion regarding supplements.

2.2.1 Vitamin B_{12}

Vitamin B_{12}, a water-soluble vitamin, must be consumed from either the diet or supplementation. Despite the widespread availability of B_{12} from fortified foods in the diet, the CDC reports that data from the National Health and Nutrition Examination Survey (NHANES) 2001–04 estimates that 1 in 31 adults aged over 51 will have low vitamin B_{12} levels ($<200\,\mathrm{pg\,mL}^{-1}$) due to malabsorption, without overt signs of B_{12} deficiency (CDC, 2009). Other causes of vitamin B_{12} deficiency include achlorhydria, vegetarian diets, and bacterial overgrowth. The aging process decreases the body's ability to cleave the vitamin from its protein carrier, thereby resulting in less bioavailability. Additionally, older adults may not consume enough or any animal proteins, the primary source of vitamin B_{12}, because of difficulties with dentition and dysphagia, as well as expense. The requirement for vitamin B_{12} is similar for all adults across the life cycle. Both the IOM and the National Institutes of Health Office of Dietary Supplements recommend that older adults be encouraged to consume B_{12}-fortified foods (IOM, 1998; NIH, 2010). Synthetic vitamin B_{12}, which is utilized to fortify foods and dietary supplements, is easily absorbed making this a good source of B_{12} for individuals over 50 years of age.

Although elevated folate levels may be desirable to reduce the risk for cardiovascular disease by diminishing homocysteine levels, concerns have arisen that elevated folate concentrations will mask hematologic symptoms of vitamin B_{12} deficiency (Cuskelly et al., 2007). A more in-depth review follows in the discussion on folate.

Depression is often undetected in older adults and can affect self-care, ability to eat, and compliance with medical treatments. Symptoms of depression include weight loss or weight gain, boredom, lack of interest in activities, increased sleeping, inability to perform activities of daily living, memory problems, and other nonspecific complaints. Nutrition risk for men has been associated with higher depression scores, longer hospitalizations, and poor appetite (D'Anci and Rosenberg, 2004). Nutrition assessments for elderly individuals who have been diagnosed or are suspected of having depression should evaluate the adequacy of the individual's vitamin B_{12} intake – measuring methylmalonic acid is more accurate. Vitamin B_{12} status should also be evaluated in individuals using medications such as histamine-2 blockers, proton-pump inhibitors, and antibiotics (Huang et al., 2006; Termanini et al., 1998).

2.2.2 Folate

Folate, also known as folic acid and folacin, is a generic term for this water-soluble B-complex vitamin. Folic acid (pteroylmonoglutamic acid) is generally used in vitamin

supplements and fortified foods. The current dietary reference intake (DRI) for folate for all nonpregnant adults is 400 μg day^{-1} (IOM, 1998).

Fortified breakfast cereals and other grain products that are fortified with folic acid are generally good dietary sources of folate and folic acid. In 1998, the US Food and Drug Administration mandated that all grain products sold in the United States be fortified with folic acid to reduce the number of infants born with neural tube defects. Following folate fortification, folate status has improved in all age groups in the United States (Pfeiffer et al., 2005; Sebastian et al., 2007). In fact, older adults exhibited the highest red-blood-cell–folate levels in a recent NHANES report (CDC, 2008).

Although these data reflect positive folate status in the United States following the mandated food fortification, concerns have arisen that excess folate intake may be associated with adverse clinical outcomes. One such concern involves folate intake and the potential for a negative impact in older adults with low B_{12} levels given that elevated folate levels may 'mask' the hematologic signs of overt vitamin B_{12} deficiency although this is rare. A 70% increase for the risk of cognitive decline in the elderly with low B_{12} levels and normal folate status has been noted; the severity of decline increased with reduced B_{12} levels and high folate concentrations [odds ratio (OR) 5.1; 95% confidence interval (CI), 2.7–9.5] (Smith et al., 2008).

Additional concerns center around the potential dual role for folate in the carcinogenesis process. Folate plays an essential role in cellular differentiation and proliferation as well as DNA repair; low folate status reportedly is associated with DNA strand breaks and alterations in DNA repair (Smith et al., 2008). Results from colorectal cancer studies reveal that timing and dose of folate ingestion may be important (Smith et al., 2008; Song et al., 2000a,b). Song et al. (2000b) noted that if folate supplementation is initiated before the presence of neoplastic foci, tumor progression is prevented. Alternatively, when folate intervention is initiated following the development of neoplastic foci, then tumor progression is enhanced. Although large clinical trials have not yet been completed investigating these observations, some evidence from human trials is available. An increased rate of deaths from cancer (OR 1.7; 95% CI, 1.06–2.72) and a trend for increased breast cancer death (OR 2.02; 95% CI, 0.88–4.72) were noted as secondary outcomes in a follow-up trial involving pregnant women consuming 5 mg day^{-1} of folic acid (Charles et al., 2004).

Recent research has focused on the role of unmetabolized folic acid (UMFA) as a potential causative factor for the adverse outcomes related to folic acid intake. Using data from a representative sample of US older adults from the NHANES 2001–02, Bailey et al. found that around 40% of older Americans have UMFA levels that do not appear to be related to folic acid intake alone, although higher rates have been detected in supplement users compared with nonusers (Bailey et al., 2010; Kalmbach et al., 2008). The impact of UMFA levels and disease risk is unknown and further research is needed to determine whether UMFA may be associated with the adverse outcomes reported with excessive folic acid exposure.

Because folate is a water-soluble vitamin, deficiency of folate can develop fairly quickly (NIH, 2009). Folate deficiency is common and results from inadequate intake (alcoholism or poor oral intake), malabsorption, increased demand (e.g., pregnancy), increased excretion (dialysis) or due to the use of some medications. Medications such as methotrexate, phenytoin, nonsterodial anti-inflammatory drugs, cholestryamine, sulfasalazine, metformin, and triamterene act as folate antagonists.

2.3 Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin essential for physiological functions including the hydroxylation of proline and lysine, for collagen synthesis and connective tissue integrity. By reacting with reactive oxygen species such as the superoxide or hydroxyl radical, vitamin C reduces oxidative damage. Nitric oxide synthase activity can also be increased through vitamin C utilization in a series of endothelial intracellular steps (Huang et al., 2000). Iron absorption and mobilization is also facilitated with vitamin C, which also plays an integral role in the metabolism of folate, tyrosine, and xenobiotics (Schleicher et al., 2009). Lastly, vitamin C may play an essential role in bone mineral density.

Obtaining vitamin C either from the diet or through supplementation is critical as humans are unable to synthesize vitamin C. Insufficient vitamin C intake is manifested as poor wound healing, petechial hemorrhages, follicular hyperkeratosis, bleeding gums, and scurvy. The efficiency of gastrointestinal absorption ranges from 80 to 90% in the face of low intake; however, absorption is significantly reduced with intakes >1 g (Simon and Hudes, 2000).

A variety of fruits and vegetables are available as rich sources of vitamin C, and the intake of five servings of both (2½ cups) daily generally provides >200 mg day^{-1}. The current DRI for vitamin C is 75 mg for women and 90 mg for men; the requirement for smokers is increased by 35 mg.

In the United States, vitamin C intake generally exceeds the DRIs for both men and women although a number of investigators have reported that vitamin C depletion is fairly common in older adults (Pfeiffer et al., 2005). In the 2003–04 NHANES, about 13% of the US population was vitamin C deficient as defined by serum levels <11.4 μM (Schleicher et al., 2009); the highest concentrations were found in younger and older participants. While vitamin C was found to have improved between NHANES III and the 2003–04 NHANES, the better vitamin C status in older persons (>60 years) was in part due to supplement use.

Although vitamin C supplements improve vitamin C status, the use of such supplements, in general, has not led to clinical benefits. Many older adults take not only multivitamin supplements daily but also an additional vitamin C supplement (Sebastian et al., 2007). Reasons for taking vitamin C supplements range from prevention of colds and

infections as well as decreasing the risk for other disease including heart disease and cancer. Despite early reports from epidemiologic studies that higher intakes of vitamin C may reduce heart disease, type 2 diabetes, and some types of cancers, prospective studies have been more mixed (Sesso et al., 2008; Song et al., 2009). Studies evaluating the benefits of foods high in vitamin C or vitamin C supplements have also shown mixed results regarding oxidative damage to the eye. The age-related eye disease study, a large, prospective, randomized controlled trial showed no benefit with 500 mg day^{-1} of vitamin C (together with vitamin E, zinc, and β-carotene) on the development or progression of cataracts (Clemons et al., 2004). Alternatively, the Blue Mountains Eye study found that increasing vitamin C consumption (both from diet and supplementation) was associated with a significant reduction in 10-year risk of nuclear cataract (P for trend was 0.045) (Tan et al., 2008).

2.4 Vitamin D

Vitamin D insufficiency is now considered a global epidemic; more than one third of the US population, particularly older adults, have insufficient levels of vitamin D (Cherniak et al., 2008). A multitude of factors are contributory in older adults, including a reduction in cutaneous production with skin exposure to UVB radiation, decreased hepatic and renal activation, reduced outdoor exposure, seasonal changes, use of sunscreen, skin pigmentation, use of antiepileptic agents, malabsorption, cholestatic conditions, obesity, medications that either impair vitamin D activation or enhance clearance, and poor dietary intake.

As summarized in Table 4.3, hypovitaminosis D has been associated with an increased risk for a number of medical conditions including coronary heart disease (CAD), inflammation and endothelial cell dysfunction, hypertension, stroke, diabetes mellitus (DM), certain types of cancers, autoimmune and respiratory disorders, osteoporosis, sarcopenia, neurological disorders as well as musculoskeletal conditions (Cherniak et al., 2008). In a meta-analysis conducted by Autier and Gandini (2007), supplemental intakes of vitamin D [ranging from 400 to 800 IU day^{-1}] were associated with a 7% reduction in total mortality from any cause [risk ratio (RR) 0.93; 95% CI, 0.87–0.99]. Additionally, Martins

Table 4.3 Potential Consequences of Insufficient Vitamin D Intake

- Cardiovascular disease
- Certain cancers
- Decrease bone mineralization
- Decreased immunity
- Increased risk for neuromuscular dysfunction
- Increased risk for falls
- Decreased cognitive performance
- Secondary hyperparathyroidism

et al. (2007) found that the adjusted prevalence of hypertension (OR 1.30), diabetes (OR 1.98), obesity (OR 2.29), and elevated serum triglyceride levels (OR 1.47) was significantly increased in the first compared with the fourth quartile of serum 25-hydroxyvitamin D levels ($P < .001$ for all). On the other hand, others have reported that supplementation with vitamin D failed to show a reduction for CAD, DM, certain cancers, and hypertension (De Boer et al., 2008; Hsia et al., 2007; Pittas et al., 2010; Wactawski-Wende et al., 2006).

Single measurement likely does not reflect vitamin D status throughout the year as levels tend to be higher in the summer and fall months compared to winter and spring. While serum 25-hydroxycholecalciferol levels do not reflect the body's total vitamin D stores, this measurement is the most common indicator currently utilized for assessing vitamin D status (Chu et al., 2010). While there has been recent debate about the optimal serum levels of vitamin D, many experts recommend serum concentrations greater than 32 mg dL^{-1} to reduce the risk for secondary hyperparathyroidism and osteomalacia (Holick, 2007). The role of intraindividual variations and disease risk requires further investigation.

The current recommendations set by the IOM for vitamin D intake for healthy older adults is > 51 years old $= 600$ IU day^{-1}; > 70 years old $= 800$ IU day^{-1}; although others recommend intakes of 800–2000 IU day^{-1} for individuals residing in temperate latitudes to achieve serum levels > 30 mg dL^{-1} (Holick, 2007; Linus Pauling Institute, 2010).

In summary, vitamin D insufficiency may be linked to the prevalence of a variety of medical conditions with the strongest evidence to date for a role of vitamin D in reducing the risk for osteoporosis. A meta-analysis examining the level of vitamin D necessary for the prevention of hip fractures found that an intake of 800 IU day^{-1} by elderly women was associated with a lower incidence of both overall fractures (23% less) and hip fractures (26% less) (National Osteoporosis Foundation, 2003). Further studies are needed to better understand the role of vitamin D in addition to optimal doses and serum levels needed for both disease prevention and treatment. Vitamin D deficiency can be managed through pharmacologic vitamin D3 doses; 50 000 IU once per week for 8 weeks followed by supplementation with D2 twice a month (Roux et al., 2008).

2.5 Vitamin E

Vitamin E is a fat-soluble vitamin that describes a family of eight antioxidants and four tocopherols (α, β, γ, and δ-tocotrienol). Structures are similar except that the tocotrienol structure contains double bonds on the isoprenoid units. α-Tocopherol is the only form of vitamin E that is actively maintained in the human body and thus is the form of vitamin E found in the greatest quantities in the blood and tissues. Similar to the absorption needs of other fat-soluble vitamins, the absorption of vitamin E requires the presence of fat as well as adequate biliary and pancreatic function. Efficiency of absorption ranges from 20 to 70%.

Although vitamin E deficiency is rare, individuals with malabsorptive conditions, including Crohn's disease, cystic fibrosis, and abetalipoproteinemia (a rare inherited disorder of fat metabolism leading to poor absorption of dietary fat and vitamin E), and compromised biliary function may be at risk for developing vitamin E deficiency. Vitamin E deficiency is characterized by neurological symptoms including impairments in balance and coordination. Peripheral neuropathy, weakness, and retinopathy are also the symptoms associated with vitamin E deficiency.

The potential for toxicity of vitamin E is very low; it is considered to be the least toxic of all the fat-soluble vitamins. However, excessive levels could interfere with blood clotting and potentiate blood-thinning medications such as dicumarol; hence, a recommended upper limit of 1000 mg day^{-1} (1500 IU of α-tocopherol) has been established (IOM, 2000).

The current RDA for vitamin E is 22.5 IU day^{-1} for adults. Reportedly, the intake of vitamin E-containing foods by consumers in the United States is low, with the majority of Americans not meeting the current dietary recommendations (Ahuja et al., 2004; Maras et al., 2004; Sebastian et al., 2007). Conversely, vitamin E supplements are regularly consumed, at high doses, by a wide variety of people in the United States for the prevention and treatment of cardiovascular disease and cancer. While this may seem a plausible solution to correct the widespread insufficient intake of vitamin E reported in the United States, data from well-designed clinical studies are not available to support this practice.

3. DIETARY SUPPLEMENTS

Many older adults reportedly consume a multitude of dietary supplements on a daily basis for a variety of reasons including disease prevention, disease treatment, or just to feel better (Sebastian et al., 2007). Many times these individuals are generally adequately nourished if not overnourished since 30% of older adults. However, ingestion of dietary supplements may also play a critical role in health maintenance and disease prevention. Owing to dietary preferences, dietary intolerances (e.g., lactose intolerance), and need for medications (e.g., proton-pump inhibitors), dietary supplement use may be necessary to fill gaps in micronutrient intake when diet alone is inadequate (Sebastian et al., 2007). Furthermore, many older adults are taking diuretic therapies, which increase the urinary excretion of several micronutrients including thiamine, potassium, magnesium, and calcium. Dietary intake of these micronutrients in the elderly population is generally insufficient (Marian and Sacks, 2009). Additionally, achieving optimal vitamin D intake through diet alone is almost impossible – particularly for older adults where intake in general may be suboptimal – therefore, daily vitamin D supplementation is likely necessary to obtain sufficient levels associated with any of the possible benefits as previously discussed.

Despite the potential benefits that may be derived from consuming dietary supplements, adverse effects have also been reported. In a meta-analysis by Bjelakovic et al. (2004), the use of high-dose antioxidants was associated with an increase in mortality (RR, 1.16; 95% CI, 1.05–1.29) in trials with a low bias; particularly the ingestion of vitamin E, when consumed either alone or in combination with other antioxidants. Similar results have been reported for β-carotene and vitamin A supplement use. Moreover, Bjelakovic et al. surmise that between 10 and 20% of Americans and Europeans may be utilizing such supplements, thus the impact may be considerable. Miller et al. recommend that given the weight of such evidence, excess supplement use by older individuals should be avoided to avoid potential adverse outcomes (Miller et al., 2005). Assessment of dietary intake, medications, and lifestyle habits should be thoroughly evaluated in order to determine if dietary supplementation is warranted.

4. CONCLUSION

The presence of chronic conditions is common in older adults and can have a variety of negative effects on nutritional and functional status as well as morbidity and mortality. Undernutrition is common in hospitalized and institutionalized older adults and can be a prognostic indicator of outcomes. Nutrition interventions are associated with improving not only health but also quality of life in this population. While consumption of a nutrient-dense diet can meet daily micronutrient needs for most Americans, potential challenges with achieving an adequate dietary intake may arise with this population due to dentition, cognition, functional status, economics, education level, medication usage, and a variety of other factors that in general affect dietary intake. Therefore, dietary supplements should be considered in order to meet the established DRIs to prevent potential deficiencies from developing as well as to correct any existing deficiencies that may be present. All clinicians caring for older adults should screen for the presence or potential for malnutrition at each clinic visit. Individuals at high risk include those with more than one chronic illness, taking a variety of medications, have a low functional status, and poor social support. A number of community-based programs are available that can promote adequate nutrient intake as well as socialization, which may improve performance status and quality of life.

REFERENCES

Ahuja, J.K., Goldman, J.D., Moshfegh, A.J., 2004. Current status of vitamin E nutriture. Annals of the New York Academy of Sciences 1031, 387–390.

Autier, P., Gandini, S., 2007. Vitamin D supplementation and total mortality. Archives of Internal Medicine 167, 1730–1737.

Bailey, R.L., Mills, J.L., Yetley, E.A., et al., 2010. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged >60 y in the United States. The American Journal of Clinical Nutrition 92, 383–389.

Bjelakovic, G., Nikolova, D., Simonetti, R.G., Gluud, C., 2004. Antioxidant supplements for preventing gastrointestinal cancers. Cochrane Database of Systematic Reviews 4, CD004183–389.10.1002/14651858.CD004183.pub2.

CDC – Centers for Disease Control and Prevention, 2008. Blood Folate Levels: The latest NHANES Results. http://www.cdc.gov/nchs/data/databriefs/db06.htm (accessed 31 July 2010).

CDC – Centers for Disease Control and Prevention, 2009. Vitamin B12 Deficiency. http://www.cdc.gov/ncbddd/b12/history.html (accessed 15 January 2011).

CDC – Centers for Disease Control and Prevention, 2011. National Center for Health Statistics. http://www.cdc.gov/nchs/pressroom/ (accessed 30 November 2010).

Charles, D., Ness, A.R., Campbell, D., Davey Smith, G., Hall, M.H., 2004. Taking folate in pregnancy and risk of maternal breast cancer. British Medical Journal 329, 1375–1376.

Cherniak, E.P., Florez, H., Roos, B.A., et al., 2008. Hypovitaminosis D in the elderly: from bone to brain. The Journal of Nutrition, Health and Aging 12, 366–373.

Chu, M.P., Alagiakrishnan, K., Sadowski, C., 2010. The cure of ageing: vitamin D – magic or myth? Postgraduate Medical Journal 86, 608–616.

Clemons, T.E., Kurinij, N., Sperduto, R.D., 2004. Associations of mortality with ocular disorders and an intervention of high-dose antioxidants and zinc in the age-related eye disease study: AREDS report no. 13. Archives of Ophthalmology 122, 716–726.

Cuskelly, G.J., Mooney, K.M., Young, I.S., 2007. Folate and vitamin B_{12}: friendly or enemy nutrients for the elderly. Proceedings of the Nutrition Society 66, 548–558.

D'Anci, K.E., Rosenberg, I.H., 2004. Folate and brain function in the elderly. Current Opinion in Clinical Nutrition and Metabolic Care 7, 659–664.

De Boer, I.H., Ticker, L.F., Connelly, S., et al., 2008. Calcium plus vitamin D supplementation and the risk of incident diabetes in WHI. Diabetes Care 31, 701–707.

Drewnowski, A., Shultz, J.M., 2001. Impact of aging on eating behaviors, food choices, nutrition, and health status. The Journal of Nutrition, Health and Aging 5, 75–79.

Feskanich, D., Weber, P., Willett, W.C., et al., 1999. Vitamin K intake and hip fractures in women: a prospective study. The American Journal of Clinical Nutrition 69, 74–79.

Fletcher, R.H., Fairfield, K.M., 2002. Vitamins for chronic disease prevention in adults: clinical applications. JAMA: The Journal of the American Medical Association 287, 3127–3129.

Gripe, M.I., Mets, T.F., Vercruysse, A., et al., 1995. Food odor thresholds in relation to age, nutritional, and health status. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 50A, B407–B414.

Grune, T., Liet, G., Palou, A., et al., 2010. Beta-carotene is an important vitamin A source for humans. The Journal of Nutrition 140, 2268S–2285S.

Harvard Kennedy School, 2006. Paul Hodge on the "Graying of America". John F. Kennedy School of Government. http://www.hks.harvard.edu/news-events/news/articles/ (accessed 30 March 2011).

Holick, M.F., 2007. Vitamin D deficiency. The New England Journal of Medicine 357, 266–281.

Hsia, J., Heiss, G., Ren, H., et al., 2007. Women's Health Initiative Investigators. Calcium/vitamin D supplementation and cardiovascular events. Circulation 115, 846–854.

Huang, H.Y., Caballero, B., Chang, S., et al., 2006. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. Annals of Internal Medicine 145, 372–385.

Huang, A., Vita, J.A., Venema, R.C., Keaney Jr., J.F., 2000. Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. The Journal of Biological Chemistry 275, 17399–17406.

IOM – Institute of Medicine Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington D.C.

IOM – Institute of Medicine, Food and Nutrition Board, 1998. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B_6, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. National Academy Press, Washington D.C.

Kalmbach, R.D., Choumenkovitch, S.F., Troen, A.M., et al., 2008. Circulating folic acid in plasma: relation to folic acid fortification. The American Journal of Clinical Nutrition 88, 763–768.

Linus Pauling Institute Micronutrient Research for Optimum Health, 2010. Vitamin D. http://lpi.oregonstate.edu/infocenter/vitamins/vitaminD/ (accessed 17 March 2011).

Maras, J.E., Bermudez, O.I., Qiao, N., Bakun, P.J., Boody-Alter, E.L., Tucker, K.L., 2004. Intake of alpha-tocopherol is limited among US adults. Journal of the American Dietetic Association 104, 567–575.

Marian, M., Sacks, G., 2009. Micronutrients and older adults. Nutrition in Clinical Practice 24, 179–195.

Martins, D., Wolf, M., Pan, D., et al., 2007. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine 167, 1159–1165.

Melhus, H., Michaelsson, K., Holmberg, L., Wolk, A., Ljunghall, S., 1999. Smoking, antioxidant vitamins, and the risk of hip fracture. Journal of Bone and Mineral Research 14, 129–135.

Melhus, H., Michaelsson, K., Kindmark, A., et al., 1998. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk of hip fracture. Annals of Internal Medicine 129, 770–778.

Merck Online Medical Library, 2010a. Chapter 11: Continuity of Care: Integration of Services. http://www.merckmanuals.com/professional/sec23.html (accessed 11 February 2011).

Merck Online Medical Library, 2010b. Vitamin A. http://www.merckmanuals.com/professional/sec01/ch004/ch004g.html (accessed 4 December 2010).

Miller III, E.R., Pastor-Barriuso, R., Dalal, D., et al., 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Annals of Internal Medicine 142, 37–46.

National Osteoporosis Foundation, 2003. Physician's Guide to Prevention and Treatment of Osteoporosis. http://www.nof.org (accessed 10 January 2011).

NIH − National Institutes of Health, Office of Dietary Supplements, 2009. Dietary Supplement Fact Sheet: Folate. http://ods.od.nih.gov/factsheets/folate (accessed 17 March 2011).

NIH − National Institutes of Health, Office of Dietary Supplements, 2010. Dietary Supplement Fact Sheet: Vitamin B_{12}. http://ods.od.nih.gov/factsheets/vitaminb12.asp (accessed 1 August 2010).

Pfeiffer, C.M., Caudill, S.P., Gunter, E.W., Osterloh, J., Sampson, E.J., 2005. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000. The American Journal of Clinical Nutrition 82, 442–450.

Pittas, A.G., Chung, M., Trikalines, T., et al., 2010. Systematic review: vitamin D and cardiometabolic outcomes. Annals of Internal Medicine 152, 307–314.

Promislow, J.H.E., Goodman-Gruen, D., Slymen, S.J., Barrett-Connor, E., 2002. Retinol intake and bone mineral density in the elderly: the Rancho Bernardo study. Journal of Bone and Mineral Research 17, 1349–1358.

Roux, C., Bischoff-Ferrari, H.A., Papapoulos, S.E., de Papp, A.E., West, J.A., Bouillon, R., 2008. New insights into the role of vitamin D and calcium in osteoporosis management: an expert roundtable discussion. Current Medical Research and Opinion 24, 1363–1370.

Schleicher, R.L., Carroll, M.D., Ford, E.S., Lacher, D.A., 2009. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). The American Journal of Clinical Nutrition 90, 1252–1263.

Sebastian, R.S., Cleveland, L.E., Goldman, J.D., Moshfegh, A.J., 2007. Older adults who use vitamin/mineral supplements differ from nonusers in nutrient intake adequacy and dietary attitudes. Journal of the American Dietetic Association 107, 1322–1332.

Selhub, J., Troen, A., Rosenberg, J.H., 2010. B vitamins and the aging brain. Nutrition Reviews 68 (S2), S112–S118.

Sesso, H.D., Buring, J.E., Christen, W.G., et al., 2008. Vitamins E and C in the prevention of cardiovascular disease in men: the physicians' health study II randomized controlled trial. JAMA: The Journal of the American Medical Association 300, 2123–2133.

Simon, J.A., Hudes, E.S., 2000. Serum ascorbic acid and gallbladder disease prevalence among US adults: the Third National Health and Nutrition Examination Survey (NHANES III). Archives of Internal Medicine 160, 931–936.

Smith, A.D., 2007. Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12. The American Journal of Clinical Nutrition 85, 3–5.

Smith, A.D., Young-In, K., Refsum, H., 2008. Is folic acid good for everyone? The American Journal of Clinical Nutrition 87, 517–533.

Song, Y., Cook, N.R., Albert, C.M., Van Denburgh, M., Manson, J.E., 2009. Effects of vitamins C and E and β-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. The American Journal of Clinical Nutrition 90, 429–437.

Song, J., Medline, A., Mason, J.B., Gallinger, S., Kim, Y.I., 2000a. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Research 60, 5434–5440.

Song, J., Sohn, K.J., Medline, A., Ash, C., Gallinger, S., Kim, Y.I., 2000b. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. Cancer Research 60, 3191–3199.

Sriram, K., Lonchyna, V.A., 2009. Micronutrient supplementation in adult nutrition therapy: practical considerations. Journal of Parenteral and Enteral Nutrition 33, 1–15.

Tan, A.G., Mitchell, P., Flood, V.M., et al., 2008. Antioxidant nutrient intake and the long-term incidence of age-related cataract: the Blue Mountains Eye study. The American Journal of Clinical Nutrition 87, 1899–1905.

Termanini, B., Gibril, F., Sutliff, V.E., Yu, F., Venzon, D.J., Jensen, R.T., 1998. Effect of long-term gastric acid suppressive therapy on serum vitamin B12 Levels in patients with Zollinger-Ellison syndrome. The American Journal of Medicine 104, 422–430.

Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., et al., 2006. Calcium plus vitamin D supplementation and the risk of colorectal cancer. The New England Journal of Medicine 354, 684–696.

Zenuk, C., Healey, J., Donnelly, J., Vaillancourt, R., Almalki, Y., Smith, S., 2003. Thiamine deficiency in congestive heart failure patients receiving long term furosemide therapy. The Canadian Journal of Clinical Pharmacology 10, 184–188.

CHAPTER *5*

Food and Longevity Genes

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ABBREVIATIONS

AGRP Agouti-related peptide
AMPK AMP-activated protein kinase
CART Cocaine- and amphetamine-regulated transcript
CRH Corticotropin-releasing hormone
GHRH Growth hormone (GH)-releasing hormone
GnRH Gonadotropin-releasing hormone
LPS Lipopolysaccharide
mTOR Mammalian target of rapamycin
NPY Neuropeptide Y
PI3K Phosphatidylinositol 3-kinase
POMC Proopiomelanocortin
PRL Prolactin
SNS Sympathetic nervous system
TRH Thyrotropin-releasing hormone
TSH Thyroid-stimulating hormone

1. INTRODUCTION

Aging processes are diverse and can be unique to individual organisms or species. Yeasts undergo a limited number of budding, which can be used as a measure of decline with age, known as replicative lifespan. Adult nematodes, which are comprised of postmitotic cells, die because of degenerative processes in tissues; they never develop neoplasms. In mammals, many physiological functions, such as cognition, decline with age. The number of neoplastic and nonneoplastic lesions increases with advancing age, resulting in the elevation of mortality rate. Even in inbred strains of rats and mice, disease patterns and ultimate cause of death are diverse. Surprisingly, a simple reduction in caloric intake, while providing essential nutrients, consistently retards most aging and disease processes and extends lifespan in many organisms (Masoro, 2003).

Reduction-of-function mutations of *age-1* (mammalian phosphatidylinositol 3-kinase) were reported to increase lifespan in nematodes (Greer and Brunet, 2009). Single gene mutations or genetic manipulations have since been shown to prolong

lifespan not only in invertebrates but also in rodents. At present, over 20 of these genes, referred to here as longevity genes, have been reported in rodents (Shimokawa et al., 2008). The phenotypes associated with some longevity mutants resemble those of dietary restriction (DR) animals. A combination of genetic manipulations and the DR paradigm has enhanced one's understanding of signals that regulate aging and lifespan in mammals. These results indicate the possibility that compounds acting on the regulatory molecules or signals could be applied to retardation of aging and aging-related disorders in humans.

This chapter reviews the current understanding of molecular mechanisms by which DR affects aging and lifespan, with particular reference to longevity genes and signals.

2. HISTORICAL VIEW OF DR

Laboratory rodents are usually well nourished as they have free access to well-balanced foods; a feeding strategy referred to as ad libitum (AL). Compared to AL-fed animals, rodents fed a restricted diet (usually 30–40% caloric reduction as compared to AL feeding), but supplied with essential nutrients, live longer. Since McCay and colleagues reported this finding in 1935, a series of studies have revealed that many aging-dependent physiological deteriorations and diseases are delayed or eradicated in DR animals (Masoro, 2003). Although it is difficult to define acceleration or deceleration of aging, many gerontologists believe DR retards the aging process in the context of comparison with the AL feeding regimen.

Many researchers have attempted to determine the key components of the antiaging effect of DR. The suggestion that food restriction may dilute toxic substances is unlikely because most of the tested laboratory foods, semisynthetic or not, had the same effect on extension of lifespan (Masoro, 2003). Restriction of protein, fat, or minerals, but with an equivalent energy supply, had minor effects when compared to total DR. Different protein sources also exhibited some modest effects. Thus, the general consensus is that restriction of dietary energy (calories) is a key component in the effect of DR on longevity (Masoro, 2003).

The oxidative stress and damage theory is the most extensively tested hypothesis to explain the mechanisms underlying the effect of DR. It is suggested that DR retards aging and extends lifespan by decreasing oxidative damage via a reduction in the generation of reactive oxygen species (ROS) and/or enhancing protective mechanisms against oxidative stresses. Many studies have reported supportive data; however, most of the findings are indirect and correlative. In fact, experiments that aim at reducing oxidative damage by enhancing protective mechanisms (e.g., by administration of antioxidants or overexpression of ROS scavenger enzyme genes) have mostly failed to extend lifespan in rodents (Pérez et al., 2009). Although modulation of ROS generation in mitochondria, and thus regulation of mitochondrial function, could be a part of the effect of DR (Shimokawa et al., 2008), simple enhancement of protective mechanisms against oxidative stress did not mimic the effect of DR, at least in mammals.

3. NEUROENDOCRINE HYPOTHESIS OF DR

3.1 Evolutionary View of the Effect of DR

The mechanism by which DR affects the aging process may be explained from an evolutionary biology viewpoint (Holliday, 2006). It is predicted that animals have evolved physiological systems to maximize survival during periods of food shortage. When environmental food resources are plentiful, animals can grow, reproduce, and store excess energy as triacylglycerol in adipocytes. Once animals encounter a period of food shortage, seasonally or abruptly occurring in nature, they suspend growth and reproduction, while they activate defense systems such as the adrenal glucocorticoid and heat shock protein systems. Animals also shift whole-body fuel utilization from both carbohydrate and fat to almost exclusively fat.

One may posit that the antiaging effect of DR is derived from these adaptive responses to food shortage. The evolutionary viewpoint suggests the presence of key molecules favoring longevity, such as transcription factors that regulate energy flux among tissues and stress response.

3.2 Neuroendocrine Hypothesis of DR

The hypothalamic arcuate nuclei of mammals, in which two groups of neurons competitively regulate the other hypothalamic neurons, play a central role in adapting to food shortage (Schwartz et al., 2000). One group of neurons expresses neuropeptide Y (NPY) and/or agouti-related peptide (AGRP); the other expresses proopiomelanocortin (POMC) and/or cocaine- and amphetamine-regulated transcript (CART). These neurons project to the other hypothalamic nuclei and regulate neurons which contain gonadotropin-releasing hormone (GnRH), growth hormone (GH)-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH). Fasting or food shortage reduces circulating hormones such as insulin, leptin, and insulin-like growth factor 1 (IGF-1) but augments ghrelin and adiponectin (Schwartz et al., 2000; Shimokawa et al., 2008). The hypothalamic neurons have specific receptors to sense alterations in plasma concentrations of these hormones. Food shortage activates the NPY/AGRP neurons, whereas it attenuates activity of the POMC/CART neurons, leading to reduced activity of GnRH, GHRH, and TRH neurons, and activation of CRH neurons. These hypothalamic changes result in attenuation of growth, reproductive, and thermogenic activities through modulation of synthesis and secretion of pituitary hormones, while activation of the adrenal axis and autonomic nervous system could also play a role in the adaptation.

Published data support the neuroendocrine hypothesis of DR. DR rodents mostly exhibit the predicted hypothalamic and pituitary changes (Shimokawa et al., 2008). When several components of the neuroendocrine system are modified in the same direction as those induced by DR, rodent lifespan is extended. For example, overexpression of the NPY gene in rats, spontaneous mutation of the GHRH receptor in mice, and chemical ablation of TRH neurons are reported to extend lifespan (Shimokawa et al., 2008).

Ames and Snell mice, in which pituitary GH, prolactin (PRL), and thyroid-stimulating hormone (TSH) are deficient because of loss-of-function mutations of *Prop1* and *Pou1f1*, respectively, also outlived wild-type (WT) counterparts.

4. LONGEVITY GENES AND RELEVANCE TO THE EFFECT OF DR

4.1 GH/IGF-1 Axis

Longevity genes are clustered in the nutrient-sensing pathway, particularly the GH/IGF-1 and/or insulin axes (Shimokawa et al., 2008). Inhibition or disruption of GH/IGF-1 signaling, such as deletion of the GH-receptor/binding protein (GHR/BP) gene or the IGF receptor type 1 gene, consistently increases lifespan in rodents. The reduced GH/IGF-1 signaling concomitantly decreases the circulating insulin concentration and the glucose-stimulated serum insulin response. Because DR inhibits the pulsatile secretion of pituitary GH and reduces the serum concentrations of IGF-1 and insulin (Shimokawa et al., 2008), GH/IGF-1 signaling is considered to be a key mediatory pathway for the effect of DR.

However, epistatic analyses of the effect of the GH/IGF-1 axis in DR have proven inconclusive (Shimokawa, 2010). GHR/BP-KO mice, in which plasma IGF-1 levels are less than 10% of those in control WT mice, did not respond to DR with an extended lifespan, suggesting that the GH/IGF-1 axis is a key signaling mechanism in the effect of DR. In contrast, the lifespan of Ames dwarf mice, in which not only GH but also PRL and TSH are deficient, was extended in response to DR, indicating that the GH/IGF-1 axis is dispensable in the effect of DR. The different hormonal milieu between GHR/BP-KO and Ames mice may confound the effect of DR. However, GH treatment for only 6 weeks, initiated at 2 weeks of age, surprisingly negated the extended lifespan and stress-resistance traits of Ames mice. Although the GH/IGF-1 axis is not the sole signaling pathway, it is undoubtedly one of the key mediators of the effect of DR.

4.2 Molecules Downstream of the GH-IGF-1 Axis

4.2.1 Forkhead box O proteins and nuclear factor erythroid 2-related factor 2

In *Caenorhabditis elegans*, extension of lifespan caused by reduced insulin-like signaling requires DAF-16, a transcription factor that regulates genes involved in stress response and energy metabolism. DR conditions are induced in *C. elegans* by crossing with *eat-2* mutants, in which the pharyngeal pumping rate is diminished (Greer and Brunet, 2009). At present, a variety of DR regimens have been developed, including dilution of bacteria in cultures or use of chemically defined liquid medias (Greer and Brunet, 2009). Mutants of *daf-16* did not have an altered lifespan in response to DR induced by bacterial dilution, suggesting a key role for the transcription factor in the effect of DR (Greer and Brunet, 2009).

Mammalian orthologs of *daf-16* include *FoxO1*, *FoxO3a*, *FoxO4*, and, recently, *FoxO6* (van der Horst and Burgering, 2007). In mice, deletion of *FoxO3a* or *FoxO4* does not result

in a marked phenotype under AL feeding conditions (Paik et al., 2007). *FoxO1*-null mice are reported to die at the embryonic stage as a result of abnormal development of the blood vessels and heart and a conditional knockout of *FoxO1* after weaning does not produce a severe phenotype (Paik et al., 2007). Mice with triple KO of *FoxO1*, *FoxO3a*, and *FoxO4* show a high incidence of malignant tumors and a shortened lifespan (Paik et al., 2007), indicating redundancy of *FoxO* genes for inhibition of tumor-igenesis under AL conditions.

We tested the role of FoxO1 in DR using *FoxO1*-KO heterozygous (HT, +/−) mice. FoxO1-target genes include stress response and metabolic genes such as *Cdkn1a* (cell cycle arrest), *Gadd45a* (DNA repair), and *Pepck* (gluconeogenesis) (van der Horst and Burgering, 2007). *FoxO1* mRNA levels were decreased in multiple tissues by approximately 50% of those in the WT (Yamaza et al., 2010). In HT mouse tissue, the mRNA levels of *FoxO3a* and *FoxO4* were similar to those of WT mice. DR slightly upregulated the *FoxO1* mRNA levels in some, but not all, tissues; DR did not affect the expression levels of *FoxO3a* and *FoxO4*. Thus, it is unlikely that *FoxO3a* and *FoxO4* expression complements the reduced level of *FoxO1* under DR conditions. Research by the authors has indicated that the life-span of the HT mice as well as the WT mice was increased by DR (Yamaza et al., 2010). Interestingly, the proportion of mice bearing tumors at death was not reduced by DR in the HT mice, although it was significantly reduced in the WT-DR mice. Findings suggest that the well-documented antineoplastic effect of DR was diminished in the HT mice. In a subsequent study using a chemically induced skin tumor model, the authors confirmed that the inhibitory effect of DR on skin tumors was abrogated in the HT mice when compared to the WT mice, suggesting an important role for FoxO1 in the effect of DR (Komatsu et al., unpublished observation). An experiment assessing FoxO1-target gene expression revealed that DR–specific upregulation of genes involved in stress response (*Cdkn1a and Gadd45a*), inflammation (C*ox2*), and autophagy (*Atg6, Atg8, and Atg12*) after 12-*O*-tetra-decanoylphorbol-13-acetate (TPA) treatment was attenuated in the skin of HT mice. The authors' findings suggest that DR exhibits its antineoplastic effect through regulation of FoxO1-target genes in response to oxidative or genotoxic stress.

Abrogation of the antineoplastic effect of DR is also reported in nuclear factor ery-throid-derived 2-related factor 2 (*Nfe2l2*)-null mice using a chemically induced skin tu-mor model (Pearson et al., 2008). Nfe2l2 is a cap-n-collar transcription factor activated by oxidative stress and electrophilic xenobiotics (Sykiotis and Bohmann, 2010). Nfe2l2 forms heterodimers with the small musculoaponeurotic fibrosarcoma oncogene (Maf) family of proteins and binds to antioxidant response element sequences. Binding leads to the coordinated transcriptional activation of a battery of antioxidant enzymes and detoxifying proteins (Sykiotis and Bohmann, 2010).

In *C. elegans*, SKN-1 is also required for the lifespan extension brought about by reduced insulin signaling and DR (Bishop and Guarente, 2007; Tullet et al., 2008). DAF-16 and SKN-1 are the two main stress-activated cytoprotective transcription

factors in *C. elegans*. In fact, a prosurvival hormetic response to the xenobiotic juglone in *C. elegans* is mediated by DAF-16 and/or SKN-1–target genes (Przybysz et al., 2009).

Some Nfe2l2-target genes are also known to possess binding sites for FoxO1, for example, glutamate–cysteine ligase regulatory subunit (*Gclc*), heme oxygenase 1 (*Hmox1*) (Przybysz et al., 2009), and genes involved in autophagy such as *Atg6*, *Atg8*, and *Atg12* (Liu et al., 2009). In contrast, the gene coding for NAD(P)H-quinone oxidoreductase 1 (*Nqo1*) is regulated by Nfe2l2 but not by FoxO1. In a preliminary study using a chemically induced skin tumor model in FoxO1 (+/−) mice, TPA treatment led to upregulation of *Nqo1*-mRNA, but not *Gclc*, *Hmox1*, *Atg6*, *Atg8*, or *Atg12* mRNA. These results imply that full expression of FoxO1 is necessary for DR-specific induction of FoxO1- and Nfe2l2-dual-target genes in response to TPA. Additionally, *Nfe2l2* KO (+/−) mice also exhibited the diminished antineoplastic effect of DR (Pearson et al., 2008). Because *FoxO1* expression should be normally regulated in Nfe2l2 (+/−) mice, the antineoplastic effect of DR requires full expression of both FoxO1 and Nfe2l2 transcription factors.

4.2.2 Mammalian target of rapamycin

Current studies on aging and longevity in a range of laboratory organisms indicate that multiple longevity signals culminate at serine/threonine protein kinase mammalian target of rapamycin (mTOR; Zoncu et al., 2011). mTOR senses cellular energy levels by monitoring the cellular ATP:AMP ratio via AMP-activated protein kinase, growth factors such as insulin and IGF-1, amino acids via Rag GTPases, and the Wnt family signal–glycogen synthesis kinase 3 pathway. mTOR is found in two distinct protein complexes, mTORC1 and mTORC2. mTORC1, which is composed of mTOR, RAPTOR, and LST8, is sensitive to rapamycin; in contrast, mTORC2, a complex of mTOR, LST8, RICTOR, and MAPKAP1, is not inhibited by rapamycin.

Activated growth factor signaling leads to the activation of thymoma viral proro-oncogene AKT, which, in turn, phosphorylates tuberous sclerosis 2 (TSC2). This inhibits TSC1/2 GTPase-activating protein activity toward the small GTPase, Ras homolog enriched in brain (RHEB), which is required for activation of mTORC1. Active mTORC1 phosphorylates its downstream substrates eukaryotic translation initiation factor 4E-binding protein 1 and ribosomal protein S6 kinase beta-1 (p70S6K), which stimulate protein synthesis and cell growth (Zoncu et al., 2011).

Genetically or pharmacologically reduced TOR signaling is reported to extend lifespan in yeast, *C. elegans*, and *Drosophila melanogaster* (Zoncu et al., 2011). Deletion of p70S6K1 in mice is also reported to favor longevity (Selman et al., 2009). Administration of rapamycin in food has also been reported to extend lifespan in mice (Harrison et al., 2009). In this experimental setting, phosphorylated ribosomal subunit protein S6, which is a substrate of p70S6K, was significantly reduced in white adipose tissue. It remains to be elucidated if DR exhibits its effects through inhibition of mTOR; however, mTOR is considered one of the key molecules regulating aging and lifespan in mammals.

4.3 NPY Axis

Several lines of evidence support a key role for NPY in the effects of DR. As described in Section 3.2, the expression level of NPY-mRNA is augmented by DR in the arcuate nucleus of the hypothalamus. In the mammalian central nervous system, NPY is one of the most abundant and widely distributed neuropeptides, indicating the importance of NPY as a neuromodulator. Selective activation of specific receptors for NPY in organotypic cultures of rat hippocampal slices results in a neuroprotective effect against glutamate receptor-mediated neurodegeneration (Silva et al., 2003). The neuroprotective activity of NPY and its Y2 and Y5 receptor ligands against the kainite-induced excitotoxicity in primary cortical and hippocampal cultures was also demonstrated (Smiałowska et al., 2009). The Y2 receptor agonist also had a neuroprotective effect in an ischemic middle cerebral artery occlusion model. DR is also reported to protect the brain from these injuries. Therefore, NPY could be a key component of DR-induced stress resistance in the brain.

NPY has also been reported to promote proliferation of postnatal neuronal precursor cells (Hansel et al., 2001), which could contribute to attenuation of aging-dependent deterioration of cognitive function in mice. DR can also stimulate neurogenesis and enhance synaptic plasticity (Mattson et al., 2003).

NPY may be involved in stress response through suppression of the GH/IGF-1 axis. In fact, previous studies indicate that GH and GHRH secretion decreases in times of stress, such as fasting, following an initial increase in NPY levels (Luque et al., 2007). GH resistance may be one of the protective responses in animals under critical conditions, including natural aging. Long-lived transgenic dwarf rats, in which GH synthesis or secretion is moderately inhibited by overexpression of antisense GH genes (Shimokawa et al., 2008), showed resistance to lipopolysaccharide (LPS)-induced inflammatory stress (Komatsu et al., 2011). Thus, the NPY/GHRH/GH/IGF-1 axis may be a main pathway for the regulation of aging and lifespan under DR conditions.

Sympathetic innervation and peripheral NPY may also mediate the effect of DR on stress. Norepinephrine is preferentially released from the postganglionic sympathetic nerves to maintain vascular tone in the resting state. However, under conditions of high-intensity sympathetic nerve activation, i.e., stress conditions, large amounts of NPY are released into circulation (Zukowska-Grojec, 1995).

In fact, NPY is reported to modulate inflammatory or immune cell functions via the sympathetic nervous system (SNS). SNS activation results in the release of NPY concomitantly with catecholamines. In a rat air pouch model of inflammation induced by carrageenan, NPY exhibited an anti-inflammatory effect, including reduced granulocyte accumulation, attenuation of phagocytosis, and significant decreases in peroxide production (Dimitrijević et al., 2006). DR has also been reported to attenuate carrageenan-induced paw edema in mice (Klebanov et al., 1995). DR is reported to modulate LPS-induced inflammatory stress in rats (Komatsu et al., 2011). Additionally, Y2 receptor KO mice are known to be sensitive to the effect of LPS-evoked immune stress (Painsipp

et al., 2008). Collectively, many studies have demonstrated the similar protective effects of NPY and DR against inflammatory stress.

The SNS innervates white adipose tissue. An *in vitro* experiment, in which sympathetic neurons were cocultured with adipocytes, revealed that the sympathetic neurons inhibited beta-adrenoceptor–stimulated lipolysis and leptin secretion through NPY (Turtzo et al., 2001). The SNS is also reported to inhibit fat cell proliferation (Cousin et al., 1993). Suppression of lipolysis and lipogenesis and reduced leptin secretion characterize the effect of DR in white adipose tissue (Shimokawa, 2010). Therefore, peripheral NPY also regulates stress response and energy metabolism.
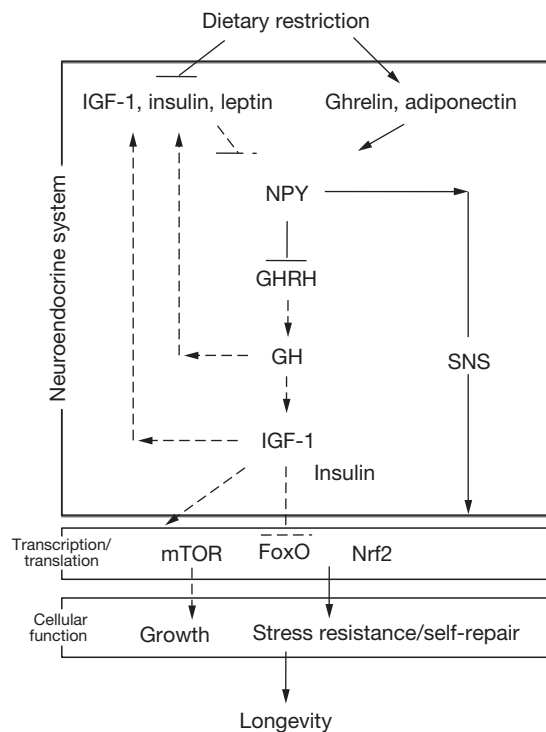
Figure 5.1 Stratified mechanisms underlying the effect of dietary restriction. At the level of the neuroendocrine system, DR augments the NPY signal in the hypothalamus and in the SNS, resulting in a new physiological milieu where the GH/IGF-1 axis is suppressed. At the transcriptional and translational levels in the cell, mTOR is inhibited because of the attenuated GH-IGF-1 and insulin signalings, and thus cell growth or anabolic processes are attenuated. The transcriptional activities of FoxO- and Nfe2l2-target genes are increased in response to oxidative stress because of the attenuated IGF-1/insulin's inhibitory signal on FoxO, producing peptides that enhance stress resistance and/or self-repair, such as DNA repair, apoptosis, and autophagy at the cellular level. These changes induced by DR lead to longevity.

Preliminary data also support the hypothesis that NPY is a key molecule in the effect of DR. The life-prolonging effect of DR was minimized in NPY (−/−) mice (Chiba et al., unpublished observation). The oxidative stress response induced by a sublethal dose of 3-nitropropionic acid was also attenuated in NPY (−/−) mice (Figure 5.1).

5. CONCLUSION

DR has been a robust nongenetic intervention favoring longevity in a range of organisms. It has been shown that the effect of DR is robust when compared to isocaloric diets devised with single foods or nutrients for longevity. Genetic and molecular analyses in both invertebrate and vertebrate models have identified key molecules mediating the effect of DR (e.g., GH, IGF-1, FOXO1, NFE2L2, MTOR, and NPY). Therefore, natural products or compounds that act on the key signal molecules of DR could be promising candidates for retardation of aging and aging-related disorders in humans.

GLOSSARY

Chemically induced skin tumor model A well-known skin tumorigenesis model. A single application of carcinogen (7,12-dimethylbenz[*a*]anthracene (DMBA)) followed by repeated treatments (usually twice a week) of the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate, leads to the development of benign papillomas in the skin of mice after several weeks. A small percentage of papillomas progresses to malignant tumors.
Longevity gene A single gene that leads to extended lifespan in organisms if the gene is spontaneously mutated or genetically manipulated.

REFERENCES

Bishop, N.A., Guarente, L., 2007. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. Nature 447, 545–549.
Cousin, B., Casteilla, L., Lafontan, M., et al., 1993. Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. Endocrinology 133, 2255–2262.
Dimitrijević, M., Stanojević, S., Mićić, S., et al., 2006. Neuropeptide Y (NPY) modulates oxidative burst and nitric oxide production in carrageenan-elicited granulocytes from rat air pouch. Peptides 27, 3208–3215.
Greer, E.L., Brunet, A., 2009. Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. Aging Cell 8, 113–127.
Hansel, D.E., Eipper, B.A., Ronnett, G.V., 2001. Neuropeptide Y functions as a neuroproliferative factor. Nature 410, 940–944.
Harrison, D.E., Strong, R., Sharp, Z.D., et al., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.
Holliday, R., 2006. Food, fertility and longevity. Biogerontology 7, 139–141.
Klebanov, S., Diais, S., Stavinoha, W.B., Suh, Y., Nelson, J.F., 1995. Hyperadrenocorticism, attenuated inflammation, and the life-prolonging action of food restriction in mice. Journals of Gerontology Series A: Biological Sciences and Medical Sciences 50, B78–B82.

Komatsu, T., Trindade, L.S., Chiba, T., et al., 2011. Acute stress response modified by modest inhibition of growth hormone axis: a potential machinery of the anti-aging effect of calorie restriction. Mechanisms of Ageing and Development 132 (3), 103–109.

Liu, H.-Y., Han, J., Cao, S.Y., et al., 2009. Hepatic autophagy is suppressed in the presence of insulin resistance and hyperinsulinemia: inhibition of FoxO1-dependent expression of key autophagy genes by insulin. Journal of Biological Chemistry 284, 31484–31492.

Luque, R.M., Park, S., Kineman, R.D., 2007. Severity of the catabolic condition differentially modulates hypothalamic expression of growth hormone-releasing hormone in the fasted mouse: potential role of neuropeptide Y and corticotropin-releasing hormone. Endocrinology 148, 300–309.

Masoro, E.J., 2003. Subfield history: caloric restriction, slowing aging, and extending life. Science of Aging Knowledge Environment 2003, RE2.

Mattson, M.P., Duan, W., Guo, Z., 2003. Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. Journal of Neurochemistry 84, 417–431.

Paik, J.-H., Kollipara, R., Chu, G., et al., 2007. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. Cell 128, 309–323.

Painsipp, E., Herzog, H., Holzer, P., 2008. Implication of neuropeptide-Y Y2 receptors in the effects of immune stress on emotional, locomotor and social behavior of mice. Neuropharmacology 55, 117–126.

Pearson, K.J., Lewis, K.N., Price, N.L., et al., 2008. Nrf2 mediates cancer protection but not prolongevity induced by caloric restriction. Proceedings of the National Academy of Sciences of the United States of America 105, 2325–2330.

Pérez, V.I., Van Remmen, H., Bokov, A., Epstein, C.J., Vijg, J., Richardson, A., 2009. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8, 73–75.

Przybysz, A.J., Choe, K.P., Roberts, L.J., Strange, K., 2009. Increased age reduces DAF-16 and SKN-1 signaling and the hormetic response of *Caenorhabditis elegans* to the xenobiotic juglone. Mechanisms of Ageing and Development 130, 357–369.

Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. Nature 404, 661–671.

Selman, C., Tullet, J.M.A., Wieser, D., et al., 2009. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326, 140–144.

Shimokawa, I., 2010. Dietary restriction and aging in rodents: a current view on its molecular mechanisms. Aging and Disease 1, 1–16.

Shimokawa, I., Chiba, T., Yamaza, H., Komatsu, T., 2008. Longevity genes: insights from calorie restriction and genetic longevity models. Molecules and Cells 26, 427–435.

Silva, A.P., Pinheiro, P.S., Carvalho, A.P., et al., 2003. Activation of neuropeptide Y receptors is neuroprotective against excitotoxicity in organotypic hippocampal slice cultures. FASEB Journal 17, 1118–1120.

Smiałowska, M., Domin, H., Zieba, B., et al., 2009. Neuroprotective effects of neuropeptide Y-Y2 and Y5 receptor agonists in vitro and in vivo. Neuropeptides 43, 235–249.

Sykiotis, G.P., Bohmann, D., 2010. Stress-activated cap'n'collar transcription factors in aging and human disease. Science Signaling 3, re3.

Tullet, J.M.A., Hertweck, M., An, J.H., et al., 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132, 1025–1038.

Turtzo, L.C., Marx, R., Lane, M.D., 2001. Cross-talk between sympathetic neurons and adipocytes in coculture. Proceedings of the National Academy of Sciences of the United States of America 98, 12385–12390.

van der Horst, A., Burgering, B.M.T., 2007. Stressing the role of FoxO proteins in lifespan and disease. Nature Reviews Molecular Cell Biology 8, 440–450.

Yamaza, H., Komatsu, T., Wakita, S., et al., 2010. FoxO1 is involved in the antineoplastic effect of calorie restriction. Aging Cell 9, 372–382.

Zoncu, R., Efeyan, A., Sabatini, D.M., 2011. mTOR: from growth signal integration to cancer, diabetes and ageing. Nature Reviews Molecular Cell Biology 12, 21–35.

Zukowska-Grojec, Z., 1995. Neuropeptide Y. A novel sympathetic stress hormone and more. Annals of the New York Academy of Sciences 771, 219–233.

Diet, Social Inequalities, and Physical Capability in Older People

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Across the world, the number of older people is rising. For example, in the US, there are currently 37 million residents aged 65 years and older. They represent 12.6% of the population – a figure projected to rise to 20% by 2030 (Kamp et al., 2010). Since the increase in life expectancy may not be matched by a rise in health expectancy (Kaiser et al., 2010), the maintenance of independence and quality of life are major challenges facing aging populations. Central to independence is physical capability – that is the ability to perform the physical tasks of everyday living, in order to function independently in older age. This is highlighted by the fact that in community-dwelling adults, poor performance on simple measures of physical capability, such as hand-grip strength, predicts mortality (Cooper et al., 2010). While losses of muscle mass and strength are expected components of aging, the rate of decline is not spread evenly across the population, with social inequalities observed in grip strength, physical functioning, and falls in older age (Syddall et al., 2009). These variations suggest that modifiable behavioral factors, such as diet and lifestyle, may be important influences on physical function and also potentially key to preventative strategies to optimize physical capability in older people. This chapter discusses the importance of dietary quality and nutrition for the promotion of physical capability in older age and also focuses on the particular challenges faced by socially disadvantaged older people.

1. DIET AND NUTRITION IN OLDER AGE

On average, food intake falls by around 25% between 40 and 70 years of age (Nieuwenhuizen et al., 2010), and some studies estimate that 16–18% of older adults in the community have daily energy intakes below 1000 kcal (Murphy, 2008). In comparison with younger adults, older adults eat more slowly, are less hungry, less thirsty, consume smaller meals, and snack less (Nieuwenhuizen et al., 2010). The mechanisms for the 'anorexia of aging' are not fully understood, but there may be a range of influences that lead to age-related reductions in appetite and food consumption (Murphy, 2008). In a recent review, Nieuwenhuizen et al. (2010) summarize the physiological,

psychological, and social factors that affect dietary intake in older age. The physiological factors include loss of taste and olfaction, increased sensitivity to the satiating effects of meals, chewing difficulties, and impaired gut function. The negative consequences of these changes are compounded by the effects of functional impairments that impact on ability to access and prepare food, psychological problems such as depression and dementia, as well as the social effects of living and eating alone. Low food intakes and monotonous diets put older people at risk of having inadequate nutrient intakes (Bartali et al., 2003). Thus, in a vicious cycle, aging and loss of physical capability may increase the risk of poor nutrition, and poor nutrition may contribute to further decline in the physical capability of older adults.

The exact estimates of prevalence of poor nutrition may differ according to the definitions used, but studies of community-dwelling adults consistently suggest that it is common in older age. For example, in the National Diet and Nutrition Survey in the UK, 14% of older men and women living in the community and 21% of those living in institutions, were at medium or high risk of undernutrition (Margetts et al., 2003). Although risk was based on a composite measure of low body mass index and recent reported weight loss, the men and women classified at greater risk of undernutrition had lower status for vitamins D, E, and C, and zinc, indicating a greater risk of micronutrient deficiency. In a review of 23 studies carried out using the Mini Nutritional Assessment (MNAR), the prevalence of malnutrition in community-dwelling older adults was defined as 2%, with a further 24% at risk of malnutrition (Guigoz, 2006). Estimates of the prevalence of undernutrition in older patients admitted to hospital are even greater, ranging between 12% and 72% (Heersink et al., 2010). These figures are substantial and indicate that there are significant numbers of older adults living in developed settings who currently have less than optimal nutrition.

Importantly, poor nutrition becomes increasingly common at older ages (Bartali et al., 2003). Recent figures from the Centers for Disease Control and Prevention (CDC) are consistent with this finding. They show that more than 2000 older adults die from malnutrition-related causes each year in the US, but the rate of malnutrition-based mortality changes markedly with age, increasing from 1.4 per 100 000 people aged 65–74 years, to 20.9 per 100 000 among those aged 85 years and older (Lee and Berthelot, 2010).

1.1 Disadvantage and Poor Nutrition

In addition to the effects of increasing age, material deprivation and social disadvantage increase the risk of poor nutrition among older adults and contribute to the wide variations in prevalence observed across communities. For example, in an analysis of US mortality data for the years 2000–03, while the average number of malnutrition-related deaths among older adults was 4 per county, almost a third of counties registered none (Lee and Berthelot, 2010). Lee and Berthelot (2010) identify three key risk factors for

malnutrition in older age: socioeconomic disadvantage, disability, and social isolation. Disadvantage is strongly linked to food insecurity and financial difficulties, such that insufficient resource limits or results in uncertain access to enough nutritious food to meet needs (Klesges et al., 2001; Lee and Frongillo, 2001). Disability and physical impairments in older age can impact on access, preparation, and consumption of food (Keller, 2005), while the psychosocial consequences of social isolation may also have an effect on overall energy intake (Hays and Roberts, 2006). In a principal component analysis of risk factors, Lee and Berthelot (2010) found in the first component that measures of poverty and disadvantage were clustered with disability, indicating that these factors are concentrated together. The social isolation effects of being widowed and living alone were distinct and represented in a second component. Importantly, both components identified in these analyses were independently related to malnutrition-related mortality.

2. PHYSICAL CAPABILITY IN OLDER AGE

A known characteristic of aging is the loss of muscle mass. Among adults aged over 50 years, it is estimated to amount to 1–2% per year (Thomas, 2007) and is an important contributor to declining muscle strength in older age. The syndrome of reduced muscle mass and strength or physical performance is described as sarcopenia, and it has recently been recommended that both low muscle mass and low muscle function (strength or performance) are used in its diagnosis (Cruz-Jentoft et al., 2010). There is considerable interest in understanding the etiology of sarcopenia as it is central to physical capability in older people (Evans et al., 2010) as well as being strongly predictive of future health (Sayer, 2010). The huge personal and financial costs of sarcopenia are also now recognized (Janssen et al., 2004). The most commonly used measure of muscle strength is grip strength, determined using a hand-held dynamometer, while a standardized assessment of physical performance can be obtained using a battery of tests that include walking speed, chair rises, and standing balance (Cruz-Jentoft et al., 2010).

Estimates of the prevalence of impaired physical function in older age vary as different measurement techniques have been used across studies and common cut-off points to define low muscle strength and poor performance are not yet established (Cruz-Jentoft et al., 2010). Estimates of the prevalence of sarcopenia range between 8% and 40% among people aged 60 years and older (van Kan, 2009), becoming increasingly common at older ages. For example, in the New Mexico Elder Health Survey, in which sarcopenia was defined using an appendicular skeletal muscle mass index, 13–24% of the participants aged less than 70 years were sarcopenic, but this figure rose to more than 40% of women and 50% of men above the age of 80 years (Baumgartner et al., 1998). Comparable data have come from one of the best-characterized European cohorts of older community-dwelling men and women, the InCHIANTI cohort (Lauretani et al., 2003). In this study, four different indicators of sarcopenia were assessed: hand-grip, knee-extension torque,

lower extremity muscle power, and calf muscle area. The estimates of prevalence of sarcopenia varied according to the measure used, but all showed increases in prevalence with age. Although standardized definitions of impaired physical function are still needed, however defined, sarcopenia is clearly prevalent among older people living in developed settings.

2.1 Disadvantage and Poor Physical Capability

Impaired physical capability is distributed unevenly across the population, and a number of studies show links between poorer self-reported function among older adults of low socioeconomic status (Rautio et al., 2005). Although there are fewer studies in which objective measures of physical function have been used, the findings appear consistent, such that poorer measured performance is associated with markers of poverty and disadvantage. For example, in a longitudinal study of older adults in Finland, lower income was related to slower walking speed and poorer grip strength, suggesting that disadvantage in older age affects a number of domains of physical capacity (Rautio et al., 2005). The findings of a UK study accord with this, since markers of material deprivation, such as limited car availability, were related to lower grip strength in older men and women, and with increased risk of falls in older men (Syddall et al., 2009). Importantly, social inequalities defined using these markers were more evident than those identified using occupationally defined social class and may better represent the social circumstances of older people (Syddall et al., 2009).

There are obvious parallels in the distribution of poor diet and impaired physical function across communities, such that poverty and disadvantage often coexist with disability (Lee and Berthelot, 2010) and with food insecurity (Klesges et al., 2001). It is not surprising, therefore, that food insecurity has been linked directly to physical limitations (Lee and Frongillo, 2001). However, because these factors are often interrelated, it is difficult to determine the temporal chain of events and the causal factors that may lead to more rapid age-related losses of physical function among the socially disadvantaged (Brewer et al., 2010).

3. DOES DIET AFFECT PHYSICAL CAPABILITY IN OLDER AGE?

There are two consequences of declining food intakes in older age that could be important for physical capability. First, lower energy intakes, if not matched by lower levels of energy expenditure, lead to weight loss, including loss of muscle mass (Nieuwenhuizen et al., 2010). Second, as older people consume smaller amounts of food, it may become more challenging for them to meet their nutrient needs – particularly for micronutrients. For older people with low food intakes, this highlights the importance of the quality of their diets. Although the importance of adequate nutrition to enable maintenance of

physical function in older age has been recognized for a long time, much of the research in this area is relatively new (Kaiser et al., 2010). There is now a growing evidence base that describes links between diet and muscle strength and physical capability in older age. The dietary components for which there is the most consistent evidence of effects on physical function in older age are protein, vitamin D, and antioxidant nutrients, including carotenoids, vitamin E, and selenium (Kaiser et al., 2010).

3.1 Protein

Protein is considered a key nutrient in older age (Wolfe et al., 2008). Dietary protein provides amino acids that are needed for the synthesis of muscle protein, and, importantly, absorbed amino acids have a stimulatory effect on muscle protein synthesis after feeding (Kim et al., 2010). There is some evidence that the synthetic response to amino acid intake may be blunted in older people, particularly at low intakes (Wolfe et al., 2008), and when protein is consumed together with carbohydrate (Paddon-Jones and Rasmussen, 2009). Recommended protein intakes may, therefore, need to be raised in older people in order to maintain nitrogen balance and to protect them from sarcopenic muscle loss (Wolfe et al., 2008).

While there is currently no consensus on the degree to which dietary protein requirements change in older age, there is important observational evidence that an insufficient protein intake may be an important contributor to impaired physical function. For example, in the US Health, Aging and Body Composition Study, a greater loss of lean mass over 3 years, assessed using dual-energy X-ray absorptiometry, was found among older community-dwelling men and women who had low energy-adjusted protein intakes at baseline (Houston et al., 2008). The differences were substantial, such that the participants with protein intakes in the top fifth of the distribution lost 40% less lean mass over the follow-up period when compared with those in bottom fifth. Protein and/or amino acid supplementation should, therefore, have the potential to slow sarcopenic muscle loss. However, while amino acid supplementation has been shown to increase lean mass and improve physical function (Børsheim et al., 2008), other trials have not been successful (Paddon-Jones and Rasmussen, 2009). Further work, including longer-term trials, is needed to define optimal protein intakes in older age (Paddon-Jones and Rasmussen, 2009).

3.2 Vitamin D

An association between vitamin D-deficient osteomalacia and myopathy has been recognized for many years (Hamilton, 2010), but the role of vitamin D and the extent to which it has direct effects on normal muscle strength and physical function remain controversial (Annweiler et al., 2009). The potential mechanisms that link vitamin D status to muscle function are complex and include both genomic and nongenomic roles (Ceglia,

2009; Hamilton, 2010). The vitamin D receptor (VDR) has been isolated from skeletal muscle, indicating that it is a target organ (Hamilton, 2010), and polymorphisms of the VDR have been shown to be related to differences in muscle strength (Geusens et al., 1997). At the genomic level, binding of the biologically active form of the vitamin (1,25-dihydroxyvitamin D) results in enhanced transcription of a range of proteins, including those involved in calcium metabolism (Hamilton, 2010). The nongenomic actions of vitamin D are currently less well understood (Ceglia, 2009).

Much of the epidemiological literature is consistent with the possibility that there are direct effects of vitamin D on muscle strength. For example, among men and women aged 60 years and older in NHANES III, low vitamin D status (serum 25-hydroxyvitamin D < 15 ng mL^{-1}) was associated with a four-fold increase in risk of frailty (Wilhelm–Leen et al., 2010), and in a meta-analysis of supplementation studies of older adults, Bischoff–Ferrari et al. (2009) showed that supplemental vitamin D (700–1000 IU per day) reduced the risk of falling by 19%. However, the evidence is not always consistent as some observational studies find no association between vitamin D status and physical function, and supplementation studies have not always resulted in measurable improvements in function (Annweiler et al., 2009). In a review of published studies, Annweiler and colleagues (2009) discuss the reasons for the divergence in study findings, some of which may be due to methodological differences, including a lack of consideration of confounding influences in some studies. Further evidence is needed, as the prevalence of low vitamin D status is increasing (Wilhelm–Leen et al., 2010) and, consistent with the distribution of impaired physical function, vitamin D deficiency may be more common among older people of low socioeconomic status (Hirani and Primatesta, 2005).

3.3 Antioxidant Nutrients

There is increasing interest in the role of oxidative stress in etiology of sarcopenia, and markers of oxidative damage have been shown to predict impairments in physical function in older age (Semba et al., 2007). Damage to biomolecules such as DNA, lipid, and proteins may occur when reactive oxygen species (ROS) are present in cells in excess. The actions of ROS are normally counterbalanced by antioxidant defense mechanisms that include the enzymes superoxide dismutase and glutathione peroxidase, as well as exogenous antioxidants derived from the diet, such as selenium, carotenoids, tocopherols, flavonoids, and other plant polyphenols (Kim et al., 2010; Semba et al., 2007). In older age, an accumulation of ROS may lead to oxidative damage and contribute to losses of muscle mass and strength (Kim et al., 2010).

A number of observational studies have shown positive associations between higher status of antioxidant nutrients and measures of physical function (Kaiser et al., 2010). Importantly, these associations are seen both in cross-sectional analyses and in longitudinal studies, such that poor status is predictive of decline in function.

The observed effects are striking. For example, among older men and women in the InCHIANTI study, higher plasma carotenoid concentrations were associated with a lower risk of developing a severe walking disability over a follow-up period of 6 years; after taking account of confounders that included level of physical activity and other morbidity, the odds ratio was 0.44 (95% CI 0.27–0.74) (Lauretani et al., 2008). Inverse associations have also been described for vitamin E and selenium status and risk of impaired physical function (Kaiser et al., 2010). There have been few studies to determine whether supplementation of older adults with antioxidant nutrients has beneficial effects on muscle strength or physical capability (Fusco et al., 2007). Since ROS have both physiological and pathological roles, interventions based on simple suppression of their activities may be unlikely to improve age-related declines in muscle mass and function (Jackson, 2009). However, since lower carotenoid status is more common among disadvantaged adults (Stimpson et al., 2007), this is an important question that needs to be addressed.

3.4 Foods and Dietary Patterns

Although there is significant evidence for roles of protein, vitamin D, and antioxidant nutrients in the maintenance of physical function in older age, much of it is observational. It may, therefore, be difficult to understand the relative importance of these different nutrients – particularly as dietary components are often highly correlated with each other. For example, while an antioxidant nutrient, such as β-carotene, may be causally related to variations in physical function, it may also be acting as a marker of other components of fruit and vegetables. In turn, since diets are patterned, high fruit and vegetable consumption may be indicators of other dietary differences which could be important for muscle function, such as greater consumption of fatty fish and higher intakes of vitamin D and n-3 long-chain polyunsaturated fatty acids (Robinson et al., 2008). The cumulative effects of nutrient deficiencies have been described by Semba et al. (2006), in which he estimated that each additional nutrient deficiency raised the risk of frailty in older women by almost 10%. This emphasizes the importance of diets of sufficient quality as well as quantity for older adults. Compared with the evidence that links variations in nutrient intake and status to physical function, much less is known at present about the influence of dietary patterns on physical capability in older age. However, 'healthy' diets and greater fruit and vegetable consumption have been shown to be associated with better physical function in both older (Robinson et al., 2008) and middle-aged adults (Houston et al., 2005; Tomey et al., 2008). This is consistent with the epidemiological evidence that links low nutrient intakes to impaired physical function, as healthier diets, characterized by frequent consumption of fruit and vegetables, are likely to provide greater intakes of a range nutrients, including protein, vitamin D, and antioxidant nutrients (Robinson et al., 2009).

4. PUBLIC HEALTH IMPLICATIONS OF THE LINKS BETWEEN DIET AND PHYSICAL CAPABILITY IN OLDER AGE

Epidemiological studies provide significant evidence of links between poor diet and poor physical capability – both of which are more common among socially disadvantaged older adults. To develop strategies to delay or prevent loss of physical capability and to address current inequalities in related morbidity, a better understanding is needed of the lifestyle factors that influence rate of decline and the mechanisms involved. However, the existing evidence already indicates the potential importance of diets of sufficient quality to promote physical capability in older age. This highlights the need for appropriate support, for example, in food assistance programs and other nutrition initiatives, to improve nutrition among vulnerable groups. A position statement by the American Dietetic Association, American Society for Nutrition, and Society for Nutrition Education (Kamp et al., 2010) recognizes this need, stating 'that all older adults should have access to food and nutrition programs that ensure the availability of safe adequate food to promote optimal nutritional status.

4.1 A Lifecourse Perspective

The health of older people is influenced by events throughout their lives (Kaiser et al., 2010), and achievement of optimal function may, therefore, depend on lifelong exposure to a healthy diet and lifestyle. To date, most observational and intervention studies have focused on later life, but there may be important opportunities to delay or prevent loss of physical capability in older age by intervening much earlier in the lifecourse. Muscle mass and strength in later life may reflect not only the current rate of muscle loss but also the peak attained earlier in life (Sayer, 2010). Factors that operate very early in life, such as those that influence early growth and development, may, therefore, be important contributors to physical capability in older age. Consistent with this possibility, low weight at birth predicts lower muscle mass and strength in younger and older adults (Sayer, 2010). Although little is currently known about the influence of early diet on later physical function, there is some evidence that it could be important. For example, recent data from the HELENA study show links between duration of breastfeeding and measurable differences in physical performance in later life (Artero et al., 2010), and risk of frailty has been shown to be greater in older adults who grew up in impoverished circumstances, and who experienced hunger in childhood (Alvarado et al., 2008). Further work is needed to determine the role of diet and nutrition across the lifecourse in the promotion of physical capability in older age.

5. SUMMARY

Prevention of age-related losses in muscle mass and strength is key to protecting physical capability in older age and enabling independent living. Current evidence links

insufficient intakes of protein, vitamin D, and antioxidant nutrients to poor physical function. Although much of this evidence is observational and the mechanisms are not fully understood, the high prevalence of low nutrient intakes and poor status among older adults make this a cause for concern – and highlight the need for diets of sufficient quality and quantity to enable nutrient needs to be met in older age and to ensure optimal function. Since poor nutrition and impaired physical function are more common among socially disadvantaged adults, there is a particular need for appropriate support to improve nutrition in vulnerable groups and to address these inequalities. However, as experience at earlier stages of life may also impact the loss of muscle and physical function in later life, efforts to promote physical capability in older age also need to consider the effectiveness of earlier interventions. Optimizing diet and nutrition throughout the lifecourse may be key to promoting physical capability in older age.

REFERENCES

Alvarado, B.E., Zunzunegui, M.V., Béland, F., Bamvita, J.M., 2008. Life course social and health conditions linked to frailty in Latin American older men and women. Journal of Gerontology. Series A 63, 1399–1406.

Annweiler, C., Schott, A.M., Berrut, G., Fantino, B., Beauchet, O., 2009. Vitamin D-related changes in physical performance: a systematic review. The Journal of Nutrition, Health and Aging 13, 893–898.

Artero, E.G., Ortega, F.B., Espana-Romero, V., et al., 2010. Longer breastfeeding is associated with increased lower body explosive strength during adolescence. The Journal of Nutrition 140, 1989–1995.

Bartali, B., Salvini, S., Turrini, A., et al., 2003. Age and disability affect dietary intake. The Journal of Nutrition 133, 2868–2873.

Baumgartner, R.N., Koehler, K.M., Gallagher, D., et al., 1998. Epidemiology of sarcopenia among the elderly in New Mexico. American Journal of Epidemiology 147, 755–763.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B., et al., 2009. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. British Medical Journal 339, b3692.

Børsheim, E., Bui, Q.T., Tissier, S., et al., 2008. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clinical Nutrition 27, 189–195.

Brewer, D.P., Catlett, C.S., Porter, K.N., et al., 2010. Physical limitations contribute to food insecurity and the food insecurity–obesity paradox in older adults at senior centers in Georgia. Journal of Nutrition for the Elderly 29, 150–169.

Ceglia, L., 2009. Vitamin D and its role in skeletal muscle. Current Opinion in Clinical Nutrition and Metabolic Care 12, 628–633.

Cooper, R., Kuh, D., Hardy, R., et al., 2010. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. British Medical Journal 341, c4467.

Cruz-Jentoft, A.J., Baeyens, J.P., Bauer, J.M., et al., 2010. Sarcopenia: European consensus on definition and diagnosis. Age and Ageing 39, 412–423.

Evans, W.J., Paolisso, G., Abbatecola, A.M., et al., 2010. Frailty and muscle metabolism dysregulation in the elderly. Biogerontology 11, 527–536.

Fusco, D., Colloca, G., Lo Monaco, M.R., Cesari, M., 2007. Effects of antioxidant supplementation on the aging process. Clinical Interventions in Aging 2, 377–387.

Geusens, P., Vandevyver, C., Vanhoof, J., et al., 1997. Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. Journal of Bone and Mineral Research 12, 2082–2088.

Guigoz, Y., 2006. The Mini Nutritional Assessment (MNA®). Review of the literature – what does it tell us? Journal of Nutrition Health and Aging 10, 466–485.

Hamilton, B., 2010. Vitamin D and human skeletal muscle. Scandinavian Journal of Medicine and Science in Sports 20, 182–190.

Hays, N.P., Roberts, S.B., 2006. The anorexia of aging in humans. Physiology and Behaviour 88, 257–266.

Heersink, J.T., Brown, C.J., Dimaria-Ghalili, R.A., Locher, J.L., 2010. Undernutrition in hospitalized older adults: patterns and correlates, outcomes, and opportunities for intervention with a focus on processes of care. Journal of Nutrition for the Elderly 29, 4–41.

Hirani, V., Primatesta, P., 2005. Vitamin D concentrations among people aged 65 years and over living in private households and institutions in England: population survey. Age and Ageing 34, 485–491.

Houston, D.K., Stevens, J., Cai, J., Haines, P.S., 2005. Dairy, fruit, and vegetable intakes and functional limitations and disability in a biracial cohort: the Atherosclerosis Risk in Communities Study. The American Journal of Clinical Nutrition 81, 515–522.

Houston, D.K., Nicklas, B.J., Ding, J., et al., 2008. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. The American Journal of Clinical Nutrition 87, 150–155.

Jackson, M.J., 2009. Strategies for reducing oxidative damage in ageing skeletal muscle. Advanced Drug Delivery Reviews 61, 1363–1368.

Janssen, I., Shepard, D.S., Katzmarzyk, P.T., Roubenoff, R., 2004. The healthcare costs of sarcopenia in the United States. Journal of the American Geriatrics Society 52, 80–85.

Kaiser, M.J., Bandinelli, S., Lunenfeld, B., 2010. Frailty and the role of nutrition in older people. A review of the current literature. Acta Biomedica 81 (Suppl 1), 37–45.

Kamp, B.J., Wellman, N.S., Russell, C., et al., 2010. Position of the American Dietetic Association, American Society for Nutrition, and Society for Nutrition Education: Food and nutrition programs for community-residing older adults. Journal of the American Dietetic Association 110, 463–472.

Keller, H.H., 2005. Reliance on others for food-related activities of daily living. Journal of Nutrition for the Elderly 25, 43–59.

Kim, J.S., Wilson, J.M., Lee, S.R., 2010. Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants. The Journal of Nutritional Biochemistry 21, 1–13.

Klesges, L.M., Pahor, M., Shorr, R.I., et al., 2001. Financial difficulty in acquiring food among elderly disabled women: results from the Women's Health and Aging Study. American Journal of Public Health 91, 68–75.

Lauretani, F., Russo, C.R., Bandinelli, S., et al., 2003. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. Journal of Applied Physiology 95, 1851–1860.

Lauretani, F., Semba, R.D., Bandinelli, S., et al., 2008. Carotenoids as protection against disability in older persons. Rejuvenation Research 11, 557–563.

Lee, M.R., Berthelot, E.R., 2010. Community covariates of malnutrition based mortality among older adults. Annals of Epidemiology 20, 371–379.

Lee, J.S., Frongillo, E.A., 2001. Factors associated with food insecurity among US elderly persons: importance of functional impairments. Journal of Gerontology 56B, S94–S99.

Margetts, B.M., Thompson, R.L., Elia, M., Jackson, A.A., 2003. Prevalence of risk of undernutrition is associated with poor health status in older people in the UK. European Journal of Clinical Nutrition 57, 69–74.

Murphy, C., 2008. The chemical senses and nutrition in older adults. Journal of Nutrition for the Elderly 27, 247–265.

Nieuwenhuizen, W.F., Weenen, H., Rigby, P., Hetherington, M.M., 2010. Older adults and patients in need of nutritional support: review of current treatment options and factors influencing nutritional intake. Clinical Nutrition 29, 160–169.

Paddon-Jones, D., Rasmussen, B.B., 2009. Dietary protein recommendation and the prevention of sarcopenia. Current Opinion in Clinical Nutrition and Metabolic Care 12, 86–90.

Rautio, N., Heikkinen, E., Ebrahim, S., 2005. Socio-economic position and its relationship to physical capacity among elderly people living in Jyväskylä, Finland: five- and ten-year follow-up studies. Social Science and Medicine 60, 2405–2416.

Robinson, S.M., Jameson, K.A., Batelaan, S.F., et al., 2008. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire Cohort Study. Journal of the American Geriatrics Society 56, 84–90.

Robinson, S., Syddall, H., Jameson, K., et al., 2009. Current patterns of diet in community-dwelling older men and women: results from the Hertfordshire Cohort Study. Age and Ageing 38, 594–599.

Sayer, A.A., 2010. Sarcopenia. British Medical Journal 341, c4097.

Semba, R.D., Bartali, B., Zhou, J., et al., 2006. Low serum micronutrient concentrations predict frailty among older women living in the community. Journal of Gerontology 61A, 594–599.

Semba, R.D., Ferrucci, L., Sun, K., et al., 2007. Oxidative stress and severe walking disability among older women. The American Journal of Medicine 120, 1084–1089.

Stimpson, J.P., Nash, A.C., Ju, H., Eschbach, K., 2007. Neighborhood deprivation is associated with lower levels of serum carotenoids among adults participating in the Third National Health and Nutrition Examination Survey. Journal of the American Dietetic Association 107, 1895–1902.

Syddall, H., Evandrou, M., Cooper, C., Aihie Sayer, A., 2009. Social inequalities in grip strength, physical function and falls among community dwelling older men and women. Journal of Aging and Health 21, 913–939.

Thomas, D.R., 2007. Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia. Clinical Nutrition 26, 389–399.

Tomey, K.M., Sowers, M.R., Crandall, C., et al., 2008. Dietary intake related to prevalent functional limitations in midlife women. American Journal of Epidemiology 167, 935–943.

Van Kan, G.A., 2009. Epidemiology and consequences of sarcopenia. The Journal of Nutrition, Health and Aging 13, 708–712.

Wolfe, R.R., Miller, S.L., Miller, K.B., 2008. Optimal protein intake in the elderly. Clinical Nutrition 27, 675–684.

Wilhelm-Leen, E.R., Hall, Y.N., de Boer, I.H., Chertow, G.M., 2010. Vitamin D deficiency and frailty in older Americans. Journal of Internal Medicine 268, 171–180.

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Dietary Patterns/Diet and Health of Adults in Economically Developing Countries

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ABBREVIATIONS

AIDS Acquired immune deficiency syndrome
BMI Body mass index
CAD Coronary artery disease
CVD Cardiovascular disease
HDL High-density lipoprotein
HICs High-income countries
HIV Human immunodeficiency virus
IR Insulin resistance
LDL Low-density lipoprotein
LMICs Low- and middle-income countries
MetS Metabolic syndrome
MUFA Monounsaturated fatty acid
NCDs Noncommunicable diseases
PUFA Polyunsaturated fatty acid
RRR Reduced rank regression
SFA Saturated fatty acid
SSA Sub-Saharan Africa
SSB Sugar-sweetened beverages
T2DM Type 2 diabetes mellitus
TB Tuberculosis
WC Waist circumference
WHO World Health Organization

1. INTRODUCTION

The health status of low- and middle-income countries (LMICs) has generally been characterized by communicable diseases such as respiratory infections, diarrheal diseases, tuberculosis (TB), and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), which are intricately linked to undernutrition (Uauy et al., 2009;

WHO, 2008). However, noncommunicable diseases (NCDs) including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), certain forms of cancers, and chronic respiratory conditions are on the rise and accounted for 80% of the global chronic disease mortality and 50% of the total disease burden in 2005. It is projected that 66% of the global chronic disease mortality and 55% of the disease burden in 2015 will be ascribed to NCDs in LMICs. Ensuing medical costs between 2006 and 2015 are estimated to be US$84 billion (Abegunde et al., 2007; WHO, 2005).

The epidemiologic transition occurring in LMICs is attributable at least in part to a rapid nutrition transition, which is inextricably related to globalization, urbanization, and a demographic transition. Unhealthy lifestyle factors (unhealthy diet, physical inactivity, and smoking) have fueled a rise in metabolic risk factors (blood pressure, glucose, plasma lipids, overweight, and obesity), which contribute significantly to NCDs. Consequently, most countries are experiencing a dual burden of communicable and noncommunicable diseases (Gersh et al., 2010; Uauy and Solomons, 2006; WCRF/AICR, 2007; WHO, 2009). According to a recent World Health Organization (WHO) report, six of the ten leading global causes of mortality are associated with NCDs in LMICs. These include high blood pressure, tobacco use, overweight and obesity, physical inactivity, high blood glucose, and high cholesterol levels (WHO, 2009).

Although diet is recognized as a key determinant of NCDs, epidemiological research has provided only limited information to guide the development of targeted preventive nutrition interventions to achieve clinical and public health recommendations to reduce NCDs. This is partly due to conflicting findings on the role of individual nutrients and foods (Mann, 2007; Smit et al., 2009). It is postulated that dietary patterns, which consider total diet as opposed to single nutrients/foods, may better inform the association between diet and health outcomes and aid in the formulation of targeted guidelines for the improved management of NCDs (Kant, 2010; Uauy et al., 2009).

In this chapter, we will first provide an overview of the health status of adults in LMICs, with a focus on nutrition-related NCDs. Their nutritional status will then be discussed. Finally, we will review studies on chronic diseases in relation to adult dietary patterns in LMICs.

2. HEALTH STATUS OF ADULTS IN ECONOMICALLY DEVELOPING COUNTRIES

2.1 Chronic Communicable Diseases

Infectious diseases still predominate in Africa and South Asia. HIV/AIDS and TB are the main chronic communicable diseases overall. The prevalence of HIV/AIDS in 2009 varied from 0.1% in East Asia to 5% in sub-Saharan Africa (SSA). In 2009, South-East Asia had the largest number of people (4 900 000) with TB; Latin America and the Caribbean had the least number (350 000). HIV/AIDS and TB inter-relate: HIV/AIDS is a major

risk factor for TB, while individuals with TB easily succumb to HIV infection (UNAIDS, 2010; WHO, 2010).

2.2 Chronic Noncommunicable Diseases

CVD, mainly coronary artery disease (CAD) and stroke, are the largest contributors to NCDs in LMICs. They are the leading causes of death (account for 80% of global CVD deaths) except in SSA, where HIV/AIDS is the main cause of mortality. Eastern Europe, Central Asia, the Middle East and North Africa have the highest rates of deaths (Gersh et al., 2010; WHO, 2008).

The prevalence of diabetes is highest in the Eastern Mediterranean and Middle East regions (9.3%) and lowest in Africa (3.8%). Seven of the top ten countries for numbers of people with diabetes (India, China, Russia, Brazil, Pakistan, Indonesia, Mexico) are in LMICs. Between 2010 and 2030, the numbers of adults with diabetes in LMICs will increase by 69%, mainly in India and China (36%). Africa is expected to have the highest increase in the proportion (98%) of adult diabetes cases (Shaw et al., 2010).

More than half (53%) of cancer cases and 60% of cancer deaths occur in LMICs. In 2008, incidence and mortality were highest in Western Pacific and lowest in Eastern Mediterranean. Lung cancer is the most common form of cancer in men (15% incidence rate) and the leading cause of death (accounts for 18% of total deaths in LMICs). Among women, breast cancer has the highest incidence rate (19%), but is the second leading cause of mortality (after cervical cancer) accounting for 13% of deaths in LMICs (IACR, 2008).

Interaction among NCDs and infectious diseases is likely to exacerbate the NCD epidemic. Given the adverse effects of antiretrovirals including insulin resistance, inflammation, dyslipidemia, and abdominal obesity, increased retroviral treatment may have a negative impact particularly in SSA (Gersh et al., 2010).

3. NUTRITIONAL STATUS OF ADULTS IN ECONOMICALLY DEVELOPING COUNTRIES

3.1 Malnutrition

Malnutrition, a continuum of nutritional disorders that comprise underweight (<2 standard deviations below the WHO reference median), overweight (BMI $25.0-<30 \, \mathrm{kg \, m^{-2}}$), obesity (BMI $\geq 30 \, \mathrm{kg \, m^{-2}}$), and micronutrient deficiencies, affects approximately one-third of the world's population. (Note that underweight and undernutrition are used interchangeably in available literature; see the glossary for definitions of terminology.) The prevalent malnutrition is ascribed to poor diet quality (FAO, 2010; SCN, 2010; Uauy and Solomons, 2006).

3.1.1 Overweight and obesity

Overweight and obesity affect nearly two-thirds (62%) of adults in LMICs, mainly women and low-income populations. Eastern Europe has the highest prevalence (65%) of both overweight and obese adults; South-East Asia, especially India, has the lowest prevalence (20%). China is expected to have the largest number of overweight and obese individuals in 2030 (Kelly et al., 2008; SCN, 2010; WCRF/AICR, 2007; WHO, 2008). Abdominal obesity (waist circumference (WC) >102 cm in men and >88 cm in women) is present in approximately 29% of men and 48% of women; median WC is lower in South-East Asia and higher in North and South Africa and the Middle East (Balkau et al., 2007).

3.1.2 Underweight

About 98% of global underweight children and adults (prevalence of 16%) reside in LMICs. China and India account for over 40% of underweight individuals. Asia and the Pacific have the largest number, and Africa the highest proportion, of underweight individuals (FAO, 2010).

3.1.3 Micronutrient deficiencies

Vitamin A and iodine deficiency, and anemia are the most common micronutrient deficiencies.

Vitamin A deficiency (serum retinol <20 μg dl^{-1}) is a severe public health problem (prevalence $>30\%$) in East and Central Asia as well as East, Central, and West Africa. Iodine deficiency, as indicated by goiter prevalence, affects 700 million people particularly in South-Central Asia; prevalence is lowest in the Caribbean. Approximately 40% of nonpregnant women, overall as well as in Africa and Asia have anemia (hemoglobin <12 g dl^{-1}). South-Central Asia, mainly India, has the highest prevalence ($\sim60\%$) and Central America the lowest (20%) prevalence of anemia (SCN, 2010).

3.2 Dietary Intake

Diets in many LMICs, particularly among low-income groups, are generally plant-based, predominantly starchy staples (such as corn, rice, and cassava), with minimal animal source products. As such, these food intake patterns lack dietary variety, are nutrient-poor, and tend to be of poor quality resulting in chronic nutritional deficiencies (vitamin A deficiency and anemia). The nutrition transition has further compromised diet quality. Consumption of energy and animal-source foods has increased, with concomitant reduction of some dietary deficiencies and improvement of overall nutrition, but intake of nutrient-rich foods has declined due to increased availability of refined and processed foods (SCN, 2010; WCRF/AICR, 2007).

Food and Agriculture Organization data on foods available for consumption indicate that over the last four decades, dietary energy from animal sources increased more than

twofold from 160 to 340 kcal day^{-1}, whereas energy from plant sources rose from 1900 to 2340 kcal day^{-1} in LMICs. Dietary energy available from grains especially wheat and rice slightly declined, a trend that is projected to continue into the 2030s. Fat available, derived mostly from plant oils, in particular palm oil in South–East Asia, has increased except in SSA. Over the same period, available energy for consumption increased by more than 600 kcal person^{-1} day^{-1} especially in Asia as exemplified by China ($\sim$1000 kcal person^{-1} day^{-1}). Similarly, intakes of meat as well as milk and dairy products rose by 150% and 60%, respectively in LMICs. By 2030, consumption of animal products is expected to increase by 44% (WCRF/AICR, 2007). Availability of vegetables and fruits increased in South–East Asia over the 40-year period, but has declined in East and Central Africa since the 1980s. Mean fruit intake is below the recommended minimum intake of 400 g day^{-1} and ranges from 207 g day^{-1} in Latin America and the Caribbean to 350 g day^{-1} in Eastern Mediterranean. Prevalence of low fruit intake ($<$5 servings day^{-1}) is highest in South–East Asia (80%) and lowest in Eastern Mediterranean (57%) (WCRF/AICR, 2007; WHO, 2008).

Food availability data from China, India, Vietnam, Thailand, and other South–East Asian countries show rapid increases in consumption of sugar-sweetened beverages (SSBs), which include soft drinks, fruit juices, iced tea, energy drinks, and vitamin water drinks. SSB intake is equally high in the Philippines and South Africa, and in Mexico accounts for $\sim$10% of total energy (Malik et al., 2010).

In a review on global dietary fat intake (% energy), mean total fat intake ranged from 11.1 in China to 50.7 in rural Nigeria. A similar pattern was observed for saturated fat (SFA) intake: 3.1 in China and 25.4 in rural Nigeria. Mean monounsaturated fat (MUFA) intake was lowest in China (3.5) and highest in Cameroon (16.4) as was polyunsaturated fat (PUFA) intake: 3.3 in China and 5.9 in Cameroon. The ratio of SFAs to the sum of MUFAs and PUFAs [SFAs/(MUFAs + PUFAs)] was lowest in China (0.36) and highest in India (1.14). A ratio of $>$0.5 denotes that the proportion of SFAs is unfavorable (Elmadfa and Kornsteiner, 2009).

International Population Study on Macronutrients and Blood Pressure (INTER-MAP) and INTERnational study of SALT and blood pressure (INTERSALT) data show that mean intakes of sodium in most countries, with the possible exception of Africa, Samoa, and Venezuela, are $>$100 mmol day^{-1} (2.3 g day^{-1}). In most Asian countries, mean intake is $>$200 mmol day^{-1} (Brown et al., 2009).

3.3 Nutrition-Related Lifestyle Factors

Unhealthy diet, alcohol consumption, smoking, and physical inactivity tend to cluster and interact increasing risk of disease (WHO, 2005). About 69% of men and women in Latin America and the Caribbean drink alcohol in comparison to only 6% of adults in Eastern Mediterranean. Mean alcohol intake varies from 1 g day^{-1} in Eastern

Mediterranean to 25 g day^{-1} ($\sim$1.5 drinks day^{-1}) in Eastern Europe. Smoking prevalence ranges from 9% in Africa to 37% in Eastern Europe. The prevalence of physical inactivity is lowest in Africa (11%) and highest in Eastern Mediterranean (28%) (WHO, 2009).

3.4 Mechanisms Linking Diet and Noncommunicable Diseases

Overweight and obesity are postulated to be key driving forces of the NCD epidemic. Both conditions are independent risk factors for other metabolic risk factors in addition to CVD, T2DM, as well as cancers of the esophagus (adenocarcinoma), breast (post-menopause), gallbladder, pancreas, kidney, endometrium, and colorectum (Dandona et al., 2005; WCRF/AICR, 2007; WHO, 2005). Abdominal obesity is indicated to be of greater importance in increasing morbidity than overall obesity and is also a key component of the metabolic syndrome (MetS), a constellation of three or more metabolic risk factors, which include impaired fasting glucose, low HDL-cholesterol, elevated triglycerides, elevated blood pressure, and abdominal obesity. MetS is a major risk factor for CVD and T2DM (Alberti et al., 2009; Dandona et al., 2005; Klein et al., 2007).

Obesity is hypothesized to be an inflammatory state. Adipocytes produce cytokines that promote systemic inflammation and endothelial dysfunction. Adipose tissue also produces nonesterified fatty acids that induce insulin resistance (IR), in addition to stimulating oxidative stress and inflammation. IR further enhances lipolysis, thus increasing free fatty acid production and causing a vicious circle of lipolysis, increased fatty acid production, IR, and inflammation. IR contributes to the development of dyslipidemia (low HDL-cholesterol, elevated triglycerides, and elevated small LDL-cholesterol), glucose intolerance, and elevated blood pressure in addition to exacerbating obesity. Macronutrients including fat and simple carbohydrates produce oxidative stress that stimulates the inflammatory responses in obesity. Other macro- and micro-nutrients and foods such as fiber, vitamin E, alcohol, fruits, and vegetables are anti-inflammatory and suppress oxidative stress (Dandona et al., 2005).

4. ASSOCIATION BETWEEN DIET AND NONCOMMUNICABLE DISEASES

4.1 Assessment of Diet–Disease Relationships

Epidemiologic nutrition research has traditionally focused on single nutrients and foods in assessing relationships between diet and health, an approach that has facilitated understanding of underlying mechanisms. However, this method is inherently confounded by food and nutrient collinearities and interactions, as well as inability to detect small nutrient effects. The role of specific nutrients, particularly fats and carbohydrates is controversial (Mann, 2007; Smit et al., 2009). This has generated interest in dietary patterns, which consider total diet as an exposure variable. Studies done to date, mainly

in high-income countries (HICs), show the dietary pattern approach to be effective (Kant, 2004, 2010; Newby and Tucker, 2004). There is, however, paucity of data on the association between dietary patterns and health outcomes in LMICs.

4.2 Dietary Patterns

Two types of dietary patterns are commonly used in nutritional epidemiologic research. Theoretical (a priori/hypothesis-driven) patterns are based on expert dietary guidelines, evidence-based nutrient scoring systems, or healthy traditional patterns (such as the Mediterranean diet). They measure aspects of diet, including adequacy, overconsumption, diversity, and the overall diet quality. Empirical (a posteriori/data-driven) patterns, by contrast, are derived statistically by cluster and factor analysis or reduced rank regression and define food and nutrient intake as actually consumed. Factor analysis aggregates foods into groups based on matrix correlations; subjects receive a score on each factor identified. Conversely, cluster analysis aggregates people into distinct dietary pattern groups (Kant, 2004, 2010).

A third method, Reduced Rank Regression (RRR), is a two-step hybrid of hypothesis-driven and data-driven approaches that first aggregates foods into groups (factors) that maximally explain intermediary biomarkers of disease. The resulting factor score computed for each individual is then used to predict a health outcome (Kant, 2010).

4.2.1 Strengths and limitations of dietary patterns

Dietary patterns consider all aspects of a diet and correct for the confounding inherent in single food/nutrient approaches. Cumulative effects of multiple nutrients may be sufficiently large to be detectable. Food-based patterns can generate hypotheses about key diet–disease relationships and facilitate formulation of dietary recommendations. Both types of patterns are reproducible and show evidence of stability (Iqbal et al., 2008; Kimokoti et al., 2012; Newby and Tucker, 2004). The patterns are valid and have been related to CVD, diabetes, cancers, MetS, overweight, obesity, and mortality, although findings of prospective studies and randomized clinical trials with regard to CVD and cancer incidence and mortality are discrepant. In longitudinal studies, "Healthy" patterns are consistently associated with a lower risk for CVD and all-cause mortality, whereas findings for cancer are conflicting; clinical trials have generally yielded negative findings (Kant, 2004, 2010; Newby and Tucker, 2004).

Theoretical patterns are subjective with regard to dietary variables including, cut-off points and scoring methods. Additionally, dietary guidelines across countries may lack scientific consensus. Empirical patterns are population-specific and similarly subjective regarding selection and labeling of factors and clusters. These methodological factors may hamper comparisons of studies. Furthermore, having established dietary patterns of a population, it is difficult to tease out the relative effects of individual foods/nutrients. Some of the food groupings in empirical patterns lack theory and cannot explain biologic

mechanisms. Since data–driven methods are not designed to derive patterns that predict disease, RRR is suggested as an alternative but may be less reproducible since the intermediate responses are predetermined in relation outcomes (DiBello et al., 2008; Kant, 2010; Newby and Tucker, 2004).

4.3 Studies on the Association between Dietary Patterns and Health Outcomes in Economically Developing Countries

The majority of studies from LMICs that have examined dietary patterns and risk of disease are cross-sectional (Becquey et al., 2010; Cai et al., 2007; Chen et al., 2006; Cunha et al., 2010; Denova–Gutiérrez et al., 2010; DiBello et al., 2009; Dugee et al., 2009; Esmaillzadeh and Azadbakht, 2008a, b; Esmaillzadeh et al., 2007; Flores et al., 2010; He et al., 2009; Lee et al., 2010; Nkondjock and Bizome, 2010; Rezazadeh and Rashidkhani, 2010) and retrospective (Amtha et al., 2009; Cui et al., 2007; De Stefani et al., 2009, 2010; DiBello et al., 2008; Iqbal et al., 2008; Jackson et al., 2009; Marchioni et al., 2007; Martínez-Ortiz et al., 2006; Toledo et al., 2010) conducted mostly in China, Iran, and Latin America. Only three studies are prospective (Sherafat-Kazemzadeh et al., 2010; Villegas et al., 2010).

In general, Western, Unhealthy and Modern patterns that are characterized by high intakes of refined and processed foods, soft drinks and juices, animal-fat rich foods, and hydrogenated fats confer risk for overweight and obesity (total and abdominal). Conversely, a Healthy pattern high in whole grains, vegetables, and fruits protects against overweight and obesity. A Traditional pattern, characterized based upon indigenous eating practices, is either detrimental or beneficial depending on the local foods (Table 7.1).

Similar findings are observed for studies on MetS and its components (Table 7.2). A Healthy/Prudent pattern is protective, whereas a Western/Modern pattern increases risk. A Traditional pattern is, likewise, beneficial or risk-enhancing based on the local foods.

Findings are also comparable for studies on CVD, diabetes, and cancer (Tables 7.3 and 7.4). Patterns labeled as Western, Animal Protein, Staple, New Affluence, Monotonous, Preferred, Chemical-related, Starchy, Sweet Beverages, Mixed, and Traditional, which have similar food components as well as a higher Dietary Risk Score (which denotes poor diet quality) confer risk for NCDs. Healthy, Prudent, Balanced, and Mixed patterns are protective. The Drinker pattern increases risk for most cancers.

Study findings are consistent with those of observational studies that have been conducted in HICs. A Western/Unhealthy/Empty Calorie pattern and a higher Nutritional Risk Score (indicative of poor diet quality) increase risk for CVD, diabetes, cancers, MetS, overweight, obesity, and mortality. Conversely, a Prudent/(Heart) Healthy pattern lowers risk for these health outcomes (Kant, 2004, 2010; Kimokoti et al., 2010; Millen et al., 2006; Newby and Tucker, 2004; Sonnenberg et al., 2005; Wolongevicz et al., 2009, 2010).

Table 7.1 Association between Dietary Patterns and Overweight and Obesity

Reference	Study population	Study design	Dietary pattern subgroups	Dietary assessment method	Results
Theoretical (a priori) dietary patterns					
None					
Empirical (a posteriori) dietary patterns					
Sichieri (2002)	Brazil	Cross-sectional	Three factors	FFQ (80 food items)	Traditional pattern associated with lower risk for overweight and obesity (BMI $\geq 25 \text{ kg m}^{-2}$) compared to other patterns
	2040 adults aged 20–60 years		Mixed Traditional Western		OR (95% CI): Men: 0.87 (0.77–0.99) Women: 0.86 (0.75–0.99)
Becquey et al. (2010)	Burkina Faso	Cross-sectional	Two clusters	FFQ (82 food items)	Modern pattern associated with overweight and obesity (BMI $>25 \text{ kg m}^{-2}$)
	1072 adults aged 20–65 years		Snacking Modern		Higher score versus lower score OR: 1.19 (95% CI: 1.03–136); P-trend $= .018$
Flores et al. (2010)	Mexico	Cross-sectional	Three clusters	FFQ (101 food items)	Refined foods and sweets and Diverse patterns associated with higher risk for overweight (BMI 25.0–$<30 \text{ kg m}^{-2}$) and obesity (BMI $\geq 30 \text{ kg m}^{-2}$) compared to the traditional pattern
	2006 National Health and Nutrition Survey 15 890 adults aged 20–59 years		Refined foods and sweets Traditional Diverse		OR (95% CI): 1.14 (1.02–1.26) to 1.31 (1.08–1.34)

Continued

Table 7.1 Association between Dietary Patterns and Overweight and Obesity—cont'd

Reference	Study population	Study design	Dietary pattern subgroups	Dietary assessment method	Results
Esmaillzadeh and Azadbakht (2008a)	Iran	Cross-sectional	Three factors	FFQ (168 food items)	Healthy pattern associated with lower risk for overall obesity and abdominal obesity
	486 women without CVD, diabetes, and cancer aged 40–60 years		Healthy		Quintile 5 versus Quintile 1: P-trend $< .05$
			Western		Western pattern associated with higher risk for overall obesity and abdominal obesity
			Iranian		Quintile 5 versus Quintile 1: P-trend $< .01$
Rezazadeh and Rashidkhani (2010)	Iran	Cross-sectional	Two factors	FFQ (168 food items)	Healthy pattern associated with lower risk for overall obesity and abdominal obesity
	460 women aged 20–50 years		Healthy		Quartile 4 versus Quartile 1: P-trend $< .05$ and P-trend $< .01$
			Unhealthy		Unhealthy pattern associated with higher risk for overall and abdominal obesity
					Quartile 4 versus Quartile 1: P-trend $< .01$
Dugee et al. (2009)	Mongolia	Cross-sectional	Three factors	FFQ (# of food items not available)	Transitional pattern associated with higher risk for overweight/obesity (BMI ≥ 25 kg m^{-2}) in all adults and abdominal obesity in men
	418 adults aged >25 years		Transitiona Traditionall Healthy		Traditional pattern associated with higher risk for abdominal obesity among women
					Healthy pattern associated with lower risk for abdominal obesity among all adults

| Cunha et al. (2010) | Brazil | Cross-sectional | Three factors | FFQ (82 food items) | Traditional pattern associated with lower risk for higher BMI (β: −1.14; $P<.001$) and WC (β: −14.68; $P=.003$) in women |
| | 1009 adults aged 20–65 years | | Mixed Western Traditional | | Western pattern associated with higher risk for higher BMI (β: 0.74; $P=.02$) and WC (β: 13.61; $P=.02$) among women |

Hybrid of theoretical and empirical dietary patterns

| Sherafat-Kazemzadeh et al. (2010) | Iran | Prospective | Five patterns derived by reduced rank regression | 24-h recall | Mean Change Quintile 5 versus Quintile 1 |
| | 141 adults aged ≥ 18 years and not on treatment for CVD, diabetes, hypertension, and hyperlipidemia | 6 years of follow-up | Traditional Fiber and PUFA Fiber and dairy Dairy Egg | | Traditional pattern associated with higher risk for higher BMI (P-trend $=.019$), WC (P-trend $<.001$), and WHR (P-trend $= .006$) Egg pattern associated with higher risk for higher WC (P-trend $< .001$) and WHR ($P=.021$) |

BMI, body mass index; CVD, cardiovascular disease; FFQ, food frequency questionnaire; WC, waist circumference; WHR, waist-to-hip ratio.

Table 7.2 Association between Dietary Patterns and the Metabolic Syndrome and Its Components

Reference	Study population	Study design	Dietary pattern subgroups	Dietary assessment method	Results
Theoretical (a priori) dietary patterns					
None					
Empirical (a posteriori) dietary patterns					
Esmaillzadeh et al. (2007)	Iran 521 women without CVD, diabetes, or cancer aged 40–60 years	Cross-sectional	Three factors Healthy	FFQ (168 food items)	Quintile 5 versus Quintile 1 Healthy pattern associated with lower risk for MetS, ↑WC, ↑BP, ↑Glucose, ↑TG, and ↓HDL-C (P-trend < .05 to < .01)
			Western		Western pattern associated with higher risk for MetS, ↑WC, ↑BP, ↑TG, and ↓ HDL-C (P-trend < .05 to <.01)
			Traditional		Traditional pattern associated with higher risk for ↑Glucose (P-trend < .05)
DiBello et al. (2009)	American Samoa 723 adults	Cross-sectional	Three factors Neotraditional	FFQ (42 food items)	Quintile 5 versus to Quintile 1 Neotraditional associated lower risk for ↑WC (P-trend = .03) and ↓HDL-C (P-trend = .02)
	Samoa 785 adults aged ≥ 18 years		Modern Mixed		Modern pattern associated with higher risk for MetS (P-trend = .05) and ↑TG (P-trend = .04)
Denova-Gutiérrez et al. (2010)	Mexico Health workers cohort study	Cross-sectional	Three factors Prudent	FFQ (116 food items)	Tertile 3 versus Tertile 1 Healthy pattern associated with lower risk for MetS, ↑WC, ↑BP, ↑Glucose, ↑TG, and ↓HDL-C (P-trend = .04 to < .01)
	5240 healthy adults aged 20–70 years		Western High protein/fat		High protein/fat associated with higher risk for MetS (P-trend = .04) and abdominal obesity (P-trend = .04)

BP, blood pressure; CVD, cardiovascular disease; FFQ, food frequency questionnaire; HDL-C, high density lipoprotein cholesterol; MetS, metabolic syndrome; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio.

Table 7.3 Association between Dietary Patterns and Cardiovascular Disease, Diabetes Mellitus, and Cancer

Reference	Study population	Study design	Dietary pattern subgroups / components	Dietary assessment method	Results
Theoretical (a priori) dietary patterns					
Iqbal et al. (2008)	52 countries	Case–control	Dietary risk score	FFQ (19 food items)	Poor diet quality associated with higher risk for acute myocardial infarction
	INTERHEART study		Composed of 7 CVD risk–related and protective foods		Quartile 4 versus Quartile 1
	16 407 adults; mean age by dietary pattern subgroup: 53.8–57.6 years		A higher score is indicative of poor diet quality		OR: 1.92 (95% CI: 1.74–2.11); *P*-trend < .001
Empirical (a posteriori) dietary patterns					
Martínez-Ortiz et al. (2006)	Costa Rica	Case–control	Two factors	FFQ (# of food items not stated)	Staple pattern associated with higher risk for acute myocardial infarction (MI)
	1014 adults Mean age: 57 years		Vegetable Staple		Quintile 4 versus Quintile 1 OR (95% CI) 3.53 (1.98–6.31); *P*-trend = .0002
DiBello et al. (2008)	Costa Rica	Case–control	Five factors	FFQ (135 food items)	Acute myocardial infarction
	3574 adults		Factor 1: high in vegetables, fruits, and polyunsaturated fat		Quintile 4 versus Quintile 1:
	Mean age: 58 years		Factors 2 and 3: mix of vegetables and high-fat dairy products		OR (95% CI)

Continued

Table 7.3 Association between Dietary Patterns and Cardiovascular Disease, Diabetes Mellitus, and Cancer—cont'd

Reference	Study population	Study design	Dietary pattern subgroups / components	Dietary assessment method	Results
			Factor 4: high in sugar, and palm oil Factor 5: high in alcohol, legumes, other unsaturated oil.		Factor 1: 0.72 (0.55–0.94); P-trend $= .02$ Factor 4: 1.38 (1.07–1.78); P-trend $= .006$ Factor 5: 0.69 (0.53–0.88); P-trend $= .004$
Iqbal et al. (2008)	52 countries INTERHEART study 16 407 adults; mean age by dietary pattern subgroup: 53.8–57.6 years	Case–control	Three factors Prudent Oriental Western	FFQ (19 food items)	Quartile 4 versus Quartile 1: OR (95% CI) Prudent pattern associated with lower risk for acute MI (P-trend $< .001$) Western pattern associated with higher risk for acute MI (P-trend $< .001$)
Chen et al. (2006)	Bangladesh Health effects of arsenic longitudinal study 11 116 adults aged ≥ 18 years	Cross-sectional	Three factors Balanced Animal protein Gourd and root vegetable	FFQ (39 food items)	Hypertension Quintile 5 versus Quintile 1: OR (95% CI) Balanced pattern 0.71 (0.59–0.85); ($P =$ trend $< .01$) Animal protein pattern 1.21 (1.03–1.49); ($P =$ trend $= .23$)
Nkondjock and Bizome (2010)	Cameroon 571 adults Age not available	Cross-sectional	Two factors Fruit and vegetable Meat	FFQ (# of food items not available)	Fruit and vegetable pattern associated with lower risk for hypertension Quartile 4 versus Quartile 1: P-trend $= .04$

Lee (2010)	China	Cross-sectional	Three factors	FFQ (81 food items)	Quintile 5 − Quintile 1: Difference in mean BP [mmHg (95% CI)] Fruit and milk pattern SBP: −2.9 (−3.4 to −2.4); P-trend < .001 DBP: −1.7 (−2.0 to −1.4); P-trend < .001 Effect stronger in heavy drinkers
	Shanghai Men's Health Study 39 252 men without CVD, HTN, or diabetes aged 40–74 years		Vegetable Fruit and milk Meat		
Esmaillzadeh and Azadbakht (2008b)	Iran 486 women without CVD, diabetes, and cancer aged 40–60 years	Cross-sectional	Three factors Healthy Western Iranian	FFQ (168 food items)	Quintile 5 versus Quintile 1: OR (95% CI) Healthy pattern associated with lower risk for hypertension (P-trend < .01) Western pattern associated with lower risk for hypertension (P-trend < .05)
Villegas et al. (2010)	China Shanghai Women's Health Study 64 191 women free of CVD, T2DM, cancer; 40–70 years	Prospective 6.9 years of follow-up	Three clusters Cluster 1: high in staples Cluster 2: high in dairy milk Cluster 3: mixed	FFQ (77 food items)	Cluster 2 associated with lower risk for T2DM compared to Cluster 1 OR: 0.78 (95% CI: 0.71–0.86)
He et al. (2009)	China 2002 National Nutrition and Health Survey 20 210 adults without diabetes aged 45–69 years	Cross-sectional	Four clusters Green water: rural Yellow earth: high in cereals New affluence: urban Western adopter: high in animal foods, cakes, drinks	FFQ	Glucose tolerance abnormality (T2DM, IGT, IFG) Other patterns versus green water pattern OR (95% CI) Yellow earth pattern: 1.26 (1.07–1.48) New affluence pattern: 1.45 (1.21–1.72)

Continued

Table 7.3 Association between Dietary Patterns and Cardiovascular Disease, Diabetes Mellitus, and Cancer—cont'd

Reference	Study population	Study design	Dietary pattern subgroups / components	Dietary assessment method	Results
Marchioni et al. (2007)	Brazil	Case–control	Four factors	FFQ (27 food items)	Oral cancer
	835 adults Median age Cases: 55.5 years; controls: 56.5 years		Prudent Traditional Snacks Monotonous		Tertile 3 versus Tertile 1: OR (95% CI) Traditional pattern 0.51 (0.32–0.81); *P*-trend = .14 Monotonous pattern 1.78 (1.12–2.85); *P*-trend < .001
Amtha et al. (2009)	Indonesia	Case–control	Four factors	FFQ (141 food items)	Oral cancer
	243 adults Age not available		Preferred Combination Chemical–related Traditional		Tertile 3 versus. Tertile 1: OR (95% CI) Preferred pattern: 2.17 (1.05–4.50) Combination pattern: 0.46 (0.23–0.91) Chemical–related pattern: 2.85 (1.34–6.05) Traditional pattern: 2.10 (1.02–4.30)
Toledo et al. (2010)	Brazil	Case–control	Three factors	FFQ (27 food items)	Tertile 3 versus Tertile 1:
	461 adults Age not available		Prudent Traditional Snacks		Prudent pattern (*P*-trend = .002) and Traditional pattern (*P*-trend = .02) associated with lower risk for oral–pharyngeal cancer
Hajizadeh et al. (2010)	Iran	Case–control	Two factors	FFQ	Esophageal cancer
	143 adults Age not available		Healthy Western		Higher versus Lower scores: OR (95% CI) Healthy pattern: 0.17 (0.19–0.98) Western pattern: 10.13 (8.45–43.68)

Cui et al. (2007)	China	Case–control	Two factors	FFQ (76 food items)	Breast cancer
	Shanghai Breast Cancer Study		Vegetable-soy		Quartile 4 versus Quartile 1: OR (95% CI)
	3015 women aged 25–64 years		Meat-sweet		Meat-sweet pattern All women: 1.3 (1.0–1.7); P-trend = .03 Postmenopausal women All: 1.6 (1.0–2.4); P-trend = .04 ER–Positive: 1.9 (1.1–3.3); P-trend = .03
Jackson et al. (2009)	Jamaica 408 men Age not available		Four factors Healthy Carbohydrate Sugary foods and sweet baked products Organ meat and fast food	FFQ (# of food items not available)	No association between dietary patterns and prostate cancer.
De Stefani et al. (2010)	Uruguay	Case–control	Five factors	FFQ (64 food items)	Quartile 4 versus Quartile 1:
	1035 men aged 45–89 years		Prudent Traditional Substituter Drinker Western		Traditional pattern (P-trend = .01) and Western pattern (P-trend < .0001) associated with higher risk for advanced prostate cancer
De Stefani et al. (2009)	Uruguay	Case–control	Four factors	FFQ (64 food items)	Prudent pattern associated with lower risk for cancers of the mouth/pharynx, larynx esophagus, UADT, breast, stomach, colon/rectum, and all sites (P-trend = .02 to .0001)

Continued

Table 7.3 Association between Dietary Patterns and Cardiovascular Disease, Diabetes Mellitus, and Cancer—cont'd

Reference	Study population	Study design	Dietary pattern subgroups / components	Dietary assessment method	Results
	6060 adults		Prudent		Western pattern associated with higher risk for cancers of the larynx, UADT, breast, colon, rectum, colon/rectum, bladder, and all sites (*P*-trend = .02 to .0001)
	(3528 cases, 2532 controls)		Western		Drinker pattern Men:
	Mean age by pattern subgroup:		Drinker		Associated with higher risk for cancers of the mouth/pharynx, larynx, esophagus, and UADT (*P*-trend = .02 to .0001)
	Men: 62.2–66.4 years Women: 57.7–66.4 years		Traditional		Women: Associated with lower risk for rectal (*P*-trend = .01) and colorectal (*P*-trend = .04) cancers Traditional pattern associated with higher risk for cancers of the UADT, stomach colon/rectum (*P*-trend = .04 to .002)
Cai et al. (2007)	China Shanghai Men's Health Study 61 582 men aged 40–74 years	Cross-sectional	Three factors Vegetable Fruit Meat	FFQ (81 food items)	CVD, hypertension, diabetes mellitus, benign tumors Quartile 4 versus Quartile 1 OR (95% CI) **Vegetable pattern** CVD: 1.27 (1.15–1.40); *P*-trend < .001 HTN: 1.33 (1.26–1.41); *P*-trend < .001 Diabetes: 2.30 (2.07–2.56); *P*-trend < .001 Tumors: 1.35 (1.18–1.56); *P*-trend < .001

Fruit pattern
CVD: 1.02 (0.92–1.13); *P*-trend = .02
HTN: 0.83 (0.78–0.88); *P*-trend < .001
Diabetes: 0.39 (0.35–0.44); *P*-trend < .001
Meat pattern
CVD: 0.65 (0.58–0.72); *P*-trend < .001
HTN: 0.70 (0.66–0.74); *P*-trend < .001
Diabetes: 1.57 (1.40–1.76); *P*-trend < .001
Tumors: 0.86 (0.74–1.00); *P*-trend = .034

CVD, cardiovascular disease; DBP, diastolic blood pressure; ER-positive, estrogen receptor positive; FFQ, food frequency questionnaire; HTN, hypertension; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MI, myocardial infarction; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; UADT, upper aerodigestive tract (oral cavity, pharynx, esophagus, and larynx).

Table 7.4 Association between Dietary Patterns and Mortality

Reference	Study population	Study design	Dietary pattern subgroups	Dietary assessment method	Results
Theoretical (a priori) dietary patterns					
None					
Empirical (a posteriori) dietary patterns					
Zhuo and Watanabe (1999)	China 65 counties 13 000 adults aged 35–64 years	Retrospective	Six factors	Three-day dietary records	Digestive cancer mortality (esophagus, stomach, colon, rectum) Esophageal cancer mortality: associated with a dietary pattern high in tuber and salt gastric cancer mortality: less related to dietary patterns Colon cancer mortality: associated with a dietary pattern rich in sea vegetables, eggs, soy sauce, and rapeseed oil Colorectal cancer mortality: Related to a dietary pattern high in sea vegetables
Cai et al. (2007)	China Shanghai Women's Health Study 74 942 women aged 40–70 years	Prospective 5.7 years of follow-up	Three factors Vegetable-rich Fruit-rich Meat-rich	FFQ (71 food items)	All-cause and cause-specific (CVD, CAD, stroke, diabetes, cancer) mortality Quartile 4 versus Quartile 1: HR (95% CI) *Fruit-rich pattern* Total: 0.80 (0.69–0.94); P-trend $= .0090$ CVD: 0.71 (0.51–0.98); P-trend $= .0309$ Stroke: 0.53 (0.34–0.82); P-trend $= .0006$ Diabetes: 0.19 (0.08–0.48); P-trend $< .0001$

CAD, coronary artery disease; CVD, cardiovascular disease; FFQ, food frequency questionnaire.

Analyses of single foods corroborate and complement these findings. SSBs, the main constituents of Unhealthy patterns, have been related to overweight, obesity, MetS, T2DM, and CVD risk (Malik et al., 2010). Processed meat, another vital component of these diets, is linked to CAD, diabetes, and colorectal cancer. Red meat also increases risk for cancer (Micha et al., 2010; WCRF/AICR), whereas *trans* (hydrogenated) fats are associated with CAD (Smit et al., 2009). Fruits and vegetables, which characterize Healthy patterns, protect against CAD (Mente et al., 2009) and most cancers (oral, pharyngeal, laryngeal, esophageal, lung, pancreatic, gastric, prostate, and colorectal) (WCRF/AICR). Vegetables similarly lower risk for T2DM (Carter et al., 2010). Whole grains, also key components of Healthy diets, are beneficial for CAD and colorectal cancer (Mente et al., 2009; WCRF/AICR).

4.4 Nutrient Patterns

Some investigators have evaluated nutrient patterns in order to enhance the understanding of dietary factors in the etiology of cancer. In Uruguay (De Stefani et al., 2008a, 2008b), a High-meat pattern (high in protein, saturated fat, MUFA, linoleic acid, alpha-linolenic acid, heterocyclic amines, and phosphorus) increased risk for lung cancer, while an Antioxidant pattern (high in glucose, fructose, carotene, vitamin C, vitamin E, folate, flavonoids, phytosterols, reduced glutathione, and nitrate) protected against esophageal and lung cancer (Table 7.5). Findings were consistent with those obtained for gastric cancer by Palli et al. in Italy (Kant, 2004).

Studies of dietary patterns, foods, and nutrients in a "top–down" approach (starting with patterns and progressing to individual nutrients) (Jacobs and Steffen, 2003) are thus seen to be complementary in nutritional epidemiology as evident from findings in both HICs and LMICs.

5. CONCLUSION

- NCDs are a clinical and public health problem in LMICs.
- Diet is a key etiological factor of NCDs.
- Dietary assessment is complex and requires multiple components to be comprehensive; measures of dietary intake – single nutrients/foods and overall diet – complement each other.
- Dietary patterns are associated with health outcomes; the dietary pattern approach is a starting point for interventions.
- More research is needed on the relationship between dietary patterns and NCDs in LMICs.

Table 7.5 Association between Nutrient Patterns and Cancer

Reference	Study population	Study design	Nutrient pattern subgroups	Dietary assessment method	Results
De Stefani et al. (2008a)	Uruguay	Case-control	Three factors	FFQ (64 food items)	Antioxidants pattern associated with lower risk for esophageal cancer
	1170 men Age not available		High-fat Carbohydrates Antioxidants		OR: 0.39 (95% CI : 0.23–0.66)
De Stefani et al. (2008b)	Uruguay	Case-control	Three factors	FFQ (64 food items)	Tertile 3 versus Tertile 1
	1692 men aged 30–89 years		High-meat		Antioxidants pattern associated with lower risk for lung cancer (*P*-trend = .02)
			Antioxidants Carbohydrates		High-meat pattern associated with higher risk for lung cancer (*P*-trend = .02)

FFQ, food frequency questionnaire.

GLOSSARY

Demographic transition The change from high to low fertility and mortality levels.
Epidemiologic transition The change from infectious diseases to chronic diseases as dominant causes of morbidity and mortality.
Malnutrition A broad term that refers to all forms of poor nutrition. Malnutrition is caused by a complex array of factors including dietary inadequacy (deficiencies, excesses, or imbalances in energy, protein, and micronutrients), infections and sociocultural factors. Malnutrition includes undernutrition as well as overweight and obesity.
Nutrition transition A shift to diets low in fiber and high in processed foods, meat products, fats, sugars, and refined grains in association with greater tobacco use and physical inactivity.
Undernutrition exists when insufficient food intake and repeated infections result in one or more of the following Underweight for age, short for age (stunted), thin for height (wasted), and functionally deficient in vitamins and/or minerals (micronutrient malnutrition).

REFERENCES

Abegunde, D.O., Mathers, C.D., Adam, T., Ortegon, M., Strong, K., 2007. The burden and costs of chronic diseases in low-income and middle-income countries. Lancet 370, 1929–1938.

Alberti, K.G., Eckel, R.H., Grundy, S.M., et al., 2009. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120, 1640–1645.

Amtha, R., Zain, R., Razak, I.A., et al., 2009. Dietary patterns and risk of oral cancer: a factor analysis study of a population in Jakarta, Indonesia. Oral Oncology 45, e49–e53.

Balkau, B., Deanfield, J.E., Després, J.P., et al., 2007. International Day for the Evaluation of Abdominal Obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. Circulation 116, 1942–1951.

Becquey, E., Savy, M., Danel, P., et al., 2010. Dietary patterns of adults living in Ouagadougou and their association with overweight. Nutrition Journal 9, 13.

Brown, I.J., Tzoulaki, I., Candeias, V., Elliott, P., 2009. Salt intakes around the world: implications for public health. International Journal of Epidemiology 38, 791–813.

Cai, H., Zheng, W., Xiang, Y.B., et al., 2007. Dietary patterns and their correlates among middle-aged and elderly Chinese men: a report from the Shanghai Men's Health Study. The British Journal of Nutrition 98, 1006–1013.

Carter, P., Gray, L.J., Troughton, J., Khunti, K., Davies, M.J., 2010. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. BMJ 341, c4229 [doi: 10.1136/bmj.c4229].

Chen, Y., Factor-Litvak, P., Howe, G.R., Parvez, F., Ahsan, H., 2006. Nutritional influence on risk of high blood pressure in Bangladesh: a population-based cross-sectional study. The American Journal of Clinical Nutrition 84, 1224–1232.

Cui, X., Dai, Q., Tseng, M., et al., 2007. Dietary patterns and breast cancer risk in the Shanghai Breast Cancer Study. Cancer Epidemiology, Biomarkers & Prevention 16, 1443–1448.

Cunha, D.B., de Almeida, R.M., Sichieri, R., Pereira, R.A., 2010. Association of dietary patterns with BMI and waist circumference in a low-income neighbourhood in Brazil. The British Journal of Nutrition 104, 908–913.

Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., Garg, R., 2005. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 111, 1448–1454.

De Stefani, E., Boffetta, P., Fagundes, R.B., et al., 2008a. Nutrient patterns and risk of squamous cell carcinoma of the esophagus: a factor analysis in Uruguay. Anticancer Research 28, 2499–2506.

De Stefani, E., Boffetta, P., Ronco, A.L., et al., 2008b. Nutrient patterns and risk of lung cancer: a factor analysis in Uruguayan men. Lung Cancer 61, 283–291.

De Stefani, E., Deneo-Pellegrini, H., Boffetta, P., et al., 2009. Dietary patterns and risk of cancer: a factor analysis in Uruguay. International Journal of Cancer 124, 1391–1397.

De Stefani, E., Ronco, A.L., Deneo-Pellegrini, H., et al., 2010. Dietary patterns and risk of advanced prostate cancer: a principal component analysis in Uruguay. Cancer Causes & Control 21, 1009–1016.

Denova-Gutiérrez, E., Castañón, S., Talavera, J.O., et al., 2010. Dietary patterns are associated with metabolic syndrome in an urban Mexican population. The Journal of Nutrition 140, 1855–1863.

DiBello, J.R., Kraft, P., McGarvey, S.T., et al., 2008. Comparison of 3 methods for identifying dietary patterns associated with risk of disease. American Journal of Epidemiology 168, 1433–1443.

DiBello, J.R., McGarvey, S.T., Kraft, P., et al., 2009. Dietary patterns are associated with metabolic syndrome in adult Samoans. The Journal of Nutrition 139, 1933–1943.

Dugee, O., Khor, G.L., Lye, M.S., et al., 2009. Association of major dietary patterns with obesity risk among Mongolian men and women. Asia Pacific Journal of Clinical Nutrition 18, 433–440.

Elmadfa, I., Kornsteiner, M., 2009. Dietary fat intake–a global perspective. Annals of Nutrition & Metabolism 54 (Suppl 1), 8–14.

Esmaillzadeh, A., Azadbakht, L., 2008a. Major dietary patterns in relation to general obesity and central adiposity among Iranian women. The Journal of Nutrition 138, 358–363.

Esmaillzadeh, A., Azadbakht, L., 2008b. Food intake patterns may explain the high prevalence of cardiovascular risk factors among Iranian women. The Journal of Nutrition 138, 1469–1475.

Esmaillzadeh, A., Kimiagar, M., Mehrabi, Y., et al., 2007. Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. The American Journal of Clinical Nutrition 85, 910–918.

Flores, M., Macias, N., Rivera, M., et al., 2010. Dietary patterns in Mexican adults are associated with risk of being overweight or obese. The Journal of Nutrition 140, 1869–1873.

Gersh, B.J., Sliwa, K., Mayosi, B.M., Yusuf, S., 2010. Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. European Heart Journal 31, 642–648.

Hajizadeh, B., Rashidkhani, B., Rad, A.H., Moasheri, S.M., Saboori, H., 2010. Dietary patterns and risk of oesophageal squamous cell carcinoma: a case-control study. Public Health Nutrition 13, 1107–1112.

He, Y., Ma, G., Zhai, F., et al., 2009. Dietary patterns and glucose tolerance abnormalities in Chinese adults. Diabetes Care 32, 1972–1976.

Iqbal, R., Anand, S., Ounpuu, S., et al., 2008. Dietary patterns and the risk of acute myocardial infarction in 52 countries: results of the INTERHEART study. Circulation 118, 1929–1937.

Jackson, M., Walker, S., Simpson, C., McFarlane-Anderson, N., Bennett, F., 2009. Are food patterns associated with prostate cancer in Jamaican men: a preliminary report. Infection Agent Cancer 4 (Suppl 1), S5.

Jacobs Jr., D.R., Steffen, L.M., 2003. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. The American Journal of Clinical Nutrition 78, 508S–513S.

Kant, A.K., 2004. Dietary patterns and health outcomes. Journal of the American Dietetic Association 104, 615–635.

Kant, A.K., 2010. Dietary patterns: biomarkers and chronic disease risk. Applied Physiology, Nutrition, and Metabolism 35, 199–206.

Kelly, T., Yang, W., Chen, C.S., Reynolds, K., He, J., 2008. Global burden of obesity in 2005 and projections to 2030. International Journal of Obesity 32, 1431–1437.

Kimokoti, R.W., Newby, P.K., Gona, P., et al., 2010. Diet quality, physical activity, smoking status, and weight fluctuation are associated with weight change in women and men. The Journal of Nutrition 140, 1287–1293.

Kimokoti, R.W., Newby, P.K., Gona, P., et al., 2012. Stability of the Framingham Nutritional Risk Score and its component nutrients over 8 years: The Framingham Nutrition Studies. European Journal of Clinical Nutrition 66, 336–344.

Klein, S., Allison, D.B., Heymsfield, S.B., et al., 2007. Waist circumference and cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. The American Journal of Clinical Nutrition 85, 1197–1202.

Lee, S.A., Cai, H., Yang, G., et al., 2010. Dietary patterns and blood pressure among middle-aged and elderly Chinese men in Shanghai. British Journal of Nutrition 104, 265–275.

Malik, V.S., Popkin, B.M., Bray, G.A., Després, J.P., Hu, F.B., 2010. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation 121, 1356–1364.

Mann, J., 2007. Dietary carbohydrate: relationship to cardiovascular disease and disorders of carbohydrate metabolism. European Journal of Clinical Nutrition 61, S100–S111.

Marchioni, D.M., Fisberg, R.M., Francisco de Góis Filho, J., et al., 2007. Dietary patterns and risk of oral cancer: a case-control study in São Paulo, Brazil. Revista de Saúde Pública 41, 19–26.

Martínez-Ortiz, J.A., Fung, T.T., Baylin, A., Hu, F.B., Campos, H., 2006. Dietary patterns and risk of nonfatal acute myocardial infarction in Costa Rican adults. European Journal of Clinical Nutrition 60, 770–777.

Mente, A., de Koning, L., Shannon, H.S., Anand, S.S., 2009. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Archives of Internal Medicine 169, 659–669.

Micha, R., Wallace, S.K., Mozaffarian, D., 2010. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation 121, 2271–2283.

Millen, B.E., Pencina, M.J., Kimokoti, R.W., et al., 2006. Nutritional risk and the metabolic syndrome in women: opportunities for preventive intervention from the Framingham Nutrition Study. The American Journal of Clinical Nutrition 84, 434–441.

Newby, P.K., Tucker, K.L., 2004. Empirically derived eating patterns using factor or cluster analysis: a review. Nutrition Reviews 62, 177–203.

Nkondjock, A., Bizome, E., 2010. Dietary patterns associated with hypertension prevalence in the Camer-
oon defence forces. European Journal of Clinical Nutrition 64, 1014–1021.

Rezazadeh, A., Rashidkhani, B., 2010. The association of general and central obesity with major dietary
patterns of adult women living in Tehran, Iran. Journal of Nutritional Science and Vitaminology
(Tokyo) 56, 132–138.

Shaw, J.E., Sicree, R.A., Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and
2030. Diabetes Research and Clinical Practice 87, 4–14.

Sherafat-Kazemzadeh, R., Egtesadi, S., Mirmiran, P., et al., 2010. Dietary patterns by reduced rank regres-
sion predicting changes in obesity indices in a cohort study: Tehran Lipid and Glucose Study. Asia Pacific
Journal of Clinical Nutrition 19, 22–32.

Sichieri, R., 2002. Dietary patterns and their associations with obesity in the Brazilian city of Rio de Janeiro.
Obesity Research 10, 42–48.

Smit, L.A., Mozaffarian, D., Willett, W., 2009. Review of fat and fatty acid requirements and criteria for
developing dietary guidelines. Annals of Nutrition & Metabolism 55, 44–55.

Sonnenberg, L., Pencina, M., Kimokoti, R., et al., 2005. Dietary patterns and the metabolic syndrome in
obese and non-obese Framingham women. Obesity Research 13, 153–1562.

Toledo, A.L., Koifman, R.J., Koifman, S., Marchioni, D.M., 2010. Dietary patterns and risk of oral and
pharyngeal cancer: a case-control study in Rio de Janeiro, Brazil. Cadernos de Saúde Pública
26, 135–142.

Uauy, R., Corvalan, C., Dangour, A.D., 2009. Conference on "Multidisciplinary approaches to nutritional
problems". Rank Prize Lecture. Global nutrition challenges for optimal health and well-being. The Pro-
ceedings of the Nutrition Society 68, 34–42.

Villegas, R., Yang, G., Gao, Y.T., et al., 2010. Dietary patterns are associated with lower incidence of type 2
diabetes in middle-aged women: the Shanghai Women's Health Study. International Journal of Epide-
miology 39, 889–899.

Wolongevicz, D.M., Zhu, L., Pencina, M.J., et al., 2009. Diet quality and obesity in women: the
Framingham Nutrition Studies. The British Journal of Nutrition 24, 1–7.

Wolongevicz, D.M., Zhu, L., Pencina, M.J., 2010. An obesity dietary quality index predicts abdominal obe-
sity in women: potential opportunity for new prevention and treatment paradigms. Journal of Obesity
pii, 945987.

Zhuo, X.G., Watanabe, S., 1999. Factor analysis of digestive cancer mortality and food consumption in 65
Chinese countries. Journal of Epidemiology 9, 275–284.

RELEVANT WEBSITES

http://www.fao.org – Food and Agriculture Organization, 2010. The state of food insecurity in the world.

http://www.iarc.fr – International Agency for Research on Cancer, 2008. World cancer report.

http://www.unscn.org – Standing Committee on Nutrition, 2010. 6th report on the world nutrition sit-
uation: progress in nutrition.

http://www.unscn.org – Uauy, R., Solomons, N.W., 2006. The role of the international community: forg-
ing a common agenda in tackling the double burden of malnutrition. SCN News 32, 26–39.

http://www.unaids.org – UNAIDS, The Joint United Nations Programme on HIV/AIDS, 2010. UNAIDS
report on the global AIDS epidemic 2010.

http://www.dietandcancerreport.org – World Cancer Research Fund/American Institute for Cancer Re-
search, 2007. Food, nutrition, physical activity, and the prevention of cancer: a global perspective.
Washington DC: AICR.

http://www.who.int – World Health Organization, 2005. Preventing chronic diseases: a vital investment.
WHO, Geneva.

http://www.who.int – World Health Organization, 2008. The global burden of disease: 2004 update.
WHO, Geneva.

http://www.who.int – World Health Organization, 2009. Global health risks: mortality and burden of dis-
ease attributable to selected major risks.

http://www.who.int – World Health Organization, 2010. Global tuberculosis control. WHO, Geneva.

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CHAPTER 8

Diet and Aging: Role in Prevention of Muscle Mass Loss

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ABBREVIATIONS

CR Calorie restriction
Cr Creatine
CRM Calorie restriction mimetics
EAAs Essential amino acids
EM Exercise mimetic
HMB Hydroxy-methylbutyrate
mTOR Mammalian target of rapamycin
NO Nitric oxide
OFAs Omega-3 fatty acids
OKG Ornithine α-ketoglutarate
RDA Recommended dietary allowance
ROS Reactive oxygen species

1. INTRODUCTION

Healthy aging depends on a wide range of factors, among which the preservation of muscle function plays a prominent role. The age-related loss of muscle mass and function, termed sarcopenia, consists in a steady and involuntary process, which results in impaired mobility, increased risk for fall and fractures, reduced ability to perform activities of daily living, and increased risk for chronic diseases and death (Rolland et al., 2008).

During normal aging, an individual loses muscle mass at a rate of ∼1–2% annually after the age of 50, concomitant with a decline in strength of 1.5% per year, with an acceleration to 3% yearly after the age of 60. This process results in a decrease in total muscle cross-sectional area of ∼40% between 20 and 60 years of age, being more dramatic in sedentary individuals than in physically active persons and twice as high in men than in women. Besides the loss of muscle mass, a concomitant increase in fat mass occurs, which may lead to a condition known as sarcopenic obesity that further increases the risk of disability, morbidity, and mortality (Rolland et al., 2008).

The pathophysiology of sarcopenia is extremely complex. Indeed, a multitude of processes and mechanisms may contribute to muscle aging, including reduced secretion of anabolic hormones, increased production of proinflammatory cytokines, acceleration of myocyte apoptosis, oxidative stress, mitochondrial dysfunction, loss of α-motoneurons, impaired satellite cell function, reduced physical activity, poor nutrition, and alterations in muscle responses to anabolic stimuli (Marzetti and Leeuwenburgh, 2006; Rolland et al., 2008).

Given the complex and intertwining relationship among various etiological factors, the quest for effective treatments to manage sarcopenia has been an intriguing, yet unresolved, issue for geriatricians. However, based on the current knowledge, the combination of physical exercise (both resistance and aerobic) and adequate protein and energy intake is presently considered the most effective intervention to prevent and manage sarcopenia (Morley et al., 2010).

The purpose of this chapter is to review the most recent findings regarding the role of nutritional factors in combating sarcopenia. The chapter is structured into two main sections: the first reporting current nutritional recommendations for sarcopenic persons, with particular emphasis on the guidelines developed by the Society for Sarcopenia, Cachexia, and Wasting Disease (Morley et al., 2010); the second introducing novel potential nutritional interventions to preserve muscle mass and function in the elderly.

2. CURRENT NUTRITIONAL RECOMMENDATIONS FOR THE MANAGEMENT OF SARCOPENIA

2.1 A Matter of Quality and Quantity

Aging is associated with a physiological loss of appetite ('anorexia of aging'), resulting in decreased caloric intake and weight loss, which in turn contribute to loss of muscle mass (Morley, 1997). Food intake gradually declines throughout adulthood. Indeed, from 20 to 80 years of age, the mean daily energy intake decreases up to 1200 kcal in men and 800 kcal in women, well below the estimated energy requirements recommended by the US Institute of Medicine (Trumbo et al., 2002). This reduction in food intake has been documented in virtually all large-scale study in healthy, community-dwelling older persons and may result from a number of physiological (e.g., reduced physical activity, decreased resting energy expenditure, loss of appetite, altered satiety signals) and nonphysiological causes (e.g., psychosocial factors, illnesses, medication effects; Morley, 1997). Moreover, advanced age is associated with changes in food preferences, with predilection for sweets, which are protein-poor foods.

These facts suggest that nutritional interventions based on balanced energy supplements may help prevent or reverse sarcopenia as part of a multimodal approach (Morley et al., 2010). However, although numerous studies in older persons with malnutrition and/or specific disease conditions have shown overall positive effects of

nutritional supplementation, attempts to specifically improve muscle mass and function through nutritional interventions alone have been largely unsuccessful. A major cause for these negative results is the difficulty in achieving a true nutritional supplementation in old age. Indeed, older persons prescribed with supplements tend to proportionally decrease their dietary intake, so that the total daily energy intake remains unchanged. Furthermore, the composition of supplements could fail to meet nutritional needs of elderly sarcopenic patients.

Indeed, a nutritional intervention for sarcopenia should provide (a) adequate caloric intake; (b) nutrients appropriate to each individual, based on age, sex, genetic background, metabolic profile, health status, physical activity levels, and eventual concomitant therapies; and (c) adequate quality and quantity of nutrients at the right time.

To this aim, the Society for Sarcopenia, Cachexia, and Wasting Disease has recently convened an expert panel to develop nutritional recommendations for the prevention and management of sarcopenia. The panel highlighted the importance of an adequate intake of several nutrients, namely, proteins and amino acids, especially leucine, (possibly) creatine, and vitamin D (Morley et al., 2010).

2.2 Proteins and Amino Acids

Although muscle protein synthesis is regulated by a complex interplay among a host of factors, adequate bioavailability of dietary-derived amino acids represents an essential prerequisite (Paddon-Jones and Rasmussen, 2009). Older persons are at high risk for inadequate protein intake. Indeed, 32–41% of women and 22–38% of men over the age of 50 ingest less protein than the recommended dietary allowance (RDA; $0.8 \, \mathrm{g \, kg^{-1} \, day^{-1}}$), and virtually no older adult consumes the highest acceptable macronutrient distribution range for protein (35% of energy intake; Kerstetter et al., 2003). Furthermore, the aged muscle displays a reduced anabolic response to dietary proteins. Hence, many researchers have argued that the current RDA for protein does not protect older adults from sarcopenia.

As a whole, epidemiologic data suggest that an increase in protein intake beyond $0.8 \, \mathrm{g \, kg^{-1} \, day^{-1}}$ may be beneficial to preserve muscle mass in late life. Indeed, to prevent or hamper muscle loss, it is recommended to consume between 1.0 and $1.5 \, \mathrm{g \, kg^{-1} \, day^{-1}}$ of protein, spreading this amount equally throughout the day (Morley et al., 2010). Hence, a moderate serving of protein (25–30 g) should be included in each meal, in order to provide a greater 24 h muscle anabolic response (Paddon-Jones and Rasmussen, 2009).

The quality of protein is also an important factor to be taken into consideration. Essential amino acids (EAAs) appear to be the primary stimulus for protein synthesis, and leucine, by activating the mammalian target of rapamycin (mTOR) pathway, is considered the strongest regulator of muscle protein turnover. Although aging is associated

with a reduced ability of muscle to respond to low doses ($\sim$7.5 g) of EAAs, higher doses (i.e., 10–15 g, with at least 3 g of leucine) are capable of overcoming this anabolic resistance and stimulate protein synthesis to a similar extent as in young subjects (Paddon-Jones and Rasmussen, 2009). Thus, older adults should be encouraged to consume protein sources containing relatively high proportions of EAAs (high-quality proteins), such as lean meat-based and dairy products, and leucine-rich foods such as legumes (soybeans, cowpea, lentils), beef, and fish. It is also suggested that a leucine-enriched balanced EAA mix may be added to the diet, in particular if the person is engaged in physical exercise.

Common concerns related to high protein intake in the elderly have not been substantiated by most studies. Rather, in many cases, beneficial effects on the systems studied have been documented. Nevertheless, plasma concentrations of five branched-chain and aromatic amino acids (leucine, isoleucine, valine, tyrosine, and phenylalanine) have recently shown to predict diabetes in the late middle-aged individuals followed for 12 years (Wang et al., 2011).

Hence, additional trials are needed to establish the optimal intake, type and timing of protein, and amino acid supplementation and to determine the effects of chronic supplementation in older adults.

2.3 Creatine

Creatine (Cr) is a guanidine compound produced endogenously from reactions involving amino acids, such as glycine, arginine and methionine, or ingested through the consumption of meat and fish. Cr has long been considered a potential ergogenic aid, because of its role as a temporal and spatial energy buffer, proton buffer, and by increasing muscle concentrations of phosphocreatine, which is necessary for high-power exercise. Indeed, Cr has emerged as a candidate to slow down the rate of muscle wasting with age, particularly when combined with resistance-training regimens (Tarnopolsky et al., 2007).

Although physiological adaptations occurring with aging may reduce the effectiveness of Cr supplementation, studies in older people have provided some evidence of positive effects of Cr supplementation, alone or with linolenic acid or proteins, on muscle mass and strength, endurance, and overall physical function (Morley et al., 2010; Tarnopolsky et al., 2007).

However, long-term studies on the effects of Cr on sarcopenia are necessary to establish the optimal dosing and timing of supplementation and to determine if the combination with proteins, amino acids, or their metabolites provides additional benefits.

2.4 Vitamin D

Vitamin D supplementation is receiving recognition as a potential strategy against sarcopenia. Vitamin D levels decline progressively with aging, partly as a result of reduced

sunshine exposure and impaired capacity of the aged skin to synthesize vitamin D under the influence of UV light. Other factors may also be involved, including the reduced renal conversion of vitamin D to its active form. Studies have reported extremely low vitamin D levels in most older persons. Indeed, 40–100% of U.S. and European community-dwelling older adults have vitamin D deficiency or insufficiency (25(OH)D levels < 30 ng ml^{-1} or < 75 nmol l^{-1}), and the situation is possibly even worse in institutionalized elderly, as a result of poorer dietary intake, decreased sun exposure, and higher rates of comorbidity (Holick, 2007).

Low vitamin D levels are associated with reduced muscle strength, functional decline, and increased risk for falls (Holick, 2007; Visser et al., 2003). In contrast, vitamin D supplementation improves muscle function, reduces the incidence of falls, and possibly impacts muscle fiber composition and morphology in vitamin D-deficient older adults (Morley et al., 2010).

The richest dietary sources of vitamin D_3 (cholecalciferol) are oily fish (e.g., salmon, mackerel, and herring) and liver oils from cod, tuna, and shark. Sun-dried mushrooms also contain variable amounts of vitamin D_2 (ergocalciferol). Older adults should, therefore, be encouraged to consume these foods; however, vitamin D supplementation is usually necessary to achieve the RDA (800 IU (20 mcg) for adults older than 70 years) in the absence of adequate sunlight exposure (Holick, 2007).

It is currently recommended to measure levels of 25(OH) vitamin D in all sarcopenic patients, and vitamin D (either D_2 or D_3) should be supplemented to all persons with plasma concentrations < 100 nmol l^{-1} (40 ng ml^{-1}; Morley et al., 2010).

Further studies are needed to completely understand the actions of vitamin D on the aging muscle. Research should also identify the genetic, metabolic, and pharmacologic profile of older individuals most likely to respond favorably to vitamin D supplementation.

2.5 Antioxidants

Supplementation with antioxidants to manage sarcopenia is worth a particular mention, because it is a paradigm of how a too simplistic way of addressing a complex issue could lead to controversial and equivocal results. The well-known free radical theory of aging postulates that an imbalance between the production of reactive oxygen species (ROS) and endogenous antioxidant defenses occurs during the aging process, resulting in higher rates of ROS-mediated cellular damage, especially in those tissues characterized by elevated oxidant generation, such as the skeletal muscle (Fusco et al., 2007; Musaro et al., 2010).

However, recent evidence suggests that ROS also act as important signaling molecules in muscle contraction and adaptation. Moderate exercise induces the production of low levels of ROS, which in turn activate specific cellular pathways that stimulate mitochondrial biogenesis and cellular antioxidant and repair capacity (Musaro et al., 2010). This adaptive response is preserved in older individuals (Safdar et al., 2010).

Hence, the indiscriminate use of antioxidant supplementation could suppress physiologically important ROS actions and blunt the beneficial effects of physical exercise (Hawley et al., 2011).

In an exhaustive review, Fusco et al. (2007) pointed out critical issues that need to be addressed before antioxidant supplementation is advised or rejected. The authors especially stressed the need for a better understanding of oxidative damage–related processes and their relationship with pro- and antioxidant factors, as well as the necessity of reliable markers of oxidative damage and antioxidants levels, a clearer picture of the network existing among different antioxidant molecules, and the identification of a therapeutic window during which antioxidant supplementation may be beneficial. This information is critical to establish the profile of individuals who could benefit from antioxidant supplementation, the best combination of antioxidant substances for older subjects, and the length of exposure to supplementation necessary for the achievement of beneficial effects.

In conclusion, even if antioxidant supplementation is increasingly adopted in Western countries, current evidence does not allow one to recommend this practice to prevent sarcopenia. Nevertheless, older persons should still be advised to consume foods rich in antioxidants because they also contain a vast array of vitamins, minerals, and fibers.

3. NEW POSSIBLE ACTORS IN THE NUTRITIONAL STRUGGLE AGAINST SARCOPENIA

3.1 Amino Acid Metabolites and Precursors: Hydroxy-Methylbutyrate and Ornithine α-Ketoglutarate

Hydroxy-methylbutyrate (HMB) is a leucine metabolite that is receiving increasing attention as a potential nutritional supplement for sarcopenia. Its effects on muscle metabolism closely resemble those of leucine. HMB may attenuate muscle protein breakdown by downregulating proteolytic pathways and stimulate protein synthesis through the mTOR pathway. Within myocytes, HMB is thought to be metabolized to hydroxymethylglutaryl–CoA, providing a large supply of cholesterol for cell membrane synthesis and, hence, maintenance of sarcolemmal integrity (Kim et al., 2010).

HMB has recently emerged as a promising dietary supplement to help reverse deficits in net anabolism in the sarcopenic muscle following exercise training. Indeed, HMB administered to older adults engaged in resistance training led to larger gains in lower (13% vs. 7%) and upper (13% vs. 11%) body strength than placebo (Vukovich et al., 2001). Furthermore, HMB, in association with lysine and arginine, improved physical function, strength, and lean body mass and increased protein turnover rate in sedentary elderly (Baier et al., 2009).

Ornithine α-ketoglutarate (OKG) is a precursor of biologically active amino acids such as glutamine, arginine, and proline, as well as other compounds, including polyamines, citrulline, and ketoisocaproate, that are potential regulators of skeletal muscle

protein metabolism and hemodynamics. OKG is also a powerful secretagog of anabolic hormones, such as insulin and growth hormone (Walrand, 2010). Anabolic actions of oral or intravenous OKG supplementation have been demonstrated in several pathological conditions associated with hypercatabolism and muscle wasting (malnutrition, burn injury, and abdominal surgery) in animal models and humans. Furthermore, OKG has been shown to improve nutritional status and quality of life, as well as to reduce healthcare costs when given to elderly patients soon after hospital discharge (Walrand, 2010).

Further studies are necessary to determine HMB and OKG mechanisms of action, as well as the effects of their supplementation in sarcopenic elderly.

3.2 Omega-3 Fatty Acids

Omega-3 fatty acids (OFAs) are essential nutrients with many potential health benefits. Indeed, OFAs, in particular eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3), possess anti-inflammatory properties, decrease triglyceride levels, lower blood pressure, reduce the growth rate of atherosclerotic plaques, decrease the risk for cardiovascular disease, and may protect against bone loss (Fetterman and Zdanowicz, 2009). Some evidence suggests that fish-derived OFAs might also help preserve muscle mass and function. For example, in patients undergoing surgery for nonmetastatic oesophageal cancer, increased OFA intake blunted the loss of total body and limb fat-free mass (Ryan et al., 2009). Moreover, an independent relationship between fatty fish consumption (the best dietary source of OFAs) and grip strength has been reported in older adults (Robinson et al., 2008). In addition, OFA supplementation has recently been shown to increase the rate of muscle protein synthesis during hyperinsulinemia–hyperaminoacidemia in older adults, most likely because of greater activation of the mTOR pathway (Smith et al., 2011).

These preliminary data represent an encouraging basis for future research on the role of OFAs in human muscle protein metabolism and suggest that an adequate intake of these compounds may represent a novel nutritional intervention against sarcopenia.

3.3 Nitrate (-Rich Foods)

Until recently, nitrate and nitrite were considered inert end products of nitric oxide (NO) metabolism and potentially toxic dietary constituents. However, in the past decade, studies have shown that these inorganic anions play a key role in several biological processes, including regulation of blood flow and pressure, cell signaling, glucose homeostasis, and tissue responses to hypoxia (Lundberg et al., 2008).

Diet is a major source of nitrate, especially vegetables such as celery, cress, chervil, lettuce, red beetroot, spinach, and rocket. Food-derived nitrate is actively taken up by salivary glands, excreted in saliva, and reduced to nitrite by oral cavity commensal bacteria residing in the oral cavity. Several mechanisms are then responsible for nitrite reduction to NO.

The effects of dietary nitrates on the aging muscle have not yet been investigated; however, some processes involved in the pathogenesis of sarcopenia could be targeted by these nutrients. For example, dietary nitrates increase gastric NO levels, which might reduce age-related early satiety, thus relieving one of the components of anorexia of aging. Moreover, insulin resistance is mechanistically associated with endothelial dysfunction as well as reduced muscle perfusion and anabolic signaling. Dietary nitrates, by increasing NO availability, could improve endothelial function and nutrient supply, restoring the normal protein anabolic response to insulin in the aged muscle. Furthermore, Larsen and colleagues (2011) showed that dietary inorganic nitrates enhance muscle mitochondrial bioenergetics, which may be harnessed as a countermeasure to sarcopenia.

Future studies will have to elucidate whether dietary inorganic nitrates offer a nutritional aid for the prevention and treatment of sarcopenia.

3.4 Calorie Restriction Mimetics, Exercise Mimetic, and Gymnomimetics

A wealth of evidence indicates that physiological stressors such as moderate calorie restriction (CR) and regular physical activity exert beneficial effects on overall health and muscle function also in advanced age (Marzetti et al., 2008). Intriguingly, recent studies suggest that a broad range of bioactive substances from plant sources may mimic the signaling events underpinning some of the effects of CR and exercise. Examples of CR mimetics (CRM) and exercise mimetic (EM) include resveratrol, quercetin, epigallocatechin-3-gallate, and nootkatone. Experimental models have shown that these phytochemicals could mimic CR and exercise through the activation of peroxisome proliferator-activated receptor-gamma coactivator-1α, sirtuin 1, and AMP-activated protein kinase (Hawley et al., 2011). However, the effects of these agents on the aged human muscle and their safety have not yet been established.

Although no single agent will probably reproduce all health benefits of CR or exercise, CRM and EM may be useful in those persons who are unable or unwilling to engage in dietary restriction or regular exercise training. To achieve this goal, it is necessary to adopt a comprehensive scientific approach elucidating the complex responses to stressors and integrating cell, tissue, organ, and systems adaptations to acute and chronic conditions. Such an approach has recently been epitomized through the characterization of changes in plasma levels of a wide range of metabolites in response to aerobic exercise (Lewis et al., 2010). Interestingly, incubation of cultured myotubes with a combination of metabolites whose plasma concentrations increased in response to exercise upregulated the expression of nur77, an orphan nuclear receptor induced in skeletal muscle after exercise as well as a transcriptional regulator of glucose and lipid metabolism-related genes. Notably, this 'gymnomimetic' effect was not observed when individual metabolites were used.

Despite these provocative findings, the field of CRM, EM, and gymnomimetics is still in its infancy, and much more research is needed to achieve a better understanding of all

properties of these compounds and stressor-activated metabolic pathways they are supposed to mimic, as well as their safety and potential therapeutic applications.

3.5 The Gut Factor

Humans are complex biological 'superorganisms,' in which vast, diverse, and dynamic microbial ecosystems have coevolved performing important roles in the definition of the host (human) physiology. In this human–microbe hybrid, the approximately 10^{14} gut microorganisms, collectively named gut microbiota, significantly contribute to the host's health status, influencing nutrient bioavailability, glucose and lipid metabolism, drug metabolism and toxicity, and immune system function (Kinross et al., 2011).

Substantial interindividual variability exists within the human gut microbiota and numerous host factors exert selective pressure on the microbiome, including host genome, diet, age, and eventual pharmacological interventions. Moreover, dysregulated host–microbiota interactions have been directly implicated in the etiopathogenesis of a number of disease conditions, such as obesity, cardiovascular disease, inflammatory bowel diseases, and autism (Kinross et al., 2011).

The aging process deeply affects the structure and function of the human gut microbiota and its homeostasis with the host's immune system, resulting in greater susceptibility to systemic infections, malnutrition, side effects of medications, and possibly contributing to the progression of chronic diseases and frailty (Tiihonen et al., 2010).

Probiotics, prebiotics, and their combinations may alleviate common gastrointestinal disorders in the elderly by modulating microbial activity and immune status. Comprehensive approaches based on the combination of different 'omic' sciences ((meta)genomics, microbiomics, trascriptomics, proteomics, metabolomics) are receiving considerable interest, as they could shed new light on the reciprocal influence between age-related changes in the gut microbiota and the physiology of older individuals, as well as identify possible targets for pharmaconutritional interventions aimed at improving the wellness of older adults.

As for sarcopenia, a better understanding of the symbiotic relationship between the gut microbiota and the aging organism is of utmost importance to design intervention strategies. Indeed, the gut microbiota could contribute to etiopathogenesis of sarcopenia, being involved in the regulation of inflammatory and redox status, splanchnic extraction of nutrients, fat mass deposition, and insulin sensitivity. In addition, the gut microbiota may deeply influence (or be influenced by) the bioavailability and bioactivity of most nutritional factors proposed as remedies against sarcopenia. For instance, colonic microbiota could modulate the metabolic fate of dietary polyphenols and other candidate CRM and EM, by converting these compounds into bioactive substances (van Duynhoven et al., 2011).

Given the importance of the gut microbiota in the regulation of human physiology, more research is warranted to explore the potential role of its manipulation in the management of sarcopenia.

4. CONCLUDING REMARKS: TOWARDS A SYSTEMS-BASED WAY OF THINKING SARCOPENIA

Sarcopenia is the prototype of a complex condition, with a plethora of intertwining etiological factors and pathogenetic mechanisms, ranging from systemic to cellular changes, giving rise to a multitude of phenotypes with different degrees of functional impairment and clinical correlates. This heterogeneity hinders the achievement of a general consensus on the definition itself of sarcopenia, further complicating the design of clinical trials and the development of effective therapeutic strategies. The only way to address this complexity is indeed through a complex approach, a paradigm shift toward a systems-based way of thinking sarcopenia. Sophisticated 'omic' platforms and informatics tools have recently been developed to characterize the phenotype of a person from his/her genetic background to the (nearly) complete repertoire of molecules representing crucial metabolic processes (metabolomics) of the human–microbe hybrid. This integrated multiomics approach, combined with appropriate functional and imaging measurements, should be the starting point to achieve a clearer definition of sarcopenia and address the critical issue of the lack of reliable diagnostic and prognostic markers for this condition. Such a multidimensional vision would also provide a fundamentally different perspective on human diet management. For example, nutrigenomics and metabolomics may define the individual nutritional and metabolic status, providing critical information on the ways the nutrients are harnessed by the body and the effects of nutrient consumption on the organism physiology. This knowledge is crucial for designing effective dietary interventions matching the actual requirements of sarcopenic elderly and, more generally, drive an individual's metabolic phenotype to a healthier direction.

The future of nutritional intervention in chronic diseases, including sarcopenia, also relies on a complex systemic way of thinking the functional effects of food, moving from traditional studies on single dietary agents to the global effect of diet on the individual's physiology. This will allow for tailoring the dietary recommendations to the subject's needs both in health and disease.

REFERENCES

Baier, S., Johannsen, D., Abumrad, N., Rathmacher, J.A, Nissen, S., Flakoll, P., 2009. Year–long changes in protein metabolism in elderly men and women supplemented with a nutrition cocktail of beta-hydroxy-beta-methylbutyrate (HMB), L-arginine, and L-lysine. Journal of Parenteral and Enteral Nutrition 33 (1), 71–82.

Fetterman Jr., J.W., Zdanowicz, M.M., 2009. Therapeutic potential of n–3 polyunsaturated fatty acids in disease. American Journal of Health-System Pharmacy 66 (13), 1169–1179.

Fusco, D., Colloca, G., Lo Monaco, M.R., Cesari, M., 2007. Effects of antioxidant supplementation on the aging process. Clinical Interventions in Aging 2 (3), 377–387.

Hawley, J.A., Burke, L.M., Phillips, S.M., Spriet, L.L., 2011. Nutritional modulation of training–induced skeletal muscle adaptations. Journal of Applied Physiology 110 (3), 834–845.

Holick, M.F., 2007. Vitamin D deficiency. The New England Journal of Medicine 357 (3), 266–281.

Kerstetter, J.E., O'Brien, K.O., Insogna, K.L., 2003. Low protein intake: the impact on calcium and bone homeostasis in humans. Journal of Nutrition 133 (3), 855S–861S.

Kim, J.S., Wilson, J.M., Lee, S.R., 2010. Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants. Journal of Nutritional Biochemistry 21 (1), 1–13.

Kinross, J.M., Darzi, A.W., Nicholson, J.K., 2011. Gut microbiome-host interactions in health and disease. Genome Medical 3 (3), 14.

Larsen, F.J., Schiffer, T.A., Borniquel, S., et al., 2011. Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metabolism 13 (2), 149–159.

Lewis, G.D., Farrell, L., Wood, M.J., et al., 2010. Metabolic signatures of exercise in human plasma. Science Translational Medicine 2 (33), 33ra37.

Lundberg, J.O., Weitzberg, E., Gladwin, M.T., 2008. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nature Reviews. Drug Discovery 7 (2), 156–167.

Marzetti, E., Leeuwenburgh, C., 2006. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Experimental Gerontology 41 (12), 1234–1238.

Marzetti, E., Lawler, J.M., Hiona, A., Manini, T., Seo, A.Y., Leeuwenburgh, C., 2008. Modulation of age-induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle. Free Radical Biology and Medicine 44 (2), 160–168.

Morley, J.E., 1997. Anorexia of aging: physiologic and pathologic. American Journal of Clinical Nutrition 66 (4), 760–773.

Morley, J.E., Argiles, J.M., Evans, W.J., et al., 2010. Nutritional recommendations for the management of sarcopenia. Journal of the American Medical Directors Association 11 (6), 391–396.

Musaro, A., Fulle, S., Fano, G., 2010. Oxidative stress and muscle homeostasis. Current Opinion in Clinical Nutrition and Metabolic Care 13 (3), 236–242.

Paddon-Jones, D., Rasmussen, B.B., 2009. Dietary protein recommendations and the prevention of sarcopenia. Current Opinion in Clinical Nutrition and Metabolic Care 12 (1), 86–90.

Robinson, S.M., Jameson, K.A., Batelaan, S.F., et al., 2008. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. Journal of American Geriatrics Society 56 (1), 84–90.

Rolland, Y., Czerwinski, S., Abellan Van Kan, G., et al., 2008. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. Journal of Nutrition, Health and Aging 12 (7), 433–450.

Ryan, A.M., Reynolds, J.V., Healy, L., et al., 2009. Enteral nutrition enriched with eicosapentaenoic acid (EPA) preserves lean body mass following esophageal cancer surgery: results of a double-blinded randomized controlled trial. Annals of Surgery 249 (3), 355–363.

Safdar, A., Hamadeh, M.J., Kaczor, J.J., Raha, S., Debeer, J., Tarnopolsky, M.A., 2010. Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. PLoS One 5 (5), e10778.

Smith, G.I., Atherton, P., Reeds, D.N., et al., 2011. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. American Journal of Clinical Nutrition 93 (2), 402–412.

Tarnopolsky, M., Zimmer, A., Paikin, J., et al., 2007. Creatine monohydrate and conjugated linoleic acid improve strength and body composition following resistance exercise in older adults. PLoS One 2 (10), e991.

Tiihonen, K., Ouwehand, A.C., Rautonen, N., 2010. Human intestinal microbiota and healthy ageing. Ageing Research Reviews 9 (2), 107–116.

Trumbo, P., Schlicker, S., Yates, A.A., Poos, M., 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Journal of the American Dietetic Association 102 (11), 1621–1630.

van Duynhoven, J., Vaughan, E.E., Jacobs, D.M., et al., 2011. Metabolic fate of polyphenols in the human superorganism. Proceedings of the National Academy of Sciences of the United States of America 108 (Suppl. 1), 4531–4538.

Visser, M., Deeg, D.J., Lips, P., 2003. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. Journal of Clinical Endocrinology and Metabolism 88 (12), 5766–5772.

Vukovich, M.D., Stubbs, N.B., Bohlken, R.M., 2001. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. Journal of Nutrition 131 (7), 2049–2052.

Walrand, S., 2010. Ornithine alpha-ketoglutarate: could it be a new therapeutic option for sarcopenia? Journal of Nutrition, Health and Aging 14 (7), 570–577.

Wang, T.J., Larson, M.G., Vasan, R.S., et al., 2011. Metabolite profiles and the risk of developing diabetes. Nature Medicine 17 (4), 448–453.

Dietary Calories on Cardiovascular Function in Older Adults

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1. INTRODUCTION

Aging is associated with several biological changes in the digestive tract, including changes in chewing, salivary flow and gastric acidity, as well as functional disturbances in the liver and pancreas (Laugier et al., 1991; Popper, 1986). These changes in both the endocrine and exocrine systems are barely perceptible in the gastrointestinal tract, because the secretory and absorptive ability of digestive tract cells is so high that clinically detectable manifestations occur only after normal function is reduced by at least 10% (Shamburek and Farrar, 1990). On the other hand, because the digestive system is also considered an endocrine system, elderly individuals' ability to secrete gastrointestinal hormones (GIH) needs to be properly evaluated. It is important to separate the changes resulting from aging itself from changes that arise from the diseases that affect individuals in this age group.

It is known that during the digestive process, several GIH with vasoactive actions are released, but it is unlikely that changes in serum levels of these hormones can be clinically detectable with aging. However, other concomitant situations that enhance the actions of GIH, such as diseases affecting the nervous plexus of the digestive tract, may occur in the elderly (Saffrey, 2004).

The vasoactive actions of GIH are not limited to their place of release; they can also influence systemic vascular actions that affect ventricular systolic function. Splanchnic circulation, for example, is responsible for 40% of the total peripheral resistance (TPR) placed on the left ventricle (LV), which suggests that the hormones that promote the dilatation or constriction of these vessels can directly modify systolic volume (VS), heart rate (HR), cardiac output (CO), TPR, and systemic arterial pressure (SAP) of individuals during the digestive process. However, such changes are often imperceptible in healthy young and old people, because humoral mechanisms and nervous reflexes are triggered simultaneously and the blood pressure remains constant.

There is evidence that aging is associated with changes in various neuronal systems. In elderly individuals at rest, there is a decline in cerebral blood flow and a decrease in the intensity of the neural response from metabolic needs and other stimulatory agents (Groschel et al., 2007). So, since older patients have dysautonomia, arising from the aging

or degenerative diseases and metabolic disorders, the neuronal response to GIH during food intake may be insufficient to maintain systemic hemodynamic variables at preingestion levels. In these circumstances, a reduction in SAP may occur.

2. GASTROINTESTINAL HORMONES WITH SYSTEMIC VASOACTIVE ACTIONS

GIH are distributed throughout the digestive tract, but mainly in the small intestine. The small intestine is considered to be the largest endocrine organ in the human body. Although its products are classified as hormones, they do not always function as substances that target cells at distant locations after they are released into the bloodstream. In fact, these peptides are often considered paracrine or autocrine, and may also serve as neurotransmitters. Below, we list some of the major GIH with vasoactive properties.

2.1 Vasoactive Intestinal Polypeptide

Vasoactive intestinal polypeptide (VIP) is a powerful vasodilator that increases intestinal blood flow and promotes relaxation in the smooth muscles of the vessels and secretion of the digestive epithelial cells. VIP belongs to a family of intestinal peptides that also includes glucagon and secretin. VIP is expressed mainly in the mesenteric innervation and in the central nervous system. In combination with nitric oxide (NO), it is a nonadrenergic and noncholinergic component of nervous transmission in intestine. When this peptide is infused directly into the coronary circulation in humans, it reduces coronary vascular resistance by 46% compared to pretreatment values (Smitherman et al., 1989). Recent studies show that VIP is able to increase coronary flow and ventricular contractile strength and it helps to reduce mean arterial blood pressure. On an equimolar basis, this peptide has a vasodilatory action 50–100 times more potent than that of acetylcholine (Henning and Sawmiller, 2001). VIP is released in response to nerve stimulation, cholinergic agonists, serotonin, dopamine agonists, the prostaglandins PGE and PGD, and growth factors (Henning and Sawmiller, 2001).

2.2 Calcitonin Gene-Related Peptide

This neuropeptide is produced by the small intestine's enteroendocrine cells. It is released in response to glucose and gastric acid secretion, and can cause marked vasodilation of the vessels in the stomach, splanchnic region, and peripheral circulation. Its action appears to be through the release of NO. It is part of the family of peptides that includes calciotonin, amylin, and adrenomedullin (Kapoor et al., 2003).

2.3 Neuropeptide Y

Neuropeptide Y (NPY) is synthesized and secreted by pancreatic cells in response to impulses from neurons in the central and peripheral nervous system. It inhibits

glucose-stimulated insulin secretion and is present in the myenteric and submucosal nervous plexuses in the digestive tract. Increases in NPY levels are observed after sympathetic stimulation. Intravascular administration of NPY is associated with marked vasoconstriction of the splanchnic circulation; however, this effect is not affected by adrenergic blockers. In healthy individuals, the infusion of progressively higher doses of NPY results in increased SAP, but myocardial perfusion, CO, and pulmonary artery pressure remain unchanged (Ullman et al., 2002).

2.4 Other Hormones

The majority of hormones secreted during the digestive process can also alter hemodynamics indirectly by affecting the absorption of liquids and electrolytes, thereby modifying volemia and the response to sympathetic and parasympathetic stimulation. Some of these hormones are involved in regulating the release of other GIH and affect the speed of gastric emptying and intestinal motility increasing or decreasing the absorption time for liquids and nutrients. Amylin, galanin, gastrin, ghrelin, somatostatin, glucagon, glucagon–like peptide-1, and glucagon–like peptide-2 are some of the hormones secreted after food ingestion.

In particular, insulin can contribute to important cardiocirculatory changes following food intake. Insulin, a hormone secreted by beta pancreatic cells, helps to metabolize glucose and inhibit glycogenolysis and gluconeogenesis. It also increases the transport of glucose into fat and muscle tissue, increases the glycolysis in these tissues and stimulates glycogen synthesis. Insulin has vasodilatory properties through the endothelial production of NO (Steinberg et al., 1994). In addition to this vasodilatory action, insulin also has the capacity to influence sodium retention by acting directly on the kidney's proximal convoluted tubule (Vallon et al., 1999).

3. FOOD INTAKE AND SYSTEMIC HEMODYNAMIC CHANGES IN THE ELDERLY

As described above, several GIH are secreted during the digestive process, many of which have direct and indirect effects on the cardiocirculatory apparatus. Physiologically, after the ingestion of food, splanchnic vessels dilate, increasing the blood flow to the digestive tube, and catecholamine levels and the HR increase in comparison to baseline values (Kooner et al., 1989). In healthy elderly individuals, SAP shows small fluctuations during the digestive process (Oberman et al., 2000). In an attempt to maintain constant SAP levels despite splanchnic vasodilation, vessels in other regions increase their resistance in response to food intake. This process represents an ideal interaction between hormones and the nervous system. However, the aging process is associated with changes in these systems' self-regulation, including reduced baroreflex activity and delayed gastric emptying (Matsukawa et al., 1996). Such changes, when present in the elderly, may attenuate reflexive responses in these systems and influence hemodynamics after the ingestion of food.

In the elderly, various hypotheses have been proposed to explain variations in post-prandial arterial blood pressure, including excessive splanchnic pooling, smaller increases in HR, worsening autonomic nervous system functions, decreases in intravascular volume and the release of GIH with improperly vascular action. These changes can contribute to reductions in arterial blood pressure, which results in a wide variety of symptoms such as angina pectoris, stroke, falls, nausea, and dizziness (Jansen and Lipsitz, 1995; Visvanathan et al., 2005).

Ventricular systolic function was evaluated carefully in 17 elderly individuals with prior clinical histories of hypertension and without postural hypotension. These patients were studied immediately after the ingestion of a meal with 700 kcal. The composition of the ingested food was 40% protein, 30% lipids, and 30% carbohydrates. Throughout the 60 min following ingestion, changes in CO, HR, SAP, and TPR were monitored through noninvasive methods. A significant reduction in systolic blood pressure levels (approximately -7 mmHg) was observed, and the lowest SAP values occurred in the 15 min after the end of ingestion. Parallel increases were noted in HR and CO, with the biggest value of CO occurring at 45 min and representing an increase of $0.9 \, \text{l min}^{-1}$ over preingestion values. A significant drop in TPR was also observed. From this evaluation, it can be concluded that despite clear changes in left ventricular function, SAP levels were minimally reduced (Ferreira Filho et al., 2007).

It is possible that compensatory mechanisms do not respond appropriately to hemodynamic changes generated by food in the digestive tract in elderly individuals with several associated comorbidities or dysautonomias. This may be detected through postprandial hypotension with all of the clinical symptoms described above.

4. FOOD CATEGORY AND HEMODYNAMIC RESPONSE

Different types of food can generate different hemodynamic responses when ingested. Thus, elderly hypertensive people were offered meals rich in lipids, proteins, and carbohydrates. Each meal was served on different, consecutive days, and each contained 700 kcal. The high-protein-content meal (63%) did not change SAP, CO, TPR, or HR during the 60-min observation period. The carbohydrate-rich meal (96%) reduced TPR and increased CO, whereas SAP did not change during the observation period. Finally, the lipid-rich meal (75%) produced greater TPR reductions and CO increases than the carbohydrate-rich meal. We conclude that although each meal contained the same number of calories, different food categories cause different cardiovascular responses, with fat-rich food causing the largest changes. In the same study, SAP did not change across the three categories analyzed. This finding demonstrates that in elderly people without clinically detectable dysautonomias, large reductions in vascular resistance were balanced by increases in CO (Ferreira-Filho et al., 2009).

The gastric emptying rate is lower in the elderly and can cause hemodynamic changes at different times compared to those observed in younger people. GIH that are only secreted when there is food in the duodenum could be secreted later in older than in younger individuals. Normally, fatty foods delay gastric emptying, and in this way systemic hemodynamic changes caused by fat intake might be noticed later than those caused by carbohydrate-rich foods (Collins et al., 1991; de Hoon et al., 2003). Some GIH and other peptides are detectable in the first phase of digestion, because they prepare the body for the digestive process, whereas others are secreted when the meal moves from the stomach to the small intestine. Thus, after eating, the hemodynamic response can vary depending on these factors.

Despite evidence that the same caloric quantity in different food categories causes different hemodynamic changes, few studies have evaluated whether ingestion of progressively higher concentrations of glucose produces different magnitudes of cardiocirculatory system response. It is known that the hemodynamic response does not correlate with blood glucose levels. Although glucose ingestion may result in SAP decreases, intravenous infusion has no effect on pressure levels, indicating that this response is mediated by the gastrointestinal tract (Visvanathan et al., 2005). In patients with autonomic changes, a venous hypertonic glucose solution produced more pronounced hemodynamic responses than isocaloric glucose infusion (Mathias, 1991).

5. INGESTION OF WATER AND FOOD WITH ZERO CALORIES

The ingestion of water can cause changes in some systemic hemodynamic variables. In contrast to individuals who consume proteins, carbohydrates, and lipids, which reduce TPR and increase CO, individuals drinking water exhibit what is called the gastropressor response. This response consists of systemic vasoconstriction and, consequently, an increase in SAP. Moreover, the HR does not increase. Some authors attribute these effects to gastric distension with increased sympathetic activity or to increased vagal activity on the heart (Routledge et al., 2002). Other studies suggest that the hypo-osmolar environment of the digestive tract after the ingestion of water could be responsible for the resulting gastropressor effect (Raj et al., 2006).

Food with restricted or zero calories quantities do not cause changes in the hemodynamic system. Supplementary studies developed in our laboratory using thoracic cardiac impedance showed that baseline values for CO, TPR, HR, and SAP were maintained after the ingestion of 400 ml of diet gelatin in elderly and young normotensive and hypertensive subjects.

6. POSTPRANDIAL HYPOTENSION

In the vast majority of cases studied, postprandial hypotension (PH) is associated with patients with autonomic failure, but patients with dopamine beta hydroxylase deficiencies,

i.e., with their sympathetic nervous system intact and without the capacity to synthesize norepinephrine, do not present with PH. This suggests that other transmitters or cotransmitters may block the hypotensive response after meals. Dopamine's vasoconstrictive effects on the splanchnic circulation represent an important way of maintaining SAP after eating. Dopamine antagonists such as, metoclopramide, reduce SAP, which indicates the presence of a dopaminergic pressor effect. Practical recommendations for controlling PH have included division of meals and small daily portions to avoid major drops in SAP, but the caloric content and the type of food offered at each meal may be critical in episodes of PH. Furthermore, splitting of meals could prolong hypotensive episodes.

Hemodynamic changes in PH are different from those reported in what is called dumping syndrome (DS). In DS, an observed drop in plasma volume and a large increase in the flow through the superior mesenteric artery along with an increase in sympathetic activity results in tachycardia, sweating, and other signs of a sympathetic aid response to maintain systemic pressure levels (Mathias, 1991). In elderly individuals specifically, there are no reports in the literature comparing the differences in both the hemodynamic and clinical presentations of these two syndromes.

7. CONCLUSIONS

Clearly, it is possible to verify changes in CO and reductions in peripheral resistance just minutes after ingestion in normal individuals, while the SAP remains constant. When dysautonomias are present, these modifications occur when the compensating mechanisms are not sufficiently adequate. For this reason, SAP levels may fall, resulting in clinical symptoms. Foods with caloric content caused greater changes in systemic hemodynamic parameters than foods with zero or reduced caloric content.

REFERENCES

Collins, P.J., Houghton, L.A., Read, N.W., et al., 1991. Role of the proximal and distal stomach in mixed solid and liquid meal emptying. Gut 32 (6), 615–619.

de Hoon, J.N., Pickkers, P., Smits, P., Struijker-Boudier, H.A., Van Bortel, L.M., 2003. Calcitonin gene-related peptide: exploring its vasodilating mechanism of action in humans. Clinical Pharmacology and Therapeutics 73 (4), 312–321.

Ferreira Filho, S.R., de Castro Rodrigues, F.F., Oliveira, P.C., Nery, M.A., 2007. Systemic hemodynamic changes in older hypertensive patients after drinking water or eating a meal. Hypertension 49 (5), e31.

Ferreira-Filho, S.R., Ferreira, A.C., Oliveira, P.C., et al., 2009. Systemic hemodynamic changes in elderly hypertensive patients after ingesting foods with lipid, protein, and carbohydrate contents. Journal of Clinical Hypertension (Greenwich, Conn.) 11 (5), 271–276.

Groschel, K., Terborg, C., Schnaudigel, S., et al., 2007. Effects of physiological aging and cerebrovascular risk factors on the hemodynamic response to brain activation: a functional transcranial Doppler study. European Journal of Neurology 14 (2), 125–131.

Henning, R.J., Sawmiller, D.R., 2001. Vasoactive intestinal peptide: cardiovascular effects. Cardiovascular Research 49 (1), 27–37.

Jansen, R.W., Lipsitz, L.A., 1995. Postprandial hypotension: epidemiology, pathophysiology, and clinical management. Annals of Internal Medicine 122 (4), 286–295.

Kapoor, K., Arulmani, U., Heiligers, J.P., et al., 2003. Effects of BIBN4096BS on cardiac output distribution and on CGRP–induced carotid haemodynamic responses in the pig. European Journal of Pharmacology 475 (1–3), 69–77.

Kooner, J.S., Raimbach, S., Watson, L., Bannister, R., Peart, S., Mathias, C.J, 1989. Relationship between splanchnic vasodilation and postprandial hypotension in patients with primary autonomic failure. Journal of Hypertension. Supplement 7 (6), S40–S41.

Laugier, R., Bernard, J.P., Berthezene, P., Dupuy, P., 1991. Changes in pancreatic exocrine secretion with age: pancreatic exocrine secretion does decrease in the elderly. Digestion 50 (3–4), 202–211.

Mathias, C.J., 1991. Postprandial hypotension. Pathophysiological mechanisms and clinical implications in different disorders. Hypertension 18 (5), 694–704.

Matsukawa, T., Sugiyama, Y., Mano, T., 1996. Age–related changes in baroreflex control of heart rate and sympathetic nerve activity in healthy humans. Journal of the Autonomic Nervous System 60 (3), 209–212.

Oberman, A.S., Gagnon, M.M., Kiely, D.K., Nelson, J.C., Lipsitz, L.A., 2000. Autonomic and neurohumoral control of postprandial blood pressure in healthy aging. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 55 (8), M477–M483.

Popper, H., 1986. Aging and the liver. Progress in Liver Diseases 8, 659–683.

Raj, S.R., Biaggioni, I., Black, B.K., et al., 2006. Sodium paradoxically reduces the gastropressor response in patients with orthostatic hypotension. Hypertension 48 (2), 329–334.

Routledge, H.C., Chowdhary, S., Coote, J.H., Townend, J.N., 2002. Cardiac vagal response to water ingestion in normal human subjects. Clinical Science (London, England) 103 (2), 157–162.

Saffrey, M.J., 2004. Ageing of the enteric nervous system. Mechanisms of Ageing and Development 125 (12), 899–906.

Shamburek, R.D., Farrar, J.T., 1990. Disorders of the digestive system in the elderly. The New England Journal of Medicine 322 (7), 438–443.

Smitherman, T.C., Popma, J.J., Said, S.I., Krejs, G.J., Dehmer, G.J., 1989. Coronary hemodynamic effects of intravenous vasoactive intestinal peptide in humans. The American Journal of Physiology 257 (4 Pt 2), H1254–H1262.

Steinberg, H.O., Brechtel, G., Johnson, A., Fineberg, N., Baron, A.D., 1994. Insulin–mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. The Journal of Clinical Investigation 94 (3), 1172–1179.

Ullman, B., Pernow, J., Lundberg, J.M., Astrom, H., Bergfeldt, L., 2002. Cardiovascular effects and cardiopulmonary plasma gradients following intravenous infusion of neuropeptide Y in humans: negative dromotropic effect on atrioventricular node conduction. Clinical Science (London, England) 103 (6), 535–542.

Vallon, V., Richter, K., Blantz, R.C., Thomson, S., Osswald, H., 1999. Glomerular hyperfiltration in experimental diabetes mellitus: potential role of tubular reabsorption. Journal of the American Society of Nephrology 10 (12), 2569–2576.

Visvanathan, R., Chen, R., Garcia, M., Horowitz, M., Chapman, I., 2005. The effects of drinks made from simple sugars on blood pressure in healthy older people. The British Journal of Nutrition 93 (5), 575–579.

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Mediterranean Lifestyle and Diet: Deconstructing Mechanisms of Health Benefits

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ABBREVIATIONS

BMD Bone mineral density
CHD Coronary heart disease
CVD Cardiovascular disease
DHA Docosahexaenoic acid
EPA Eicosapentaenoic acid
MD Mediterranean diet
METS Metabolic syndrome
ML Mediterranean lifestyle
MUFAs Monounsaturated fat acids
n-3 ω–3
PUFAs Polyunsaturated fatty acids

1. INTRODUCTION

Longevity and health are determined by genetic and epigenetic factors. Human evolution has resulted in the final phenotype, although different factors may still modulate morbid conditions and their related risk factors. Diet, exercise, and lifestyle are major determinants of health and longevity. The Mediterranean lifestyle (ML) is one which was once the traditional way of life found around the Mediterranean Sea. It includes physical work and spending leisure time outdoors. Currently, it is well known that this physical and diet pattern provides many health advantages, reducing hypertension risk and cardiovascular disease (CVD), diabetes, some cancer types, depression, and cognitive decline (Pérez-López et al., 2009). The ML was the life of poor people who were hard physical workers and had few staple foods.

The Mediterranean diet (MD) concept was created by Ancel Keys to include the common characteristics of poverty, although not related to a common specific diet

component, but rather to a drastic reduction of saturated fat and use of vegetable oils instead. Thus, although there are some common characteristics, there is no unique MD: Spaniards love pork, but North Africans do not; some use olive oil and others lard. Therefore, there are many variants of the MD. In 2010, the UNESCO declared the MD as an Intangible Cultural Heritage of Humanity after the proposal of four countries: Spain, Greece, Italy, and Morocco.

For centuries, Greek, Roman, Spanish, and individuals from other civilizations followed a diet based on simple natural foods including olive oil, legumes, nonrefined cereals, vegetables and fruits, moderate amount of fish and few red meat, low-moderate amounts of dairy products, and nuts and wine during meals in moderate quantities. The MD is based on monounsaturated fatty acids (MUFAs) found in olive oil and nuts and ω-3 (n-3) polyunsaturated fatty acids (PUFAs) found in fatty fish (sardines, salmon, tuna, and trout). Instead of solid fats such as butter or margarine, Mediterranean people use olive oil in almost everything they eat, including salads, vegetables, fish, pastas, breads, and even cakes and pastries.

Unfortunately, at the present time, few Mediterranean people (South of Europe and North of Africa) follow what was called the MD. In addition, lifestyle has changed and exercise, natural foods, and equilibrated diet components are not followed. Instead, a lot of precooked or fast-food is usually consumed, leisure time at outdoors and exercise have been reduced, and detrimental indoor activities been increased, including the use of television, electronic games, and Internet. Time devoted to select natural products and prepare meals has been reduced due to work organization. Thus, the prevalence of overweight/obesity and age-related morbidities has increased to figures found in other latitudes. In fact, Mediterranean people currently have some of the worst diets in Europe, and half of the individuals from Italy, Portugal, Greece, and Spain are overweight. The purpose of this chapter is to describe the traditional Mediterranean way of life and its diet components and the mechanisms by which they help maintain health and reach longevity.

2. OLIVE OIL

Olive (*Olea europaea*) cultivation is widespread at the Mediterranean region and is important for humans and environment. Freshly picked olive fruit is not palatable since it has phenolic compounds and oleuropein that makes the fruit taste bitter, but not unhealthy. Olive oil is the natural juice that may be directly consumed after pressed from the fruit, rich in MUFAs – mainly oleic acid – and in antioxidants such as phenols and vitamin E. No other natural oil has a large amount of MUFA as olive oil. Olives and olive oil also contain other compounds with anticancer properties, such as squalene and terpenoids.

Micronutrients of olives and olive oil have beneficial properties on cardiovascular risk factors, cancer, and age-related cognitive decline. It is the main element of the MD as a

source of energy, cooking, and seasoning. There are several categories of olive oil, although extra virgin olive oil (from the first pressing of the olives) is particularly rich in antioxidants. The virgin oil is obtained after the second pressing, pure oil is that undergoing some processing (e.g. filtering and refining), and extra light oil undergoes considerable processing and retains only a mild olive flavor.

Olive oil ingestion has a protective function on the digestive tract, activating pancreatic hormone and bile secretion, hence reducing gallstone formation risk. Olive oil may also reduce human cancer incidence. Different studies performed in Southern Europe suggest that olive oil consumption has a favorable effect in reducing breast, digestive tract, and upper aerodigestive tract cancers (Pelucchi et al., 2011). Differences were especially significant when comparing aerodigestive cancer risk in subjects consuming mainly olive oil versus those consuming mainly butter. High olive oil consumption has a protective effect as compared to women with the lowest olive oil consumption.

Regular olive oil consumption reduces blood pressure probably through the actions of oleic acid, α-tocopherol, polyphenols, and other phenolic compounds that are not present in other oils. It has been postulated that oleic acid acts on G-protein-associated cascades that regulate adenylyl cyclase and phospholipase C (Terés et al., 2008).

MD consumption correlates with a lower risk for the metabolic syndrome (METS). Phenolic compounds have antioxidant, antithrombotic, and anti-inflammatory properties. They prevent lipoperoxidation, induce favorable lipid profile changes (lowering total cholesterol and low-density lipoprotein cholesterol [LDL-C] while increasing high-density lipoprotein cholesterol [HDL-C]), and improve endothelial function. Olive oil does not favor obesity and is well tolerated by diabetics (Pérez-Martínez et al., 2011). In addition, virgin olive oil may reduce platelet aggregation sensitivity, factor plasma levels, and tissue factor expression in mononuclear cells and a concomitant increase in fibrinolytic activity, reducing plasma activator inhibitor type 1 (Pérez-Jiménez et al., 2006).

Some components of the MD have been positively associated with bone health. After adjusting for several confounding factors, an MD with high consumption of fish and olive oil and low intake of red meat was linked to higher spine and total bone mineral density (BMD) (Kontogianni et al., 2009). Osteoporotic fracture reduction in relation to these changes remains to be determined.

3. MODERATE RED WINE CONSUMPTION

Light-to-moderate red wine consumption during meals is associated with cardiovascular risk reduction in middle-aged and elderly subjects, including coronary heart disease, stroke, and total mortality. Benefits of this type of wine consumption are lost when subjects expose to heavier drinking. The benefits of light-to-moderate alcohol consumption have also been reported with beer and spirits. Cardioprotective effects of moderate wine consumption are due to anticoagulation, an increase in HDL-C, and decreased platelet

aggregation and LDL-C and plasma apolipoprotein(a) levels. The mechanism of cardiovascular protection has been related to genetic variation of hepatic alcohol dehydrogenase 3. This enzyme decreases ethanol metabolism rate. Homozygous for the slow-oxidizing allele indirectly have high HDL-C levels and lower myocardial infarction risk (Hines et al., 2001).

The coexistence of cardiovascular risk factors with unanticipated low incidence of coronary disease has been associated with low-to-moderate red wine consumption. This phenomenon is known as the 'French paradox' and is more pronounced for red wine than for other alcoholic drinks and inputted to the antioxidants present in wine. Red wine contains active compounds called polyphenols, especially resveratrol, which act on cardiovascular end points. Indeed, they produce – *in vitro* – biochemical changes associated with decreased arterial damage, reduced angiotensin II activity, increased nitric oxide secretion, reduced platelet aggregation, and neuroprotective activities. However, there are other effects less well defined such as decreased LDL oxidation and antisenescence actions on myocytes (Opie and Lecour, 2007). However, both nongenomic and genomic actions may be mediated by proteins specifically targeted by resveratrol.

Resveratrol has shown anticancer properties, inhibiting the *in vitro* proliferation of a wide variety of human tumor cells. Although it has anticancer activity in animal models, evidence of its effects in humans is scarce (Athar et al., 2007). Resveratrol is envisioned as cancer chemopreventive agent, reducing adipogenesis and viability in maturing preadipocytes through transcriptional factor downregulation and mitochondrial genetic modulation. In addition, resveratrol increases lipolysis and reduces lipogenesis in mature adipocytes. Castrated aged rats reduce weight gain and inhibit bone loss when supplemented with a daily combination of resveratrol, vitamin D, quercetin, and genistein (Baile et al., 2011).

Reports indicate that moderate alcohol consumption is associated with higher BMD in men and postmenopausal women, although high liquor intake is related to lower BMD (Tucker et al., 2009). The trend for stronger associations between BMD and beer or wine, as compared to liquor with higher alcohol content, indicates that bone benefits may be related to other constituents different from ethanol.

4. FRUIT AND VEGETABLES

Fresh vegetables and fruits are essentials in the MD. Fruit, vegetables, and whole grains allow feeling full faster and longer and will moderate glycemia and caloric intake. These products have been associated with a lower incidence of heart disease, diabetes, and many cancers. The traditional MD includes vegetables, including tomatoes, broccoli, peppers, capers, spinach, eggplant, mushrooms, white beans, lentils, and chick peas. Several observational studies have reported the advantage of consuming fresh vegetables, fruits, and legumes. In an Italian prospective study, high consumption of leafy vegetables and olive

oil was inversely associated with coronary heart disease (CHD), whereas no association was found for fruit consumption (Bendinelli et al., 2011).

Reduction of cancer risk has been associated with fruit and vegetable consumption accompanied with low meat and carbohydrate intake (La Vecchia, 2004). Subjects in the highest tertile of vegetable and fruit consumption have low relative risks (between 0.3 and 0.7) for different types of cancer as compared to those in the lowest tertile. It seems that these MD components have antioxidants and other micronutrients active against cancer cells. Despite this, the main protective component is still unknown. Fruit and vegetable consumption also has positive effects over bone health (spine and femoral neck) in younger and older age groups (Prynne et al., 2006). In these individuals, however, it remains to be determined if this effect is related per se to the diet or rather to their lifestyle.

Mortality risk and four dietary patterns were investigated in a prospective Italian study followed up for a median of 6.2 years (Masala et al., 2007). The pattern that included a high proportion of olive oil, raw vegetables, soups, and poultry was inversely associated with an overall lower mortality rate in both crude and adjusted models. On the contrary, a diet pattern including pasta, tomato sauce, red meat, processed meat, added animal fat, white bread, and wine was associated with an increased overall mortality.

5. CEREALS AND LEGUMES

Cereals and legumes have healthy components such as those found in vegetables and are easy to store for prolonged periods of time. Cereals and legumes are rich in phenolic acids, flavonoids, tannins, lignans, alkylresorcinols, and other active compounds. In addition, total grain consumption provides all the biochemical beneficial components. Maintaining grains close to their original forms reduces starch digestion and eventually reduces glycemia spikes and insulin resistance risk (McKeown et al., 2002).

Whole-grain consumption decreases the risk of CVD, stroke, type 2 diabetes (T2D), METS, and gastrointestinal cancers (Jones, 2006). CHD incidence and risk are reduced 20% and 40%, respectively, in individuals who usually consume whole grains as compared to those who rarely ever consume (Flight and Clifton, 2006). Several studies have failed to establish an association between fiber-alone consumption and CHD events, seeming that the beneficial effects are related to whole grains.

The analysis of randomized controlled trials related to whole-grain food consumption or diets failed to show benefits on CHD mortality, CHD events, or morbidity. Oatmeal consumption is associated with total cholesterol and LDL-C level reduction (Kelly et al., 2007). However, the majority of studies included in the meta-analysis addressed short-term treatments.

Prevention of T2D with whole-grain consumption has been analyzed. Priebe et al. (2008) reviewed the association between whole-grain or cereal consumption and T2D

incidence. It was found that one prospective study reported a preventive effect of whole-grain or cereal fiber consumption over T2D development.

The effect of legume consumption over depressed mood has been studied in women, according to menopausal status (Li et al., 2010). In women with menstrual function, legume consumption seemed to have a deleterious trend over depressed mood. However, moderate consumption of legumes had a significant protective effect in women in the menopausal transition, whereas no association was found in either postmenopausal women or men.

6. THE ω-3 FATTY ACIDS

The n–3 PUFAs are present in oily fish and nuts and have been related to health benefits including cardiovascular risk, hypertension, certain types of cancer, and neurological disorders such as Alzheimer's disease and macular degeneration. Epidemiological studies have reported that n–3 PUFA intake may protect from CVD. Prospective studies have shown that fish or fish oil consumption containing n–3 PUFA acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) decreases cardiovascular-related mortality. Randomized studies have confirmed that EPA plus DHA consumption is protective at daily doses of <1 g (Breslow, 2006). EPA and DHA display antiarrhythmic and antiatherosclerotic effects that may be beneficial in CVD prevention. Involved mechanisms include lowering plasma triaglycerols, blood pressure, platelet aggregation, and inflammation, all of which improve vascular reactivity (von Schacky, 2007). Short-term treatment (6 weeks) of overweight dyslipidaemic men and treated-hypertensive patients with daily 4 g of EPA and DHA reduced *in vivo* oxidant stress measured as a fall in human plasma and urine isoprostane levels (Mas et al., 2010).

In individuals with hypercholesterolemia, marine n–3 fatty acid supplements improve systemic artery endothelial function. Brachial-artery-flow-mediated dilatation (assessed by ultrasound) significantly improved after a 4-month supplementation with marine n–3 fatty acids (4 g day^{-1}). In this study, endothelial-dependent dilatation was not affected (Goodfellow et al., 2000).

Nuts are rich in unsaturated fatty acids, fiber, vitamin E, plant sterols, L-arginine, and other healthy nutrients. They are easy to store and consumed as snacks. Eating 50 g of nuts per day such as almonds, hazelnuts, walnuts, pistachios, and peanuts may reduce cardiovascular risk. It seems that all nuts share health benefits, although some are more heart protective. Indeed, nuts have n–3, which are cardioprotective and reduce cardiovascular risk and health-related mortality (Ros et al., 2010). In menopausal women, soy nut consumption reduced vasomotor symptoms (Welty et al., 2007).

The possible benefits of nut n–3 fatty acid consumption over triglyceride levels, inflammation, and endothelial function have been studied in healthy subjects with moderate hypertriglyceridemia (Skulas-Ray et al., 2011). In this randomized study,

EPA + DHA daily doses of 0.85 versus 3.4 g were compared. EPA + DHA at the higher dose significantly lowered triglycerides, but neither doses improved endothelial function or inflammatory status over the 8-week supplementation period (Skulas-Ray et al., 2011).

Endothelial dysfunction plays a significant role in atherogenesis. Inconsistent results have been found in relation to n-3 PUFA supplementation and endothelial function in healthy subjects. Contrarily, markers of endothelial dysfunction improve in overweight, dyslipidaemic, and diabetic patients (Egert and Stehle, 2011).

7. SUN AND LEISURE TIME: VITAMIN D, SEROTONIN, AND FRIENDS

The Mediterranean region is characterized by bright sunny days and good weather, with few climatic oscillations in comparison to other regions closer to the poles. Good weather invites people to spend leisure time outdoors. Sunlight promotes the synthesis of vitamin D and serotonin, which improves physical and emotional status. Vitamin D has been associated with better quality of life, less prevalence of comorbid conditions, and less frailty (Pérez-López et al., 2011).

The traditional ML includes the happy company of family and friends. Currently, it is known that health and happiness are influenced by large social networks (Fowler and Christakis, 2009). Lack of social support or social integration has been associated with mortality, CHD, and other negative vital outcomes. Friendship is a value for help and emotional support. In addition, friend behavior and lifestyle may influence individuals to adopt healthy or risky behavior.

In young women, physical exercise and living with a partner were positively associated to serum 25-hydroxyvitamin D (25(OH)D) levels, whereas in older women, physical activity, vitamin D intake, and urban dwelling positively associated with serum 25 (OH)D. At the same time, BMD significantly related to serum 25(OH)D (Pasco et al., 2009). In this sense, vitamin D may be seen as a healthy lifestyle marker (Pérez-López et al., 2011).

8. FINAL REMARKS

The interaction between genome characteristics and the environment have caused specie evolution, life duration, and the health–disease duet. The Human Genome Project and new technologies may provide breakthrough biological approaches to preserve health, increase longevity, and reduce morbidity of age-related pathologies, including CVD, cancer, and degenerative diseases. Natural, simple food, and exercise, like the ML, may be one of the healthiest ways of living and maintaining quality of life and vital satisfaction. Functional food design will be carried out once proper identification of active products contained in healthy meals is performed.

GLOSSARY

Mediterranean diet A diet based on natural products, olive oil, fish and nuts, vegetables, and fruits.

Mediterranean legumes They are part of the *Leguminosae* botanical family and are among the first cultivated plants in the Mediterranean basin. Legumes or beans are among the plants with the richest protein content. Amino acids in beans are complementary to those found in cereals and are the first foods cultivated in archeological sites.

Olive oil Oil obtained from the fruits (olives) of the *Olea europaea*, a traditional tree of the Mediterranean basin. It is used for cooking and in pharmaceuticals, soap, and cosmetics.

Resveratrol A natural phenol produced by several plants; found in the skin of red grapes. The high amounts found in red wine may explain the health benefits of drinking a small quantity of wine with meals.

Sunlight Sunlight is the primary earth energetic source, it increases serotonin and vitamin D synthesis and favors leisure activities.

ω-3 fatty acids A type of unsaturated fat present in fish (salmon, tuna, sardines, mackerel, and trout) and nuts (walnuts, almonds, hazelnuts, and pistachios).

REFERENCES

Athar, M., Back, J.H., Tang, X., et al., 2007. Resveratrol: a review of preclinical studies for human cancer prevention. Toxicology and Applied Pharmacology 224, 274–283.

Baile, C.A., Yang, J.Y., Rayalam, S., et al., 2011. Effect of resveratrol on fat mobilization. Annals of the New York Academy of Sciences 1215, 40–47.

Bendinelli, B., Masala, G., Saieva, C., et al., 2011. Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. The American Journal of Clinical Nutrition 93, 275–283.

Breslow, J.L., 2006. n-3 fatty acids and cardiovascular disease. The American Journal of Clinical Nutrition 83, 1477S–1482S.

Egert, S., Stehle, P., 2011. Impact of n-3 fatty acids on endothelial function: results from human interventions studies. Current Opinion in Clinical Nutrition and Metabolic Care 14, 121–131.

Flight, I., Clifton, P., 2006. Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. European Journal of Clinical Nutrition 60, 1145–1159.

Fowler, J.H., Christakis, N.A., 2009. Dynamic spread of happiness in a large social network: longitudinal analysis over 20 years in the Framingham Heart Study. British Medical Journal 337, a2338.

Goodfellow, J., Bellamy, M.F., Ramsey, M.W., Jones, C.J., Lewis, M.J., 2000. Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. Journal of the American College of Cardiology 35, 265–270.

Hines, L.M., Stampfer, M.J., Ma, J., et al., 2001. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. The New England Journal of Medicine 344, 549–555.

Jones, J.M., 2006. Grain-based foods and health. Cereals Foods World 51, 108–113.

Kelly, S.A., Summerbell, C.D., Brynes, A., Whittaker, V., Frost, G., 2007. Wholegrain cereals for coronary heart disease. Cochrane Database of Systematic Reviews 2, CD005051.

Kontogianni, M.D., Melistas, L., Yannakoulia, M., et al., 2009. Association between dietary patterns and indices of bone mass in a sample of Mediterranean women. Nutrition 25, 165–171.

La Vecchia, C., 2004. Mediterranean diet and cancer. Public Health Nutrition 7, 965–968.

Li, Y., Dai, Q., Tedders, S.H., Arroyo, C., Zhang, J., 2010. Legume consumption and severe depressed mood, the modifying roles of gender and menopausal status. Public Health Nutrition 13, 1198–1206.

Mas, E., Woodman, R.J., Burke, V., et al., 2010. The omega-3 fatty acids EPA and DHA decrease plasma F(2)-isoprostanes: results from two placebo-controlled interventions. Free Radical Research 44, 983–990.

Masala, G., Ceroti, M., Pala, V., et al., 2007. A dietary pattern rich in olive oil and raw vegetables is associated with lower mortality in Italian elderly subjects. The British Journal of Nutrition 98, 406–415.

McKeown, N.M., Meigs, J.B., Liu, S., Wilson, P.W.F., Jacques, P.F., 2002. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. The American Journal of Clinical Nutrition 76, 390–398.

Opie, L.H., Lecour, S., 2007. The red wine hypothesis: from concepts to protective signalling molecules. European Heart Journal 28, 1683–1693.

Pasco, J.A., Henry, M.J., Nicholson, G.C., Brennan, S.L., Kotowicz, M.A., 2009. Behavioural and physical characteristics associated with vitamin D status in women. Bone 44, 1085–1091.

Pelucchi, C., Bosetti, C., Negri, E., Lipworth, L., La Vecchia, C., 2011. Olive oil and cancer risk: an update of epidemiological findings through 2010. Current Pharmaceutical Design 17, 805–812.

Pérez-Jiménez, F., Lista, J.D., Pérez-Martínez, P., et al., 2006. Olive oil and haemostasis: a review on its healthy effects. Public Health Nutrition 9, 1083–1088.

Pérez-López, F.R., Chedraui, P., Haya, J., Cuadros, J.L., 2009. Effects of the Mediterranean diet on longevity and age-related morbid conditions. Maturitas 64, 67–79.

Pérez-López, F.R., Chedraui, P., Fernández-Alonso, A.M., 2011. Vitamin D and aging: beyond calcium and bone metabolism. Maturitas 69, 27–36.

Pérez-Martínez, P., García-Ríos, A., Delgado-Lista, J., Pérez-Jiménez, F., López-Miranda, J., 2011. Mediterranean diet rich in olive oil and obesity, metabolic syndrome and diabetes mellitus. Current Pharmaceutical Design 17, 769–777.

Priebe, M.G., van Binsbergen, J.J., de Vos, R., Vonk, R.J., 2008. Whole grain foods for the prevention of type 2 diabetes mellitus. Cochrane Database of Systematic Reviews 1, CD006061.

Prynne, C.J., Mishra, G.D., O'Connell, M.A., et al., 2006. Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. The American Journal of Clinical Nutrition 83, 1420–1428.

Ros, E., Tapsell, L.C., Sabaté, J., 2010. Nuts and berries for heart health. Current Atherosclerosis Reports 12, 397–406.

Skulas-Ray, A.C., Kris-Etherton, P.M., Harris, W.S., et al., 2011. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. The American Journal of Clinical Nutrition 93, 243–252.

Terés, S., Barceló-Coblijn, G., Benet, M., et al., 2008. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. PNAS 105, 13811–13816.

Tucker, K.L., Jugdaohsingh, R., Powell, J.J., et al., 2009. Effects of beer, wine, and liquor intakes on bone mineral density in older men and women. The American Journal of Clinical Nutrition 89, 1188–1196.

von Schacky, C., 2007. Omega-3 fatty acids and cardiovascular disease. Current Opinion in Clinical Nutrition and Metabolic Care 10, 129–135.

Welty, F.K., Lee, K.S., Lew, N.S., Nasca, M., Zhou, J.R., 2007. The association between soy nut consumption and decreased menopausal symptoms. Journal of Women's Health (2002) 16, 361–369.

FURTHER READING

Brown, L., van der Ouderaa, F., 2007. Nutritional genomics: food industry applications from farm to fork. The British Journal of Nutrition 97, 1027–1035. http://journals.cambridge.org/download.php?file=%2FBJN%2FBJN97_06%2FS0007114507691983a.pdf&code=db8f662bba89e08d90aecae20ceafeb1.

Hidalgo, C.A., Blumm, N., Barabasi, A.L., Christakis, N.A., 2009. A dynamic network approach for the study of human phenotypes. [PMC free article] PLoS Computational Biology 5, e1000353. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2661364/pdf/pcbi.1000353.pdf.

Iacoviello, L., Santimone, I., Latella, M.C., de Gaetano, G., Donati, M.B., 2008. Nutrigenomics: a case for the common soil between cardiovascular disease and cancer. Genes and Nutrition 3, 19–24. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2311494/pdf/12263_2008_Article_79.pdf.

Lee, W.N., Go, V.L., 2005. Nutrient-gene interaction: tracer-based metabolomics. The Journal of Nutrition 135, 3027S–3032S. http://jn.nutrition.org/content/135/12/3027S.full.pdf.

Loscalzo, J., Kohane, I., Barabasi, A.L., 2007. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. Molecular Systems Biology 3, 124 [PMC free article].

Lusis, A.J., Weiss, J.N., 2010. Cardiovascular networks: systems-based approaches to cardiovascular disease. Circulation 121, 157–170. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2836123/pdf/nihms175360.pdf.

Sanz de Galdeano, A., 2007. An economic analysis of obesity in Europe: health, medical care and absenteeism costs. FEDEA, Madrid 2007 http://www.fedea.es/pub/papers/2008/dt2007-38.pdf.

RELEVANT WEBSITES

http://www.the-aps.org – Ancel Keys. The American Physiological Society.
http://www.dieta-mediterranea.com – Dieta Mediterránea.
http://www.eufic.org – European Food Information Council (EUFIC).
http://www.internationaloliveoil.org – International Olive Oil Council.
http://www.mediterraneandiet.gr – The Mediterranean Diet.
http://www.oldwayspt.org – The Oldways Mediterranean Diet Pyramid.
http://www.unesco.org – UNESCO. The Mediterranean diet.

Creatine and Resistance Exercise: A Possible Role in the Prevention of Muscle Loss with Aging

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Sarcopenia, defined as the age-related loss of muscle mass (Thompson, 2009), has a detrimental effect on muscle strength (Evans, 1995), bone health (Candow and Chilibeck, 2010), and metabolic rate, leading to an increase in fat mass (i.e., sarcopenic obesity) and an impaired ability to perform tasks of daily living. Approximately one in four adults over 70 years of age will experience rapid muscle and strength loss (Hepple, 2003). It is estimated that by the year 2040, there will be at least 8–13 million Americans of 85 years of age or older (Booth et al., 2000). The cost of health associated with this growing population is enormous. For example, over $300 billion is spent annually for treating sarcopenic-related symptoms (Booth et al., 2000).

Mechanistically, muscle and force loss with aging may be partially caused by a reduction in muscle fiber number (Trappe, 2001), although fiber atrophy, especially among type II fibers, is also involved (Larsson et al., 2001). A further fast-to-slow transformation process resulting in an increased number of intermediate slow-twitch muscle fibers (i.e., type IIa) is also evident, which inevitably decreases muscle strength (Hepple, 2003). Regarding muscle contraction, the age-related slowing of twitch properties in motor units of both fast-twitch and slow-twitch muscle fibers is thought to be caused by alterations in sarcoplasmic reticulum functionality. Aging has a negative effect on sarcoplasmic reticulum protein function (Larsson et al., 2001), resulting in a decline in maximum contractile force in skeletal muscle. At the cellular level, there is also a significant decrease in myosin per unit of muscle with aging (Marx et al., 2002). Furthermore, aging also appears to influence the activity and function of satellite cell. Satellite cells are mononucleated cells that have a definitive lifespan (for review, see Brack and Rando, 2007). Once activated (i.e., mechanical stimuli from resistance exercise), satellite cells produce muscle precursor cells to form new myofibrils. An attenuation of satellite cell proliferation could potentially limit aging muscle accretion, especially if satellite cell proliferation is exhausted during a continuous lifespan of repeated cycles of atrophy and regrowth. It has been shown recently that there is a substantial attenuation in satellite cell number and function in type II, but not in type I, fibers of the vastus lateralis in older adults (Verdijk et al., 2007),

indirectly suggesting that the reduction in satellite cell attenuation with aging is fiber specific, which may help to explain the reduction in type II muscle fibers. An increase in oxidative stress may also contribute to the deterioration of muscle tissue with aging (for review, see Johnston et al., 2008). There is a progressive cellular decline with aging due to an increase in mutagenic oxygen radicals (i.e., reactive oxygen species), leading to cellular senescence (Johnston et al., 2008). Continuous oxidative damage over time with aging may exhaust antioxidant systems resulting in cellular stress. Coincidently, cytokines are released in response to chronic inflammation and stress (i.e., resistance exercise), and aging results in an accelerated increase in the release of the proinflammatory cytokines that has a negative effect on aging muscle biology (Bautmans et al., 2005).

1. CREATINE AND AGING

Creatine is a nitrogen-containing compound naturally produced in the body or found in the diet primarily from red meat and seafood (Wyss and Kaddurah-Daouk, 2000). Creatine is primarily produced in a two-step process starting in the kidney and finishing in the liver but can also be synthesized entirely in the pancreas or liver. Very little creatine is retained at the site of production. The majority of creatine is transported from areas of synthesis (i.e., liver, kidney, and pancreas) to areas of storage and utilization (i.e., skeletal muscle) (Persky and Brazeau, 2001). Skeletal muscle creatine content is dependent on muscle fiber composition (Persky and Brazeau, 2001). Type II muscle fibers have high levels of free creatine (Cr) and phosphocreatine (PCr). With aging, there is a progressive decline in skeletal muscle mass (i.e., type II fibers) and strength (Evans, 1995). Speculation exists that reduced high-energy phosphate metabolism may play a role in these metabolic changes with age. Since 90–95% of PCr is typically found in skeletal muscle and PCr is needed to maintain the ATP/ADP ratio during resistance exercise (Greenhaff et al., 1994), a progressive decrease in skeletal muscle with age would be associated with a reduction in PCr. Moller et al. (1980) and Campbell et al. (1999) showed that older adults had significantly lower PCr stores compared to younger adults. An increase in intramuscular creatine from creatine supplementation should theoretically increase PCr resynthesis during resistance exercise and have a favorable effect on muscle accretion with aging. To support this hypothesis, Brose et al. (2003) found a significant increase in intramuscular total creatine, strength, and lean tissue mass in older adults from creatine supplementation during 14 weeks of resistance training, and Chrusch et al. (2001) reported a significant increase in lean tissue mass following 12 weeks of creatine supplementation and resistance training in older men. Mechanistically, creatine may influence muscle hypertrophy through an increase in cellular hydration status (Balsom et al., 1995), myogenic transcription factors (i.e., MRF-4 and myogenin; Willoughby and Rosene, 2003), satellite cell activity (Olsen et al., 2006), and anabolic hormone secretion (i.e., IGF-I; Burke et al., 2008) or by reducing protein catabolism (Candow et al., 2008; Parise et al., 2001).

2. STRATEGIC CREATINE SUPPLEMENTATION

It is well known that a single bout of intense resistance exercise will stimulate muscle protein turnover (i.e., protein catabolism and protein synthesis; Phillips, 2004). Although the signaling pathways for stimulating muscle protein synthesis (i.e., MTOR) are increased after exercise, it appears that this anabolic response is delayed in the postabsorptive period (Phillips, 2004). Research indicates that the strategic ingestion of creatine (i.e., before and after resistance exercise) may influence aging muscle protein kinetics. A few studies have shown that creatine supplementation, both before and after resistance exercise, have positive effects on muscle mass and strength with aging (for review, see Candow and Chilibeck, 2008). For example, consuming creatine immediately before and immediately after supervised resistance exercise sessions (leg press, chest press, lat pull down, shoulder press, leg extension, leg curl, biceps curl, triceps extension, and calf press; 3 days week^{-1}, 10 weeks) resulted in greater whole-body muscle size (ultrasound; elbow and knee flexor and extensors, ankle plantar-flexors and dorsi-flexors; 2.0 cm) compared to placebo (0.8 cm) and resistance exercise in healthy older males (59–77 years of age; Candow et al., 2008). By comparing the effects of creatine supplementation immediately before and immediately after resistance exercise in older adults, Candow et al. (unpublished findings) also found a significant increase in muscle mass and strength, independent of the timing of ingestion. Creatine supplementation has also been shown to decrease whole-body protein breakdown (approximate plasma leucine rate of appearance) in young men (Parise et al., 2001), suggesting that creatine exhibits anticatabolic effects on muscle and whole-body proteins. Furthermore, by comparing the effects of creatine ingestion before and after resistance exercise training (10 weeks) to creatine ingestion in the morning and evening of training days, Cribb and Hayes (2006) showed that creatine ingestion before and after exercise resulted in significantly greater intramuscular creatine content, lean tissue mass, and muscle cross-sectional area of type II fibers. Although it is difficult to compare results across studies, it has been theorized that these positive results from creatine ingestion before and after exercise may be due to an increase in blood flow and delivery of creatine to exercising muscles (Harris et al., 1992), an upregulation of the kinetics involved in creatine transport (Robinson et al., 1999), and an increase in Na^+-K^+ pump function during muscle contraction (Robinson et al., 1999) (Table 11.1).

3. SAFETY OF CREATINE FOR OLDER ADULTS

Research examining the potential health risks associated with creatine supplementation in older adults is minimal. Common adverse effects typically reported include involve nausea, vomiting, diarrhea, excessive thirst, and GI track complications (Persky and Rawson, 2007). However, these symptoms are usually based on anecdotal reports from young adults who adapt to a creatine 'loading' protocol where they ingest 20 g of creatine (5 g for four times) per day for 5–7 days and then ingest approximately 5–7 g day^{-1}

Table 11.1 Summary of Studies Involving Creatine Supplementation on Body Composition and Muscle Performance in Older Adults

Study	Population	Dosage	Main findings
Bemben et al. (2010)	Male (48–72 years) CR: 11, PL: 10	CR: 5 g, 14 weeks	↔ Lean tissue mass, strength
Bermon et al. (1998)	Male/female (70 years) CR: 16; PL: 16	CR: 20 g, 5 days Maintenance: 3 g, 47 days	↔ Lower limb muscle volume
Brose et al. (2003)	Male/female (68 years) CR: 14; PL: 14	CR: 5 g, 98 days	↑ Fat-free mass
Candow et al. (2008)	Male (66 years) CR: 23; PL: 12	CR: 0.1 g kg^{-1}, 30 days	↑ Muscle hypertrophy
Chrusch et al. (2001)	Male (71 years) CR: 16; PL: 14	CR loading: 0.3 g kg^{-1}, 5 days Maintenance: 0.07 g kg^{-1}, 79 days	↑ Fat-free mass, strength
Eijnde et al. (2003)	Male (64 years)	CR: 5 g, 52 weeks CR: 23; PL: 23	↔ Fat-free mass
Gotshalk et al. (2008)	Female (63 years) CR: 15; PL: 12	CR: 0.3 g kg^{-1}, 7 days	↑ Fat-free mass, strength
Gotshalk et al. (2002)	Male (65 years) CR: 10; PL: 8	CR: 0.3 g kg^{-1}, 7 days	↑ Fat-free mass
Jakobi et al. (2001)	Male (72 years) CR: 7; PL: 5	CR: 20 g, 5 days	↔ Force production
Rawson et al. (1999)	Male (74 years) CR: 10; PL: 10	CR: 20 g, 10 days Maintenance: 4 g, 20 days	↔ Fat-free mass
Rawson and Clarkson, (2000)	Male (69–78 years)	CR: 20 g day^{-1}, 5 days	↔ Isometric/isokinetic strength
Tarnopolsky et al. (2007)	Male/female (70 year) CR: 21, PL: 18	CR: 5 g day^{-1}, 6 months	↑ Fat-free mass, strength
Wiroth et al. (2001)	Male (70 years) CR: 7, PL: 7	CR: 15 g day^{-1}, 5 days	↑ Cycling power and work performed

CR, creatine;, PL, placebo.

thereafter. However, in two recent studies by the author using a creatine side effects questionnaire, creatine supplementation during 10–12 weeks of resistance exercise resulted in no adverse effects (Candow et al., unpublished findings, 2008). It is important to note that creatine supplementation only occurred on training days in these two studies.

Initial weight gain from creatine supplementation may be the result of enhanced intramuscular creatine stores (Francaux and Poortmans, 1999), as creatine has the ability to regulate osmosis within the myocyte and could potentially elevate intracellular osmolarity (i.e., water retention). Speculation exists, that is, a rapid increase in cellular hydration, could lead to muscle cramping and muscle strains over time. However, in our most recent study, creatine supplementation only on resistance exercise training days (three times per week) had no effect on body mass (Candow et al., unpublished findings). Previously, in older men (59–72 years of age) who supplemented with creatine 7 days), no reports of muscle cramping were observed (Gotshalk et al., 2002).

Creatine is a nitrogen-containing amine compound that is readily converted to creatinine and excreted through the kidneys (Francaux and Poortmans, 1999). An increase in dietary creatine may cause unwanted stress to kidney function. However, Poortmans and Francaux (1999) showed that creatine supplementation (10 months to 5 years) at various doses (1–80 g day^{-1}) had no effect on plasma albumin, urinary creatinine, or urea. It has been recently shown that creatine supplementation had no effect on kidney function in healthy older adults, as assessed by urinary microalbumin (Candow et al., unpublished findings).

4. SUMMARY

Resistance exercise is a simple and an effective strategy to maintain or increase muscle mass and strength with aging, which may lead to a greater quality of life. In addition to resistance exercise, nutrition is another important variable that has a direct impact on aging muscle biology. The ingestion of creatine monohydrate, combined with resistance exercise, has a greater impact on aging muscle and strength over resistance exercise alone. Recent evidence suggests that ingesting creatine in close proximity to resistance exercise (before and after) has a positive effect on muscle mass and strength. Future research should continue to determine the mechanistic actions of how creatine influences muscle protein kinetics, especially in aging adults.

REFERENCES

Balsom, P.D., Soderlund, K., Sjodin, B., 1995. Skeletal muscle metabolism during short duration high-intensity exercise: influence of creatine supplementation. Acta Physiologica Scandinavica 154, 303–310.
Bautmans, I., Njemini, R., Vasseur, S., et al., 2005. Biochemical changes in response to intensive resistance exercise training in the elderly. Gerontology 51, 253–265.

Bemben, M.G., Witten, M.S., Carter, J.M., Eliot, K.A., Knehans, A.W., Bemben, D.A., 2010. The effects of supplementation with creatine and protein on muscle strength following a traditional resistance training program in middle-aged and older men. The Journal of Nutrition, Health & Aging 14 (2), 155–159.

Bermon, S., Venembre, P., Sachet, C., Valour, S., Dolisi, C., 1998. Effect of creatine monohydrate ingestion in sedentary and weight-trained older adults. Acta Physiologica Scandinavica 164, 147–155.

Booth, F.W., Gordon, S.E., Carlson, C.J., Hamilton, M.T., 2000. Waging war on modern chronic diseases: primary prevention through exercise biology. Journal of Applied Physiology 273, 774–787.

Brack, A.S., Rando, T.A., 2007. Intrinsic changes and extrinsic influences of myogenic stem cell function during aging. Stem Cell Reviews 3, 226–237.

Brose, A., Parise, G., Tarnopolsky, M.A., 2003. Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. Journal of Gerontology: Biological and Medical Sciences 58, 11–19.

Burke, D.G., Candow, D.G., Chilibeck, P.D., et al., 2008. Effect of creatine supplementation and resistance-exercise training on muscle insulin-like growth factor in young adults. International Journal of Sport Nutrition and Exercise Metabolism 18, 389–398.

Campbell, W.W., Barton Jr., M.L., Cyr-Campbell, D., et al., 1999. Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. American Journal of Clinical Nutrition 70 (6), 1032–1039.

Candow, D.G., Chilibeck, P.D., 2008. Timing of creatine or protein supplementation and resistance training in the elderly. Applied Physiology, Nutrition and Metabolism 33, 184–190.

Candow, D.G., Chilibeck, P.D., 2010. Review: potential effect of creatine supplementation for improving properties of aging bone health. The Journal of Nutrition, Health & Aging 14 (2), 149–153.

Candow, D.G., McLeod, K.M., Zello, G.A., et al., (unpublished findings). Creatine application strategies during resistance-training in healthy older adults.

Candow, D.G., Little, J.P., Chilibeck, P.D., et al., 2008. Low-dose creatine combined with protein during resistance training in older men. Medicine and Science in Sports and Exercise 40, 1645–1652.

Chrusch, M.J., Chilibeck, P.D., Chad, K.E., Davison, K.S., Burke, D.G., 2001. Creatine supplementation combined with resistance training in older men. Medicine and Science in Sports and Exercise 33, 2111–2117.

Cribb, P.J., Hayes, A., 2006. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. Medicine and Science in Sports and Exercise 38, 1918–1925.

Eijnde, B.O., Van Leemputte, M., Goris, M., et al., 2003. Effects of creatine supplementation and exercise training on fitness in males 55 to 75 years old. Journal of Applied Physiology 95, 818–828.

Evans, W.J., 1995. What is sarcopenia? Journal of Gerontology: Biological and Medical Sciences 50, 5–8.

Francaux, M., Poortmans, J.R., 1999. Effects of training and creatine supplement on muscle strength and body mass. European Journal of Applied Physiology 80, 165–168.

Gotshalk, L.A., Volek, J.S., Staron, R.S., Denegar, C.R., Hagerman, F.C., Kraemer, W.J., 2002. Creatine supplementation improves muscular performance in older men. Medicine and Science in Sports and Exercise 34, 537–543.

Gotshalk, L.A., Kraemer, W.J., Mendonca, M.A., et al., 2008. Creatine supplementation improves muscular performance in older women. European Journal of Applied Physiology 102, 223–231.

Greenhaff, P.D., Bodin, K., Soderland, K., Hultman, E., 1994. Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. American Journal of Physiology 266, 725–730.

Harris, R.C., Soderland, K., Hultman, E., 1992. Elevation of creatine in resting and exercise muscle of normal subjects by creatine supplementation. Clinical Sciences 83, 367–374.

Hepple, R.T., 2003. Sarcopenia-a critical perspective. Science of Aging Knowledge Environment 46, pe31.

Jakobi, J.M., Rice, C.L., Curtin, S.V., Marsh, G.D., 2001. Neuromuscular properties in older men following acute creatine supplementation. European Journal of Applied Physiology 84, 312–328.

Johnston, A.P., De Lisio, M., Parise, G., 2008. Resistance training, sarcopenia, and the mitochondrial theory of aging. Applied Physiology, Nutrition and Metabolism 33, 191–199.

Larsson, L., Yu, F., Hook, P., Ramamurthy, B., Marx, J.O., Pircher, P., 2001. Effects of aging on regulation of muscle contraction at the motor unit, muscle cell, and molecular levels. International Journal of Sport Nutrition and Exercise Metabolism 11, 28–43.

Marx, J.O., Kraemer, W.J., Kolluri, R., Pircher, P., Larsson, L., 2002. The effects of aging on myosin heavy chain concentration in skeletal muscle fibers of young and old men. Journal of Gerontology: Biological and Medical Sciences 57, 232–238.

Moller, P., Bergstrom, J., Furst, P., Hellstrom, K., 1980. Effect of aging on energy-rich phosphagens in human skeletal muscles. Clinical Science (London) 58 (6), 553–555.

Olsen, S., Aagaard, P., Kadi, F., et al., 2006. Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. The Journal of Physiology 1, 525–534.

Parise, G., Mihic, S., MacLennan, D., Yarasheski, K.E., Tarnopolsky, M.A., 2001. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. Journal of Applied Physiology 91, 1041–1047.

Persky, A.M., Brazeau, G.A., 2001. Clinical pharmacology of the dietary supplement creatine monohydrate. Pharmacological Reviews 53, 161–176.

Persky, A.M., Rawson, E.S., 2007. Safety of creatine supplementation. Subcellular Biochemistry 46, 275–289.

Phillips, S.M., 2004. Protein requirements and supplementation in strength sports. Journal of Nutrition 20, 689–695.

Poortmans, J.R., Francaux, M., 1999. Long-term oral creatine supplementation does not impair renal function in healthy athletes. Medicine and Science in Sports and Exercise 31, 1108–1110.

Rawson, E.S., Clarkson, P.M., 2000. Acute creatine supplementation in older men. International Journal of Sport Nutrition and Exercise Metabolism 21, 71–75.

Rawson, E.S., Wehnert, M.L., Clarkson, P.M., 1999. Effects of 30 days of creatine ingestion in older men. European Journal of Applied Physiology 80, 139–144.

Robinson, T.M., Sewell, D.A., Hultman, E., Greenhaff, P.L., 1999. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. Journal of Applied Physiology 87, 598–604.

Tarnopolsky, M., Zimmer, A., Paikin, J., et al., 2007. Creatine monohydrate and conjugated linoleic acid improve strength and body composition following resistance exercise in older adults. PLoS One 2 (10), e991.

Thompson, L.V., 2009. Age-related muscle dysfunction. Experimental Gerontology 44, 106–111.

Trappe, S., 2001. Master athletes. International Journal of Sport Nutrition and Exercise Metabolism 11, 196–207.

Verdijk, L.B., Koopman, R., Schaart, G., Meijer, K., Savelberg, H.H., van Loon, L.J., 2007. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. American Journal of Physiology, Endocrinology and Metabolism 292, E151–E157.

Willoughby, D.S., Rosene, J.M., 2003. Effects of oral creatine and resistance training on myogenic regulatory factor expression. Medicine and Science in Sports and Exercise 35, 923–929.

Wiroth, J.B., Bermon, S., Andreï, S., Dalloz, E., Hébuterne, X., Dolisi, C., 2001. Effects of oral creatine supplementation on maximal pedaling performance in older adults. European Journal of Applied Physiology 84 (6), 533–539.

Wyss, M., Kaddurah-Daouk, R., 2000. Creatine and creatinine metabolism. Physiology Reviews 80, 1107–1213.

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Exercise in the Maintenance of Muscle Mass: Effects of Exercise Training on Skeletal Muscle Apoptosis

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ABBREVIATIONS

AIF Apoptosis inducing factor
AP-1 Activating protein-1
Apaf-1 Apoptosis protease activating factor-1
ARC Apoptosis repressor with caspase-associated recruitment domain
Bad Bcl-2 antagonist of apoptosis
Bak Bcl-2 homologous antagonist/killer
Bax Bcl-2 associated protein X
Bcl-2 B-cell lymphoma-2
Bcl-X$_L$ B-cell lymphoma X long isoform
Bid BH3 interacting domain death agonist
Bok Bcl-2 related ovarian killer
CES Chronic electrical stimulation
cFLIP FADD-like interleukin-1 beta converting enzyme inhibitory protein
cIAP-1,2 Cellular inhibitor of apoptosis protein-1,2
dATP Deoxyadenosine triphosphate
endoG Endonuclease G
FADD Fas associated death domain
IκBα IkappaB-alpha
IL-1, 6, 15 Interleukin-1, 6, 15
MOMP Mitochondrial outer membrane permeabilization
NF-κB Nuclear factor-kappa B
Omi/HtrA2 High temperature requirement A2
Puma p53-upregulated modulator of apoptosis
RIP1 Receptor interacting protein-1
Smac/Diablo Second mitochondrial activator of caspases/direct IAP binding protein with low pI
TNFR1, 2 Tumor necrosis factor receptor 1,2
TNF-α Tumor necrosis factor alpha
TRADD TNF receptor associated death domain
TRAF-2 TNF receptor associated factor-2
XIAP X-linked inhibitor of apoptosis protein

1. INTRODUCTION

Sarcopenia, the loss of muscle mass and function, is a well-known aspect of the aging process. It is estimated that the loss of muscle mass in humans during aging equates to $\sim 40\%$ between the ages of 20 and 80 (Lexell et al., 1988). With the rising elderly population, sarcopenia is quite prevalent with 45% of the elderly US population having moderate to severe sarcopenia (Janssen et al., 2004). The loss in mass is due to both a decrease in fiber number as well as the cross-sectional area of existing fibers. Potential mechanisms contributing to the loss of muscle mass include mitochondrial dysfunction, activation of proteolytic pathways, which may be in response to oxidative stress, hormonal adaptations, and loss in neurological innervations, all of which have been shown to activate apoptotic pathways in various cell types. Hence, apoptosis had been hypothesized and then shown to play a role in the pathogenesis of sarcopenia (Baker and Hepple, 2006; Dirks and Leeuwenburgh, 2002, 2004; Pistilli et al., 2006). Furthermore, there is a strong inverse correlation between the amount of apoptosis in aged muscle and the muscle weight, suggesting that apoptosis plays an important role in sarcopenia (Baker and Hepple, 2006; Marzetti et al., 2009; Pistilli et al., 2006). Apoptosis has classically been defined as programmed cell death or cell suicide and, therefore, theorized to contribute to the loss in fiber number. However, recent research has shown that activation of apoptotic pathways is involved in myofibrillar protein degradation and plays a role in atrophy of muscle fibers (Du et al., 2004), likely in the absence of fiber death. Thus, it is likely that activation of apoptotic pathways during aging contributes to both the loss in fibers as well as atrophy of the remaining fibers.

Due to the consequences of sarcopenia, including increased risk of immobility, disability, and mortality (Janssen et al., 2004; Melton et al., 2000; Rantanen et al., 1999), preventive measures are of importance. Preventative strategies include nutritional measures, hormonal interventions, and exercise training. Both aerobic exercise training and resistance training are known to be beneficial for muscle function and overall health. Due to the important role of apoptosis in sarcopenia, the effects of exercise training on this topic are of interest. Very little has been published on the effects of resistance training on skeletal muscle apoptosis; therefore, this chapter will focus on the beneficial effects of aerobic exercise training on skeletal muscle apoptosis.

2. MECHANISMS OF APOPTOSIS

Apoptosis is executed by specific cellular signaling pathways and is, therefore, characterized by specific biochemical and morphological events. Some of these identifying features of apoptosis include chromatin condensation, DNA fragmentation into mono- and oligonucleosomes, cellular shrinkage, and membrane blebbing forming apoptotic bodies, which are engulfed by macrophages or neighboring cells. The two major pathways extensively described include the intrinsic (mitochondrion-mediated) and extrinsic (receptor-mediated) apoptotic signaling (see Figure 12.1).
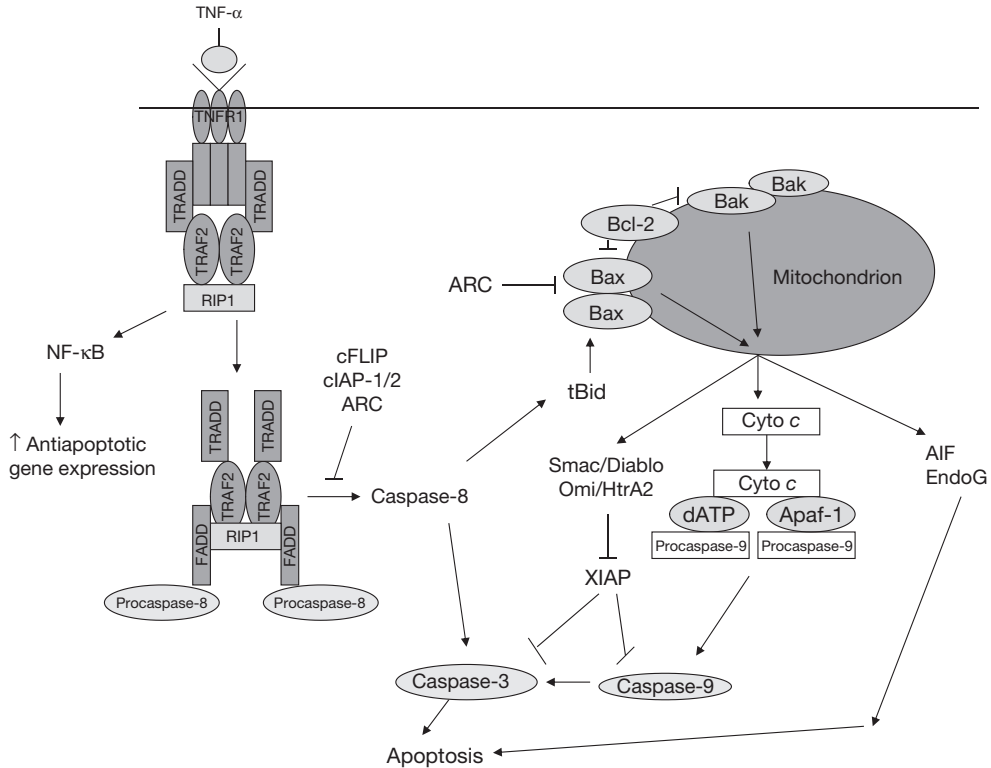
Figure 12.1 Mitochondrial- and receptor-mediated signaling (see text for description).

2.1 Mitochondrion-Mediated Signaling

Mitochondria play a central role in initiating apoptosis. Upon stimulation, mitochondria can release cytochrome c and other proapoptotic proteins such as Smac/DIABLO (second mitochondria–derived activator/direct IAP-binding protein with low pI), Omi/HtrA2 (high temperature requirement A2), apoptosis inducing factor (AIF), and endoG (endonuclease G) into the cytosol via mitochondrial outer membrane permeabilization (MOMP) (Chipuk and Green, 2008). Once released, cytochrome c forms a complex, known as the apoptosome, with procaspase-9, Apaf-1 (apoptotic protease-activating factor-1), and deoxyadenosine triphosphate (dATP) (Li et al., 1997). Formation of the apoptosome allows for close proximity of procaspase-9 and, therefore, dimerization. Once dimerized, procaspase-9 molecules are cleaved to form the more stable enzyme, caspase-9. The active enzyme can cleave and activate effector caspases, such as procaspase-3, which leads to the typical morphological features of apoptosis. This process is highly regulated at a number of levels. First, MOMP is regulated by the Bcl-2 (B-cell lymphoma/leukemia-2) family of proteins. This family consists of a number of

proteins, which are antiapoptotic or pro-apoptotic. For example, Bcl–2 and Bcl–X$_L$ (Bcl–2 related gene, long isoform) protect against MOMP while Bax (Bcl–2 associated x protein), Bak (Bcl–2 antagonist killer 1), Bad (Bcl–2 antagonist of cell death), Bid (BH3 interacting domain death agonist), Bok (Bcl–2 related ovarian killer), Noxa, and Puma (p53–upregulated modulator of apoptosis) favor MOMP. It is thought that Bax and Bak can homo–oligomerize and form a pore in the outer mitochondrial membrane to promote MOMP (Chipuk et al., 2010). The remaining members of the Bcl–2 family regulate this process by complex protein interactions with each other and possibly with non–Bcl–2 family proteins (Chipuk et al., 2010). A second level of regulation involves the inhibition of caspases by members of the inhibitor of apoptosis protein (IAP) family. Specifically, X chromosome-linked IAP (XIAP) can bind to caspase-9 and –3 to inhibit their enzyme activity (Silke et al., 2002). Lastly, the mitochondria can release Smac/Diablo and Omi/HtrA2 via MOMP, along with cytochrome *c*, to relieve the inhibition exerted by XIAP, so apoptosis can be executed (Suzuki et al., 2001; Vaux and Silke, 2003).

Mitochondria can also release pro–apoptotic proteins, AIF, and endoG, which can function independently from the caspase cascade. Upon MOMP, both of these proteins participate in apoptosis by translocating to the nucleus to induce chromatin condensation and large-scale DNA fragmentation in a caspase–independent manner (Li et al., 2001; Susin et al., 2000). Caspases can be activated simultaneously but are not required for large-scale DNA fragmentation and nuclear apoptosis induced by AIF and endoG (Li et al., 2001; Susin et al., 2000).

2.2 Receptor-Mediated Signaling

Cytokines can induce apoptosis in some cell types via their interaction with specific receptors of the tumor necrosis factor receptor (TNFR) superfamily. Tumor necrosis factor alpha (TNF-α) is a cytokine that can elicit a broad spectrum of responses and is the most studied cytokine relating to skeletal muscle atrophy and apoptosis. Exposure of cells to TNF-α most commonly causes activation of nuclear factor-kappa B (NF-κB) and activating protein–1 (AP-1) resulting in the expression of genes involved in cell survival and acute and chronic inflammatory responses (Baud and Karin, 2001). However, TNF-α is capable of inducing apoptosis. TNF-α signals via two membrane receptors: TNFR1 and TNFR2. These receptors are homologous in their extracellular domains but are structurally different in their cytoplasmic domains. TNFR1 contains a death domain, whereas TNFR2 does not. TNFR1 mediates signaling for both apoptosis and cell survival while TNFR2 mostly transduces signals favoring cell survival. Ligand binding to TNFR1 can induce apoptosis in an effector cell via the activation of procaspase-8, which cleaves and activates effector caspases, such as procaspase-3, initiating cellular destruction (Micheau and Tschopp, 2003). Alternatively, binding of TNF-α to TNFR1 can induce an antiapoptotic response mediated through the transcription factor NF-κB (Micheau and

Tschopp, 2003). Evidence suggests that cytokines can increase the levels of antiapoptotic proteins. It was shown that the stimulation of NF-κB and its subsequent transcriptional activity determines the cells' fate (Micheau and Tschopp, 2003). Specifically, cells with suppressed NF-κB signals undergo TNF-α–induced apoptosis due to low expression levels of antiapoptotic proteins. Binding of TNF-α to TNFR1 leads to recruitment of adaptor proteins to the cytoplasmic domain of TNFR1. Adaptor proteins include TNFR associated death domain (TRADD), TNFR associated factor-2 (TRAF2), receptor interacting protein-1 (RIP1), and fas associated death domain (FADD). To activate NF-κB, thereby promoting cell survival, TRADD associates with TNFR1 and is able to recruit RIP1 and TRAF2 to form a TNFR1-TRADD-RIP1-TRAF2 complex. This leads to activation of NF-κB, with consequent expression of inflammatory and antiapoptotic proteins. However, under conditions where NF-κB is suppressed, TRADD, TRAF2, and RIP1 dissociate from TNFR1 to translocate to the cytosol where they recruit FADD and procaspase-8, which leads to apoptosis (Micheau and Tschopp, 2003). Apoptosis mediated via TNFR1 can be inhibited by cFLIP (cellular FADD-like interleukin-1β–converting enzyme inhibitory protein) and cellular IAP 1 and 2 (cIAP-1 and cIAP-2), which interfere with the activation of procaspase-8.

Once apoptosis is initiated via activation of procaspase-8, the activation of the mitochondrion-mediated signaling may occur but is downstream from caspase-8 activation. Active caspase-8 activates tBid, which leads to stimulation of Bax and Bak (Chipuk et al., 2010). Some cell types require activation of the mitochondrion-mediated signaling via tBid to execute apoptosis and others do not, depending on their expression of XIAP.

Apoptosis repressor with CARD domain (ARC) is an antiapoptotic protein that inhibits both mitochondrial-mediated and receptor-mediated apoptosis. ARC has been shown to interact with caspase-2 and -8 and also interacts with Bax inhibiting cytochrome c release (Gustafsson et al., 2004; Koseki et al., 1998).

In summary, mitochondria play a central role in the execution of apoptosis. Mitochondria can release proteins that function to activate the caspase cascade or proteins that can directly induce chromatin condensation and DNA fragmentation in a caspase-independent manner. Apoptosis can also be induced in response to TNF-α via TNFR1 and caspase-8 activation, particularly under conditions where NF-κB is suppressed. Both the mitochondrial- and receptor-mediated pathways are regulated at multiple levels in order to maintain precise control over cell death and survival when challenged by a changing environment such as occurs *in vivo* with aging and exercise training.

3. EFFECTS OF AEROBIC EXERCISE TRAINING ON SKELETAL MUSCLE APOPTOSIS

Aerobic exercise training results in many beneficial adaptations, including adaptations in apoptotic signaling leading to a greater resistance to apoptosis. Studies have shown that

the rate of apoptosis decreases in response to exercise training (Marzetti et al., 2008; Song et al., 2006). For example, it has been shown that white gastrocnemius from 24-month old rats subjected to 12 weeks of treadmill exercise had significantly lower levels of cleaved caspase-3 and mono- and oligo-nucleosomes, compared to age-matched controls and similar to their young (3-month) counterparts (Song et al., 2006). Likewise, 4 weeks of treadmill training in 28-month-old rats was able to bring back levels of cleaved caspase-3 and mono- and oligo-nucleosomes in the extensor digitorum longus (EDL) to youthful levels (Marzetti et al., 2008). In the soleus, there was no age or exercise effect on the apoptotic index or the levels of cleaved caspase-3 (Marzetti et al., 2008). Thus, it appears that exercise training protects against apoptosis in apoptotic–prone aging muscle, as shown in the muscles with primarily type II muscle fibers such as the white gastrocnemius and EDL. However, there does not seem to be a significant effect of exercise training on the basal levels of apoptosis in muscle from young animals, which are not apoptotic prone (Marzetti et al., 2008; Siu et al., 2004; Song et al., 2006).

Exercise training results in decreased basal levels of apoptosis and/or increased resistance to apoptosis in response to a stimulus in skeletal muscle likely due to adaptations in both the mitochondrial- and receptor-mediated signaling pathways. Exercise-induced adaptations in each pathway are discussed in the next section.

3.1 Adaptations in the Mitochondrial-Mediated Apoptotic Signaling Pathway in Response to Exercise Training

Exercise training causes adaptations in several proteins regulating the mitochondrial-mediated pathway. First, exercise training leads to adaptations in proteins that regulate cytochrome c release in both aged and young skeletal muscle. Exercise training alters the Bax/Bcl-2 ratio in aging muscle. In both, the white gastrocnemius and the soleus of aged animals, the Bax/Bcl-2 ratio increases compared to young counterparts (Song et al., 2006). Twelve weeks of treadmill training reversed the age-related increase in both muscles (Song et al., 2006). Exercise training may also lead to adaptations in young muscle that result in increased resistance to cytochrome c release in response to an apoptotic stimulus. It was shown that 8 weeks of treadmill training in 3-month-old rats resulted in a decrease in the Bax/Bcl-2 ratio in the soleus muscle due to increased protein levels of Bcl-2 (Siu et al., 2004). Seven days of chronic electrical stimulation (CES) did not have an effect on the Bax/Bcl-2 ratio in the tibialis anterior (Adhihetty et al., 2007). However, despite having no effect on the Bax/Bcl-2 ratio, CES did increase the resistance to cytochrome c release in response to H_2O_2 in isolated mitochondria (Adhihetty et al., 2007). It was shown that H_2O_2-induced cytochrome c release in isolated intermyofibrillar mitochondria was reduced when taken from muscle that was subjected to CES compared to muscle that was unstimulated. However, CES did not alter the cytochrome c response in isolated subsarcolemmal mitochondria (Adhihetty et al., 2007). The resistance to stimulated cytochrome c release may be due to the CES–induced upregulation of

ARC that was also shown to occur (Adhihetty et al., 2007). Treadmill training also was shown to upregulate ARC (Siu et al., 2005a). Thus, an exercise-training stimulus results in adaptations that likely increase the resistance to cytochrome c release by the mitochondria.

Secondly, exercise training affects regulatory proteins downstream of cytochrome c release. Protein levels of Apaf-1 and XIAP have been shown to be affected by exercise. Eight weeks of treadmill training of young animals led to an increased expression of XIAP and decreased expression of Apaf-1 in the soleus muscle (Siu et al., 2004, 2005a). Both of these exercise–induced adaptations would seemingly increase the resistance to apoptosis. Reduced levels of Apaf-1 would compromise formation of the apoptosome and activation of caspase-9, while increased levels of XIAP would inhibit activity of caspase-9 and –3. It has been shown that aging muscle is associated with increased protein levels of both Apaf-1 and XIAP (Chung and Ng, 2006; Dirks and Leeuwenburgh, 2004; Siu et al., 2005b). However, the effects of exercise training in aged muscle on the expression of these proteins are currently unknown.

Mitochondrial release of AIF and EndoG induces chromatin condensation and DNA fragmentation in a caspase-independent manner. Eight weeks of treadmill training had no effect on AIF expression in the soleus of young animals (Siu et al., 2004). However, 7 days of CES led to an increase in the expression of AIF in the tibialis anterior of young animals (Adhihetty et al., 2007). Since AIF is proapoptotic, it may be surprising that CES would stimulate AIF expression. However, reports have identified AIF as an important regulator of oxidative phosphorylation and energy homeostasis in skeletal muscle and may also have antioxidant properties (Cande et al., 2004; Joza et al., 2005; Vahsen et al., 2004). AIF mutant mice show significant skeletal muscle atrophy and weakness associated with severe defect in complex I of the respiratory chain, increased levels of oxidative stress, and lactic acidemia (Joza et al., 2005). Therefore, the CES–induced AIF expression may be due to its role in energy production in response to an exercise stimulus. These studies suggest that the effects of an exercise-training stimulus on the expression of AIF may be mode specific and/or muscle-type specific. It was reported that 4 weeks of treadmill training of young or old animals did not alter cytosolic levels of AIF or EndoG in the EDL or soleus (Marzetti et al., 2008). H_2O_2-stimulated AIF release from subsarcolemmal and intermyofibrillar mitochondria was also studied. Seven days of CES increased the susceptibility for H_2O_2-stimulated AIF release in subsarcolemmal mitochondria but had no effect in intermyofibrillar mitochondria (Adhihetty et al., 2007). AIF release from mitochondria has been shown to be dependent upon cleavage from the inner mitochondrial membrane via calpain to enable release into the cytosol (Polster et al., 2005). CES was shown to increase calpain levels by twofold in subsarcolemmal mitochondria, although it did not reach statistical significance ($p = 0.07$). In contrast, CES had no effect on calpain expression in intermyofibrillar mitochondria (Adhihetty et al., 2007). Hence, the authors suggest that the exercise-induced

susceptibility to AIF release from subsarcolemmal mitochondria may be calpain-dependent (Adhihetty et al., 2007). The rationale behind the CES-induced adaptations leading to enhanced AIF release is currently unknown. In summary, exercise training does not appear to affect cytosolic levels of EndoG in young or old animals. The effects of exercise training on the stimulated AIF release from mitochondria may be population-dependent, increasing susceptibility in the subsarcolemmal population. Furthermore, the expression of AIF in response to exercise training may be dependent upon mode of exercise and/or may be a muscle-specific effect.

3.2 Adaptations in the Receptor-Mediated Apoptotic Signaling Pathway in Response to Exercise Training

Exercise training modulates many aspects of receptor-mediated apoptotic signaling. Aging has been associated with elevated plasma and/or skeletal muscle levels of TNF-α (Bruunsgaard et al., 1999; Marzetti et al., 2009; Phillips and Leeuwenburgh, 2005) and has been associated with lower muscle mass and strength (Visser et al., 2002). Exercise training has been utilized as a possible means of modulating plasma and/or skeletal muscle TNF-α levels (Bruunsgaard, 2005; Lambert et al., 2008; Lira et al., 2009). Individuals self-reporting a high level of physical activity have lower plasma TNF-α levels than sedentary individuals (Bruunsgaard, 2005). Twelve weeks of exercise training decreased skeletal muscle TNF-α gene expression in obese elderly persons (Lambert et al., 2008). Eight weeks of treadmill training reduced TNF-α gene expression in the soleus and EDL of young rats (Lira et al., 2009). Hence, exercise training appears to reduce levels of plasma and skeletal muscle TNF-α gene expression.

Skeletal muscle TNFR1 expression levels are altered by age and exercise. Aging increases TNFR1 in old EDL but not soleus (Marzetti et al., 2008). The increased TNFR1 expression in aged EDL may result in the preferential sarcopenia of this fast muscle compared to the slow soleus. The elevated TNFR1 expression levels are not fixed, as exercise training restores TNFR1 expression to youthful levels in the aged EDL (Marzetti et al., 2008).

TNFR1 activation can result in the assembly of the TRADD-TRAF2-RIP1-FADD death inducing signaling complex and subsequent caspase-8 activation. The effects of exercise training on the expression of TRADD, TRAF2, RIP1, and FADD are currently unknown; however, it was shown that aging and exercise affect caspase-8 activation. Indeed, in aged fast muscle, EDL, cleaved caspase-8 levels are elevated, but not in the slow soleus muscle (Marzetti et al., 2008). Exercise training restores cleaved caspase-8 levels to that found in EDL of young animals (Marzetti et al., 2008). cFLIP inhibits recruitment of procaspase-8 to the death-inducing signaling complex, thereby inhibiting apoptosis. Siu et al. (2005a) found that exercise training does not alter cFLIP protein expression in young slow soleus. It has been shown that aging also does not alter the cFLIP expression

(Marzetti et al., 2009; Siu et al., 2005b). The effects of exercise on other inhibitors of caspase-8, such as cIAP-1/2, are unknown.

As discussed previously, activation of TNFR1 leads to caspase-8 cleavage, usually under conditions where NF-κB is suppressed. Aging and exercise training affect activation of NF-κB and some of its known regulators. The effects of aging on NF-κB DNA binding activity has been shown to be muscle specific, preferentially affecting muscles with predominantly white fast fibers (Phillips and Leeuwenburgh, 2005; Song et al., 2006). It was shown that aging decreased DNA binding activity in the white gastrocnemius and superficial vastus lateralis, while there was no effect in the soleus (Phillips and Leeuwenburgh, 2005; Song et al., 2006). Treadmill training was shown to reverse these effects of aging on DNA binding activity in the white gastrocnemius back to youthful levels (Song et al., 2006). The effects of aging and exercise training on NF-κB DNA binding activity may be due to altered regulation of IkappaB alpha (IκBα) with age and exercise. IκBα is an inhibitor, which holds NF-κB inactive. Upon stimulation, IκBα is phosphorylated and degraded, which leads to translocation of NF-κB to the nucleus and transcription of antiapoptotic genes. It was shown that IκBα phosphorylation in the gastrocnemius decreased with age, which is consistent with decreased activation of NF-κB (Song et al., 2006). Exercise training resulted in increased levels of phosphorylated IκBα (Song et al., 2006). In contrast, human vastus lateralis muscle contained elevated levels of phosphorylated IκBα compared to younger counterparts (Buford et al.). Further, older physically active men had lower levels of phosphorylated IκBα than sedentary aged-matched counterparts (Buford et al., 2010). The discrepancy could be due to differences between human and rodent muscle and/or due to external factors difficult to control in human studies. In summary, aged fast-type muscle (rodent) is associated with decreased phosphorylation of IκBα and NF-κB DNA binding activity, which may be a contributing factor to elevated levels of caspase-8 cleavage and apoptosis. Exercise training reverses these effects leading to increased resistance to apoptosis, likely due to upregulation of antiapoptotic proteins via NF-κB.

4. CONCLUSION

Aerobic training increases resistance against apoptosis in aging skeletal muscle. It appears that exercise training leads to a decreased level of apoptosis in aging muscle, particularly fast-type muscle, and induces protective adaptations in regulatory proteins in both the mitochondrial- and receptor-mediated signaling pathways. More is known about the effects of aging and exercise training on the mitochondrial-mediated pathway. It has been shown that exercise training leads to a decrease in the Bax/Bcl-2 ratio and an increase in ARC expression and also increases the resistance to stimulated cytochrome *c* release from mitochondria. Exercise training can also increase the expression of XIAP and decrease the expression of Apaf-1. Exercise training does not appear to affect basal levels of cytosolic AIF

or EndoG; however, it may affect stimulated release of AIF from mitochondria. Furthermore, there is some evidence that an exercise stimulus (CES) may increase the expression of AIF.

Aging and exercise training affect the receptor-mediated pathway. Aging skeletal muscle has been associated with elevated gene expression of TNF-α and TNFR1, predominantly in fast-type muscle. Exercise training was shown to reverse these effects. Cleaved caspase-8 has also been shown to increase in aged fast-type muscle and attenuated by exercise training. Exercise training did not affect the expression of cFLIP. Phosphorylation of IκBα and NF-κB DNA binding activity has been shown to decrease in fast-type muscle with aging and was attenuated by exercise training. Thus, exercise training provides a beneficial effect in providing protection against apoptosis in apoptotic-prone muscle. Currently, it is not known if activation of these pathways during aging and prevention with exercise training affects preferentially fiber number or size. Future research will delineate the contribution to each of these catabolic processes underlying the pathogenesis of sarcopenia.

REFERENCES

Adhihetty, P.J., Ljubicic, V., Hood, D.A., 2007. Effect of chronic contractile activity on SS and IMF mitochondrial apoptotic susceptibility in skeletal muscle. American Journal of Physiology, Endocrinology and Metabolism 292, E748–E755.

Baker, D.J., Hepple, R.T., 2006. Elevated caspase and AIF gene expression correlate with progression of sarcopenia during aging in male F344BN rats. Experimental Gerontology 41, 1149–1156.

Baud, V., Karin, M., 2001. Signal transduction by tumor necrosis factor and its relatives. Trends in Cell Biology 11, 372–377.

Bruunsgaard, H., 2005. Physical activity and modulation of systemic low-level inflammation. Journal of Leukocyte Biology 78, 819–835.

Bruunsgaard, H., Andersen-Ranberg, K., Jeune, B., Pedersen, A.N., Skinhoj, P., Pedersen, B.K., 1999. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 54, M357–M364.

Buford, T.W., Cooke, M.B., Manini, T.M., Leeuwenburgh, C., Willoughby, D.S., 2010. Effects of age and sedentary lifestyle on skeletal muscle NF-kappaB signaling in men. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 65A, 532–537.

Cande, C., Vahsen, N., Metivier, D., et al., 2004. Regulation of cytoplasmic stress granules by apoptosis-inducing factor. Journal of Cell Science 117, 4461–4468.

Chipuk, J.E., Green, D.R., 2008. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends in Cell Biology 18, 157–164.

Chipuk, J.E., Moldoveanu, T., Llambi, F., Parsons, M.J., Green, D.R., 2010. The BCL-2 family reunion. Molecular Cell 37, 299–310.

Chung, L., Ng, Y.C., 2006. Age-related alterations in expression of apoptosis regulatory proteins and heat shock proteins in rat skeletal muscle. Biochimica et Biophysica Acta 1762, 103–109.

Dirks, A., Leeuwenburgh, C., 2002. Apoptosis in skeletal muscle with aging. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 282, R519–R527.

Dirks, A.J., Leeuwenburgh, C., 2004. Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12. Free Radical Biology & Medicine 36, 27–39.

Du, J., Wang, X., Miereles, C., et al., 2004. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. Journal of Clinical Investigation 113, 115–123.

Gustafsson, A.B., Tsai, J.G., Logue, S.E., Crow, M.T., Gottlieb, R.A., 2004. Apoptosis repressor with caspase recruitment domain protects against cell death by interfering with Bax activation. Journal of Biological Chemistry 279, 21233–21238.

Janssen, I., Baumgartner, R.N., Ross, R., Rosenberg, I.H., Roubenoff, R., 2004. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. American Journal of Epidemiology 159, 413–421.

Joza, N., Oudit, G.Y., Brown, D., et al., 2005. Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. Molecular and Cellular Biology 25, 10261–10272.

Koseki, T., Inohara, N., Chen, S., Nunez, G., 1998. ARC, an inhibitor of apoptosis expressed in skeletal muscle and heart that interacts selectively with caspases. Proceedings of the National Academy of Sciences of the United States of America 95, 5156–5160.

Lambert, C.P., Wright, N.R., Finck, B.N., Villareal, D.T., 2008. Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons. Journal of Applied Physiology 105, 473–478.

Lexell, J., Taylor, C.C., Sjostrom, M., 1988. What is the cause of the ageing atrophy? Total number, size, and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. Journal of Neurological Sciences 84, 275–294.

Li, L.Y., Luo, X., Wang, X., 2001. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412, 95–99.

Li, P., Nijhawan, D., Budihardjo, I., et al., 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91, 479–489.

Lira, F.S., Koyama, C.H., Yamashita, A.S., et al., 2009. Chronic exercise decreases cytokine production in healthy rat skeletal muscle. Cell Biochemistry and Function 27, 458–461.

Marzetti, E., Carter, C.S., Wohlgemuth, S.E., et al., 2009. Changes in IL-15 expression and death-receptor apoptotic signaling in rat gastrocnemius muscle with aging and life-long calorie restriction. Mechanisms of Ageing and Development 130, 272–280.

Marzetti, E., Groban, L., Wohlgemuth, S.E., et al., 2008. Effects of short-term GH supplementation and treadmill exercise training on physical performance and skeletal muscle apoptosis in old rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 294, R558–R567.

Melton III, L.J., Khosla, S., Crowson, C.S., O'connor, M.K., O'Fallon, W.M., Riggs, B.L., 2000. Epidemiology of sarcopenia. Journal of American Geriatrics Society 48, 625–630.

Micheau, O., Tschopp, J., 2003. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114, 181–190.

Phillips, T., Leeuwenburgh, C., 2005. Muscle fiber specific apoptosis and TNF-{alpha} signaling in sarcopenia are attenuated by life-long calorie restriction. The FASEB Journal 19, 668–670.

Pistilli, E.E., Jackson, J.R., Alway, S.E., 2006. Death receptor-associated pro-apoptotic signaling in aged skeletal muscle. Apoptosis 11, 2115–2126.

Polster, B.M., Basanez, G., Etxebarria, A., Hardwick, J.M., Nicholls, D.G., 2005. Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. Journal of Biological Chemistry 280, 6447–6454.

Rantanen, T., Guralnik, J.M., Foley, D., et al., 1999. Midlife hand grip strength as a predictor of old age disability. Journal of the American Medical Association 281, 558–560.

Silke, J., Hawkins, C.J., Ekert, P.G., et al., 2002. The anti-apoptotic activity of XIAP is retained upon mutation of both the caspase 3- and caspase 9-interacting sites. The Journal of Cell Biology 157, 115–124.

Siu, P.M., Bryner, R.W., Martyn, J.K., Alway, S.E., 2004. Apoptotic adaptations from exercise training in skeletal and cardiac muscles. The FASEB Journal 18 (10), 1150–1152.

Siu, P.M., Bryner, R.W., Murlasits, Z., Alway, S.E., 2005a. Response of XIAP, ARC, and FLIP apoptotic suppressors to 8 wk of treadmill running in rat heart and skeletal muscle. Journal of Applied Physiology 99, 204–209.

Siu, P.M., Pistilli, E.E., Alway, S.E., 2005b. Apoptotic responses to hindlimb suspension in gastrocnemius muscles from young adult and aged rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 289, R1015–R1026.

Song, W., Kwak, H.B., Lawler, J.M., 2006. Exercise training attenuates age-induced changes in apoptotic signaling in rat skeletal muscle. Antioxidants & Redox Signaling 8, 517–528.

Susin, S.A., Daugas, E., Ravagnan, L., et al., 2000. Two distinct pathways leading to nuclear apoptosis. The Journal of Experimental Medicine 192, 571–580.

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., Takahashi, R., 2001. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Molecular Cell 8, 613–621.

Vahsen, N., Cande, C., Briere, J.J., et al., 2004. AIF deficiency compromises oxidative phosphorylation. EMBO Journal 23, 4679–4689.

Vaux, D.L., Silke, J., 2003. Mammalian mitochondrial IAP binding proteins. Biochemical and Biophysical Research Communications 304, 499–504.

Visser, M., Pahor, M., Taaffe, D.R., et al., 2002. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 57, M326–M332.

Taurine and Longevity – Preventive Effect of Taurine on Metabolic Syndrome

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ABBREVIATIONS

AII Angiotensin II
CVD Cardiovascular diseases
DOCA Deoxycorticosterone acetate
HDL High density lipoproteins
HOCl Hypochlorous acid
ICAM-1 Intercellular adhesion molecule-1
LDL Low-density lipoproteins
MPO Myeloperoxidase
NAFLD Nonalcoholic fatty liver disease
NASH Nonalcoholic steatohepatitis
NF-κB Nuclear factor-κB
SHR Spontaneously hypertensive rat
STZ Streptozotocin
TauCl Taurine chloramine
VLDL Very low-density lipoproteins

1. INTRODUCTION

Taurine is a ubiquitous sulfur-containing amino acid present in most mammalian tissue and is involved in many important physiological functions (Huxtable, 1992). Unlike common amino acids, taurine is not incorporated into proteins and found in the free form. The intracellular level of taurine is regulated both by uptake of taurine through the taurine transporter and by endogenous synthesis from methionine and cysteine. Taurine body pool is regulated by renal reabsorption. When dietary intake of taurine or taurine synthesis is reduced, urinary taurine excretion declines due to increase in renal tubular reabsorption of taurine.

Fish and shellfish are rich in taurine and are a major source of protein for the Japanese people. The association between fish consumption and risk of cardiovascular diseases

(CVD) has been extensively studied. Worldwide epidemiological study indicated that taurine intakes estimated by 24-h urinary taurine excretion were inversely related to CVD mortality, particularly coronary heart disease mortality (Yamori et al., 1996, 2009). Thus, taurine in fish is the key nutrient responsible for CVD prevention and may contribute to the longevity of the Japanese because of the inverse association of the average life expectancy with CVD mortalities. Taurine is proven to protect against tissue damage, which is likely to be associated with prevention of a variety of diseases. Although the underlying mechanisms responsible for the various physiological and pharmacological effects of taurine are not clearly understood, its osmoregulatory, antioxidant, membrane–stabilizing, Ca^{2+} regulatory, and immunomodulating action seem to be involved (Huxtable, 1992).

The metabolic syndrome is a cluster of several cardio–metabolic risk factors, including abdominal obesity, hyperglycemia, dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and hypertension (NCEP-ATP guidelines, 2001). The prevalence of metabolic syndrome is increasing in current societies and the condition is now very common among the aging population. The individual components of metabolic syndrome and metabolic syndrome as a whole increase the risk of heart failure, CVD mortality, and all–cause mortality. Many studies in animal models and humans suggest that taurine prevents or ameliorates these components of metabolic syndrome.

This chapter will focus on possible role of taurine in pathogenesis of metabolic syndrome. In addition, immunomodulating effect of taurine as a mechanism responsible for beneficial role of taurine in the prevention of metabolic syndrome will also be discussed because low-grade systemic inflammation is known to be involved in developing obesity, insulin resistance, and CVD (Hotamisligil, 2006).

2. EFFECT OF TAURINE ON HYPERTENSION

Antihypertensive effect of taurine was first demonstrated in genetically hypertensive rats, spontaneously hypertensive rat (SHR), and stroke-prone SHR. Taurine supplementation suppressed the development of hypertension in SHR, concomitantly with the decrease in heart rate and plasma catecholamine levels (Nara et al., 1978). Taurine attenuated hypertension and salt intake induced by preoptically rennin injection in SHR (Abe et al., 1987). In deoxycorticosterone acetate (DOCA)-salt rats, dietary taurine reduced blood pressure and urinary adrenaline excretion, and increased urinary salt excretion (Fujita and Sato, 1986). Cerebroventricular administration of taurine had decreased blood pressure in SHR and DOCA-salt rats (Inoue et al., 1986). Antihypertensive effect of taurine was also noted in hypertension models induced by high-fructose (Anuradha and Balakrishnan, 1999), ethanol (Harada et al., 2000), and salt (Ideishi et al., 1994). In contrast to antihypertensive effect in hypertensive rats, taurine had no effect in normotensive rats such as Wistar-Kyoto rats. These studies suggest that antihypertensive

effect of taurine is mainly due to the suppression of sympathetic nerve activity (Fujita and Sato, 1986; Nara et al., 1978).

In spite of many studies in animals, there are only a few data available in humans. Six grams of taurine per day for 1 week significantly reduced systolic and diastolic blood pressure in a double blind, placebo-controlled trial of borderline hypertensive young patients (Fujita et al., 1987). Similar to animal experiments, taurine intake (6 g day^{-1}) decreased urinary norepinephrine excretion, but did not significantly lower blood pressure in young normotensive Japanese (Mizushima et al., 1996). Smaller amount of taurine, 3 g day^{-1}, decreased mildly elevated blood pressure significantly in 2 months in Tibetans living at the foot of Mt. Everest, whose 24-h urinary taurine excretion was nearly one-tenth of the Japanese because of their strict discipline not to eat fish at all (Yamori et al., 1996). Worldwide epidemiological study, WHO-CARDIAC (*Car*diovascular *Di*seases and *A*limentary *C*omparison) study revealed that greater means of 24-h urinary taurine excretion in worldwide populations were associated with significantly lower blood pressure and slower heart rates (Yamori et al., 2009). Moreover, individuals excreting enough amount of taurine in 24-h urine over the world average had significantly lower systolic and diastolic blood pressure, in average as well as lower CVD risks than those excreting less taurine below the average (Yamori et al., 2010b). In relation to salt-sensitive hypertension, in individuals excreting greater amount of salt in 24-h urine blood pressure in average was significantly higher in those with higher heart rate than with lower heat rate. Among those with higher heart rate, higher 24-h urinary taurine excretion was associated with lower blood pressure, indicating possible neural involvement of salt-sensitive hypertension (Yamori et al., 2010a).

Angiotensin II (AII) plays an important homeostatic role in blood pressure regulation, water and salt balance, and tissue growth control under physiologic conditions. Taurine has been shown to antagonize the action of AII. Taurine inhibits the actions of AII on Ca^{2+} transport, protein synthesis, cell growth, and apoptosis, and thereby ameliorates the adverse effects of AII including cardiac hypertrophy, volume overload, and myocardial remodeling (Schaffer et al., 2000). Conversely, taurine deficiency by taurine transport inhibitor, β-alanine, stimulates AII-induced cell apoptosis in neonatal cardiomyocytes.

Taurine has been reported to ameliorate the age-related decline of saline volume-induced diuresis and natriuresis in the unilaterally nephrectomized rats (Mozaffari and Schaffer, 2002). Antihypertensive effect of taurine may partially be attributed to taurine-induced natriuresis.

3. EFFECT OF TAURINE ON ATHEROSCLEROSIS

Antiatherosclerotic effect of taurine has been reported in various types of animal models, including mice (Kondo et al., 2001; Murakami et al., 1999a), rats (Murakami et al., 1996), rabbits (Murakami et al., 2002a), and quails (Murakami et al., 2010). In diet-induced

atherosclerotic models, taurine prevents the development of atherosclerosis, concomitantly with a marked reduction in plasma lipid levels. Taurine prevented the elevation of plasma low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) cholesterol and triglycerides in animals fed a high–cholesterol/high–fat diet (Murakami et al., 1996, 2010). Taurine increased the lowered plasma high density lipoproteins (HDL)-cholesterol. Amelioration on plasma lipid profiles is closely related to antiatherosclerotic effect of taurine, since hyperlipidemia is a major risk factor for the development of atherosclerosis. In contrast, antiatherosclerotic effect of taurine is not accompanied by the reduction in plasma lipid levels in genetic models of hyperlipidemia (Kondo et al., 2001; Murakami et al., 2002a). Taurine reduced oxidation products in the serum and aorta, suggesting the involvement of antioxidative effect of taurine in the suppression of atherosclerosis.

Endothelial cells play an important regulatory role in the circulation as a physical barrier and as a source of regulatory substances such as nitric oxide, endothelin-1, and prostacyclin. Dysfunction of these mechanisms for regulating vascular function therefore is closely involved in the pathophysiology of hypertension and atherosclerosis. It should be noted that the level of taurine in endothelial cells is much higher than other parts of vessels, suggesting some significant role of taurine in endothelial cells (Terauchi et al., 1998).

LDL, oxidized LDL, and high glucose induce expression of cell surface adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (ICAM-1), and damage endothelial cells leading to cell death. These changes can be prevented in the presence of taurine. Taurine protects against endothelial dysfunction induced by native LDL *in vivo*, or by oxidized LDL *in vitro* (Tan et al., 2007). Taurine prevents high-glucose-induced endothelial cell apoptosis (Wu et al., 1999). Taurine prevents hyperglycemia-induced ICAM-1 expression, endothelial cell apoptosis, along with prevention of cardiac dysfunction in rats (Casey et al., 2007). In addition, taurine also prevents lipopolysaccharide (LPS)-induced rolling and adhesion of leukocytes *in vivo* (Egan et al., 2001). Taurine administration rescues endothelial dysfunction including reduced vasodilatory response, increase leukocyte-endothelial cell interaction, upregulation of adhesion molecules, and lectin-like oxidized low-density lipoprotein receptor-1, in diabetic animal models (Wang et al., 2008). Beneficial effects of taurine on endothelial cell function were demonstrated also in smokers and type 1 diabetic subjects (Fennessy et al., 2003).

4. EFFECT OF TAURINE ON DYSLIPIDEMIA

An elevated LDL cholesterol level is a major risk factor for CVD, and several randomized clinical trials have shown that lowering LDL cholesterol levels results in a substantial reduced CVD morbidity and mortality.

Effect of taurine on cholesterol levels of plasma and liver has been extensively studied. Taurine supplementation improves high-cholesterol/high-fat diet-induced hyperlipidemia in rats (Murakami et al., 1996; Yamamoto et al., 2000; Yokogoshi et al., 1999), mice (Murakami et al., 1999b), hamsters (Murakami et al., 2002b), and quails (Murakami et al., 2010). Taurine decreases plasma levels of non-HDL cholesterol (LDL + VLDL cholesterol) and triglycerides in animals fed a high-cholesterol/high-fat diet. In contrast, the effect of taurine on plasma HDL-cholesterol depends on experimental conditions.

As taurine is involved in conjugation of bile acid, the stimulation of bile acid synthesis is postulated as the major mechanism underlying cholesterol-lowering action of taurine. Taurine stimulates hepatic bile acid production and increases fecal bile acid excretion. Cholesterol-lowering effect of taurine is accompanied by mRNA expression and enzymatic activity of cytochrome P450 7A1 (CYP7A1), a rate-limiting enzyme of bile acid synthesis (Murakami et al., 1996; Yokogoshi et al., 1999). Loss of bile acids from the enterohepatic circulation results in derepression of CYP7A1 activity and an increase in bile acid synthesis. The liver compensates for the loss of cholesterol by increasing cholesterol synthesis and by upregulating hepatic LDL receptor activity. In hamsters, cholesterol-lowering effect of taurine has been shown to be associated with upregulation of hepatic CYP7A1 activity and HMG-CoA reductase activity and stimulated hepatic LDL clearance (Murakami et al., 2002b). In addition to stimulation of bile acid secretion, taurine reduces hepatic secretion of cholesterol ester and apolipoprotein B, which is also involved in cholesterol-lowering action of taurine, in part (Yamamoto et al., 2000; Yanagita et al., 2008).

Taurine increases taurine-conjugated bile acids in human as in animals (Tanno et al., 1989). However, the effects of taurine on plasma lipid levels in human are less certain. This discrepancy may be related to the dosage and duration of taurine treatment, or difference of synthetic activity of taurine in the body. Epidemiologically, worldwide cross sectional study indicated serum total cholesterol levels in average were significantly lower in the populations and individuals excreting 24-h urinary taurine over the world average than those excreting taurine below the world average (Yamori et al., 2010b).

5. EFFECT OF TAURINE ON OBESITY

Obesity has become one of the most relevant health issues in many countries around the world within a decade. Antiobesity effect of taurine has been shown in mice and humans. Taurine supplementation to obese KK mice for 10 weeks decreases body weight gain and abdominal fat (Fujihira et al., 1970). Seven weeks-supplementation of taurine (3 g day^{-1}) to overweight subjects significantly reduced body weight, concomitantly with decrease in plasma lipids (Zhang et al., 2004).

Recent studies revealed that synthetic activity of taurine is unexpectedly high in adipose tissue (Ide et al., 2002). The mRNA expression of cysteine sulfinic acid decarboxylase, one of the rate-limiting enzymes of taurine synthesis, in rat adipose tissues is higher than those in the liver and kidney. In three T3-L1 adipocytes, activities of enzymes involved in taurine synthesis increased during adipogenic differentiation (Ueki and Stipanuk, 2009). It has been shown that taurine synthesis is decreased in white adipose tissues of obese mice due to reduction in cysteine dioxygenase expression, another rate-limiting enzyme of taurine synthesis, which results in decreased plasma taurine level (Tsuboyama-Kasaoka et al., 2006). Dietary taurine supplementation prevented obesity in both diet-induced and genetically obese mice. They also suggested that upregulation of peroxisome proliferator-activated receptor gamma coactivator-1α, peroxisome proliferator-activated receptor α, and peroxisome proliferator-activated receptor γ and their target genes by taurine may result in increased energy expenditure including fatty acid β-oxidation in white adipose tissues and thereby prevent obesity. Low plasma taurine in obese individuals was reported also in humans. In Korean female adolescents, plasma taurine level of obese subgroup is lower than normal and underweight subgroup (Lee et al., 2003). Worldwide population survey indicated that populations as well as individuals excreting higher 24-h urinary taurine had significantly lower BMI and lower prevalence of obesity than those excreting lower urinary taurine (Yamori et al., 2010b).

6. EFFECT OF TAURINE ON DIABETES

Type II diabetes is a complicated metabolic disease affecting millions of individuals worldwide. Type II diabetes and its complications contribute significantly to morbidity and mortality. In pancreas, taurine is found in high concentrations inside glucagon and somatostatin-containing cells in the pancreatic islets, suggesting that its release is necessary for an efficient modulation of insulin secretion (Bustamante et al., 2001). Previous *in vivo* and *in vitro* studies showed that taurine affects blood glucose level and insulin sensitivity. These effects are attributed to direct action on islet β-cells and indirect action on cellular function through amelioration of cellular lipid and glucose metabolism. The effect of taurine on Ca^{2+} channel and cellular Ca^{2+} levels may be responsible for insulin secretion (Ribeiro et al., 2009). Pretreatment with a single dose of taurine attenuates the elevation of serum glucose after intraperitoneal glucose loading in rats (Kulakowski and Maturo, 1984). This finding is associated with the stimulation of glucose uptake by skeletal muscle and liver, and increase in glycogen synthesis. In addition, taurine pretreatment ameliorates streptozotocin (STZ)-induced hyperglycemia, which is due to β-cell protection against STZ (Tokunaga et al., 1979). In contrast, when taurine is given after onset of diabetes, it is less effective. Taurine protects pancreatic tissue induced by glucose challenge (Kaplan et al., 2004). Chronic treatment of taurine delays the onset of diabetes

in nonobese diabetic mice, a model of type I diabetes, probably due to an altered β-cell development (Arany et al., 2004).

Taurine is also effective in the animal model of type II diabetes. Dietary taurine ameliorates insulin resistance, leading to increase in cellular uptake of glucose. Taurine supplementation modulates glucose metabolism and ameliorates insulin resistance in fructose-fed rats (Anuradha and Balakrishnan, 1999). In the Otsuka Long-Evans Tokushima Fatty rats, a model of insulin resistance and type II diabetes, taurine improves hyperglycemia and insulin resistance, concomitantly with decrease in abdominal fat, and serum and liver lipid levels (Harada et al., 2004; Nakaya et al., 2000). Taurine prevents insulin resistance and lipid peroxidation induced by glucose infusion (Haber et al., 2003). In addition to antidiabetic action, taurine has been shown to reduce mortality rate in STZ-induced diabetic rats (Di Leo et al., 2004).

In human, plasma and platelet taurine concentrations are lower in type I diabetic patients than in control subjects (Franconi et al., 1995). In type II diabetic subject or obese subject, chronic ingestion of taurine did not influence the blood glucose level, insulin secretion, or sensitivity, despite the elevated plasma taurine level (Brøns et al., 2004; Franconi et al., 1995). Meanwhile, oral taurine ingestion reduces lipid infusion–induced plasma oxidative stress marker and prevents insulin resistance in obese and overweight men (Xiao et al., 2008).

7. EFFECT OF TAURINE ON NAFLD/NONALCOHOLIC STEATOHEPATITIS

NAFLD, the most prevalent liver diseases in Western countries, is strongly associated with obesity, insulin resistance, hypertension, and dyslipidemia and is regarded as the liver manifestation of the metabolic syndrome. NAFLD represents a spectrum of disease ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and NAFLD-associated cirrhosis and end-stage liver disease.

Most of animal studies have used mice or rats fed high-fat diets or genetically altered mice. Many animal experiments showed that taurine reduces hepatic levels of triglycerides and cholesterol in diet-induced animal models including rats (Murakami et al., 1996; Yokogoshi et al., 1999), mice (Murakami et al., 1999b), and hamsters (Murakami et al., 2002b). Taurine treatment ameliorates hepatic histological change and blood profile of lipids and glucose, as well as hepatic mRNA expression of TNF-α, TGF-β, and type I procollagen in an experimental NASH model (Chen et al., 2006). Taurine also improves the fatty liver of children with obesity (Obinata et al., 1996). In fatty acid–loaded HepG2 cells, taurine inhibits the synthesis and cellular content of triglycerides and cholesterol ester (Yanagita et al., 2008).

Thus, taurine ameliorates fatty liver and NAFLD/NASH by normalizing a disturbance of hepatic lipid metabolism including stimulation of bile acid synthesis, as described above. In addition, antioxidative and anti-inflammatory effects may be important for the prevention of NAFLD/NASH by taurine.

8. EFFECT OF TAURINE ON AGING

Aging affects many pathways involved in cardiovascular homeostasis. Age-related decline in tissue taurine has been reported in animals. In the liver, kidney, and serum of F344 rats, taurine levels significantly decrease with age. The reduced biosynthesis of taurine may be partially responsible for decline in tissue taurine levels (Eppler and Dawson, 1999). In contrast, there is no clear change in tissue of SD rats. In striatum, cortex, nucleus accumbens, and cerebellum of old Wistar rat brain, taurine concentrations were significantly lower than those in young rats (Benedetti et al., 1991). It should be considered that there are strain differences of rat in the tissue level and biosynthesis of taurine (Eppler and Dawson, 1998). Taurine concentration in male C57BL/6J mice increased with age in the heart, decreased in leg muscle, and remained unchanged in the brain, liver, kidney, and blood (Massie et al., 1989).

Various biological functions are reduced with age. Taurine has been shown to counteract age-related decline of biological functions. A considerable number of experiments suggest that improvement of functional impairment in aged animals by taurine may be associated with amelioration of oxidative stress. Taurine improves the proliferative response in age-related decline of T-cells by restoration of the increment of the concentration of intracellular free calcium ion (Nishio et al., 1990). Taurine improves ozone-induced memory deficits in old rats, accompanied by decreased lipid peroxidation in the frontal cortex (Rivas-Arancibia et al., 2000). Taurine prevents age-related progressive renal fibrosis in rats (Cruz et al., 2000). Taurine improves learning and retention in aged mice (El Idrissi, 2008).

Recently, taurine transporter knockout mice (taut-/- mice) have been generated and analyzed. In tau-/- mice, taurine levels are markedly decreased in various tissues. These mice exhibit lower body weight, reduced fertility, and shorter life-span compared to wild-type mice (Warskulat et al., 2007). In addition, tau-/- mice develop age-dependent disorders including visual and cardiac dysfunctions, hepatitis, and impaired exercise capacity. These changes in tau-/- mice seem to be related to defect in osmoregulatory effect by taurine. Analysis of taurine transporter knockout mice revealed that taurine deficiency triggers and accelerates chronic hepatitis and liver fibrosis in mice beyond 1 year of age, suggesting cytoprotective effect of taurine against age-related elevation of oxidative stress.

9. IMMUNOMODULATORY EFFECT OF TAURINE

It is widely recognized that chronic inflammation is closely associated with the etiology of metabolic syndrome. Taurine has been shown to affect immune system and function (Schuller-Levis and Park, 2004). Its immunomodulatory action is thought to be exerted mainly via taurine chloramine. Leukocytes contain millimolar concentration of taurine,

which reacts with hypochlorous acid (HOCl) produced by the myeloperoxidase (MPO) and generates low toxic taurine chloramines (TauCl; Weiss et al., 1982). This is an important process for HOCl detoxification. HOCl plays a major role in host defenses against bacteria and other invading pathogens (Klebanoff and Coombs, 1992). But when produced in excess, it leads to oxidative tissue damage and contributes to the development or progression of diseases. MPO-derived HOCl has also been implicated in LDL modification and pathogenesis of atherosclerosis *in vivo* (Podrez et al., 2000). Interestingly, it is suggested that generated TauCl is a significant biological effector molecule. TauCl has been shown to suppress reactive oxygen species and inflammatory cytokines from neutrophils (Marcinkiewicz et al., 1998), macrophages (Park et al., 1993), monocytes, and other inflammatory cells (Barua et al., 2001). In studies on the mechanism of action, TauCl inhibits translocation of nuclear factor-κB (NF-κB) into the nucleus (Kanayama et al., 2002) or NF-κB signaling pathway (Barua et al., 2001).
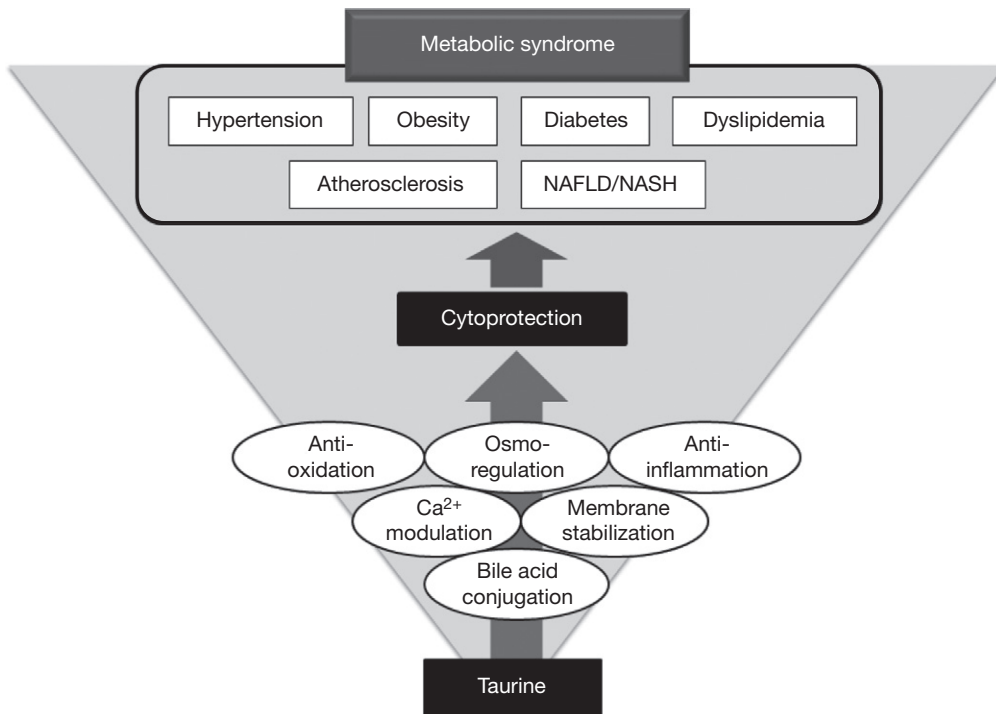
Figure 13.1 Possible mechanisms responsible for beneficial effect of taurine in prevention and amelioration of metabolic syndrome. Effects of taurine on metabolic syndrome including hypertension, obesity, diabetes, dyslipidemia, atherosclerosis, and nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) are mediated through basic physiological actions of taurine; antioxidation, osmoregulation, anti-inflammation, Ca^{2+} modulation, membrane stabilization, and bile acid conjugation.

Anti–inflammatory effect of taurine has been reported *in vivo* including human study. Thus, anti–inflammatory effect of taurine via generation of TauCl may play a crucial role in the underlying preventive mechanism of vascular diseases, obesity, insulin resistance, and hypertension.

10. CONCLUSIONS

Many studies have revealed a preventive effect of taurine on metabolic syndrome. As shown in Figure 13.1, the effect of taurine is attributable to cytoprotection through its osmoregulatory, antioxidant, membrane-stabilizing, Ca^{2+} regulatory, and immuno-modulating action. Analysis of tau-/- mice supports these effects of taurine. Worldwide epidemiological study also demonstrates that taurine intake is beneficial for CVD prevention and thus for longevity. In spite of numerous studies in cultured cells and animal models, little information is available for the effect of taurine in humans. Further clinical and intervention studies in humans will hopefully contribute to the elucidation of the essential role of taurine in longevity.

REFERENCES

Abe, M., Shibata, K., Matsuda, T., Furukawa, T., 1987. Inhibition of hypertension and salt intake by oral taurine treatment in hypertensive rats. Hypertension 10, 383–390.

Anuradha, C.V., Balakrishnan, S.D., 1999. Taurine attenuates hypertension and improves insulin sensitivity in the fructose-fed rat, an animal model of insulin resistance. Canadian Journal of Physiology and Pharmacology 77, 749–754.

Arany, E., Strutt, B., Romanus, P., et al., 2004. Taurine supplementation in early life altered islet morphology, decreased insulitis and delayed the onset of diabetes in non-obese diabetic mice. Diabetologia 47, 1831–1837.

Barua, M., Liu, Y., Quinn, M.R., 2001. Taurine chloramines inhibits inducible nitric oxide synthase and TNF-α gene expression in activated alveolar macrophages: decreased NF-κB activation and IκB kinase activity. Journal of Immunology 167, 2275–2281.

Benedetti, M.S., Russo, A., Marrari, P., Dostert, P., 1991. Effects of ageing on the content in sulfur-containing amino acids in rat brain. Journal of Neural Transmission. General Section 86, 191–203.

Brøns, C., Spohr, C., Storgaard, H., Dyeberg, J., Vaag, A., 2004. Effect of taurine treatment on insulin secretion and action, and on serum lipid levels in overweight men with a genetic predisposition for type II diabetes mellitus. European Journal of Clinical Nutrition 58, 1239–1247.

Bustamante, J., Lobo, M.V., Alonso, F.J., et al., 2001. An osmotic-sensitive taurine pool is located in rat pancreatic islet cells containing glucagon and somatostatin. American Journal of Physiology 281, E1275–E1285.

Casey, R.G., Gang, C., Joyce, M., Bouchier-Hayes, D.J., 2007. Taurine attenuates acute hyperglycaemia-induced endothelial cell apoptosis, leucocytes-endothelial cell interactions and cardiac dysfunction. Journal of Vascular Research 44, 31–39.

Chauncey, K.B., Tenner Jr., T.E., Lombardini, J.B., et al., 2003. The effect of taurine supplementation on patients with type 2 diabetes mellitus. Advances in Experimental Medicine and Biology 526, 91–96.

Chen, S.W., Chen, Y.X., Shi, J., Lin, Y., Xie, W.F., 2006. The restorative effect of taurine on experimental nonalcoholic steatohepatitis. Digestive Diseases and Sciences 51, 2225–2234.

Cruz, C.I., Ruiz-Torres, P., del Moral, R.G., Rodriguez-Puyol, M., Rodriguez-Puyol, D., 2000. Age-related progressive renal fibrosis in rats and its prevention with ACE inhibitors and taurine. American Journal of Physiology 278, F122–F129.

Di Leo, M.A., Santini, S.A., Silveri, N.G., et al., 2004. Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats. Amino Acids 27, 187–191.

Egan, B.M., Chen, G., Kelly, C.J., Bouchier-Hayes, D.J., 2001. Taurine attenuates LPS-induced rolling and adhesion in rat microcirculation. Journal of Surgical Research 95, 85–91.

El Idrissi, A., 2008. Taurine improves learning and retention in aged mice. Neuroscience Letters 436, 19–22.

Eppler, B., Dawson Jr., R., 1998. The effects of aging on taurine content and biosynthesis in different strains of rats. Advances in Experimental Medicine and Biology 442, 55–61.

Eppler, B., Dawson Jr., R., 1999. Cysteine sulfinate decarboxylase and cysteine dioxygenase activities do not correlate with strain-specific changes in hepatic and cerebellar taurine content in aged rats. Mechanisms of Ageing and Development 110, 57–72.

Fennessy, F.M., Moneley, D.S., Wang, J.H., Kelly, C.J., Bouchier-Hayes, D.J., 2003. Taurine and vitamin C modify monocyte and endothelial dysfunction in young smokers. Circulation 107, 410–415.

Franconi, F., Bennardini, F., Mattana, A., et al., 1995. Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus: effects of taurine supplementation. American Journal of Clinical Nutrition 61, 1115–1119.

Fujihira, E., Takahashi, H., Nakazawa, M., 1970. Effect of long-term feeding of taurine in hereditary hyperglycemic obese mice. Chemical and Pharmaceutical Bulletin 18, 1636–1642.

Fujita, T., Ando, K., Noda, H., Ito, Y., Sato, Y., 1987. Effects of adrenomedullary activity and taurine in young patients with borderline hypertension. Circulation 75, 525–532.

Fujita, T., Sato, Y., 1986. Changes in blood pressure and extracellular fluid with taurine in DOCA-salt rats. American Journal of Physiology 250, R1014–R1020.

Haber, C.A., Lam, T.K., Yu, Z., et al., 2003. N-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance *in vivo*: possible role of oxidative stress. American Journal of Physiology 285, E744–E753.

Harada, H., Kitazaki, K., Tsujino, T., et al., 2000. Oral taurine supplementation prevents the development of ethanol-induced hypertension in rats. Hypertension Research 23, 277–284.

Harada, N., Ninomiya, C., Osako, Y., et al., 2004. Taurine alters respiratory gas exchange and nutrient metabolism in type 2 diabetic rats. Obesity Research 12, 1077–1084.

Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. Nature 444, 860–867.

Huxtable, R.J., 1992. Physiological actions of taurine. Physiological Reviews 72, 101–163.

Ide, T., Kushiro, M., Takahashi, Y., Shinohara, K., Cha, S., 2002. mRNA expression of enzymes involved in taurine biosynthesis in rat adipose tissues. Metabolism 51, 1191–1197.

Ideishi, M., Miura, S., Sakai, T., et al., 1994. Taurine amplifies renal kallikrein and prevents salt-induced hypertension in Dahl rats. Journal of Hypertension 12, 653–661.

Inoue, A., Takahashi, H., Lee, L.C., et al., 1986. Hypotensive responses to centrally administered taurine in DOCA-salt hypertensive rats. Japanese Circulation Journal 50, 1215–1223.

Kanayama, A., Inoue, J., Sugita-Konishi, Y., Shimizu, M., Miyamoto, Y., 2002. Oxidation of IkB α at methionine 45 is one cause of taurine chloramines-induced inhibition of NF-κB activation. Journal of Biological Chemistry 277, 24049–24056.

Kaplan, B., Karabay, G., Zagyapan, R.D., et al., 2004. Effects of taurine in glucose and taurine administration. Amino Acids 27, 327–333.

Klebanoff, S.J., Coombs, R.W., 1992. Viricidal effect of polymorphonuclear leukocytes on human immunodeficiency virus-1. Role of the myeloperoxidase system. Journal of Clinical Investigation 89, 2014–2017.

Kondo, Y., Toda, Y., Kitajima, H., et al., 2001. Taurine inhibits development of atherosclerotic lesions in apolipoprotein E-deficient mice. Clinical and Experimental Pharmacology and Physiology 28, 809–815.

Kulakowski, E.C., Maturo, J., 1984. Hypoglycemic properties of taurine: not mediated by enhanced insulin release. Biochemical Pharmacology 33, 2835–2838.

Lee, M.Y., Cheong, S.H., Chang, K.J., Choi, M.J., Kim, S.K., 2003. Effect of the obesity index on plasma taurine levels in Korean female adolescents. Advances in Experimental Medicine and Biology 526, 285–290.

Marcinkiewicz, J., Grabowska, A., Bereta, J., Bryniarski, K., Nowak, B., 1998. Taurine chloramines down-regulates the generation of murine neutrophil inflammatory mediators. Immunopharmacology 40, 27–38.

Massie, H.R., Williams, T.R., DeWolfe, L.K., 1989. Changes in taurine in aging fruit flies and mice. Experimental Gerontology 24, 57–65.

Mizushima, S., Nara, Y., Sawamura, M., Yamori, Y., 1996. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. Advances in Experimental Medicine and Biology 403, 615–622.

Mozaffari, M.S., Schaffer, S.W., 2002. Chronic taurine treatment ameliorates reduction in saline-induced dieresis and natriuresis. Kidney International 61, 1750–1759.

Murakami, S., Kondo, Y., Sakurai, T., Kitajima, H., Nagate, T., 2002a. Taurine suppresses development of atherosclerosis in Watanabe heritable hyperlipidemic (WHHL) rabbits. Atherosclerosis 163, 79–87.

Murakami, S., Kondo, Y., Toda, Y., et al., 2002b. Effect of taurine on cholesterol metabolism in hamsters: up-regulation of low density lipoprotein (LDL) receptor by taurine. Life Sciences 70, 2355–2366.

Murakami, S., Kondo, Y., Tomisawa, K., Nagate, T., 1999a. Prevention of atherosclerotic lesion development in mice by taurine. Drugs under Experimental and Clinical Research 25, 227–234.

Murakami, S., Kondo-Ohta, Y., Tomisawa, K., 1999b. Improvement in cholesterol metabolism in mice given chronic treatment of taurine and fed a high-fat diet. Life Sciences 64, 83–91.

Murakami, S., Sakurai, T., Tomoike, H., et al., 2010. Prevention of hypercholesterolemia and atherosclerosis in the hyperlipidemia- and atherosclerosis-prone Japanese (LAP) quail by taurine supplementation. Amino Acids 38, 271–278.

Murakami, S., Yamagishi, I., Asami, Y., et al., 1996. Hypolipidemic effect of taurine in stroke-prone spontaneously hypertensive rats. Pharmacology 52, 303–313.

Nakaya, Y., Minami, A., Harada, N., et al., 2000. Taurine improves insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous type 2 diabetes. American Journal of Clinical Nutrition 71, 54–58.

Nara, Y., Yamori, Y., Lovenberg, W., 1978. Effect of taurine on blood pressure in spontaneously hypertensive rats. Biochemical Pharmacology 27, 2689–2692.

NCEP-ATP guidelines executive guide lines, 2001. Summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Journal of the American Medical Association 285, 2486–2497.

Nishio, S., Negoro, S., Hosokawa, T., et al., 1990. The effect of taurine on age-related immune decline in mice: the effect of taurine on T cell and B cell proliferative response under costimulation with ionomycin and phorbol myristate acetate. Mechanisms of Ageing and Development 52, 125–139.

Obinata, K., Maruyama, T., Hayashi, M., Watanabe, T., Nittono, H., 1996. Effect of taurine on the fatty liver of children with simple obesity. Advances in Experimental Medicine and Biology 403, 607–613.

Park, E., Quinn, M.R., Wright, C.E., Schuller-Levis, G., 1993. Taurine chloramines inhibits the synthesis on nitric oxide and the release of tumor necrosis factor in activated RAW 264.7 cells. Journal of Leukocyte Biology 54, 119–124.

Podrez, E.A., Abu-Soud, H.M., Hazen, S.L., 2000. Myeloperoxidase-generated oxidants and atherosclerosis. Free Radical Biology and Medicine 28, 1717–1725.

Ribeiro, R.A., Bonfleur, M.L., Amaral, A.G., et al., 2009. Taurine supplementation enhances nutrient-induced insulin secretion in pancreatic mice islets. Diabetes/Metabolism Research and Reviews 25, 370–379.

Rivas-Arancibia, S., Dorado-Martinez, C., Borgonio-Perez, G., et al., 2000. Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environmental Research 82, 7–17.

Schaffer, S.W., Lombardini, J.B., Azuma, J., 2000. Interaction between the actions of taurine and angiotensin II. Amino Acids 18, 305–318.

Schuller-Levis, G.B., Park, E., 2004. Taurine and its chloramines: modulators of immunity. Neurochemical Research 29, 117–126.

Tan, B., Jiang, D.J., Huang, H., et al., 2007. Taurine protects against low-density lipoprotein-induced endothelial dysfunction by the DDAH/ADMA pathway. Vascular Pharmacology 46, 338–345.

Tanno, N., Oikawa, S., Koizumi, M., et al., 1989. Effect of taurine administration on serum lipid and biliary lipid composition in man. Tohoku Journal of Experimental Medicine 159, 91–99.

Terauchi, A., Nakazawa, A., Johkura, Y., Yan, L., Usuda, N., 1998. Immunohistochemical localization of taurine in various tissues of the mouse. Amino Acids 15, 151–160.

Tokunaga, H., Yoneda, Y., Kuriyama, K., 1979. Protective actions of taurine against streptozotocin-induced hyperglycemia. Biochemical Pharmacology 28, 2807–2811.

Tsuboyama-Kasaoka, N., Shozawa, C., Sano, K., et al., 2006. Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 147, 3276–3284.

Ueki, I., Stipanuk, M.H., 2009. 3T3-L1 adipocytes and rat adipose tissue have a high capacity for taurine synthesis by the cysteine dioxygenase/cysteinesulfinate decarboxylase and cysteamine dioxygenase pathway. Journal of Nutrition 139, 207–214.

Wang, L.J., Yu, Y.H., Zhang, L.G., et al., 2008. Taurine rescues vascular endothelial dysfunction in streptozocin-induced diabetic rats: correlated with downregulation of LOX-1 and ICAM-1 expression on aortas. European Journal of Pharmacology 597, 75–80.

Warskulat, U., Heller-Stilb, B., Oermann, E., et al., 2007. Phenotype of the taurine transporter knockout mouse. Methods in Enzymology 428, 439–458.

Weiss, S.J., Klien, R., Slivka, A., Wei, M., 1982. Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. Journal of Clinical Investigation 70, 598–607.

Wu, Q.D., Wang, J.H., Fennessy, F., Redmond, H.P., Bouchier-Hayes, D., 1999. Taurine prevents high-glucose-induced human vascular endothelial cell apoptosis. American Journal of Physiology 277, C1229–C1238.

Xiao, C., Giacca, A., Lewis, G.F., 2008. Oral taurine but not N-acetylcysteine ameliorates NEFA-induced impairment in insulin sensitivity and β cell function in obese and overweight, non-diabetic men. Diabetologia 51, 139–146.

Yamamoto, K., Yoshitama, A., Sakono, N., et al., 2000. Dietary taurine decreases hepatic secretion of cholesterol ester in rats fed a high-cholesterol diet. Pharmacology 60, 27–33.

Yamori, Y., Liu, L., Ikeda, K., et al., 2001. WHO-Cardiovascular Disease and Alimentary Comparison (CARDIAC) Study Group. Distribution of twenty-four hour urinary taurine excretion and association with ischemic heart disease mortality in 24 populations of 16 countries: results from the WHO-CARDIAC study. Hypertension Research 24, 453–457.

Yamori, Y., Liu, L., Mori, M., et al., 2009. Taurine as the nutritional factor for the longevity of the Japanese revealed by a world-wide epidemiological survey. Advances in Experimental Medicine and Biology 643, 13–25.

Yamori, Y., Nara, Y., Ikeda, K., et al., 1996. Is taurine a preventive nutritional factor of cardiovascular diseases or just a biological marker of nutrition? Advances in Experimental Medicine and Biology 403, 623–629.

Yamori, Y., Taguchi, T., Hamada, A., et al., 2010a. Taurine in health and diseases consistent evidence from experimental and epidemiological studies. Journal of Biomedical Science 17 (Suppl. 2), S6.

Yamori, Y., Taguchi, T., Mori, M., et al., 2010b. Low cardiovascular risks in the middle aged males and females excreting greater 24-hour urinary taurine and magnesium in 41 WHO-CARDIAC study populations in the world. Journal of Biomedical Science 17 (Suppl. 1), S21.

Yanagita, T., Han, S.Y., Hu, Y., et al., 2008. Taurine reduces the secretion of apolipoprotein B100 and lipids in HepG2 cells. Lipids in Health and Disease 7, 38–43.

Yokogoshi, H., Mochizuki, H., Nanami, K., et al., 1999. Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. Journal of Nutrition 129, 1705–1712.

Zhang, M., Bi, L.F., Fang, J.H., et al., 2004. Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. Amino Acids 26, 267–271.

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Preventing the Epidemic of Mental Ill Health: An Overview

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ABBREVIATIONS

AA Arachidonic acid
DHA Docosahexaenoic acid
WHO World Health Organization

1. INTRODUCTION

At the end of the seventeenth century, mental ill health was of little significance and was little discussed. At the end of the eighteenth century, it was perceived as probably increasing and was of some concern. At the end of the nineteenth century, it was perceived as an epidemic and was a major concern, and at the end of the twentieth century, it was simply accepted as part of the fabric of life (Torrey and Miller, 2002). Now, in the twenty first century, the cost of brain disorders has overtaken those of any other health burden (Crawford et al., 2009; Wang et al., 2010). The consequences, in terms of mental disability, impose a disproportionately high cost on health services and society because of its life-long impact. For example, in England (a country that is part of the United Kingdom of Great Britain and Northern Ireland), the costs associated with mental health problems were estimated to be £105.2 billion during 2010 (Centre for Mental Health, 2010). It should be noted that £105.2 billion during 2010 was greater than the whole of the funding for England's publicly run National Health Service (NHS); so, it is a huge cost, and during 2010, it was about 9.6% of gross value added (GVA) (Harker, 2012; Office for National Statistics, 2011).

Leading scientist professor Steve Jones said the hope that genetic research could provide a cure for a host of common diseases (genetic disorders are rare) has proved to be a false dawn, and that we have wandered into a blind alley, and it might be better that we come out of it and start again. In most cases, hundreds of genes are responsible, and often they have less effect than other factors such as diet, lifestyle, and the environment (Jones, 2009). Children with the genetic disorder, phenylketonuria, have been protected from

Figure 14.1 A bowl of steamed mussels, *Mytilus edulis*. Edible bivalves, especially cooked wild bivalves such as mussels, oysters and clams, are some of the most nutritious foods for humans on the planet (Anthony A. Robson ©).

severe mental decline, not from the human genome project, but from nutrition and health management. The view of diet being a major driver of health and disease dates back to Sir Robert McCarrison's studies in India, early last century. Eating adequate amounts of foods rich in bioavailable brain nutrients, for example, shellfish[1], such as mussels (Figure 14.1) and oysters (which contain nutrients including protein, docosahexaenoic acid (DHA), arachidonic acid (AA), iodine, iron, copper, zinc, manganese, and selenium as well as a variety of antioxidants including vitamins; see Table 14.1) promotes health and helps prevent diet-induced mental ill health today (e.g., Robson, 2009). This overview highlights the major changes that are urgently needed in order to promote health and help prevent the epidemic of mental ill health (for an overview of preventing non-communicable diseases, see Robson, 2013b). After all, disease prevention, in the long run, is far less costly than treatment.

2. HUMAN DIET

Agriculture introduced foods as staples for which the human genome had little evolutionary experience. More importantly, food-processing procedures were developed, particularly following the Industrial Revolution, which allowed for quantitative and qualitative food and nutrient combinations that had not previously been encountered over the course of human evolution. Cooking oils, cereals, dairy products, refined sugars, fatty meats, alcohol, NaCl salt, and combinations of these foods fundamentally altered several

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[1] There have been misleading warnings about mercury in seafood. In the absence of a major methylmercury spill into a localized marine environment, selenium in seafood reacts with all the mercury in seafood to produce a safe compound. The actual risks of mercury exposure from the consumption of foods from mercury-contaminated terrestrial and freshwater ecosystems have gone unrecognized for too long (Berry and Ralston, 2008).

Table 14.1 Nutrient Density of Some of the Most Nutritious Brain Foods (Value per 100 g)

	DHA (g)	AA (g)	I[a]	Fe[b] (mg)	Cu (mg)	Zn (mg)	Mn (mg)	Se (μg)	Vitamin A (μg_RAE)	B12 (μg)	B6 (mg)	C (mg)	D[a] (μg)	Folate (μg)
Oyster meat, wild, cooked (15169)[c]	0.584	0.160	200	11.99	7.569	181.61	0.697	71.6	54	35.02	0.118	6.0	0.14	14
Mussel meat, cooked (15165)[c]	0.506	0.140	160	6.72	0.149	2.67	6.800	89.6	91	24.00	0.100	13.6	0.12	76
Clam meat, cooked (15159)[c]	0.146	0.082	150	27.96	0.688	2.73	1.000	64.0	171	98.89	0.110	22.1	0.12	29
Snapper fish, cooked (15102)	0.273	0.044	40	0.24	0.046	0.44	0.017	49.0	35	3.50	0.460	1.6	0.12	6
Egg, poached (01131)[d]	0.037[e]	0.141	36.6	1.83	0.102	1.10	0.039	31.6	139	1.28	0.121	0	0.67	35
Salmon, Atlantic, cooked (15209)	1.429	0.342	6.5	1.03	0.321	0.82	0.021	46.8	13	3.05	0.944	0	7.4	29
Lamb brain, cooked (17186)	0.590	0.270	4	1.68	0.210	1.36	0.059	12.0	0	9.25	0.110	12.0	0.12	5

Entries retrieved from the USDA National Nutrient Database for Standard Reference, Release 22 (2009) and are identified by a five-digit nutrient database number in parentheses.

[a] Data from the Australian Food, Supplement and Nutrient Database (AUSNUT) 2007 (Available from http://www.foodstandards.gov.au/).

[b] Two billion people, over 30% of the World's population, are anemic, many because of iron deficiency. World Health Organization, 2009. Micronutrient deficiencies: iron deficiency anaemia. http://www.who.int/nutrition/topics/ida/en/print.html.

[c] 100 g provides 100% or more of the adult RDA for iodine. Institute of Medicine, 2001. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutrition Board. National Academy Press, Washington, DC

[d] Vitamin B12 in eggs is poorly absorbed relative to other foods containing B12. Watanabe, F., 2007. Vitamin B-12 sources and bioavailability. Experimental Biology and Medicine 232, 1266–1274

[e] Sum EPA + DPA + DHA = 0.195 g (DPA – docosapentaenoic acid ω-3 is an intermediary between EPA and DHA), in raw eggs from hens on a diet enriched in DHA (Data valid on 8th October 2010 - product: Marks & Spencer plc UK free range omega-3 eggs).

key nutritional characteristics of ancestral human diets and ultimately had far-reaching effects on health and well being. As these foods gradually displaced the minimally processed, but often cooked, wild foods in human diets, they adversely affected the following dietary indicators: (1) fatty acid composition, (2) energy density, (3) macronutrient composition, (4) micronutrient density, (5) acid–base balance, (6) sodium (as NaCl)–potassium ratio, and (7) fiber content (Cordain et al., 2005; Robson, 2009). Wild foods known to be consumed by hunter-gatherers have higher nutrient concentrations than their domesticated counterparts (Brand-Miller and Holt, 1998; Eaton and Konner, 1985), including the muscle meat of wild animals (First Data Bank, 2000).

3. GENERAL EFFECTS OF DIET ON THE HUMAN BRAIN

The developing human brain, between 24 and 42 weeks of gestation, is particularly vulnerable to nutritional insults because of the rapid trajectory of several neurological processes, including synapse formation and myelination. Conversely, the young brain is remarkably plastic and, therefore, more amenable to repair after nutrient repletion. On balance, the brain's vulnerability to nutritional insults outweighs its plasticity, not only while the nutrient is in deficit, but also after repletion (Georgieff, 2007). Adverse neurodevelopmental outcomes in children are associated with inadequate maternal consumption of brain nutrients during pregnancy and lactation (e.g., Helland et al., 2003; Hibbeln et al., 2007). Breastfeeding, in comparison to feeding breast milk substitutes such as infant formula, has a wide range of health benefits for mothers and children (e.g., Gartner et al., 2005; Horta et al., 2007). A significant positive effect of breastfeeding on cognitive ability in children has been found over and above the expected positive effect of maternal education (Bartels et al., 2009). The current World Health Organization (WHO) recommendation for breastfeeding is that all infants should be exclusively breastfed for the first 6 months of life, and receive nutritionally adequate and safe complementary foods while breastfeeding continues for up to 2 years of age or beyond (World Health Organization, 2002). The WHO recommendations have been adopted and endorsed by many countries; yet barely one in three infants is exclusively breastfed during the first 6 months of life (World Health Organization, 2010).

Increasing evidence suggests that depression, bipolar disorder, cognitive decline, age-related macular degeneration, Alzheimer's disease, aggression, hostility and antisocial behavior relate to a lack of brain nutrients in the human diet (Crawford et al., 2009). Of course, alcohol and other drug abuse also have deleterious effects on the brain. Furthermore, obesity is common among women of reproductive age. Although obesity alone confers increased disease risk, obesity in pregnancy presents added health problems and increases the incidence of premature births and low birth weight babies (e.g., Rajasingam et al., 2009). There is creditable data that the increase in bipolar disorder has recently been most rapid among children (Moreno et al., 2007). Poor maternal

nutrition and living conditions have been causatively linked to low birth weight regardless of socioeconomic status, ethnicity, or smoking habit (Doyle et al., 1989; Rees et al., 2005; Wynn et al., 1994). Low birth weight is the strongest predictor of the risk for chronic ill health, including brain disorders, heart disease, stroke and diabetes along with learning and numeracy difficulties, behavioral problems, low skill level, restricted job opportunities, and crime (e.g., Barker, 2004). As birth weight falls (mostly premature deliveries), the incidence of severe neurodevelopmental disorders rises sharply from about 1 in 1000 live births to over 200 in 1000 live births below 1.5 kg (UK Office for National Statistics data). Yet, non-communicable brain disorders and other degenerative non-communicable diseases are rare or nonexistent in hunter-gatherers eating a late Paleolithic diet, that is, a low-energy-dense diet with a wild plant-to-animal energy intake ratio ∼1:1, with fish and shellfish providing a significant proportion of the animal component (Eaton et al., 2010).

4. THE MOST IMPORTANT BRAIN NUTRIENTS

All brain nutrients are important for neurogenesis and development (the adult human brain contains regions where continuous neurogenesis occurs (van de Berg et al., 2010)), but certain nutrients have greater effects on brain health than do others. Iodine deficiency is the most common cause of preventable brain damage in the World. Iodine deficiency disorders include mental retardation, hypothyroidism, goiter, and varying degrees of other growth and developmental abnormalities (de Benoist et al., 2008). The WHO estimates that over 30% of the world's population (2 billion people) has insufficient iodine intake (de Benoist et al., 2008). The global problem of iodine deficiency primarily affects people not regularly consuming shellfish, fish, seaweed, plants grown in iodine-rich soil, animals fed iodine-rich foods, or iodized table salt. However, plant-based diets rich in staples like cassava or soybeans (the basis of many vegan and vegetarian food products) contain goiterogens, which inhibit iodine absorption (Cunnane et al., 2007). Further, the key minerals needed for brain development and function are more bioavailable from shellfish and fish than from plant-based diets where their absorption is impaired by phytates and other antinutrients.

Fetal protein-energy malnutrition results in intrauterine growth retardation which is associated with a high prevalence of subsequent neurodevelopmental abnormalities characterized by cognitive disabilities (Spinillo et al., 1993). DHA is a potent neurobiological agent that is important for synaptogenesis, membrane function, and myelination. DHA deficiency is associated with many changes in brain function including alterations in learning and memory, auditory and olfactory responses to stimuli, reductions in the size of neurons, changes in nerve growth factor levels, delayed cell migration in the developing brain, and an increase in depressive and aggressive behavior (Crawford et al., 2009). AA is the precursor of a number of different endocannabinoids and eicosanoids which play important roles in the normal homeostasis of brain function (Tassoni et al.,

2008). Iron deficiency at any time in life may disrupt metabolic processes and subsequently change cognitive and behavioral functioning. Iron treatment has been found to normalize cognitive function in young women (Murray-Kolb and Beard, 2007). Copper deficiency alters, in particular, the developing cerebellum causing long-term effects on motor function, balance, and coordination (Georgieff, 2007). Manganese deficiency, which may enhance susceptibility to convulsions, appears to affect manganese homeostasis in the brain, probably followed by alteration of neural activity (Takeda, 2003). Selenium mediates its effects on brain and behavior development through thyroid hormone metabolism; folate and choline mediate their effects through one-carbon metabolism, DNA methylation, and neurotransmitter synthesis. Life-long selenium deficiency is associated with lower cognitive function (Gao et al., 2007). Folate deficiency can lead to neurological disorders, such as depression and cognitive impairment (Gomez-Pinilla, 2008). Zinc deficiency alters autonomic nervous system regulation. Severe zinc deficiency during gestation can lead to overt fetal brain malformations, and suboptimal zinc nutrition during gestation can have long-term effects on the offspring's nervous system (Adamo and Oteiza, 2010).

Vitamin A is particularly important during periods of rapid growth, both during pregnancy and in early childhood. Vitamin A derivatives, retinoids, control the differentiation of neurones, and a role has been suggested in memory, sleep, depression, Parkinson's disease, and Alzheimer's disease (Tafti and Ghyselinck, 2007). Furthermore, vitamin A plays a critical role in visual perception and a deficiency causes blindness (Benton, 2008). Impaired cognitive function is associated with the inadequate provision of vitamin B12 throughout life (Benton, 2008).

In many instances, it is likely that a diet deficient in one brain nutrient will be deficient in others. Nutrients do not function in isolation. It is possible that a beneficial response to the supplementation of a single deficient brain nutrient, for example, choline, has not been observed because the functioning of other aspects of a chain of necessary reactions has been inhibited by other deficiencies (Benton, 2008). It is also important to emphasize that consuming diets that are excessively rich or deficient in brain nutrients at any time in life may cause disease or premature death (Church et al., 2009; Georgieff, 2007). Thus, primary prevention of mental ill health starts, crucially, with optimal adult nutrition before the inception of pregnancy, includes breastfeeding and continues throughout the life of the newborn (Robson, 2009). Diet, lifestyle and environment do not just affect a person's health, they also determine the health of their children and possibly the health of their grandchildren (Marsh, 2012; Pembrey et al., 2006).

5. ENERGY DENSITY AND NUTRIENT DENSITY

Human food production should be linked to human nutritional requirements as its first priority (Robson, 2012, 2013a). Thus, the high-energy-density and low nutrient density that characterize the modern diet must be overcome simultaneously (Robson, 2011, 2012, 2013b). People can develop paradoxical nutritional deficiency from eating high-energy-dense foods

with a poor nutrient content (Robson, 2009). The finding that people with a low-energy-dense diet (<1.6 kcal g^{-1}) have the lowest total intakes of energy, even though they consume the greatest amount of food, has important implications for promoting compliance with a healthy diet (Ledikwe et al., 2006). A farmed and/or processed food that is not both low-energy-dense and of high-nutrient-density is of poor dietary quality compared to the low-energy-dense foods of high nutrient density that humans should eat: the most nutritious cooked wild plant and animal foods for humans (Eaton et al., 2010: Robson, 2006, 2010a, 2011).

Processed low-fat foods can have a deleteriously high-energy-density (c.f. Robson, 2013a). The emphasis on just reducing dietary fat (Farhang, 2007; Hsieh and Ofori, 2007) must be refocused on reducing the positive imbalance between the intake and the expenditure of food energy. Low fat, high carbohydrate, cereal-based products are often of high-energy-density. For example, a Masterfoods Twix® chocolate biscuit bar: 56% carbohydrate and 2.2% water $= 5.5$ kcal g^{-1}, Kellogg's Special K®: 71% carbohydrate and 3% water $= 3.8$ kcal g^{-1}, white bread: 51% carbohydrate and 36% water $= 2.7$ kcal g^{-1}, while roasted wild water buffalo meat: 0% carbohydrate and 69% water $= 1.3$ kcal g^{-1}, shrimp meat cooked in moist heat: 0% carbohydrate and 77% water $= 1.0$ kcal g^{-1} and boiled celery: 4% carbohydrate and 94% water $= 0.2$ kcal g^{-1} (c.f. Table 14.2).

Processed food products of plant origin such as chocolate bars, biscuits, fruit bars, and cereal bars have a high-energy-density principally because they have a low water content (Robson, 2011, 2012, 2013a). Self-assembled, water-filled, edible nanotubes that self-organize into a more complex structure, possibly a 3D network of nanocellulose, could be incorporated into many processed foods to lower their energy density to <1.6 kcal g^{-1} (c.f. Norton et al., 2009; Robson, 2012). Nanocellulose is composed of nanosized cellulose fibrils (fiber diameter: 20–100 nm), and has a water content of up to 99% and the same molecular formula as plant cellulose (Klemm et al., 2006). The water inside the nanosized cellulose fibrils could contain flavor with few calories, for example, a cup of tea without milk $= 0.01$ kcal g^{-1}. The shape and supramolecular structure of the nanocellulose can be regulated directly during biosynthesis to produce fleeces, films/patches, spheres, and tubes (Klemm et al., 2011). Other edible materials can strongly adhere to the surface and the inside of nanocellulose structures such as fleeces to form edible composites (Chang et al., 2012). Taste sensation per mouthful could be improved by adding flavoring substances processed on the nanoscale (increased surface area in contact with taste and smell receptors) to edible composites (Ultrafine food technology: Eminate Limited, Nottingham, United Kingdom). Durethan® KU2-2601 packaging film produced by Bayer Polymers, Germany, is a nanocomposite film enriched with silicate nanoparticles, which is designed to prevent the contents from drying out and prevent the contents from coming into contact with oxygen and other gases. Durethan® KU2-2601 can prevent food spoilage (Neethirajan and Jayas, 2011) and thus the water content of dehydrated plant-based food products can be increased without reducing product shelf life. Therefore, nanocellulose is expected to be widely used a as a nature-based food additive (Chang, et al., 2012; Klemm, et al., 2011).

Table 14.2 Energy Density, Water, Protein, and Carbohydrate Content of a Selection of Foods (Value per 100 g)

	Energy (kcal)	Water (g)	Protein (g)	Carbohydrate (g)
Oil, soybean[a] (04044)[b]	884	0.00	0.00	0.00
Chocolate, dark (19904)[b]	598	1.37	7.79	45.90
Twix® bar, Masterfoods (42183)[b]	550	2.20	7.30	56.00
Potato chips (19811)	536	1.90	7.00	52.90
Oat breakfast bar (43100)[b]	464	4.10	9.80	66.70
Rice cake (42204)	392	5.80	7.10	81.10
Corn pops®, Kellogg's (08068)[b]	378	3.00	3.70	90.00
Bread, white (18069)[b]	266	36.44	7.64	50.61
Ice cream, vanilla (19089)[b]	249	57.20	3.50	22.29
Beef sirloin, roasted (13953)[b]	211	62.63	26.05	0.00
Salmon, Atlantic, farmed, cooked (15237)[b]	206	64.75	22.10	0.00
Chicken meat, roasted (05013)[c]	190	63.79	28.93	0.00
Salmon, Atlantic, wild, cooked (15209)[c]	182	59.62	25.44	0.00
Mussel meat, cooked (15165)[c]	172	61.15	23.80	7.39
Beef brain, cooked (13320)[d]	151	74.86	11.67	1.48
Clam meat, cooked (15159)[d]	148	63.64	25.55	5.13
Egg, poached (01131)	142	75.54	12.52	0.78
Oyster meat, eastern, wild, cooked (15169)[d]	137	70.32	14.10	7.82
Moose meat, wild, roasted (17173)[d]	134	67.83	29.27	0.00
Water buffalo meat, wild, roasted (17161)[d]	131	68.81	26.83	0.00
Shrimp meat, cooked (15151)[d]	99	77.28	20.91	0.00
Pear, raw (09252)[d]	58	83.71	0.38	15.46
Broccoli, cooked (11091)[d]	35	89.25	2.38	7.18
Spinach, boiled (11458)[d]	23	91.21	2.97	3.75
Watercress, raw (11591)[d]	11	95.11	2.30	1.29

Entries retrieved from the USDA National Nutrient Database for Standard Reference, Release 22 (2009) and are identified by a five-digit nutrient database number in parentheses.
[a] Soybean oil provides 20% of all calories in the median USA diet.Gerrior, S., Bente, L., 2002. Nutrient content of the U.S. food supply, 1909–99: a summary report. US Department of Agriculture, Home Economics report no. 55, Washington, DC
[b] High-energy-density >2 kcal g^{-1}.
[c] Medium energy density 2.0–1.6 kcal g^{-1}.
[d] Low-energy-density <1.6 kcal g^{-1}. Ledikwe, J.H., Blanck, H.M., Kettel Khan, L., et al., 2006. Dietary energy density is associated with energy intake and weight status in US adults. American Journal of Clinical Nutrition 83, 1362–1368

The bioavailable nutrient content including cofactors of processed foods should be based on the nutritional value of the most nutritious cooked wild foods for humans and can be increased using existing bioactive encapsulation (Robson, 2010a, 2011). Aquatic biotechnology can provide the food industry with sufficient amounts of all

the nutrients needed for mass-scale optimal human brain nutrition including protein, DHA, AA, iodine, iron, copper, zinc, manganese, and selenium as well as a variety of antioxidants including vitamins (Harun et al., 2010; Liu et al., 2012; Ortiz et al., 2006). Reducing particle size using nanotechnology can further improve the properties of bioactive compounds (e.g., DHA), such as delivery, solubility, prolonged residence time in the gastrointestinal tract, and efficient absorption through cells (Chen et al., 2006).

A reduction in liquid calorie intake has been found to have a greater effect on weight loss than a reduction in solid calorie intake (Chen et al., 2009). Sugar-sweetened beverages (SSBs) require little digestion. Glucose and fructose can be directly absorbed into the bloodstream without digestion. Reducing the energy density of processed foods, including SSBs and simultaneously increasing the cost of their assimilation makes them more akin to foods consumed by late Palaeolithic humans. The energetic cost of the assimilation of processed foods can be increased by increasing their protein and fiber content (both protein and fiber can be produced on a mass scale using aquatic biotechnology) (Eaton et al., 2010; Robson, 2010a, 2011). Protein has more than three times the thermic effect of either fat or carbohydrate (Crovetti et al., 1998), and protein has a greater satiety value than fat or carbohydrate (Crovetti et al., 1998; Stubbs, 1998). A high-protein diet (protein and carbohydrate intake both being approximately one third of total energy intake, (Eaton et al., 2010)) is of vital importance as a weight-loss strategy for the overweight or obese and for weight maintenance (Robson, 2009; Veldhorst et al., 2008). Clinical trials have shown that calorie-restricted, high-protein diets are more effective than are calorie-restricted, high-carbohydrate diets in promoting (Baba et al., 1999; Layman, 2003; Skov et al., 1999) and maintaining (Westerterp-Plantenga et al., 2004) weight loss in overweight subjects, while producing less hunger and more satisfaction (Johnston et al., 2004). Furthermore, high-protein diets have been shown to improve metabolic control in patients with type 2 diabetes (Odea, 1984; Odea et al., 1989; Seino et al., 1983). Food-grade protein-based nanotubes (Graveland-Bikker and De Kruif, 2006) may be used to increase the protein content of processed foods that are currently high in fat or high in carbohydrate. Functional foods and drinks are required to simultaneously satisfy the human "sweet tooth" and almost completely remove added sugars such as glucose, fructose and sucrose from the diet (Eaton et al., 2010). Savoury food and drinks can be sweetened by adding fruit to them or adding calorie-free Purefruit™ (Tate & Lyle) monk fruit (*Siraitia grosvenorii*) extract (Robson, 2013a). PUREFRUIT™ is approximately 200 times sweeter than sugar and has exceptional stability.

Cooking has obvious beneficial effects by increasing food safety and improving diet quality (Carmody and Wrangham, 2009). However, cooking can reduce the water content of a high-energy-dense processed food and, thus, further increase its deleteriously high-energy-density, especially if it is cooked twice. For example, toasting whole-wheat bread increases its energy density from 2.5 to 3.1 kcal g^{-1} as water content decreases by 14% (data calculated from USDA National Nutrient Database for Standard Reference). Nanoscale science and

technology are now enabling us to understand many natural and unnatural processes. Studying nanostructures at the cell and DNA level gives us insight into the working of these processes and how to manipulate, prevent, and/or enhance them for the benefit of mankind.

6. ROADMAPPING THE FUTURE

There are more humans on Earth than can be sustained by the natural world. Thus, the nutritional value of processed and farmed foods will be increasingly based on the nutritional value of the late Paleolithic human diet to help prevent diet–induced mental ill health, because unbiased observers agree that nutritional advice from conventional sources, whether based on epidemiologic or mechanistic findings, has not affected complex degenerative disease incidence/prevalence as much as hoped (Eaton et al., 2010). Furthermore, modern animal husbandry caused the rise in the production of high fat meat with a low nutrient density[2] and it will have to be corrected because of its negative effects on animal welfare and human nutrition (Wang et al., 2010; Ametaj et al., 2010; Daley et al., 2010; Jonsson et al., 2006). Food products and wellness programs that help prevent the causal mechanisms of mental ill–health will be of great benefit to the mankind (Lands, 2009; Robson, 2010b). Emergent technologies can enhance the cleaning and management of lakes, rivers, estuaries, and coastal waters to restore and enhance CO_2 and nitrogen fixation and simultaneously provide more sustainable foods rich in bioavailable brain nutrients, for example, omnivorous shellfish (Robson, 2006), to help prevent the current epidemic of mental ill–health. Emergent technologies will change the society beyond anything that has gone before. This should, but not with any certainty, eventually slow down the spiraling increase in healthcare costs (Tolfree and Smith, 2009).

7. CONCLUSION

Mental ill health is an epidemic worldwide because of the combined effect of the modern diet and a sedentary lifestyle (e.g. Robson, 2013b). A low-energy-dense diet rich in bioavailable brain nutrients-plus-exercise is most effective for preventing mental ill-health throughout life. Obesity in pregnancy and poor human nutrition must be tackled head-on. Human food production must be linked to human nutritional requirements as its first priority. High-energy-density and low nutrient density which characterize the modern diet must be overcome simultaneously. Nanocellulose and calorie-free monk fruit extract could be used to lower the energy density of processed foods/drinks, and their bioavailable brain nutrient content including cofactors can be increased using bioactive encapsulation. Aquatic biotechnology can provide all the nutrients needed to make processed foods really nutritious. In conclusion, the nutritional value of processed and farmed foods should be

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[2] Compare the energy density and protein content of farmed and wild salmon in Table 14.2.

based on the nutritional value of the late Palaeolithic human diet to help prevent mental ill-health and other postprandial insults (Robson, 2009, Robson, 2013b).

REFERENCES

Adamo, A.M., Oteiza, P.I., 2010. Zinc deficiency and neurodevelopment: the case of neurons. BioFactors 36 (2), 117–124 10.1002/biof.91.

Ametaj, B.N., Zebeli, Q., Saleem, F., et al., 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics 6, 583–594.

Baba, N.H., Sawaya, S., Torbay, N., Habbal, Z., Azar, S., Hashim, S.A., 1999. High protein vs high carbohydrate hypoenergetic diet for the treatment of obese hyperinsulinemic subjects. International Journal of Obesity 23, 1202–1206.

Barker, D.J., 2004. The developmental origins of chronic adult disease. Acta Paediatrica 93, 26–33.

Bartels, M., van Beijsterveldt, C.E.M., Boomsma, D.I., 2009. Breastfeeding, maternal education and cognitive function: a prospective study in twins. Behavior Genetics 39, 616–622.

Benton, D., 2008. Micronutrient status, cognition and behavioral problems in childhood. European Journal of Nutrition 47 (supplement 3), 38–50.

Berry, M.J., Ralston, N.V.C., 2008. Mercury toxicity and the mitigating role of selenium. EcoHealth 5, 456–459.

Brand-Miller, J.C., Holt, S.H.A., 1998. Australian Aboriginal plant foods: a consideration of their nutritional composition and health implications. Nutrition Research Reviews 11, 5–23.

Carmody, R.N., Wrangham, R.W., 2009. The energetic significance of cooking. Journal of Human Evolution 57, 379–391.

Chang, S.-T., Chen, L.-C., Lin, S.-B., Chen, H.-H., 2012. Nano-biomaterials application: Morphology and physical properties of bacterial cellulose/gelatin composites via crosslinking. Food Hydrocolloids 27 (1), 137–144.

Chen, L.W., Appel, L.J., Loria, C., et al., 2009. Reduction in consumption of sugar–sweetened beverages is associated with weight loss: the PREMIER trial. The American Journal of Clinical Nutrition 89, 1299–1306.

Centre for Mental Health, 2010. The Economic and Social Costs of Mental Health Problems in 2009/10. Centre for Mental Health, London. (http://www.centreformentalhealth.org.uk/pdfs/Economic_and_social_costs_2010.pdf)

Chen, L., Remondetto, G.E., Subirade, M., 2006. Food protein-based materials as nutraceutical delivery systems. Trends in Food Science and Technology 17, 272–283.

Church, M.W., Jen, K.-L.C., Jackson, D.A., Adams, B.R., Hotra, J.W., 2009. Abnormal neurological responses in young adult offspring caused by excess omega-3 fatty acid (fish oil) consumption by the mother during pregnancy and lactation. Neurotoxicology and Teratology 31, 26–33.

Cordain, L., Eaton, S.B., Sebastian, A., et al., 2005. Origins and evolution of the Western diet: health implications for the 21st century. The American Journal of Clinical Nutrition 81, 341–354.

Crawford, M.A., Bazinet, R.P., Sinclair, A.J., 2009. Fat intake and CNS functioning: ageing and disease. Annals of Nutrition and Metabolism 55, 202–228.

Crovetti, R., Porrini, M., Santangelo, A., Testolin, G., 1998. The influence of thermic effect of food on satiety. European Journal of Clinical Nutrition 52, 482–488.

Cunnane, S.C., Plourde, M., Stewart, K., Crawford, M.A., 2007. Docosahexaenoic acid and shore-based diets in hominin encephalization: a rebuttal. American Journal of Human Biology 19, 578–581.

de Benoist, B., McLean, E., Andersson, M., Rogers, L., 2008. Iodine deficiency in 2007: global progress since 2003. Food and Nutrition Bulletin 29, 195–202.

Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A., Larson, S., 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9, 10.

Doyle, W., Crawford, M.A., Wynn, A.H.A., Wynn, S.W., 1989. Maternal nutrient intake and birth weight. Journal of Human Nutrition and Dietetics 2, 407–414.

Eaton, S.B., Konner, M., 1985. Paleolithic nutrition – a consideration of its nature and current implications. The New England Journal of Medicine 312, 283–289.

Eaton, S.B., Konner, M.J., Cordain, L., 2010. Diet-dependent acid load, paleolithic nutrition, and evolutionary health promotion. The American Journal of Clinical Nutrition 91, 295–297.

Farhang, B., 2007. Nanotechnology and lipids. Lipid Technology 19, 132–135.

First Data Bank, 2000. Nutritionist v Nutrition Software, Version 2.3. First Data Bank, San Bruno, CA.

Gao, S.J., Jin, Y.L., Hall, K.S., et al., 2007. Selenium level and cognitive function in rural elderly Chinese. American Journal of Epidemiology 165, 955–965.

Gartner, L.M., Morton, J., Lawrence, R.A., et al., 2005. Breastfeeding and the use of human milk. Pediatrics 115, 496–506.

Georgieff, M.K., 2007. Nutrition and the developing brain: nutrient priorities and measurement. The American Journal of Clinical Nutrition 85, 614S–620S.

Gomez-Pinilla, F., 2008. Brain foods: the effects of nutrients on brain function. Nature Reviews. Neuroscience 9, 568–578.

Graveland-Bikker, J.F., De Kruif, C.G., 2006. Unique milk protein based nanotubes: food and nanotechnology meet. Trends in Food Science and Technology 17, 196–203.

Harker, R., 2012. NHS funding and expenditure. House of Commons Library - Standard Note: SN/SG/724. http://www.parliament.uk/business/publications/research/briefing-papers/SN00724.

Harun, R., Singh, M., Forde, G.M., Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews 14, 1037–1047.

Helland, I.B., Smith, L., Saarem, K., Saugstad, O.D., Drevon, C.A., 2003. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 111, e39–e44.

Hibbeln, J., Davis, J., Steer, C., et al., 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 369, 578–585.

Horta, B.L., Bahl, R., Martinés, J.C., Victora, C.G., 2007. Evidence on the long-term effects of breastfeeding: systematic reviews and meta-analysis. World Health Organization, Geneva, Switzerland.

Hsieh, Y.H.P., Ofori, J.A., 2007. Innovations in food technology for health. Asia Pacific Journal of Clinical Nutrition S16, 65–73.

Klemm, D., Kramer, F., Moritz, S., et al., 2011. Nanocelluloses: a new family of nature-based materials. Angewandte Chemie International Edition 50 (24), 5438–5466.

Klemm, D., Schumann, D., Kramer, F., et al. (Eds.), 2006. Nanocelluloses as innovative polymers in research and application. In: Polysaccharides II, vol. 205. Springer, Berlin/Heidelberg.

Johnston, C.S., Tjonn, S.L., Swan, P.D., 2004. High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults. The Journal of Nutrition 134, 586–591.

Jonsson, T., Ahrén, B., Pacini, G., et al., 2006. A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs. Nutrition and Metabolism 3, 39.

Jones, S., 2009. Genetic 'magic bullet' cures have proven a 'false dawn'. Daily Telegraph. http://www.telegraph.co.uk/science/science-news/5190914/Genetic-magic-bullet-cures-have-proven-a-false-dawn.html (21.04.2009).

Lands, B., 2009. False profits and silent partners in health care. Nutrition and Health 20, 79–89.

Layman, D.K., 2003. The role of leucine in weight loss diets and glucose homeostasis. The Journal of Nutrition 133, 261S–267S.

Ledikwe, J.H., Blanck, H.M., Kettel Khan, L., et al., 2006. Dietary energy density is associated with energy intake and weight status in US adults. The American Journal of Clinical Nutrition 83, 1362–1368.

Liu, L., Liu, C.C., Li, J.L., 2012. Comparison of biochemical composition and nutritional value of Antarctic krill (Euphausia Superb) with several species of shrimps. Advanced Materials Research 361–363, 799–803.

Marsh, D., 2012. Darwin's passionate environmentalism or the dangerous fallacy of the 'All-sufficiency of natural selection' theory. Nutrition and Health 21, 76–90.

Moreno, C., Laje, G., Blanco, C., Jiang, H., Schmidt, A.B., Olfson, M., 2007. National trends in the outpatient diagnosis and treatment of bipolar disorder in youth. Archives of General Psychiatry 64, 1032–1039.

Murray-Kolb, L.E., Beard, J.L., 2007. Iron treatment normalizes cognitive functioning in young women. The American Journal of Clinical Nutrition 85, 778–787.

Neethirajan, S., Jayas, D.S., 2011. Nanotechnology for the food and bioprocessing industries. Food and Bioprocess Technology 4 (1), 39–47.

Norton, J., Fryer, P., Parkinson, J., Cox, P., 2009. Development and characterisation of tempered cocoa butter emulsions containing up to 60% water. Journal of Food Engineering 95 (1), 172–178.

Nurse, J., 2008. Mental health – new horizons after the national service framework. Westminster Health Forum Keynote Seminar 17th July 2008. Westminster Health Forum, London.

Odea, K., 1984. Marked improvement in carbohydrate and lipid-metabolism in diabetic Australian aborigines after temporary reversion to traditional lifestyle. Diabetes 33, 596–603.

Odea, K., Traianedes, K., Ireland, P., et al., 1989. The effects of diet differing in fat, carbohydrate, and fiber on carbohydrate and lipid-metabolism in type-II diabetes. Journal of the American Dietetic Association 89, 1076–1086.

Office for National Statistics (2011) Statistical Bulletin. Regional, sub-regional and local Gross Value Added 2010. http://www.ons.gov.uk/ons/rel/regional-accounts/regional-gross-value-added–income-approach-/december-2010/stb-regional-gva-dec-2011.html.

Ortiz, J., Romero, N., Robert, P., et al., 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chemistry 99, 98–104.

Pembrey, M.E., Bygren, L.O., Kaati, G., et al., 2006. Sex-specific, male-line transgenerational responses in humans. European Journal of Human Genetics 14, 159–166.

Rajasingam, D., Seed, P.T., Briley, A.L., Shennan, A.H., Poston, L., 2009. A prospective study of pregnancy outcome and biomarkers of oxidative stress in nulliparous obese women. American Journal of Obstetrics and Gynecology 200, 395 e1–9.

Rees, G., Doyle, W., Srivastava, A., Brooke, Z.M., Crawford, M.A., Costeloe, K.L., 2005. The nutrient intakes of mothers of low birth weight babies-a comparison of ethnic groups in East London, UK. Maternal & Child Nutrition 1, 91–99.

Robson, A., 2006. Shellfish view of omega-3 and sustainable fisheries. Nature 444, 1002.

Robson, A.A., 2009. Preventing diet induced disease: bioavailable nutrient-rich, low-energy-dense diets. Nutrition and Health 20, 135–166.

Robson, A.A., 2010a. Creating convenience food based on human nutritional requirements. Azo Nanotechnology 2635, 336–361.

Robson, A.A., 2010b. Nanotechnologies and food: 1st report of session 2009–10. In: House of Lords Papers 22-II 2009–10. Evidence, vol. 2. The Stationery Office, London, pp. 336–361.

Robson, A.A., 2011. Food nanotechnology: water is the key to lowering the energy density of processed foods. Nutrition and Health 20 (3–4), 231–236.

Robson, A. A., 2012. Tackling obesity: can food processing be a solution rather than a problem? Agro Food Industry Hi-Tech 23(2 monographic supplement series: Weight Management), 10–11.

Robson, A.A., 2013a. Chocolate bars based on human nutritional requirements. In: Watson, R.R., Preedy, V.R., Zibadi, S. (Eds.), Chocolate in Health and Nutrition. In: Nutrition and Health, vol. 7. pp. 143–148. Humana Press, New York. DOI http://dx.doi.org/10.1007/978-1-61779-803-0_12.

Robson, A.A., 2013b. Preventing the epidemic of non-communicable diseases: an overview. In: Watson, R.R., Preedy, V.R. (Eds.), Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease. Academic Press, San Diego

Seino, Y., Seino, S., Ikeda, M., Matsukura, S., Imura, H., 1983. Beneficial-effects of high protein-diet in treatment of mild diabetes. Human Nutrition. Applied Nutrition 37, 226–230.

Skov, A.R., Toubro, S., Ronn, B., Holm, L., Astrup, A., 1999. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. International Journal of Obesity 23, 528–536.

Spinillo, A., Stronati, M., Ometto, A., Fazzin, E., Lauzin, G., Guaschino, S., 1993. Infant neurodevelopmental outcome in pregnancies complicated by gestational hypertension and intrauterine growth-retardation. Journal of Perinatal Medicine 21, 195–203.

Stubbs, R.J., 1998. Appetite, feeding behaviour and energy balance in human subjects. The Proceedings of the Nutrition Society 57, 341–356.

Tafti, M., Ghyselinck, N.B., 2007. Functional implication of the vitamin A signaling pathway in the brain. Archives of Neurology 64, 1706–1711.

Takeda, A., 2003. Manganese action in brain function. Brain Research Reviews 41, 79–87.

Tassoni, D., Kaur, G., Weisinger, R.S., Sinclair, A.J., 2008. The role of eicosanoids in the brain. Asia Pacific Journal of Clinical Nutrition 17 (supplement 1), 220–228.

Tolfree, D., Smith, A., 2009. Roadmapping Emergent Technologies. Matador, Leicester.

Torrey, E.F., Miller, J., 2002. The Invisible Plague: The Rise of Mental Illness from 1750 to the Present. Rutgers University Press, New Jersey.

Van de Berg, D.J., Huitinga, I., Hol, E.M., 2010. Longterm quiescent cells in the aged human subventricular neurogenic system specifically express GFAP-δ. Aging Cell 9 (3), 313–326. http://dx.doi.org/10.1111/j.1474-9726.2010.00556.x.

Veldhorst, M., Smeets, A., Soenen, S., et al., 2008. Protein-induced satiety: effects and mechanisms of different proteins. Physiology and Behavior 94, 300–307.

Wang, Y., Lehane, C., Ghebremeskel, K., Crawford, M.A., 2010. Modern organic and broiler chickens sold for human consumption provide more energy from fat than protein. Public Health Nutrition 13, 400–408.

Westerterp-Plantenga, M.S., Lejeune, M., Nijs, I., van Ooijen, M., Kovacs, E.M.R., 2004. High protein intake sustains weight maintenance after body weight loss in humans. International Journal of Obesity 28, 57–64.

World Health Organization, 2002. Global strategy for infant and young child feeding. Report by the secretariat (A55/15, paragraph 10, page 5) 16 April 2002. World Health Organization, Geneva, Switzerland.

World Health Organization, 2010. Promoting proper feeding for infants and young children. http://www.who.int/nutrition/topics/infantfeeding/en/.

Wynn, S.W., Wynn, A.H.A., Doyle, W., Crawford, M.A., 1994. The association of maternal social class with maternal diet and the dimensions of babies in a population of London Women. Nutrition and Health 9, 303–315.

RELEVANT WEBSITES

http://www.mccarrisonsociety.org.uk/ – The McCarrison Society for Nutrition and Health.

http://www.mother-and-child.org/ – The Mother and Child Foundation – working for good health in mothers and children.

http://www.who.int/en/ – World Health Organization.

Energy Metabolism and Diet: Effects on Healthspan

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1. INTRODUCTION

Among the enormous literature on theoretical explanations of aging, the role of energy metabolism has received the most attention. For thousands of years, energy metabolism has provided an understanding of how we function today, tomorrow, and eventually when we cease to function altogether. The study of metabolism and aging has been spurred along by the intriguing association between high rates of energy expenditure (EE) in short–lived mammals and low rates of EE in long–lived mammals. Proponents of the 'rate of living theory' believe that EE per lifespan is fixed, and abundant usage will accelerate aging. While the rate of living theory is no longer accepted (Holloszy and Smith, 1986), energy metabolism and the biological processes that control the machinery are under intense study.

The amount and type of food intake can impact energy metabolism to a degree that might alter cellular processes involved in aging. A conceptual cure illustrating this path–way is depicted in Figure 15.1. This model demonstrates the inter–relatedness of energy metabolism and energy intake with aging process. Some bioactive foods have promising capability to alter sub–cellular components of energy metabolism that might feed back to the phenotype of energy balance.

1.1 Total Energy Expenditure and Aging

Total energy expenditure is comprised of three major components that include: resting metabolic rate, activity energy expenditure, and energy due to the thermic effect of food (Figure 15.2). Resting metabolic rate (RMR) can be further divided into sleeping and arousal EE, where simple arousal is associated with a 10% increase in EE above that seen during sleeping (Ravussin et al., 1986). Activity EE is composed of EE due to volitional exercise (i.e., jogging, walking for exercise, etc.) and non–exercise activity thermogenesis (NEAT). NEAT is the energy expended from spontaneous physical activities that include non–volitional movements (i.e., fidgeting) but is often defined as any EE outside formal volitional exercise programs. More details about each of these components and their re–spective changes with age are outlined in subsequent sections.
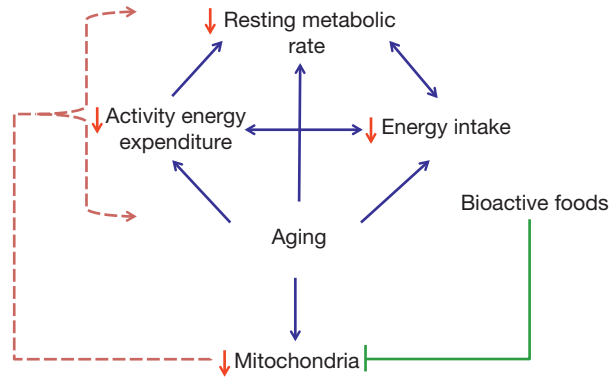
Figure 15.1 Conceptual model of how aging is associated with changes in energy metabolism and the influences of mitochondria. Bioactive foods have potential to modulate mitochondrial function that feed-forward onto components of energy metabolism.
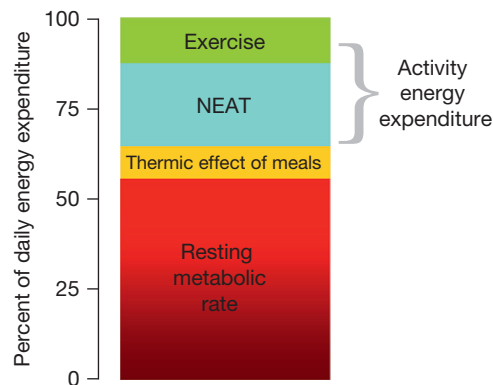
Figure 15.2 Categories of total energy expenditure. Resting metabolic rate (RMR) accounts for 60–80% of the total, thermic effect of food (TEF) metabolism uses 10% of total, activity energy expenditure (AEE) is the most variable component and can be divided into volitional exercise and non-exercise activity thermogenesis (NEAT). Exercise and NEAT can comprise 20–50% of total. *Reprinted from Manini, T.M., 2010. Energy expenditure and aging. Ageing Research Review, 9, 1–11. With permission.*

Total EE demonstrates an inverted U pattern across the lifespan. In the first two decades of life, TEE increases twofold and mirrors the increase in body mass (see Figure 15.3). These changes plateau during the next two decades of life between 17 and 40 years of age, and again this appears to mirror changes in body mass. Following the age of 40 years, TEE begins to decline quite dramatically to a point where 75 year olds experience TEE levels similar to a 7–11 year old, despite having significantly greater body mass.
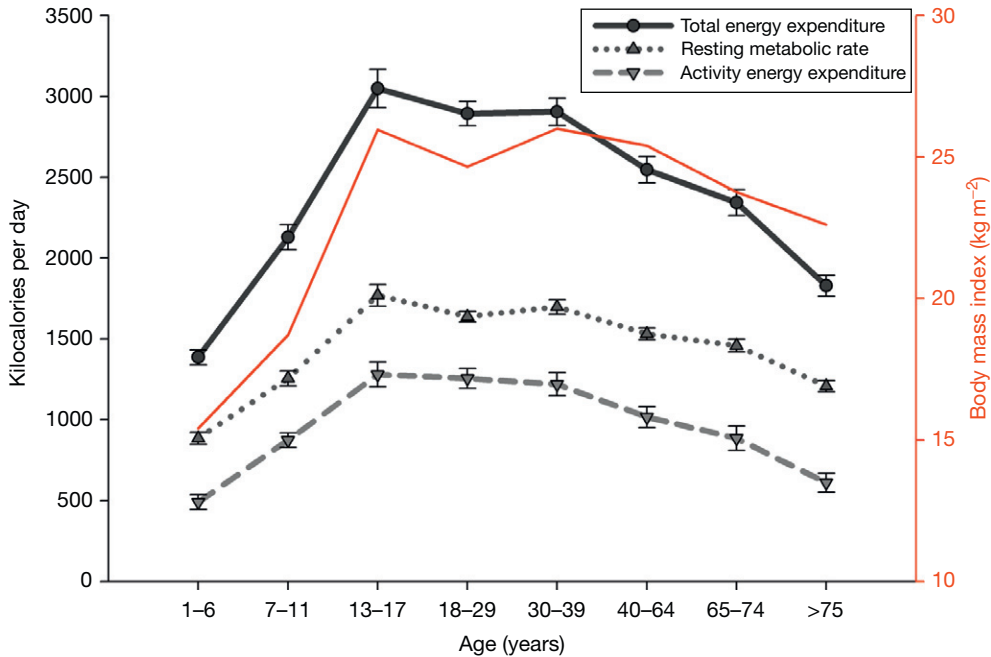
Figure 15.3 Age-related changes in categories of energy expenditure. Body mass index is also plotted as a function of age to evaluate the tracking association between energy expenditure and body mass with age. Data reproduced from Table 3 in Black et al. (1996) demonstrates a decrease in all components of energy expenditure with increasing age. *Reprinted from Manini, T.M., 2010. Energy expenditure and aging. Ageing Research Review, 9, 1–11. With permission.*

1.2 Resting Metabolic Rate and Aging

Resting metabolic rate composes 60–80% of total EE and is primarily responsible for the maintenance of organismal homeostasis. Aging is associated with a progressive decline in whole-body RMR at a rate of 1–2% per decade after 20 years of age (see Figure 15.3). This decline is closely linked with the decrease in whole body fat-free mass, which is composed of metabolically active tissues and organs. However, there is some debate whether the decrease in RMR is entirely due to changes in fat-free mass. This debate is triggered by findings that an adjustment for body composition does not fully account for differences in RMR between young and old adults (Krems et al., 2005). In other words, RMR remains significantly lower in the elderly even after correcting for body composition differences. A greater understanding of this finding can be gleaned from the following equation:

$$RMR = \Sigma(Met_i \times Mass_i)$$

where, $Mass_i$ is the mass of that organ, and Met_i is the specific metabolic rate of an in-dividual organ expressed as kcal kg^{-1} d^{-1}, which represents the metabolic rate of individual cells within that organ.

Organ masses ($Mass_i$) have been assessed since the first report in 1926 where autopsy studies were performed on approximately 2200 men and women (Bean, 1926). These data suggested a 32% reduction in liver mass, a 23% reduction in kidney mass, a 55% re-duction in spleen mass, but a 10% increase in heart mass from age 20 to 80 years. New *in vivo* data using magnetic resonance imaging suggest that mass of the brain, bone, kidney, liver, and muscle decline at relatively similar rates, between 10% and 20% from the ages of 20 to 80 years (Figure 15.4). These data suggest that the decline in the mass of most organs undoubtedly contributes to the decrease in RMR with age.

The other component of the RMR equation, Met_i, is determined by cellular fractions in tissue, mitochondrial proton leak, protein turnover, and Na^+-K^+-ATPase (Rolfe and Brown, 1997). As such, Met_i is not easily measured *in vivo*, and, thus, much of the
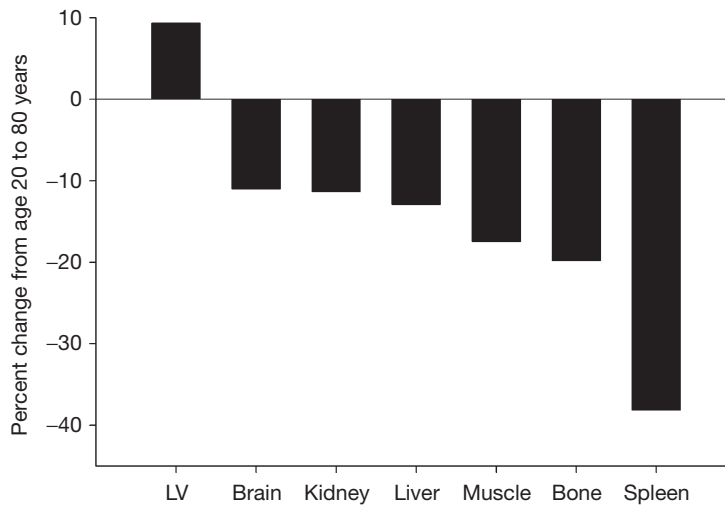
Figure 15.4 Organ mass changes from age 20 to 80 years. Predicted organ mass change from age 20 to 80 years. Regression coefficients from He et al. (2009) were used to estimate a percent change in the left ventricle (LV) of the heart, brain, kidney, liver, and spleen mass. The predicted values were derived by fitting the model for an African-American women with a height of 1.6 m and body mass of 70 kg. Kidney and spleen masses estimated with the natural logarithm were exponentiated for calculating values presented in the figure. Appendicular muscle mass was estimated using regression coefficients from Gallagher et al. (1997), where body mass and height were used to predict age-related change in African-American women. Bone mass (i.e. bone mineral content) was estimated using data in women from Rico et al. (1993). Data from Rico et al. were not adjusted for body mass and no information was given as to the race distribution of the subject sample. *Reprinted from Manini, T.M., 2009. Organ-o-penia. Journal of Applied Physiology 106, 1759–1760. With permission.*

literature relies simply on organ mass or whole body fat-free mass assuming that specific metabolic rates and the cellularity that composes it are constant across the lifespan. However, new data that assessed whole body cellularity using body cell mass as a function of fat-free mass (BCM/FFM) found that up to the age of 50 years cellularity is accurately predicted by RMR, but severely underestimates RMR after the age of 50 years. This systematic underestimation is related to a lower cell fraction of fat-free mass, indicating a low metabolic rate per unit of tissue. Therefore, the homogeneity of specific metabolic rate across the lifespan should not be assumed, and this factor may explain the remaining variance associated with age-associated declines in RMR. A conceptual model of contributors to age-related changes in RMR is illustrated in Figure 15.5.

1.3 Activity Energy Expenditure and Aging

Activity EE (AEE) is comprised of two categories: volitional exercise and non-exercise movements and are highly variable (Levin et al., 1999). It is the most difficult to measure, and thus the least studied, component of total EE with regard to aging. AEE has a strong environmental component with a large range in humans, from 262 kcal day^{-1} in demented elderly, to 1434 kcal day^{-1} in mountaineers on Mount Everest, to 6333 kcal day^{-1} in cyclists in the Tour de France. AEE is also tied to underlying genes, as evidenced in familial–based and monozygotic twin studies (Goran, 1997). These studies suggest that genetics explain 72% of the variance in AEE. These data suggest that while there is an obvious environmental influence, underlying genetic makeup partially controls AEE.
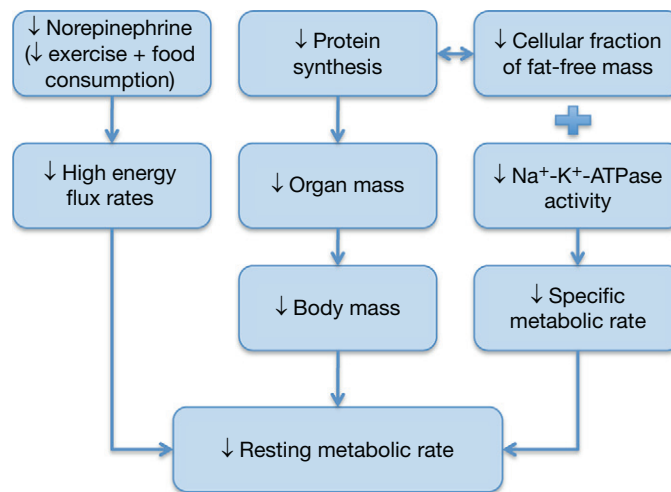
Figure 15.5 Schematic of biological control processes for age-related decrease in resting metabolic rate. *Reprinted from Manini, T.M., 2010. Energy expenditure and aging. Ageing Research Review, 9, 1–11. With permission.*
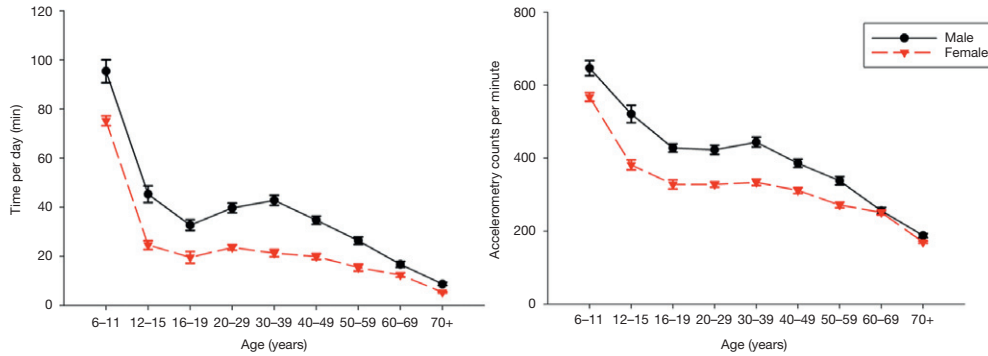
Figure 15.6 Volitional physical activity levels across the lifespan in a nationally representative sample of Americans (National Health and Nutrition Examination Survey – NHANES 2003–2004) over 7 days. Physical activity was measured by accelerometry as (a) mean counts per minute and (b) time spent in moderate or vigorous physical activity according to an activity count threshold.

Despite having a genetic component, AEE demonstrates a sharp decline with increasing age. In a 25-year longitudinal study conducted by Westerp and Meijer, AEE decreased by 9% and 25% at 60–74 and >75 years of age, respectively (Westerterp and Meijer, 2001). This reduction in AEE accounted for the major determinate of age-associated decline in total EE. Interestingly, changes in fat-free mass did not explain the reductions in AEE supporting an innate biological adaptation with age. Data on a US national representative sample has provided more evidence that aging is associated with a drastic decline in activity EE. Troiano and coworkers placed accelerometers (devices that measures amount and intensity of movement) on the hip of 4867 individuals across the age spectrum. Figure 15.6a and 15.6b illustrates data where moderate and vigorous activity (combined) of men and women decline in a rapid manner after 11 years of age and again after the age of 39 years. The amount of time performing moderate or vigorous intensity activity decreased from 42 min day^{-1} (21 min day^{-1} in women) to 8.7 min day^{-1} (5.4 min day^{-1} in women) in men from age 39 to 70+ years. This represents an approximately 75% decline in time spent performing moderate and vigorous intensity activity in both men and women. Objective data from a nationally representative sample of US persons strongly suggests that volitional physical activity declines with increasing age.

1.4 Activity Energy Expenditure and Energy Intake

Modulations in AEE have been closely linked with changes in energy intake (EI) or dietary patterns across the lifespan in both animal and human studies. Maintaining an optimal energy balance represents an adaptive response across species; therefore, reductions in AEE typically result in decreased EI. Similarly, lowering EI, as is done in caloric restriction (CR) research, typically leads to less AEE. Changes in body weight and
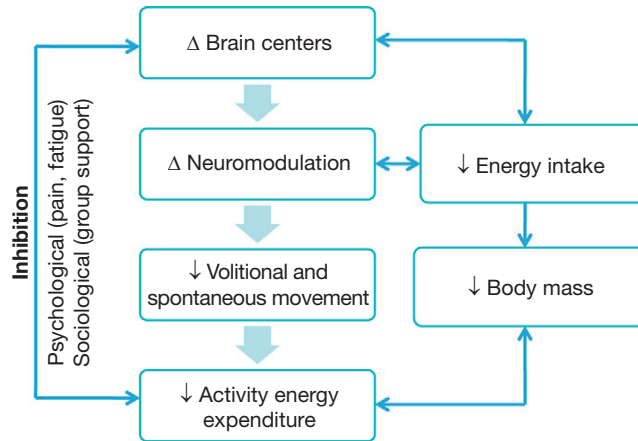
Figure 15.7 Schematic of the control processes for decreases in activity energy expenditure with age. *Modified from Manini, T. M., 2010. Energy expenditure and aging. Ageing Research Review, 9, 1–11.*

composition result when this relationship becomes unbalanced. As was described in previous sections, alterations in body weight directly influence RMR. Consequently, changes in AEE and energy metabolism across the lifespan have widespread implications, including effects on eating behavior and health status. See Figure 15.7 for a conceptual model illustrating the association between AEE and EI.

1.5 Balancing Energy Expenditure and Energy Intake: Findings from Animals and Young Adults

Previous research in animals and humans manipulating either AEE or EI illustrates that the body counters reductions on one side of the energy balance relationship by lowering the other to return to homeostasis. For example, if EI is decreased, animals typically respond by initially increasing foraging activity in response to insufficient food (Chen et al., 2005), but then significantly minimize their AEE. While the relationship between EE and EI has been evaluated within a number of different species, confounding variables, such as micronutrient deficiency resulting from CR, has been most effectively controlled in work with rodents. Though the level of restriction and diet composition varies considerably across rodent studies, animals consistently respond to reductions in EI by reducing their AEE (Ramsey et al., 2000). Long-term maintenance of reduced EI, however, eventually reduces this effect and AEE normalizes. Future research assessing the effects of CR on cellular and organ EE is required to better understand acute and chronic changes in AEE in response to reduced EI.

Similarly, research on humans demonstrates alterations in EE including both volitional exercise and NEAT are significantly related to caloric consumption. The strength

of the association between volitional exercise and EI in young adults has been debated for decades. Traditional beliefs hold that exercise increases EI, while challengers assert that changes in EI are only loosely related to bouts of exercise. Converging evidence indicates that volitional exercise does increase appetite and the physiological need to increase EI; however, the relationship is reduced by the fact that not all individuals act on these physiological responses due to environmental and social factors (Blundell and King, 1999).

NEAT levels and corresponding EI have been closely investigated in overweight and obese young adults. Findings from large-scale randomized clinical trials corroborate animal research by demonstrating reductions in EI result in significant decreases in free-living physical activity (Martin et al., 2011). Interestingly, an opposite response occurs with excess EI. Levine and colleagues (Levine et al., 1999) have illustrated that some individuals have increased NEAT with overeating that allows them to thwart gains in body mass. Activation of NEAT in response to high EI is not completely understood, although it is thought to rise from specific brain centers namely the orexins that connect brain reward regions (Garland et al., 2011), resulting in greater spontaneous physical activity, to prevent weight gain. Accordingly, Levine argues inadequate adjustments of NEAT represent a major cause of obesity, but this lack of adjustment seems to be under tight biological control.

1.6 Essential Foods: Energy Intake and Aging

Though the dynamic between AEE and EI plays an important role across all stages of the lifespan, age-related changes in AEE cause this relationship to become less stable as people age, leading to unfavorable eating behaviors, reductions in body weight, and adverse health outcomes. Both volitional exercise and NEAT tend to decline with advancing age, which provides a likely explanation for the linear decrease in caloric intake and reductions in body weight, commonly witnessed in even healthy older adults (Morley, 2001). As such, age-related reductions in energy intake are closely linked to changes in energy expenditure.

Longitudinal studies indicate average EI decreases by approximately 30% between the ages of 20 and 80 years (Hallfrisch et al., 1990), which can translate to a loss between 1000 and 1200 kcal day^{-1} for men and 600 and 800 kcal day^{-1} for women. Such pronounced age-related reductions in caloric consumption, a phenomenon known as the anorexia of aging, has been demonstrated in animal models and humans (Morley, 2003) and is often associated with undesirable changes in body mass. Additionally, older adults appear to have difficulty returning their energy balance to homeostatic levels, which is evidenced by reductions in EI that exceed the decrease in EE. Such an energy imbalance frequently results in unintentional weight loss and deleterious changes in body composition in older adults. For example, individuals in westernized countries tend to gain weight until they reach 50–60 years of age, stabilize for several years, then subsequently lose 0.5–4% of

their body weight annually (Chapman, 2010). The majority of this decrease in body weight in older adults is generally due to loss of fat-free body mass (i.e., sarcopenia). Unintentional weight loss, particularly when it involves significant reductions in lean body mass, has been associated with frailty and numerous adverse health outcomes, including mortality, malnutrition, and cachexia following the onset of illness (Morley, 2003).

Importantly, while decreased EE likely plays the predominant role in reducing EI among older adults, suggesting EE solely explains that lower caloric consumption neglects the multifaceted nature of the anorexia of aging syndrome (Morley, 2003). Most theorists agree that age-related decline in food intake is caused by a blend of social, emotional, cultural, financial, and physiologic mechanisms. Specifically, social isolation and depression become increasingly common as people age and have been significantly tied to decreased EI, particularly in long-term care setting. Physiological changes, such as increased satiety and decline in pleasurable response to food, also lead to reductions in EI. Whether these factors are involved in impaired regulation of energy balance remains unclear. While the aforementioned factors certainly contribute to changes in EI, researchers generally accept that reduction in caloric consumption is mainly a physiological condition rooted in declines in EE.

1.7 Non-essential Bioactive Foods: Effects on Sub-cellular Energy Metabolism Processes

Research has recently implicated a strong role of nutrient availability/sensing in aging and lifespan determination. For example, nearly all studies to date have shown that caloric restriction (CR) increases lifespan and improves many of the age-associated declines in cellular function in a variety of organisms. Lifespan extension by CR usually involves beneficial modulation in cellular metabolism and proliferation of mitochondrial activity (Merry, 2004). Mitochondria have a central role in cellular metabolism and their dysfunction has been associated with cardiovascular, metabolic, and neurodegenerative diseases. Furthermore, reactive oxygen species (ROS) generated from mitochondria respiration have been shown to contribute to the aging process by damaging cellular components, and ultimately leading to deterioration in cell, tissue, and organism function over time.

Although CR continues to be the most robust aging intervention in a wide variety of species, the utility of this intervention in humans is limited by the *amount* and *duration* of restriction required for many of its beneficial effects. For example, animal models typically show that 30–40% CR for a duration equivalent to at least 12–20 years in humans is effective as a pro-longevity strategy. Importantly, recent human studies have demonstrated that 25% CR for 6 months to 1 year is capable of acutely reproducing many of the metabolic and physiological responses observed in animals (Fontana, 2009). However, questions remain regarding whether the beneficial effects of CR in humans are stable and whether humans could maintain low-calorie diets for prolonged periods. As such, interest is growing in the field of CR mimetics – interventions that mimic

the metabolic, hormonal, and physiological effects of CR and produce CR-like effects on aging and lifespan without having to reduce long-term food intake. While we are unable to review all the potential natural CR surrogates, we have highlighted two bioactive non-essential foods that have generated the most evidence to date as a CR mimetic, with a focus on the metabolic mechanisms by which they exert their effects.

1.7.1 Resveratrol

Resveratrol (RSV), a natural polyphenolic compound primarily found in the skins of grapes, is a type of CR mimetic shown to exert diverse antiaging effects in animal models. Baur et al. (2006) provided the first key evidence that RSV may be a valuable tool in the regulation of energy balance, health, and longevity. The authors showed that middle-aged mice on a high-calorie diet treated with RSV for the remainder of their lives exhibited increased survival, improved insulin sensitivity, increased mitochondria in the liver, and increased activity of the metabolic regulator AMP-activated protein kinase (AMPK). Further exploring the mechanism through which RSV impacts health and longevity, Lagouge and colleagues (Lagouge et al., 2006) examined whether RSV administered in either a chow diet or high fat (HF) diet for 15 weeks impacts mitochondrial function and metabolic homeostasis in mice. RSV treatment increased mitochondrial activity in both the liver and brown adipose tissue, translating to improved aerobic capacity and increased energy expenditure. Specifically, RSV treatment of HF-fed mice increased running time and consumption of oxygen in muscle fibers during an endurance test, increased cold tolerance during a cold test, and significantly increased EE (oxygen consumption), which could not be explained by an increase in spontaneous activity. Additionally, the HF-fed mice treated with RSV were protected from the development of obesity and showed improved insulin sensitivity independent of effects on body weight. Importantly, underlying these metabolic changes in the RSV-treated mice were striking mitochondria morphological changes (i.e., mitochondrial size and mitochondrial DNA content) in the muscle and brown adipose tissue and a significant increase in gene expression of pathways related to energy homeostasis in brown adipose tissue. In line with this work, Csiszar and colleagues (Csiszar et al., 2009) found that RSV increased mitochondria mass and mitochondrial DNA content and induced mitochondrial biogenesis factors in cultured human coronary arterial endothelial cells. Collectively, these data indicate that RSV may be able to alleviate the deleterious effect of a high calorie diet on overall health and lifespan. Furthermore, RSV's ability to induce mitochondrial proliferation in several highly oxidative tissues may translate into a general shift toward a more oxidative metabolism, as seen with CR.

1.7.2 Rapamycin

Rapamycin was initially identified as a new antibiotic with strong antifungal activity, secreted by bacteria isolated from Easter Island. Rapamycin, via TOR inactivation, has been implicated in lifespan extension in simple organisms (i.e., yeast and flies) and

recently in mammals (i.e., mice) (Harrison et al., 2009). Harrison et al. was the first to demonstrate that dietary-encapsulated rapamycin administered late in life extends the lifespan of genetically intact mammalian species of both genders. Specifically, rapamycin increased mean lifespan of mice by 13% for females and 9% for males, and increased life expectancy by 38% and 28% for females and males, respectively. These results are consistent with the hypothesis that reduced signaling of the mTORC1 protein complex of the mammalian target of rapamycin (mTOR) extends chronological lifespan in mammalian species (Cota, 2009). mTOR is an intracellular nutrient-sensing protein that regulates autophagy, cell growth, and the transcription of many genes involved in metabolic and biosynthetic pathways. mTOR inhibition has been implicated as an underlying mechanism for lifespan extension by caloric restriction, whereas unrestrained up-regulation of mTOR signaling has been associated with cancer, inflammation, and diabetes. While the underlying mechanisms of the age-related effects of mTOR have not been fully elucidated, influences on mitochondrial function likely play an important role. Evidence shows that mTOR activity is tightly correlated with mitochondrial metabolism in mammalian cells, and that disruption of the mTORC1 complex by rapamycin lowers mitochondrial membrane potential and oxidative capacity (Schieke et al., 2006). Furthermore, Pan and Shadel (2009) revealed that inhibition of TOR by rapamycin signaling extends the lifespan of yeast by increasing mitochondrial translation rates and cellular mitochondrial oxygen consumption (i.e., respiration). This, more efficient mitochondrial respiration, decreases ROS production, thereby limiting damage to cellular components. Rapamycin is also well-known to block TOR in humans (Drummond et al., 2009); however, whether this leads to the same health and longevity effects found in animals remains to be seen.

1.7.3 Other potential CR mimetic bioactive foods

The National Institute on Aging has developed a multi-institutional program to evaluate substances that may extend lifespan and delay disease and dysfunction in mice (Interventions Testing Program). This program has investigated approximately 12 agents, including RSV and rapamycin. Other promising bioactive foods currently being tested are curcumin and green tea extract. Previous research has shown that curcumin, widely used as a spice and herbal medicine in Asia, extends the lifespan of two different strains of *Drosophila melanogaster* flies (Lee et al., 2010). This effect was accompanied by decreased expression of several age-related genes, including TOR. Green tea is a widely consumed beverage in Asia, previously shown to have a variety of cardiovascular and metabolic health-promoting effects in humans and animal models (see Wolfram, 2007 for a review). Green tea's beneficial health effects are attributed to its rich source of polyphenols and, in particular, to its most abundant polyphenolic compound, a catechin known as EGCG. Recent randomized cross-over studies have shown that green tea extract increases 24-h energy expenditure and fat oxidation in healthy and overweight adults. While these studies offer promising results, the effects of green tea on aging and longevity are yet to be elucidated.

In sum, the last decade has witnessed great progress in the identification of foods that may alleviate disease and promote healthy aging. In particular, emerging bioactive foods, such as RSV and rapamycin, may be capable of eliciting beneficial outcomes that are similar to those of RSV, potentially through the modulation of mitochondrial redox metabolism. The NIA's Interventions Testing Program should continue to provide major advances in the field of CR mimetics, and new bioactive food candidates will continue to emerge. Nevertheless, whether these bioactive foods alleviate disease and increase lifespan in humans ultimately remains unknown and the answer likely remains decades away. However, the results are promising and suggest that finding foods which mimic the CR response has the potential to at least significantly improve the health of humans.

2. CONCLUDING THOUGHTS

This chapter has reviewed a spectrum research that spans from the intact human to subcellular components that control energy metabolism. It is clear that all components of energy metabolism tend to decline with increasing age. This association is tightly intertwined with changes in energy intake and body mass. The role of bioactive foods on energy metabolism is yet to be fully revealed, but there are some compounds that hold promise for modifying age-related changes in energy metabolism at the sub-cellular level. Future research will continue to evaluate how the manipulation of energy metabolism can alter healthspan.

GLOSSARY

Activity energy expenditure The amount of energy that is expended during both volitional and non-volitional activities.
Bioactive foods Foods that are not essential in the diet, but might provide biological effects on the function of cells.
Essential foods Macro- and micro-nutrients that are absolutely essential for cell growth, maintenance, and repair.
Healthspan The amount of life spent without comorbidities and disability. This term differs from lifespan in that it measures active life-expectancy.
Resting metabolic rate The amount of energy that is expended at rest without sleeping.
Total energy expenditure The daily amount of energy that a human expends. It is composed of resting metabolic rate, thermic affect of food and activity energy expenditure.

REFERENCES

Baur, J.A., Pearson, K.J., Price, N.L., et al., 2006. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444, 337–342.
Bean, B., 1926. Composite study of weight of vital organs in man. American Journal of Physical Anthropology 9, 293–317.

Black, A.E., Coward, W.A., Cole, T.J., Prentice, A.M., 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. European Journal of Clinical Nutrition 50, 72–92.

Blundell, J.E., King, N.A., 1999. Physical activity and regulation of food intake: current evidence. Medicine & Science in Sports & Exercise 31, S573–S583.

Chapman, I.M., 2010. Obesity paradox during aging. Interdisciplinary Topics in Gerontology 37, 20–36.

Chen, D., Steele, A.D., Lindquist, S., Guarente, L., 2005. Increase in activity during calorie restriction requires Sirt1. Science 310, 1641.

Cota, D., 2009. Mammalian target of rapamycin complex 1 (mTORC1) signaling in energy balance and obesity. Physiology and Behavior 97, 520–524.

Csiszar, A., Labinskyy, N., Pinto, J.T., et al., 2009. Resveratrol induces mitochondrial biogenesis in endothelial cells. American Journal of Physiology – Heart and Circulatory Physiology 297, H13–H20.

Drummond, M.J., Fry, C.S., Glynn, E.L., et al., 2009. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. The Journal of Physiology 587, 1535–1546.

Fontana, L., 2009. The scientific basis of caloric restriction leading to longer life. Current Opinion in Gastroenterology 25, 144–150.

Gallagher, D., Visser, M., De Meersman, R.E., et al., 1997. Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. Journal of Applied Physiology 83, 229–239.

Garland Jr., T., Schutz, H., Chappell, M.A., et al., 2011. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. Journal of Experimental Biology 214, 206–229.

Goran, M.I., 1997. Genetic influences on human energy expenditure and substrate utilization. Behavior Genetics 27, 389–399.

Hallfrisch, J., Muller, D., Drinkwater, D., Tobin, J., Andres, R., 1990. Continuing diet trends in men: the Baltimore Longitudinal Study of Aging (1961-1987). Journal of Gerontology 45, M186–M191.

Harrison, D.E., Strong, R., Sharp, Z.D., et al., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.

He, Q., Heshka, S., Albu, J., et al., 2009. Smaller organ mass with greater age, except for heart. Journal of Applied Physiology 106 (6), 1780–1784.

Holloszy, J.O., Smith, E.K., 1986. Longevity of cold-exposed rats: a reevaluation of the "rate-of-living theory" Journal of Applied Physiology 61, 1656–1660.

Krems, C., Luhrmann, P.M., Strassburg, A., Hartmann, B., Neuhauser-Berthold, M., 2005. Lower resting metabolic rate in the elderly may not be entirely due to changes in body composition. European Journal of Clinical Nutrition 59, 255–262.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., et al., 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127, 1109–1122.

Lee, K.S., Lee, B.S., Semnani, S., et al., 2010. Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in Drosophila melanogaster. Rejuvenation Research 13, 561–570.

Levin, S., Jacobs Jr., D.R., Ainsworth, B.E., Richardson, M.T., Leon, A.S., 1999. Intra-individual variation and estimates of usual physical activity. Annals of Epidemiology 9, 481–488.

Levine, J.A., Eberhardt, N.L., Jensen, M.D., 1999. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 283, 212–214.

Martin, C.K., Das, S.K., Lindblad, L., et al., 2011. Effect of calorie restriction on the free-living physical activity levels of nonobese humans: results of three randomized trials. Journal of Applied Physiology 110, 956–963.

Merry, B.J., 2004. Oxidative stress and mitochondrial function with aging–the effects of calorie restriction. Aging Cell 3, 7–12.

Morley, J.E., 2001. Decreased food intake with aging. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 56 (Spec No 2), 81–88.

Morley, J.E., 2003. Anorexia and weight loss in older persons. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 58, 131–137.

Pan, Y., Shadel, G.S., 2009. Extension of chronological life span by reduced TOR signaling requires down-regulation of Sch9p and involves increased mitochondrial OXPHOS complex density. Aging (Albany, NY) 1, 131–145.

Ramsey, J.J., Harper, M.E., Weindruch, R., 2000. Restriction of energy intake, energy expenditure, and aging. Free Radical Biology & Medicine 29, 946–968.

Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L., Bogardus, C., 1986. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. The Journal of Clinical Investigation 78, 1568–1578.

Rico, H., Revilla, M., Villa, L.F., Alvarez de Buergo, M., 1993. Age-related differences in total and regional bone mass: a cross-sectional study with DXA in 429 normal women. Osteoporosis International 3, 154–159.

Rolfe, D.F., Brown, G.C., 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiological Reviews 77, 731–758.

Schieke, S.M., Phillips, D., Mccoy Jr., J.P., et al., 2006. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. Journal of Biological Chemistry 281, 27643–27652.

Westerterp, K.R., Meijer, E.P., 2001. Physical activity and parameters of aging: a physiological perspective. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 56A, 7–12.

Wolfram, S., 2007. Effects of green tea and EGCG on cardiovascular and metabolic health. Journal of the American College of Nutrition 26, 373S–388S.

RELEVANT WEBSITES

http://www.nia.nih.gov – National Institute on Aging (NIH).

http://www.who.int – World Health Organization (WHO).

http://www.senescence.info - senescence.info: An Educational and Informational Resource on the Science of Aging.

http://www.fightaging.org – Fight Aging!

Nutritional Hormetins and Aging

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ABBREVIATIONS

ARE Antioxidant response element
ELS Essential lifespan
HSR Heat shock response
Keap Kelch-like ECH-associated protein
Nrf2 Nuclear factor erythroid-2-realtged factor
SR Stress response

1. INTRODUCTION

Modulation of human health and longevity through nutrition is one of the longest running themes in the history of anti-aging. While dreams of the perfect food for eternal youth and immortality may still occupy the minds of some, modern scientific knowledge has opened up novel approaches toward understanding and utilizing nutrition in a more realistic and rational way. One such modern approach is hormesis and hormetins for healthy aging and longevity, which is based on the observations that low levels of potentially toxic substances can have health beneficial effects by the induction and stimulation of maintenance and repair systems. Such a phenomenon of mild stress-induced health benefits is known as physiological hormesis (Calabrese, 2004; Mattson and Calabrese, 2010), and any condition which causes physiological hormesis is termed as a hormetin (Rattan, 2008). Nutritional hormetins are the hormesis-inducing components of the food, which bring about their beneficial effects by activating one or multiple pathways of stress response (SR) (Rattan, 2012a).

However, in order to fully appreciate the application of nutritional hormetins to modulate aging and longevity, it is important first to have a brief overview of the current status of one's understanding of the biological basis of aging, which will be followed by the discussion of nutritional hormetins in aging research and interventions.

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2. UNDERSTANDING THE BIOLOGICAL PRINCIPLES OF AGING

The biological bases of aging are well understood, and a distinctive framework has been established, which can be the basis for developing effective interventions. There are four major biological principles of aging and longevity:

1. *Evolutionary life history principle*: Aging is an emergent phenomenon seen primarily in the period of survival beyond the natural lifespan of a species, termed 'essential life-span' (ELS) (Rattan, 2000, 2006).

2. *Non-genetic principle*: There is no fixed and rigid genetic program, which determines the exact duration of survival of an organism, and there are no real gerontogenes whose sole function is to cause aging (Rattan, 2006).

3. *Differential principle*: The progression and rate of aging are different in different species, organisms within a species, organs and tissues within an organism, cell types within a tissue, sub-cellular compartments within a cell type, and macromolecules within a cell (Rattan, 2006).

4. *Molecular mechanistic principle*: Aging is characterized by a stochastic occurrence, accumulation, and heterogeneity of damage in macromolecules, leading to the shrinkage of the homeodynamic space and the failure of maintenance and repair pathways (Rattan, 2006, 2007; Holliday and Rattan, 2010; Rattan, 2012b).

Thus, aging is an emergent and epigenetic meta-phenomenon beyond ELS, which is not controlled by a single mechanism. Although, individually, no tissue, organ, or system becomes functionally exhausted even in very old organisms, it is their combined interaction and interdependence that determines the survival of the whole. All living systems have the intrinsic homeodynamic ability to respond, to counteract, and to adapt to the external and internal sources of disturbance. A wide range of molecular, cellular, and physiological pathways of repair are well known, and these range from multiple pathways of nuclear and mitochondrial DNA repair to free radical counteracting mechanisms, protein turnover and repair, detoxification mechanisms, and other processes including immune responses and stress responses. All these processes involve numerous genes whose products and their interactions give rise to a "homeodynamic space" or the "buffering capacity", which is the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state. Aging, senescence, and death are the final manifestations of a progressive shrinking of the homeodynamic space (Holliday and Rattan, 2010, Rattan, 2006, 2007).

3. FROM UNDERSTANDING TO INTERVENTION

As a biomedical issue, the biological process of aging underlies all major human diseases. Although the optimal treatment of each and every disease, irrespective of age, is a social and moral necessity, preventing the onset of age-related diseases by intervening in the

basic process of aging is the best solution for improving the quality of human life in old age. According to the principles of aging and longevity described above, therapeutic interventions against aging need to be primarily preventive in terms of slowing down the rate and extent of shrinkage of the homeodynamic space.

A critical component of the homeodynamic property of living systems is their capacity to respond to stress. In this context, the term "stress" is defined as a signal generated by any physical, chemical, or biological factor (stressor), which, in a living system, initiates a series of events in order to counteract, adapt, and survive. Table 16.1 gives a list of main molecular SRs, their potential stressors, and various effectors, which are integral to the organismic property of homeodynamics.

Based on the involvement of one or more molecular SRs, higher-order (cellular, organ, and body level) SRs are manifested, which include apoptosis, inflammation, and hyperadrenocorticism leading to increased levels of circulating corticosterones in the body. Not all pathways of the SR respond to every stressor, and although there may be some overlap, generally, SR pathways are quite specific. The specificity of the response is mostly determined by the nature of the damage induced by the stressor and the variety of downstream effectors involved. For example, cytoplasmic induction of protein denaturation by heat, heavy metals, and antibiotics will initiate the so-called heat shock response (HSR) by inducing the synthesis of heat shock proteins (HSP) followed by the activation of proteasome-mediated protein degradation (Liberek et al., 2008; Verbeke et al., 2001). However, unfolded proteins in the endoplasmic reticulum (ER) will induce unfolded protein response (UPR) and will initiate the induction of synthesis of a totally different set of proteins and their downstream effectors (Banhegyi et al., 2007; Yoshida, 2007). Similarly, whereas oxidative damage to proteins will generally initiate Nrf2-mediated antioxidant response, damage to DNA by free radicals or other agents will result in the activation

Table 16.1 Major Pathways of Stress Response in Human Cells

Stress response	Stressors	Effectors
Heat shock response (HSR)	Heat, heavy metals, antibiotics, protein denaturation	Heat shock proteins, proteasome, and other proteins
Unfolded protein response (UPR)	Unfolded and misfolded proteins in endoplasmic reticulum	Chaperones and co-chaperones
Autophagic response	Food limitation, hypoxia, damaged organelles	Lysosomes
DNA-repair response	Radiation, oxidants, free radicals	DNA-repair enzymes
Antioxidant response	Free radicals, reactive oxygen species, pro-oxidants	Nrf-2, heme oxygenase, FOXO
Sirtuin response	Energy depletion	Sirtuins
NFκB inflammatory response	Pathogens, allergens, damaged macromolecules	Cytokines, nitric oxide synthase

of DNA repair enzymes. In the same vein, whereas nutritional deprivation and low energy levels will activate autophagy and FOXO-sirtuin pathways, infections and antigenic challenge will generally initiate pro-inflammatory NFκB response.

However, often, the source of activation (stressor) cannot be easily identified and may involve more than one stressor and their effectors. Examples of such SR include early inflammatory SR and neuroendocrinal SR, which lead to the synthesis and release of interleukins and corticoid hormones, respectively. Similarly, pathways involving NF-κB, Nrf2, FOXO, sirtuins, and heme-oxygenase (HO) activation may involve more than one type of stressors and stress signals, including pro-oxidants, free radicals, reactive oxygen species (ROS), and nutritional components. But most importantly, an appropriate and optimal SR is an essential aspect of successful homeodynamics and continued survival.

4. STRESS, HORMESIS, AND HORMETINS

The consequences of SR can be both harmful and beneficial depending on the intensity, duration, and frequency of the stress and on the price paid in terms of energy utilization and other metabolic disturbances. But the most important aspect of SR is that it is not monotonic with respect to the dose of the stressor; rather, it is almost always characterized by a non-linear biphasic relationship. Several meta-analyses performed on a large number of papers published in the fields of toxicology, pharmacology, medicine, and radiation biology have led to the conclusion that the most fundamental shape of the dose response is neither threshold nor linear but is U- or inverted U-shaped depending on the endpoint being measured (Calabrese, 2008; Calabrese et al., 2007). This phenomenon of biphasic dose response was termed as hormesis (Southam and Ehrlich, 1943), and the science and study of hormesis is now termed as hormetics (Rattan, 2012a).

The key conceptual features of hormesis are the disruption of homeodynamics, the modest overcompensation, the re-establishment of homeodynamics, and the adaptive nature of the process. An example of stress-induced hormesis is the well-documented beneficial effects of moderate exercise as a hormetic agent, which initially increases the production of free radicals, acids, and aldehydes (Radak et al., 2008). Another frequent observation in studies of hormesis is that a single hormetic agent, such as heat shock or physical activity, can improve the overall homeodynamics of cells and enhance other activities such as tolerance to other stresses, by initiating a cascade of processes resulting in a biological amplification and eventual beneficial effects (Mattson, 2008; Rattan, 2008).

Hormesis in aging is defined as the life-supporting beneficial effects resulting from the cellular responses to single or multiple rounds of mild stress. Various mild stresses that have been reported to delay aging and prolong longevity in cells and animals include temperature shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise, and food restriction. All such compounds, which bring about biologically beneficial effects by causing mild stress and thus stimulating defense

pathways, are termed as hormetins (Rattan, 2008; Rattan and Demirovic, 2010; Rattan et al., 2009). Hormetins may be categorized as: (1) physical hormetins, such as exercise, heat, and radiation; (2) psychological hormetins, such as mental challenge and focused attention or meditation; and (3) biological and nutritional hormetins, such as infections, micronutrients, spices, and other sources.

5. NUTRITIONAL HORMETINS

Among different types of hormetins, nutritional hormetins, and especially those derived from plant sources, have generated much scientific interest for their health beneficial effects. This is because of the realization that not all chemicals found in plants are beneficial for animals in a simple and straightforward manner, but rather they cause molecular damage by virtue of their electrochemical properties and have a typical biphasic hormetic dose response (Balstad et al., 2011). Although the exact nature of the initial molecular damage caused by such compounds may not be easily identified, an activation of one or more SRs, as listed in the Table 16.1, is a good indicator of the primary action of the compound.

For example, the antioxidant response by the activation of Nrf2 transcription factor follows the electrophilic modification/damage of its inhibitor protein Keap1, which then leads to the accumulation, heterodimerization, nuclear translocation, and DNA binding of Nrf2 at the antioxidant response element (ARE), resulting in the downstream expression of a large number of the so-called antioxidant genes, such as heme oxygenease HO-1, superoxide dismutase, glutathione, and catalase (Balstad et al., 2011; Calabrese et al., 2008, 2010). Some well-known phytochemicals, which strongly induce Nrf2-mediated SR, include curcumin, quercetin, genistein, and eugenol (Balstad et al., 2011; Lima et al., 2011). A similar induction of SR involving Nrf2 has also been reported for various food extracts, such as coffee, turmeric, rosemary, broccoli, thyme, clove, and oregano (Balstad et al., 2011; Demirovic and Rattan, 2011). Screening for other inducers of Nrf2 SR in natural compounds isolated from nutritional sources, or synthetic compounds with nutritional utility, and in complex and multiple food extracts will discover novel hormetins useful for healthy aging and longevity.

Another SR pathway, which has been studied in detail and can be the basis for identifying novel nutritional hormetins, is the so-called HSR. Induction of proteotoxic stress, such as protein misfolding and denaturation, initiates HSR by the intracellular release of the heat shock transcription factor (HSF) from their captor-proteins, followed by its nuclear translocation, trimerization, and DNA binding for the expression of several heat shock proteins – HSPs (Liberek et al., 2008; Verbeke et al., 2001). A wide range of biological effects then occur which involve HSPs and include protein repair, refolding, and selective degradation of abnormal proteins leading to the cleaning up and an overall improvement in the structure and function of the cells. Various phytochemicals and

nutritional components have been shown to induce HSR and have health beneficial effects, including anti-aging and longevity-promoting effects. Some examples of nutritional hormetins involving HSR are phenolic acids, polyphenols, flavanoids, ferulic acid (Barone et al., 2009; Son et al., 2008), geranylgeranyl, rosmarinic acid, kinetin, zinc (Berge et al., 2008; Son et al., 2008; Sonneborn, 2010), and the extracts of tea, dark chocolate, saffaron, and spinach (Wieten et al., 2010). Further screening of animal and plant components, for their ability to induce HSR, will identify other potential hormetins.

Other pathways of SR, which are involved in initiating hormetic effects of nutritional components, are the NFkB, FOXO, sirtuins, DNA repair response, and autophagy pathways. Resveratrol and some other mimetics of calorie restriction work by the induction of one or more of these SR pathways (Longo, 2009; Sonneborn, 2010). Hormesis may also be an explanation for the health beneficial effects of numerous other foods and food components, such as berries, garlic, Gingko, and other fruits and vegetables. Discovering novel nutritional hormetins, by putting potential candidates through a screening process for their ability to induce one or more SR pathways in cells and organisms, can be a promising strategy. Finally, understanding the hormetic and interactive mode of action of natural and processed foods is a challenging field of research and has great potential in developing nutritional and other lifestyle modifications for aging intervention and therapies.

REFERENCES

Balstad, T.R., Carlsen, H., Myhrstad, M.C., et al., 2011. Coffee, broccoli and spices are strong inducers of electrophile response element-dependent transcription in vitro and in vivo – studies in electrophile response element transgenic mice. Molecular Nutrition & Food Research 55, 185–197.

Banhegyi, G., Baumeister, P., Benedetti, A., et al., 2007. Endoplasmic reticulum stress. Annals of the New York Academy of Sciences 1113, 58–71.

Barone, E., Calabrese, V., Mancuso, C., 2009. Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. Biogerontology 10, 97–108.

Berge, U., Kristensen, P., Rattan, S.I.S., 2008. Hormetic modulation of differentiation of normal human epidermal keratinocytes undergoing replicative senescence in vitro. Experimental Gerontology 43, 658–662.

Calabrese, E.J., 2004. Hormesis: from marginalization to mainstream: a case for hormesis as the default dose-response model in risk assessment. Toxicology and Applied Pharmacology 197, 125–136.

Calabrese, E.J., 2008. Hormesis and medicine. British Journal of Clinical Pharmacology 66, 594–617.

Calabrese, E.J., Bachmann, K.A., Bailer, A.J., et al., 2007. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. Toxicology and Applied Pharmacology 222, 122–128.

Calabrese, V., Cornelius, C., Mancuso, C., et al., 2008. Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. Neurochemical Research 33, 2444–2471.

Calabrese, V., Cornelius, C., Dinkova-Kostova, A.T., Calabrese, E.J., Mattson, M.P., 2010. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxidants & Redox Signaling 13, 1763–1811.

Demirovic, D., Rattan, S.I.S., 2011. Curcumin induces stress response and hormetically modulates wound healing ability of human skin fibroblasts undergoing ageing in vitro. Biogerontology 12, 437–444.

Holliday, R., Rattan, S.I.S., 2010. Longevity mutants do not establish any "new science" of ageing. Biogerontology 11, 507–511.

Liberek, K., Lewandowska, A., Zietkiewicz, S., 2008. Chaperones in control of protein disaggregation. EMBO Journal 27, 328–335.

Lima, C.F., Pereira-Wilson, C., Rattan, S.I., 2011. Curcumin induces heme oxygenase-1 in normal human skin fibroblasts through redox signaling: Relevance for anti-aging intervention. Molecular Nutrition & Food Research 55, 430–442.

Longo, V.D., 2009. Linking sirtuins, IGF-I signaling, and starvation. Experimental Gerontology 44, 70–74.

Mattson, M.P., 2008. Hormesis defined. Ageing Research Reviews 7, 1–7.

Mattson, M.P., Calabrese, E. (Eds.), 2010. Hormesis – a revolution in biology, toxicology and medicine. Springer, New York.

Radak, Z., Chung, H.Y., Koltai, E., Taylor, A.W., Goto, S., 2008. Exercise, oxidative stress and hormesis. Ageing Research Reviews 7, 34–42.

Rattan, S.I.S., 2000. Biogerontology: the next step. Annals of the New York Academy of Sciences 908, 282–290.

Rattan, S.I.S., 2006. Theories of biological aging: genes, proteins and free radicals. Free Radical Research 40, 1230–1238.

Rattan, S.I.S., 2007. Homeostasis, homeodynamics, and aging. In: Birren, J. (Ed.), Encyclopedia of Gerontology. 2nd edn. UK, Elsevier Inc.

Rattan, S.I.S., 2008. Hormesis in aging. Ageing Research Reviews 7, 63–78.

Rattan, S.I.S., 2012a. Rationale and methods of discovering hormetins as drugs for healthy ageing. Expert Opinion on Drug Discovery 7, 439–448.

Rattan, S.I.S., 2012b. Biogerontology − from here to where? The Lord Cohen Medal lecture-2011. Biogerontology 13, 83–91.

Rattan, S.I.S., Demirovic, D., 2010. Hormesis can and does work in humans. Dose-Response 8, 58–63.

Rattan, S.I.S., Fernandes, R.A., Demirovic, D., Dymek, B., Lima, C.F., 2009. Heat stress and hormetin-induced hormesis in human cells: effects on aging, wound healing, angiogenesis and differentiation. Dose-Response 7, 93–103.

Son, T.G., Camandola, S., Mattson, M.P., 2008. Hormetic dietary phytochemicals. Neuromolecular Medicine 10, 236–246.

Sonneborn, J.S., 2010. Mimetics of hormetic agents: stress-resistance triggers. Dose-Response 8, 97–121.

Southam, C.M., Ehrlich, J., 1943. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. Phytopathology 33, 517–524.

Verbeke, P., Fonager, J., Clark, B.F.C., Rattan, S.I.S., 2001. Heat shock response and ageing: mechanisms and applications. Cell Biology International 25, 845–857.

Wieten, L., van der Zee, R., Goedemans, R., et al., 2010. Hsp70 expression and induction as a readout for detection of immune modulatory components in food. Cell Stress & Chaperones 15, 25–37.

Yoshida, H., 2007. ER stress and diseases. FEBS Journal 274, 630–658.

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The Health Benefits of the Ayurvedic Anti-Aging Drugs (*Rasayanas*): An Evidence-Based Revisit

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ABBREVIATIONS

ACP Acid phosphatase
ADP Adenosine diphosphate
ALP Alkaline phosphatase
ALT Alanine aminotransferase
AST Aspartate aminotransferase
CAT Catalase
CCl$_4$ Carbon tetrachloride
GM-CSF Granulocyte macrophage-colony stimulating factor
GPx Glutathione peroxidase
GSH Gluatathione
GST Glutathione S-transferase
IFN-γ Interferon-gamma
IL-2 Interleukin-2
PMA Phorbol-12-myristate-13-acetate
ROS Reactive oxygen species
SOD Superoxide dismutase

1. INTRODUCTION

With advances in the field of medicine, the mean survival age of human beings has increased, leading to a proportionate raise in the percentage of geriatric population. Estimates are that in the year 2050, the number of people 60 years or older will increase from the currently 1 in 10 to 1 in 5 and that the ratio of people aged 65 years or older to those aged 15–64 years will double in developed nations and triple in developing nations (Datta et al., 2011). This increase in the population of the older population, combined with possible lesser number of caretakers, will greatly affect the global healthcare system.

Aging or senescence is fundamentally a complex process in which a progressive decline in the efficiency of physiological processes ensues and is manifested within an organism at genetic, molecular, cellular, biochemical, organ, physiological, and system
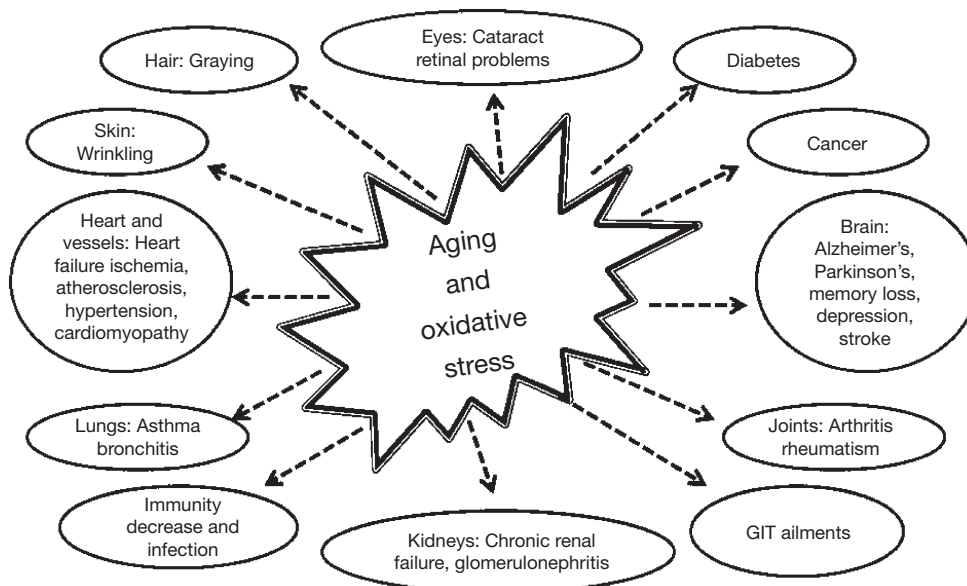
Figure 17.1 Common diseases seen with aging and due to or aggravated by oxidative stress.

levels. There is also a decrease in the ability of cells to recover from a physical or a mutagenic damage. This leads to deterioration of physical function, reduction in fecundity, and loss of vitality and will concomitantly increase the risk of various diseases (Figure 17.1) and can eventually cause death (Datta et al., 2011; Halliwell and Gutteridge, 2007).

2. HYPOTHESIS OF AGING

Although the fundamental mechanisms for aging are still poorly understood, various hypotheses have been proposed, such as the error catastrophe and genomic instability theory, the neuroendocrine theory, the free radical theory, the membrane dysfunction theory, the hayflick limit theory, the mitochondrial decline theory, the cross-linking (glycation) theory, and the inflammatory theory. Of these, the free radical theory proposed by Denham Harman is the most accepted and observations from both preclinical and clinical studies have substantiated the hypothesis (Halliwell and Gutteridge, 2007).

Free radical theory proposes that excess generation of free radicals causes oxidative damage to biomolecules and a progressive and irreversible accumulation of products of oxidation, decrease in the cellular levels or activities of antioxidant systems. This in combination with the increased inflammatory responses, apoptosis, altered cell signaling,

and defective tissue renewal contribute to impaired physiological function, increased incidence of disease, and decrease in lifespan (Halliwell and Gutteridge, 2007).

The balance among reactive oxygen species (ROS) production, cellular antioxidant defenses, activation of stress-related signaling pathways, and the production of various gene products, as well as the effect of aging on these processes, determines whether a cell exposed to an increase in ROS will be destined for survival or death. Mitochondrial DNA is considered the most vulnerable candidate for oxidative damage as the mitochondria are constantly exposed to high oxygen pressure, and the genetic mechanisms that protect the DNA from damage are lacking or deficit in mitochondria. DNA repair enzymes, the third line defense against oxidative stress, decline with age (Halliwell and Gutteridge, 2007).

Numerous studies have shown that oxidative DNA damage accumulates in the brain, muscle, liver, kidney, and long-lived stem cells. These accumulated DNA damages are the likely cause of the decline in gene expression and loss of functional capacity observed with increasing age. Oxidative stress is also implicated in the etiopathogenesis of many age-related diseases and clinical complications such as atherosclerosis, diabetes mellitus, muscular dystrophy, and neurodegeneraive diseases such as Alzheimer's disease and Parkinson's disease (Halliwell and Gutteridge, 2007).

Tissue repair and regeneration are essential for longevity in complex animals and often depend on the proliferation of unspecialized cells known as stem or progenitor cells. In many tissues, importantly the muscle, the regenerative capacity of stem cells decreases with age, and these changes are thought to trigger age-related symptoms and diseases. Additionally, due to failure or decrease in the repair and regeneration mechanisms, the damaged tissues will not undergo repair and regeneration effectively and the accumulation of defective cells will lead/contribute to the pathogenic process (Halliwell and Gutteridge, 2007).

3. *AYURVEDA* AND AGING

Ayurveda, which when translated literally means *science of life,* is the traditional system of Indian medicine and dates back to 5000 BC. It is an integral part of Indian culture and *materia medica* and remains an influential system of medicine, especially in the Indian subcontinent. The concept and treatment principles of *Ayurveda* are different from that of modern medicine. While modern medicine is evidence based and makes use of a distinct well-defined chemical entity for treatment, the emphasis in *Ayurveda* is mainly on disease prevention and promotion of good health, by following the proper life style and adopting measures that rejuvenate the cells of the body. Additionally, the disease-preventive and health-promotive approach of *Ayurveda* takes into consideration the whole body, mind, and spirit while dealing with the maintenance of health (Datta et al., 2011; Sharma and Dash, 1998).

In *Ayurveda*, rejuvenation of the cells is addressed in the *Rasayana Shastra*. In colloquial terms *Rasayana* means "the path of juice" or "juice-incorporate" (*Rasa*=juice and *Ayana*=path), or *Elixir vitae*. In *Ayurveda*, consumption of specific *Rasayanas* at appropriate times and in the recommended pattern is supposed to increase the *Rasa* in the cell and body, which, in modern terms, may be interpreted as to rejuvenate the system. According to Charaka Samhita, a *Rasayana* is a drug that promotes intelligence, memory, freedom from disease, longevity, strength of the senses, and great increase in the strength of the digestive system (Sharma and Dash, 1998). Administration of *Rasayana* is supposed to nourish the blood, lymph, muscles, adipose tissue, bones, bone marrow, and semen. On account of this, it prevents any kind of degenerative changes and illness, increases life span, and arrests the signs of aging. Studies suggest that *Rasayana* therapy acts by modulating the neuro-endocrino-immune systems and in turn rejuvenating the complete functional dynamics of the organs by delaying aging and enhancing intelligence, memory, strength, youth, luster, sweetness of voice, and vigor (Rege et al., 1999; Vayalil et al., 2002a).

Rasayana is deemed beneficial for nearly all diseases, with a special emphasis on the disorders of aging, when the body is deprived of adequate nutrition and vigor by means of optimization or homeostasis. It is used either to rejuvenate the general health of the body or to aid the body in attaining its maximum functional potential (Sharma and Dash, 1998). *Rasayana* is beneficial to people irrespective of their age, sex, or ethnicity. *Rasayana* treatment defers old age and, when taken in good health, optimizes all aspects of the physiology and maintains youthfulness and vigor, as well as vitality of the body. This has a major role in increasing the vitality and keeping diseases at bay. The health benefits of consuming *Rasayana* drugs are cumulative with time and regularity, and are devoid of side effects, even when taken indefinitely (Sharma and Dash, 1998).

4. TYPES OF *RASAYANA* DRUGS AND SOME OF THEIR COMPOSITION

The *Rasayana* drugs or preparations used to achieve the benefits are customarily a complex mixture of medicinal plants with miniscule amounts of minerals, pearls, corals, gems, and *Shilajit* (mineral exudates) (Sharma and Dash, 1998). Many plants have been extensively used as *Rasayana* drugs in *Ayurveda* for the management of neurodegenerative diseases, as rejuvenators, immunomodulators, aphrodisiac, and nutritional supplements (Sharma and Dash, 1998).

Some of the most commonly used plants are *Emblica officinalis* (Indian gooseberry, *Amla*), *Tinospora cordifolia* (*Guduchi*), *Asparagus racemosus* (*Shatavari*), *Withania somnifera* (*Ashwagandha*), *Terminalia chebula* (*Haritaki*), *Terminalia belerica* (*Bibhitaki*), *Boerhavia diffusa* (*Punarnava*), *Aloe vera* (*Kumari*), *Bacopa monnieri* (*Mandukaparni*), *Glycyrrhiza glabra* (*Yashtimadhu*), and *Picrorhiza kurroa* (*Katuki*) (Sharma and Dash, 1998).

Several recipes for *Rasayana* are presented in *Ayurveda* and depending on the composition of the plants and their ratio, they may be organ/tissue-specific like for brain, heart,

Table 17.1 Organ-specific effect of the *Rasayana* plants and drugs in the prevention of aging in the Ayurvedic system of medicine (Sharma and Dash, 1998)

Scientific names	Sanskrit name	Action
Phyllanthus emblica or *Emblica officinalis*	Amalaki	Eye, heart, respiratory system, and immunomodulator
Terminalia chebula	Harithaki	GI tract, cardioprotective, and immunomodulator
Tinospora cordifolia	Amrutha	Modulator
Boerhavia diffusa	Punarnava	Kidney, heart, and hematopoietic stimulator
Bacopa monnieri	Mandukaparni	Brain
Convolvulus pluricaulis	Shankhapushpi	Brain
Asparagus racemosus	Shathavari	Reproductive system
Glycyrrhiza glabra	Yashtimadhu	Brain
Withania somnifera	Ashwagandha	Brain and nervous system, and immunomodulator
Picrorhiza kurroa	Katuki	Skin and gastro intestinal system
Piper longum	Pippali	GI tract
Acorus calamus	Vacha	GI tract
Embelia Ribes	Vidanga	Worm infestation in children
Semecarpus anacardium	Bhallataka	Skin
Rasayanas drugs	**Action**	
Amritaprasham	Respiratory system, hemopoietic	
Narasimha Rasayana	Hair, skin, and speech, intellectual power	
Triphala	GI tract, immunomodulator, hematopoietic	
Anwala Churma	Digestive system, immunomodulator	
Brahmi Rasayana	Brain	
Amalkadi Ghrita	Brain, immunomodulator	
Chyavanaprash	Respiratory system, immunomodulator	
Brahma Rasayana	Brain	

reproductive organs, etc. or for general/whole body use (Sharma and Dash, 1998) (Table 17.1). Some of the famous *Rasayana* formulations are the *Triphala*, *Chyawanprash*, *Amalaki Rasayana*, *Amrit Rasayana*, *Brahma Rasayana, Ashwagandha Rasayana*, *Narasimha Rasayana*, *Amritaprasham*, *Anwala churna*, *Brahmi Rasayana*, and *Amalkadi Ghrita* (Sharma and Dash, 1998). In the following section the validated pharmacological observations of some of the *Ayurvedic Rasayana* drugs are addressed. Additionally in Table 17.2, the composition of the *Rasayana* drugs are also enlisted.

4.1 Chyavanaprasha

Chyavanaprasha, named after its inventor Rishi (sage) Chyavana, is one of the oldest and most popular Ayurvedic preparations. It is the foremost of all herbal rejuvenating tonics and the most commonly used *Rasayana* drug in India and abroad. It is a tridoshic rasayana and due to its numerous nutritional properties is regarded as "the elixir of life" (Sharma

Table 17.2 Composition of various *Rasayana* drugs

Rasayana	Composition	Reference
Amritaprasham	*Holostemma annulare, Vigna vexillata, Phaseolus adenanthus, Glycyrrhiza glabra, Zingiber officinale, Asparagus racemosus, Boerhavia diffusa, Sida retusa, Clerodendrum serratum, Mucuna pruriens, Hedychium spicatum, Phyllanthus niruri, Piper longum, Vitis vinifera, Emblica officinalis, Pueraria tuberosa, Saccharum officinarum, Piper nigrum, Cinnamomum zeylanica, Elettaria cardamomum, Garcinia morella,* and *Mesua ferrea.*	Vayalil et al. (2002b)
Ashwaganda Rasayana	*Withania somnifera, Pueraria tuberosa, Hemidesmus indicus, Cyminum cuminum, Aloe barbadensis, Vitis vinifera, Elettaria cardamomum, Zingiber officinale, Piper nigrum,* and *Piper longum.*	Vayalil et al. (2002b)
Brahma Rasayana	*Emblica officinalis, Terminalia chebula, Uraria picta, Desmodium gangeticum, Gmelina arborea, Solanum nigrum, Tribulus terrestris, Aegle marmelos, Premna tomentosa, Stereospermum suaveolens, Oroxylom indicum, Sida rhombifolia, Boerhavia diffusa, Ricinus communis, Vigna vexillata, Phaseolus adenanthus, Asparagus racemosus, Holostemma annulare, Leptadenia reticulata, Desmostachya bipinnata, Saccharum officinarum, Oryza malampuzhensis, Cinnamomum iners, Elettaria cardamomum, Cyperus rotundus, Curcuma longa, Piper longum, Aquilaria agallocha, Santalum album, Centella asiatica, Mesua ferrea, Clitoria ternate, Acorus calamus, Scirpus grossus, Glycyrrhiza glabra,* and *Embelia ribes.*	Vayalil et al. (2002b)
Chyavanaprasha Chyawanprash chyavanaprasha chyavanaprash chyawanaprash	*Emblica officinalis, Bambusa arundinacea, Aegle marmelos, Clerodendron phlomidis, Oroxylum indicum, Gmelina arborea, Stereospermum suaveolens, Sida cordifolia, Desmodium gangeticum, Uraria picta, Teramnus labialis, Piper longum, Tribulus terrestris, Solanum indicum, Solanum xanthocarpum, Pistacia integerrima, Phaseolus trilobus, Phyllanthus niruri, Vitis vinifera, Leptadenia reticulata, Inula racemosa, Aquilaria agallocha, Tinospora cordifolia, Terminalia chebula, Ellettaria cardamomum, Cinnamomum cassia, Cinnamomum iners, Habenaria intermedia, Microstylis wallichii, Microstylis muscifera, Mesua ferrea, Hedychium spicatum, Cyperus rotundus, Boerhavia diffusa, Polygonatum verticillatum, Nymphaea alba, Santalum album, Pueraria tuberosa, Adhatoda vasica, Roscoea alpina, Martynia diandra,* and *Sesamum indicum.*	Jagetia et al. (2004a)
Narasimha Rasayana	*Acacia catechu, Plumbago zeylanica, Xylia dolabriformis, Pterocarpus marsupium, Embelia ribes, Semecarpus anacardium, Eclipta alba, Terminalia chebula, Emblica officinalis,* and *Terminalia belerica.*	Vayalil et al. (2002b)
Triphala	*Emblica officinalis, Terminalia chebula,* and *Terminalia belerica*	Jagetia et al. (2002)

Table 17.2 Composition of various *Rasayana* drugs—cont'd

Rasayana	Composition	Reference
Anwala churna	*Emblica officinalis*	Vasudevan and Parle, 2007
Amalkadi Ghrita	*Emblica officinalis*, *Glycyrrhiza glabra*, and cow's ghee (clarified butter fat)	Achliya et al. (2004)
Brahmi rasayana	*Bacopa monnieri*	Shukia et al. (1987)

and Dash, 1998). The first documented evidence of this formulation is observed in the Ayurvedic text Charaka Samhita. According to Charaka, the high priest of *Ayurveda*, Chyawanprash is used to treat cough, dyspnea, voice problems, and cardiac problems (Sharma and Dash, 1998).

Animal studies have shown that Chyavanaprasha possesses radioprotective effects and protects mice against radiation-induced sickness and mortality (Jagetia et al., 2004a), decreases carbon tetrachloride-induced liver damage in rats (Jose and Kuttan, 2000), reduces tumor growth (ascites and solid tumor volume), and concomitantly increases the life span of tumor-bearing mice (Jose et al., 2001). Clinical studies have also shown that Chyavanaprasha is an anabolic agent and benefits people of all ages and health (Sharma and Dash, 1998). Consumption of 20 g of Chyawanprash twice a day for 2 months by bidi smokers decreased their cough, increased their appetite, and helped them gain body weight (Yadav et al., 2003).

4.2 Brahma Rasayana

In *Ayurveda, Brahma Rasayana* is a brain-specific geriatric tonic and its regular consumption is supposed to improve resilience to mentally demanding chores, to promote mental clarity, improve memory, cognition, and reduce the symptoms of aging, such as wrinkling and graying of hair (Joseph et al., 1999; Sharma and Dash, 1998). Administering *Brahma Rasayana* to cancer patients undergoing chemo- or radiotherapy caused a rapid increase in the levels of lymphocytes and neutrophils. It reduced the total number of consecutive days of leukopenia, neutropenia, and lymphopenia. In total, these observations indicate that *Brahma Rasayana* accelerated the recovery of the hemopoietic system and decreased the levels of serum lipid peroxidation (Joseph et al., 1999).

Experimental studies have also shown that *Brahma Rasayana* is myeloprotective and increases the total leukocyte count, percentage of polymorphonuclear cells, bone marrow cellularity, α-esterase positive cells, and number of spleen colonies in mice exposed to ionizing radiation. It also enhanced the levels of IFN-γ, IL-2, and GM-CSF in the serum of both normal and irradiated mice. These results suggest that the proliferation

of stem cells induced by *Brahma Rasayana* in irradiated mice may be related to stimulation of cytokine production (Rekha et al., 1998, 2000, 2001c).

Brahma Rasayana is reported to scavenge hydroxyl, superoxide, nitric oxide, and lipid peroxides uninitiated in rat liver homogenate *in vitro*. It also inhibited generation of lipid peroxides by the Fe^{2+}–ascorbate and Fe^{3+}–Adenosine diphosphate (ADP)–ascorbate system with rat liver homogenate *in vitro*. Oral administration of *Brahma Rasayana* (50 mg/dose/animal) inhibited the PMA–induced superoxide generation in mice peritoneal macrophages. A dose–dependent inhibition of the nitrite production in peritoneal macrophages of mice was also observed as at 10 and 50 mg/dose/animal 25.2 and 37.8% inhibition was observed (Rekha et al., 2001a). *Brahma Rasayana* increased the levels of superoxide dismutase (SOD), catalase (CAT), and tissue and serum levels of glutathione (GSH) with a concomitant decrease in the lipid peroxides in the liver (Rekha et al., 2001b).

Studies have also shown that *Brahma Rasayana* enhances the *in vivo* antioxidant status in cold-stressed (Ramnath and Rekha, 2009) and heat-stressed (Ramnath et al., 2009) chickens. Recently, Guruprasad et al. (2010) have observed that feeding older mice with *Brahma Rasayana* for 8 consecutive weeks did not induce mutagenesis. Additionally it was also observed that *Brahma Rasayana* marginally increased the sperm count and increased the number of mitotic cells, validating the rejuvenating effect. Feeding *Brahma Rasayana* protected mice from radiotoxic effects, reduced the loss of organ weight (spleen, liver and kidney) and body weight, decreased the levels of serum and liver lipid peroxides, alkaline phosphatase (ALP), and alanine amino transaminase (ALT) (Vayalil et al., 2002a). Together, all these observations validate the rejuvenating properties of *Brahma Rasayana* and suggest its usefulness to humans.

4.3 *Ashwagandha Rasayana*

Ashwagandha Rasayana, which contains *W. somnifera* (Ashwagandha), is a widely acclaimed Ayurvedic drug for people of all ages. It has revitalizing action on the nerves, the bone marrow, and reproductive organs. Regular consumption is believed to retard senescence, rectify abnormalities of the sense organs, hypotrophy of muscles, to rejuvenate the reproductive organs, and to increase fertility. It is also useful in stress, weakness, nervous exhaustion, sexual debility, geriatric problems, memory loss, muscular weakness, insomnia, and cough. It is also supposed to inhibit aging and catalyze the anabolic processes of the body (Sharma and Dash, 1998).

Preclinical studies have shown that *Ashwagandha Rasayana* possesses radioprotective effects and reduces the radiation-induced emaciation, decrease in the organ weight and to decrease radiation-induced increase in serum transaminase levels and lipid peroxidation (Vayalil et al., 2002a). Innumerable studies have shown that *W. somnifera* increases longevity by promoting physical and mental health and to rejuvenate the body in

debilitated conditions. Ashwagandha is also useful in epilepsy, stress, and neurodegenerative diseases such as Parkinson's and Alzheimer's disorders, tardive dyskinesia, cerebral ischemia, and contributes to the general well-being (Kulkarni and Dhir, 2008).

4.4 *Narasimha Rasayana*

Narsimha Rasayana, consisting of over ten medicinal plants, is supposed to be potent in reversing aging, improving immune system, and to increase sexual vigor and potency (Sharma and Dash, 1998). It contains *Semecarpus anacardium*, *Eclipta alba*, *T. chebula*, *E. officinalis*, and *T. belerica* that are rasayana plants and possess myriad anti-aging and pharmacological properties. Preclinical studies have shown that feeding mice with 50 mg kg^{-1} b. wt. of *Narasimha Rasayana* 5 days prior to radiation arrested the ill effects of radiation. When compared to the radiation alone cohorts, administering *Narsimha Rasayana* before exposure to radiation increased body weight and organ weight, and decreased the levels of serum and tissue lipid peroxides, serum alkaline phosphatase, and serum alanine amino transaminase (Vayalil et al., 2002a).

4.5 Triphala

Triphala (in Sanskrit tri = three and phala = fruits) is another important Ayurvedic medicinal preparation (Baliga, 2010; Sharma and Dash, 1998). It is an antioxidant-rich herbal formulation and possesses diverse beneficial properties that range from gastroprotection to immune strengthening. In Ayurvedic practice, triphala is used for gastric disorders like dyspepsia, poor food assimilation, cleansing of colon, constipation, gastrointestinal tract, and colon tonifier (Nadkarni, 1976). It is also used in cardiovascular diseases, eye diseases, poor liver function, large intestine inflammation, and ulcerative colitis (Mukherjee et al., 2006). Triphala has been reported to assist in weight loss, to treat anemia, jaundice, constipation, cough, asthma, fever, chronic ulcers, leucorrhoea, and pyorrhea (Baliga, 2010; Mukherjee et al., 2006). Triphala is reported to be radioprotective when administered through both intraperitoneal (Jagetia et al., 2002, 2004a) and oral routes (Sandhya et al., 2006).

Triphala is reported to be a scavenger of free radicals (Jagetia et al., 2004a; Naik et al., 2005; Sabu and Kuttan, 2002; Vani et al., 1997), inhibitor of oxidant-induced lipid peroxidation (Deep et al., 2005; Naik et al., 2005; Sabu and Kuttan 2002; Vani et al., 1997), inhibit DNA damage (Naik et al., 2005; Sandhya et al., 2006); antimutagenic (Arora et al., 2003, 2005a; Kaur et al., 2002); anti-inflammatory (Rasool and Sabina, 2007); immunomodulatory (Srikumar et al., 2005, 2006); increase antioxidant molecule glutathione (Deep et al., 2005), antioxidant enzymes (Deep et al., 2005; Sandhya et al., 2006), and the Phase II detoxification enzyme GST (Deep et al., 2005) and inhibit superoxide-induced hemolysis of red blood cells (Vani et al., 1997). All these properties may have contributed to the observed beneficial effects.

4.6 Amritaprasham

According to Ayurvedic practitioners, regular intake of Amritaprasham early morning is supposed to improve strength, stamina, and retard aging (Sharma and Dash, 1998). It is also reported to be useful in the treatment of chronic fever, cough, bronchial asthma, burning sensation, and seminal abnormalities such as azoospermia, oligospermia, erectile dysfunction, and menstrual disorder. It is indicated for urinary disorders, hemorrhoids, gastrointestinal (GI) disorders, epistaxis, anorexia, thirst, vomiting, and loss of consciousness. It is supposed to improve strength and provide hemopoietic stimulatory action (Sharma and Dash, 1998). Amritaprasham is also reported to possess radioprotective effects and to ameliorate the ill effects of radiation (Vayalil et al., 2002a).

4.7 Anwala Churna

Anwala churna consisting of dried *E. officinalis* Gaertn powder is a potent Ayurvedic preparation with myriad pharmacological properties. Amla is a potent rejuvenator and useful in stalling degenerative and senescence process, to promote longevity, enhance digestion, to treat constipation, reduce fever, purify the blood, reduce cough, alleviate asthma, strengthen the heart, benefit the eyes, stimulate hair growth, enliven the body, and to enhance intellect. Studies have shown that oral administration of Anwala churna (50, 100 and 200 mg kg^{-1}) to both young and aged mice for 15 consecutive days caused a dose-dependent improvement in memory, reversed the scopolamine- and diazepam-induced amnesia, and reduced the levels of both brain cholinesterase activity and total cholesterol levels (Vasudevan and Parle, 2007).

4.8 Amalkadi Ghrita

Amalkadi Ghrita made of *E. officinalis*, *G. glabra*, and cow's ghee (clarified butter fat) is an important Ayurvedic formulation useful in the treatment of liver disorders. Preclinical studies have shown that Amalkadi Ghrita (100 and 300 mg kg^{-1}, p.o.) possesses hepatoprotective activity against CCl_4-induced hepatic damage in rats and the results were comparable to that of silymarin used as positive control. When compared to the controls, administering Amalkadi Ghrita reduced the levels of serum aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), and bilirubin. The histopathological studies further confirmed the protective effects as Amalkadi Ghrita-treated cohorts exhibited almost normal architecture (Achliya et al., 2004).

4.9 *Brahmi Rasayana*

Brahmi Rasayana consisting of Brahmi (*Bacopa monniera*) is an important medhya rasayana (neurotonic). In *Ayurveda*, regular consumption of this *Rasayana* is supposed to improve memory, learning ability, and concentration. It is also prescribed in mental disorders,

epilepsy, mania, hysteria, memory loss, and depression (Shukia et al., 1987). Preclinical studies have shown that *Brahmi Rasayana* possesses anti–inflammatory effects comparable to that of indomethacin and mediates the effect possibly by interfering with the action and/or synthesis of prostaglandins and probably by stabilizing the lysosomal membranes (Jain et al., 1994). Studies have also shown that *Brahmi Rasayana* possesses sedative effect, prolongs the hypnotic action of pentobarbitone, and causes a variable blockade of con–ditioned avoidance response. It also offers protection against electroshock seizures and chemoconvulsions. It was also effective in antagonizing haloperidol–induced catalepsy, suggesting involvement of the gamma–aminobutyric acid (GABA)-ergic system in me–diating the beneficial effects (Shukia et al., 1987).

5. MECHANISMS RESPONSIBLE FOR THE BENEFICIAL EFFECTS

The exact mechanism of action responsible for the beneficial effects of these *Rasayana* drugs is unknown. As these formulations contain many plants with diverse pharmacolog–ical properties, it is logical to expect that myriad protective mechanisms are concomi–tantly operating. Some of the studied and reported mechanisms are explained in the following sections (Figure 17.2, Table 17.3).

5.1 Free Radical Scavenging

Excess generation of free radicals, like superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^\bullet$), nitric oxide (NO), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2),
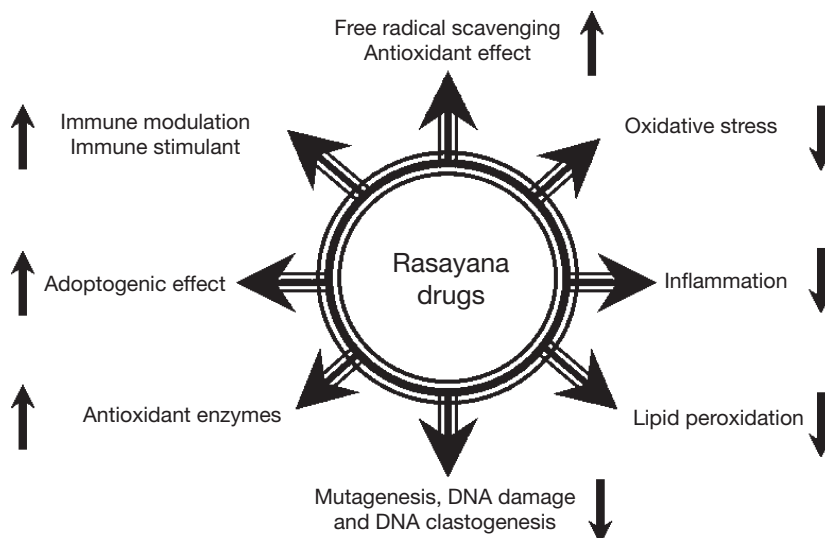
Figure 17.2 Biochemical targets responsible for anti-aging and other beneficial uses of the *Ayurvedic Rasayana* drugs. Arrows up = increase: Arrows down = decrease.

Table 17.3 Plants with various pharmacological properties, which are an integral part of the *Rasayana* drugs

Pharmacological properties	Plants	Reference
Free radical scavenging	*Emblica officinalis, Withania somnifera, Terminalia chebula, Terminalia belerica, Asparagus racemosus, Tinospora cordifolia, Ocimum sanctum, Curcuma longa, Zingiber officinale, Aegle marmelos, Oroxylum indicum, Sida cordifolia, Tribulus terrestris, Phyllanthus niruri, Vitis vinifera, Ellettaria cardamomum, Cinnamomum cassia, Cyperus rotundus, Boerhavia diffusa, Santalum album, Adhatoda vasica, Sesamum indicum, Cinnamomum zeylanica, Glycyrrhiza glabra, Centella asiatica, Acorus calamus, Embelia ribes, Hemidesmus indicus, Cyminum cuminum,* and *Aloe barbadensis.*	Arora et al. (2005b)
Antioxidant	*Asparagus racemosus, Ocimum sanctum, Podophyllum hexandrum, Tinospora cordifolia, Hippophae rhamnoides, Zingiber officinalis, Centella asiatica, Syzygium cumini, Ligusticum wallichii,* and *Vitis vinifera.*	Arora et al. (2005b)
Anti-inflammatory	*Glycyrrhiza glabra, Allium sativum, Aloe vera, Tinospora cordifolia, Hippophae rhamnoides, Curcuma longa, Centella asiatica, Syzygium cumini, Ocimum sanctum, Moringa oleifera, Zingiber officinale,* and *Eleutherococcus senticosus*	Arora et al. (2005b)
Antimutagenic and prevention of DNA damage	*Ocimum sanctum, Curcuma longa, Zingiber officinale, Aegle marmelos, Phyllanthus niruri, Mentha piperita, Tinospora cordifolia, Emblica officinalis, Terminalia chebula,* and *Terminalia belerica*	Arora et al. (2005b)
Immunomodulatory and Adaptogenic activities	*Emblica officinalis, Withania somnifera, Viscum album, Ocimum sanctum,* and *Tinospora cordifolia*	Arora et al. (2005b)

causes damage to cell structures, including lipids and membranes, proteins, and DNA. Scientific studies have shown that phytochemicals and antioxidant molecules are effective in nullifying the free radical–induced damage and delay the pathogenic process. *In vitro* studies suggest that *Brahma Rasayana* scavenge Fe^{2+}–ascorbate and Fe^{3+}–ADP–ascorbate-induced lipid peroxidation, and scavenge the hydroxyl, superoxide, and nitric oxide generated *in vitro*. It also inhibited the PMA-induced superoxide generation in mice peritoneal macrophages and nitrite production in peritoneal macrophages (Rekha et al., 2001a). Triphala and Chyavanaprash have also been observed to scavenge nitric oxide *in vitro* (Jagetia et al., 2004a,2004b). *Rasayana* drugs are composite herbal formulations and many plants, which are an integral part of *Rasayana* preparations, possess free radical scavenging and antioxidant effects (Arora et al., 2005a; Naik et al., 2003; Vayalil et al., 2002a,2002b).

5.2 Inhibition of Lipid Peroxidation

The polyunsaturated fatty acids (PUFAs) are vulnerable to peroxidative attack and the damage it inflicts on the cell membranes leads to various pathogenic process. *In vitro* studies have shown that both alcoholic and aqueous extracts of *Brahma Rasayana* inhibit enzymatic- and nonenzymatic-induced microsomal lipid peroxidation in a concentration-dependent manner (Rekha et al., 2001a,2001b,2001c). *Brahma Rasayana* treatment decreased the radiation-induced increase in the serum lipid peroxidation in cancer treatment (Joseph et al., 1999) and serum and liver lipid peroxidation in the chickens subjected to heat stress (Ramnath et al., 2009). Triphala is also observed to decrease the radiation-induced lipid peroxidation *in vitro* (Naik et al., 2003) and in normal animals (Deep et al., 2005).

5.3 Increase in Antioxidant Enzymes

The antioxidant enzymes SOD, GPx, and CAT cooperate or in a synergistic method work to protect cell against oxidative stress and prevent or delay the pathological changes. Studies have shown that Triphala and *Brahma Rasayana* increase the levels of glutathione and antioxidant enzymes and protect against the oxidative stress (Deep et al., 2005; Rekha et al., 2001b; Sandhya et al., 2006). The administration of aqueous extract of the medicinal plants, like *Aegle marmelos*, *T. chebula*, and *E. officinalis*, that are an integral part of many *Rasayana* formulations have been reported to effectively modulate the oxidative stress and enhance the antioxidant status in rodents (Bhattacharya and Muruganandam, 2003; Deep et al., 2005; Singh et al., 2000).

5.4 Antimutagenic Activities

Mutagenesis is an important step in the process of carcinogenesis and its prevention is of great importance in preventing cancer. There is increasing evidence that many phytochemicals, plants, and their compound formulations can act as inhibitors of mutagenesis and carcinogenesis (Arora et al., 2003, 2005a; Kaur et al., 2002). Kaur et al. (2002) have reported that aqueous chloroform and acetone extracts of Triphala were observed to possess antimutagenic effects against both direct and indirect mutagens in the Ames histidine reversion assay. The extracts inhibited the mutagenicity induced by both direct- and indirect-acting mutagens, but the inhibition was greater for S9-dependent mutagens (Kaur et al., 2002). The acetone and chloroform extracts were observed to be better than the aqueous extracts in the TA98 and TA100 tester strains of *Salmonella typhimurium*. Maximum inhibition was observed for the acetone extract (Kaur et al., 2002).

Triphala and its individual constituents are also reported to prevent γ-radiation-induced DNA strand break formation in the plasmid DNA (pBR322) *in vitro* (Naik et al., 2005). Feeding of Triphala also observed to have inhibited the radiation-induced DNA strand breaks in leukocytes and splenocytes of mice exposed to whole body

irradiation of 7.5 Gy (Sandhya et al., 2006). Studies by Yadav et al. (2003) have shown that consumption of Chyawanprash by bidi smokers decreased the genotoxic risk caused by tobacco mutagen when compared with untreated bidi smokers; consumption of Chyawanprash decreased the mitotic index, chromosomal aberrations, sister chromatid exchanges, and satellite associations (Yadav et al., 2003).

5.5 Anti-inflammatory Effects

Anti-inflammatory drugs, especially nonsteroidal forms, have great importance in modern medical practice. Studies have shown that *Rasayana* drugs and some of their constituent plants are potent inhibitors of inflammation as shown by reduction in paw edema induced by carrageenan and various experimentally induced inflammatory reactions (Jain et al., 1994; Rekha et al., 1997) and alleviate rheumatoid arthritis (Rekha et al., 1997). *Brahmi Rasayana* is also reported to possess anti-inflammatory effects comparable to that of indomethacin (Jain et al., 1994). Triphala is shown to be effective in preventing the Freund's adjuvant-induced arthritis and inflammation in mice. The effect was observed to be better than that of indomethacin, and the levels of lysosomal enzymes, tissue marker enzymes, glycoproteins, and paw thickness were significantly altered in the Triphala group to near normal conditions (Rasool and Sabina, 2007).

5.6 Hemopoietic Stimulation

Scientific studies indicate that the basal hematopoiesis is maintained throughout life but is weakened in advanced age to cope with hematological stress. Exposure to ionizing radiation decreases the hematopoiesis in mammals and is a good model to study the effect of drugs in both protecting and stimulating hematopoiesis when subjected to hematological stress (Arora et al., 2005b; Baliga, 2010). Preclinical studies suggest that the *Rasayana* drugs possess hemopoietic stimulatory function against cytotoxic effects of anticancer agents. Triphala, *Brahma Rasayana, Narasimha Rasayana, Ashwaganda Rasayana*, Amritaprasham, and Chyawanprash have been observed to attenuate the radiation-induced damage to the hemopoietic system (Vayalil et al., 2002a,2002b). The plants *Acanthopanax senticosus, E. officinalis, Ocimum sanctum, W. somnifera, T. cordifolia*, and *B. diffusa* provide total-body radiation protection by stimulating hemopoiesis (Arora et al., 2005b).

5.7 Immune Modulation

The immune system, which is the primary defense mechanism in the body against various pathogens and cancer, grows weaker with age. Immune response can be modulated by immune modulators and studies indicate that certain phytochemicals possess immunostimulatory (immunopotentiation or strengthening of immune reactions) effects. Clinical studies have shown that administration of *Brahma Rasayana* increased the activity of lymphocytes and increase in serum granulocyte macrophage-colony stimulating factor

(GM-CSF) was observed (Joseph et al., 1999). Triphala has been reported to possess immunomodulatory activities and to stimulate the neutrophil functions in the immunized rats and stress–induced suppression in the neutrophil functions (Srikumar et al., 2005).

5.8 Adaptogenic and Antistress Properties

An adaptogen increases the power of resistance against physical, chemical, or biological noxious agents and has a normalizing influence on the body. A number of plants possess adaptogenic activity due to diverse classes of chemical compounds. The plant adaptogens reduce reactivity of host defense systems and decrease damaging effects of various stressors due to increased basal level of mediators involved in the stress response (Ramnath et al., 2009).

Oral administration of Triphala is reported to significantly prevent the noise (Srikumar et al., 2006) and cold stress (Dhanalakshmi et al., 2007)–induced behavioral and biochemical abnormalities in albino rats. Studies have also shown that *Brahma Rasayana* enhances the *in vivo* antioxidant status in cold-stressed and heat-stressed chickens (Ramnath and Rekha, 2009; Ramnath et al., 2009). Studies have shown that the *Rasayana* plants *W. somnifera*, *E. officinalis*, *A. racemosus*, and *T. cordifolia* also possess adaptogenic effects and contributed to the observed effects (Table 17.2) (Arora et al., 2005b; Ramnath et al., 2009; Rege et al., 1999; Suresh and Vasudevan, 1994).

6. CONCLUSIONS

Scientific studies carried out in the past decade have conclusively shown that *Ayurvedic Rasayana* drugs are beneficial in various conditions. Scientific studies carried in the recent past have shown that the *Rasayana* drugs Chyavanprasha, Triphala, *Brahma Rasayana, Ashwagandha Rasayana, Brahmi Rasayana, Narasimha Rasayana*, Amalkadi Ghrita, Anwala churna, and Amritaprasham are effective in various ailments and stress. The mechanism of action of herbal drugs differs in many respects from that of synthetic drugs and can be characterized as a polyvalent action and interpreted as additive or, in some cases, potentiating. A combination of factors, such as free radical scavenging, prevention of lipid peroxidation, inhibition of DNA damage, and protection, as well as rapid regeneration of bone marrow stem cell increase/restoration of antioxidants, would have also contributed to the beneficial effects.

Most of the published observations for the various pharmacological properties of the *Rasayana* drugs have been with experimental animals and help in justifying their applicability to humans. Additionally, these *Rasayana* drugs have been consumed by the inhabitants in Indian subcontinent since time immemorial and the nontoxic nature of these drugs gives immense advantage in initiating human studies. For optimal use and application, detailed studies are required to understand the maximum permissible dose and toxic effects of each of these *Rasayana* drugs with healthy human volunteers. The

results of these experiments will be of immense use for future clinical trials aimed at both treatment and prevention of aging and associated ailments.

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REFERENCES

Achliya, G.S., Wadodkar, S.G., Dorle, A.K., 2004. Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. Journal of Ethnopharmacology 90, 229–232.

Arora, S., Brits, E., Kaur, S., et al., 2005a. Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX tests. Journal of Environmental Pathology, Toxicology, and Oncology 24, 193–200.

Arora, R., Gupta, D., Chawla, R., et al., 2005b. Radioprotection by plant products: present status and future prospects. Phytotherapy Research 19, 1–22.

Arora, S., Kaur, K., Kaur, S., 2003. Indian medicinal plants as a reservoir of protective phytochemicals. Teratogenesis, Carcinogenesis, and Mutagenesis 1, 295–300.

Baliga, M.S., 2010. Triphala, ayurvedic formulation for treating and preventing cancer: a review. Journal of Alternative and Complementary Medicine 16, 1301–1308.

Bhattacharya, S.K., Muruganandam, A.V., 2003. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. Pharmacology, Biochemistry, and Behavior 75, 547–555.

Datta, H.S., Mitra, S.K., Paramesh, R., Patwardhan, B., 2011. Theories and management of aging: modern and ayurveda perspectives. Evidence-Based Complementary and Alternative Medicine 2011, 528527.

Deep, G., Dhiman, M., Rao, A.R., Kale, R.K., 2005. Chemopreventive potential of Triphala (a composite Indian drug) on benzo(a)pyrene induced forestomach tumorigenesis in murine tumor model system. Journal of Experimental & Clinical Cancer Research 24, 555–563.

Dhanalakshmi, S., Devi, R.S., Srikumar, R., Manikandan, S., Thangaraj, R., 2007. Protective effect of Triphala on cold stress-induced behavioral and biochemical abnormalities in rats. Yakugaku Zasshi 127, 1863–1867.

Guruprasad, K.P., Mascarenhas, R., Gopinath, P.M., Satyamoorthy, K., 2010. Studies on Brahma Rasayana in male swiss albino mice: chromosomal aberrations and sperm abnormalities. Journal of Ayurveda and Integrative Medicine 1, 40–44.

Halliwell, B., Gutteridge, J.M.C., 2007. Aging, nutrition, disease and therapy: a role for antioxidants? In: Free radicals in biology and medicine. 4th edition Oxford Press, oxford, pp. 614–677.

Jagetia, G.C., Baliga, M.S., Malagi, K.J., Sethukumar, Kamath M., 2002. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation. Phytomedicine 9, 99–108.

Jagetia, G.C., Malagi, K.J., Baliga, M.S., Venkatesh, P., Veruva, R.R., 2004a. Triphala, an ayurvedic rasayana drug, protects mice against radiation-induced lethality by free-radical scavenging. Journal of Alternative and Complementary Medicine 10, 971–978.

Jagetia, G.C., Rao, S.K., Baliga, M.S., Babu, K.S., 2004b. The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: a preliminary study. Phytotherapy Research 18, 561–565.

Jain, P., Khanna, N.K., Trehan, N., Pendse, V.K., Godhwani, J.L., 1994. Antiinflammatory effects of an ayurvedic preparation, Brahmi Rasayan, in rodents. Indian Journal of Experimental Biology 32, 633–636.

Jose, J.K., Kuttan, R., 2000. Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash. Journal of Ethnopharmacology 72, 135–140.

Jose, J.K., Kuttan, G., Kuttan, R., 2001. Antitumour activity of *Emblica officinalis*. Journal of Ethnopharmacology 75, 65–69.

Joseph, C.D., Praveenkumar, V., Kuttan, G., Kuttan, R., 1999. Myeloprotective effect of a non-toxic indigenous preparation Rasayana in cancer patients receiving chemotherapy and radiation therapy. A pilot study. Journal of Experimental & Clinical Cancer Research 18, 325–329.

Kaur, S., Arora, S., Kaur, K., Kumar, S., 2002. The *in vitro* antimutagenic activity of Triphala – an Indian herbal drug. Food and Chemical Toxicology 40, 527–534.

Kulkarni, S.K., Dhir, A., 2008. *Withania somnifera*: an Indian ginseng. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32, 1093–1105.

Mukherjee, P.K., Rai, S., Bhattacharya, S., et al., 2006. Clinical studies of 'Triphala' – a well known phytomedicine from India. Iranian Journal of Pharmacology and Therapeutics 5, 51–54.

Nadkarni, A.K., 1976. K.M. Nadkarni's Indian Materia Medica. Popular Prakashan Pvt. Ltd., Bombay, India.

Naik, G.H., Priyadarsini, K.I., Bhagirathi, R.G., et al., 2005. *In vitro* antioxidant studies and free radical reactions of Triphala, an ayurvedic formulation and its constituents. Phytotherapy Research 19, 582–586.

Naik, G.H., Priyadarsini, K.I., Satav, J.G., et al., 2003. Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. Phytochemistry 63, 97–104.

Ramnath, V., Rekha, P.S., 2009. Brahma Rasayana enhances *in vivo* antioxidant status in cold-stressed chickens (*Gallus gallus domesticus*). Indian Journal of Pharmacology 41, 115–119.

Ramnath, V., Rekha, P.S., Sujatha, K.S., 2009. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by Brahma Rasayana. Evidence-Based Complementary and Alternative Medicine 5, 77–84.

Rasool, M., Sabina, E.P., 2007. Anti-inflammatory effect of the Indian ayurvedic herbal formulation Triphala on adjuvant-induced arthritis in mice. Phytotherapy Research 21, 889–894.

Rege, N.N., Thatte, U.M., Dahanukar, S.A., 1999. Adaptogenic properties of six rasayana herbs used in ayurvedic medicine. Phytotherapy Research 13, 275–291.

Rekha, P.S., Kuttan, G., Kuttan, R., 1997. Antioxidant and anti-inflammatory activity of Brahma Rasayana. Amala Research Bulletin 17, 68–72.

Rekha, P.S., Kuttan, G., Kuttan, R., 1998. Immunopotentiating activity of Brahma Rasayana. Amala Research Bulletin 18, 85–90.

Rekha, P.S., Kuttan, G., Kuttan, R., 2000. Effect of herbal preparation, Brahma Rasayana, in amelioration of radiation induced damage. Indian Journal of Experimental Biology 38, 999–1002.

Rekha, P.S., Kuttan, G., Kuttan, R., 2001a. Antioxidant activity of brahma rasayana. Indian Journal of Experimental Biology 39, 447–452.

Rekha, P.S., Kuttan, G., Kuttan, R., 2001b. Effect of Brahma Rasayana on antioxidant system after radiation. Indian Journal of Experimental Biology 39, 1173–1175.

Rekha, P.S., Kuttan, G., Kuttan, R., 2001c. Effect of Brahma Rasayana on antioxidant systems and cytokine levels in mice during cyclophosphamide administration. Journal of Experimental & Clinical Cancer Research 20, 219–223.

Sabu, M.C., Kuttan, R., 2002. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of Ethnopharmacology 81, 155–160.

Sabu, M.C., Kuttan, R., 2004. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian Journal of Physiology and Pharmacology 48, 81–88.

Sandhya, T., Lathika, K.M., Pandey, B.N., et al., 2006. Protection against radiation oxidative damage in mice by Triphala. Mutation Research 609, 17–25.

Sharma, R.K., Dash, B., 1998. Carka Samhita, vol. II. Chowkamba Sanskrit Series Office, Varanasi, India.

Shukia, B., Khanna, N.K., Godhwani, J.L., 1987. Effect of Brahmi Rasayan on the central nervous system. Journal of Ethnopharmacology 21, 65–74.

Singh, R.P., Banerjee, S., Rao, A.R., 2000. Effect of *Aegle marmelos* on biotransformation enzyme systems and protection against free-radical-mediated damage in mice. Journal of Pharmacy and Pharmacology 52, 991–1000.

Srikumar, R., Jeya Parthasarathy, N., Sheela Devi, R., 2005. Immunomodulatory activity of Triphala on neutrophil functions. Biological & Pharmaceutical Bulletin 28, 398–403.

Srikumar, R., Parthasarathy, N.J., Manikandan, S., Narayanan, G.S., Sheeladevi, R., 2006. Effect of Triphala on oxidative stress and on cell–mediated immune response against noise stress in rats. Molecular and Cellular Biochemistry 283, 67–74.

Suresh, K., Vasudevan, D.M., 1994. Augmentation of murine natural killer cell and antibody dependent cellular cytotoxicity activities by *Phyllanthus emblica*, a new immunomodulator. Journal of Ethnopharmacology 44, 55–60.

Vani, T., Rajani, M., Sarkar, S., Shishoo, C.J., 1997. Antioxidant properties of the ayurvedic formulation Triphala and its constituents. International Journal of Pharmacognosy 35, 313–317.

Vasudevan, M., Parle, M., 2007. Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.): an ayurvedic preparation. Physiology and Behavior 91, 46–54.

Vayalil, P.K., Kuttan, G., Kuttan, R., 2002a. Rasayanas: evidence for the concept of prevention of diseases. The American Journal of Chinese Medicine 30, 155–171.

Vayalil, P.K., Kuttan, G., Kuttan, R., 2002b. Protective effects of Rasayanas on cyclophosphamide and radiation–induced damage. Journal of Alternative and Complementary Medicine 8, 787–796.

Yadav, J.S., Thakur, S., Chadha, P., 2003. Chyawanprash Awaleha: a genoprotective agent for bidi smokers. International Journal of Human Genetics 3, 33–38.

CHAPTER *18*

Selenium, Selenoproteins, and Age-Related Disorders

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1. INTRODUCTION

Over the last century, advances in nutrition and public health have contributed to a rapid growth of the elderly population. Although aging is an inevitable biological process, recent breakthroughs in the understanding of the longevity pathways shed light on further extension of lifespan through dietary-intervention approaches. In particular, the efficacy of calorie restriction has recently been confirmed in primates, in which lifespan is extended and age-related phenotypes and pathologies are attenuated (Colman et al., 2009). Furthermore, mid-age and old mice administrated with rapamycin, an inhibitor of the target of rapamycin (TOR) nutrient-sensing pathway, show extended lifespan (Harrison et al., 2009). These results point to the significance of nutritional control of aging intervention via bioactive food components.

Another popular theory of aging is based on free radical accumulation. Although proposed by Denham Harman in 1950s, the free radical theory of aging is still not yet definitively proven or disproven. Free radicals can be generated endogenously from normal metabolic processes including energy production in mitochondria and immune response in macrophages, as well as exogenously including environmental exposures and excessive tobacco and alcohol consumption. Free radicals contain unpaired electrons that are bioactive to oxidize DNA, proteins, and lipids. Free radicals can be quenched by bioactive food antioxidants such as the selenium-dependent glutathione peroxidases, and DNA and protein repair proteins can act as a second line of defense. Interestingly, the maximum lifespan in vertebrates is negatively correlated with the rate of free radical production, and lipid peroxidation increases in the elderly (Finkel and Holbrook, 2000). Of note, the aging theories of free radicals and DNA damage merge when one considers the accumulation of oxidative DNA damage during the aging process.

2. SELENIUM

Selenium was discovered by Berzelius in 1817. Early research focused on selenium toxicity until 1957, when the essentiality of this element in animals was established. In 1972, selenium was found to be a cofactor for glutathione peroxidase. Selenium is a trace mineral essential for a spectrum of biological and physiological functions. Selenium is widely distributed in inorganic forms in the soil and in organic forms in certain foods such as Brazil nuts and seafood. Wide variations have been found in selenium status in different parts of the world. The reported symptoms of selenium deficiency in animals include inflammation and injury of the muscles, degeneration of the pancreas, and abnormal coloration. The human Keshan disease is a cardiovascular disease and is endemic to those who rely on foods grown on the selenium-deficient land in Northeast China. The etiology of Keshan disease is the increased susceptibility of heart muscle to Coxsackievirus B_3 due to selenium deficiency. Kashin–Beck disease is a chronic osteoarthropathy occurring in young children due to dietary deficiency in both selenium and iodine. Based on a mouse model engineered to carry selenoprotein deficiency in osteo-chondroprogenitors, it is convincing that selenoproteins play critical roles in preventing the bone pathologies (Downey et al., 2009). Organic selenium compounds have high bioavailability but are also toxic in large doses. In this regard, nanoencapsulation of selenium compounds has been considered to modulate selenium bioavailability and toxicity.

Selenium can regulate metabolic functions through selenium–containing proteins. The immune response and maintenance of tissue homeostasis can possibly be influenced by the selenium status (Carlson et al., 2009). The heavy metal toxicity can be decreased interaction with selenium. Selenium can also improve the production of sperm and sperm movement and alleviate cardiovascular diseases. Long-term epidemiological studies indicate a reverse relationship between selenium intake and cancer risk. The Nutritional Prevention of Cancer (NPC) Trial was the first double-blind, placebo-controlled intervention trial in a Western population. The NPC Trial concludes that daily selenium intake at a supranutritional level significantly decreases risks of prostate, colon, and lung cancer in skin cancer patients (Clark et al., 1996). Furthermore, the role of selenium in suppressing prostatic intraepithelial neoplasia has been inferred. Nonetheless, the prematurely terminated Selenium and Vitamin E Cancer Prevention (SELECT) Trial (due to diabetic complications) does not support prostate cancer prevention by selenomethionine alone or in combination with vitamin E (Lippman et al., 2009).

3. SELENOPROTEINS

Selenium substitutes sulfur in cysteine (Cys), forming the 21st amino acid, selenocysteine (Sec). Sec is more active than Cys because of the lower pK_a value and stronger

nucleophilicity. Excessive selenium compounds and Sec can result in rapid NADPH oxidation and accumulation of ROS. Therefore, there is no free pool of Sec. Selenoprotein contains Sec that is incorporated during the translation process when Sec-tRNA^{Sec} decodes the in-frame UGA codon on selenoprotein mRNA. Selenoprotein mRNA also contains a Sec insertion sequence (SECIS) that forms a stem-loop structure in the 3-untranslated region. SECIS is associated with Sec-specific elongation factor and SECIS-binding protein 2 (SBP2), which works in concert with Sec-tRNA^{Sec} to avoid UGA being recognized as a stop signal. Mammalian cells can use both organic and inorganic forms of selenium for Sec incorporation. The key selenium metabolite, selenide, can be generated by reducing selenite in the glutaredoxin and thioredoxin systems. Selenophosphate synthetase 2 (SPS2) converts selenide into monoselenophosphate, which is the active selenium donor for the formation of Sec-tRNA^{Sec}.

Selenoproteins do not exist in all species. There are selenoproteins in archaea, bacteria, and most eukaryotes, but not in yeast and higher plants. Computational sequence analyses of the entire genome conclude that there is a total of 25 and 26 selenoproteins in humans and mice, respectively (Kryukov et al., 2003). The majority of selenoproteins are involved in redox regulation. The selenium-dependent glutathione peroxidases (GPX1–4 and GPX6) and thioredoxin reductases (TrxR1-3) directly suppress oxidative stress. Moreover, the expression of GPX2 can be upregulated through the redox-sensitive transcription factor Nrf2/Keap1, and selenoprotein H (SelH) is proposed as a sensor of nuclear oxidative stress. Selenoproteins have been implicated in many metabolic and functional pathways such as aging, cancer, and virus infection (Lu and Holmgren, 2009), but functions of many of the newly identified selenoproteins are not well characterized.

The deficit of dietary selenium can dramatically affect the expression of selenoproteins to various degrees and in a tissue-specific manner. For example, GPX1 is more sensitive than other selenoproteins to body selenium fluctuation, and dietary selenium deficiency suppresses GPX1 expression to a greater extent in liver and heart than in testis in the rat (Brigelius-Flohe, 1999).

3.1 Glutathione Peroxidases (GPXs)

Selenium-dependent GPXs in humans include the ubiquitous GPX1, the gastrointestinal GPX2, the plasma GPX3, phospholipid hydroperoxide GPX4, and the olfactory epithelium- and embryonic tissue-specific GPX6 (Arthur, 2000). GPX1–3 are tetrameric enzymes and prefer to reduce hydrogen peroxide. GPX1 is the most abundant selenoprotein and a major metabolic form of body selenium against acute oxidative stress (Cheng et al., 1998). Unlike GPX1, the expression of GPX2 and GPX3 is tissue-specific. GPX2 can suppress oxidative stress and intestinal mucosa inflammation. The extracellular GPX3 is synthesized in liver, kidney, lung, and heart and secreted to plasma. GPX1 and GPX3 are indicators of body

selenium status. GPX4 is a monomeric enzyme whose preferred substrates include phospholipid and cholesterol hydroperoxides. There are three isoforms of GPX4 according to their subcellular localization: cytosol, mitochondria, and nuclear. Only cytosolic GPX4 is essential for embryonic development and cell survival. Mitochondrial and nuclear GPX4 are structural components of the sperm mitochondrial capsule in mature spermatozoa and involved in sperm maturation and male fertility (Ursini et al., 1999).

The link between GPX proteins and certain diseases is evidenced. Erythrocyte GPX1 level is decreased in Alzheimer's patients, which may account for an increased susceptibility of the neurons to oxidative stress (Vural et al., 2010). Another age-related disease, Parkinson's disease, also exhibits lowered GPX and antioxidant activities in peripheral blood, elevated 8-oxo-G level, and neurodegeneration. Moreover, the GPX1 Pro1981-Leu single nucleotide polymorphism (SNP) is shown to be associated with lung cancer. In many cancer cells, GPX1 and GPX4 are downregulated, and overexpression of GPX3 can inhibit prostate cancer cell growth. Interestingly, mice deficient in both GPX1 and GPX2 spontaneously develop ileocolitis and intestinal cancer.

3.2 Thioredoxin Reductases (TrxRs)

There are three TrxRs in mammals: the cytosolic TrxR1, the mitochondrial TrxR2, and the thioredoxin–glutaredoxin reductase (TrxR3) highly expressed in testis. Interestingly, expression of TrxR1 is high in neuronal tissues (Soerensen et al., 2008). Under dietary selenium deficiency, the expression of TrxRs is prioritized in the brain, suggesting a critical role of TrxRs in the brain. Mice with null deletion of TrxR1 or TrxR2 are embryonic lethal, while the essentiality of TrxR3 is unknown.

TrxRs use thioredoxin nucleoside diphosphate as a substrate, and the enzymatic activity is regulated by dietary selenium. Decreased TrxR activity is associated with ROS accumulation and the etiology of Alzheimer's and Parkinson's diseases. As such, TrxRs are proposed as potential therapeutic targets for ROS-associated neurodegenerative diseases. On the other hand, since tumor cells may take electrons from the Trx system, TrxRs have emerged as new targets for anticancer drug development. TrxRs may optimize malignant cell growth during tumorigenesis and have been found to be overexpressed in many aggressive tumors (Park et al., 2006).

3.3 Iodothyronine Deiodinases (DIOs)

The DIO family includes DIO1–3, which are involved in the regulation of thyroid hormones thyroxine (T4), 3,5,3′-triiodothyronine (T3), and reverse triiodothyronine (rT3) (Bianco and Kim, 2006). The expression and function of DIOs are tissue-specific. DIO1 regulates T3 production in the thyroid glands and the circulating level of T4. Mice with DIO1 knockout display abnormal concentrations of thyroid hormones. DIO2 is expressed in the thyroid, central nervous system, pituitary, and skeletal muscle, where

this selenoprotein regulates T3 circulation and production. DIO2 knockout mice exhibit disrupted auditory functions, thermogenesis, and brain development. DIO3 is expressed in fetal tissues, placenta, neonatal brain, and skin to locally control the deiodination process. DIO3 knockout mice show reduced viability, growth retardation, impaired fertility, reduced T3, and increased T4 levels.

There are a few human diseases associated with defective expression of DIOs and thyroid hormone metabolism. Individuals with Graves' hyperthyroidism show increased T3 and DIO1 levels, suggesting the possibility of treating the disease by DIO1 inhibition. In humans, the expression of a truncated SBP2 is associated with abnormal thyroid hormone metabolism (Azevedo et al., 2010). Combined selenium and iodine deficiency leads to the condition of myxedematous cretinism showing increased oxidative damage and altered thyroid hormone metabolism. The link between DIO dysregulation and cancer is also evidenced. Expression of DIO1 is reduced or lost in breast cancer, and dysfunctional DIO1 and DIO2 are associated with papillary thyroid cancer (Azevedo et al., 2010).

3.4 Selenoprotein 15

Selenoprotein 15 (Sep15) is highly expressed in the thyroid, parathyroid, and prostate (Gladyshev et al., 1998). The major cellular location of Sep15 is endoplasmic reticulum, suggesting a role of this selenoprotein in protein folding and disulfide bond formation. There are a few identified Sep15 SNPs, including 811 (C/T) and 1125 (A/G) in the $3'$ UTR and 1125 (A/G) in the SECIS (Gladyshev et al., 1998). The 1125A/G polymorphic Sep15 variant may determine the Sec incorporation efficiency during translation. The Sep15 polymorphisms are highly relevant to the prostate cancer mortality. Consistently, by manipulating Sep15 expression in CT26 mouse colon cancer cells and injecting them to BALC/c mice, Irons et al. (2010) conclude that Sep15 may promote pulmonary metastasis.

3.5 Selenoprotein P

Selenoprotein P (SelP) is the second selenoprotein discovered in mammals. SelP bears 10 Sec residues and two SECIS, suggesting a critical role in the maintenance of selenium homoeostasis (Burk and Hill, 2005). SelP is the dominate selenoprotein in plasma, and the expression level decreases only under severe dietary selenium deficiency. SelP is mainly synthesized as a glycoprotein in the liver and secreted into body fluids. SelP facilitates selenium delivery from the liver to peripheral tissues, especially the brain. SelP knockout mice show decreased SelW, GPX1, and GPX4 mRNA expression in the brain and testis, implicating this selenoprotein as a selenium transporter. Moreover, SelP has been reported to reduce phospholipid hydroperoxide and protect neuronal cells

from oxidative stress. The research using postmortem tissues from Alzheimer's brain revealed high SelP expression in amyloid–β plaques and neurofibrillary tangles. Furthermore, reduced expression of SelP is linked to certain cancers. For instance, SelP SNPs enhance the incidence of prostate cancer risk in Sweden men, probably due to increased oxidative stress in the cancer cells.

3.6 Other Selenoproteins

As discussed above, SPS plays an essential role for Sec incorporation. Interestingly, SPS2, but not SPS1, is a selenoprotein and involves in monoselenophosphate synthesis. Although SPS1 is an essential gene for the regulation of glutamate level and mitochondrial function in drosophila (Shim et al., 2009), its role in mammals remains unclear. Dietary selenium status also regulates the expression of the 10-kDa selenoprotein W (SelW) in muscle, spleen, testis, and brain in rats. SelW contains a redox motif and binds glutathione, thus being considered as an antioxidant enzyme. Overexpression of SelW in Chinese hamster ovary (CHO) cells and H1299 human lung cancer cells resulted in resistance to H_2O_2 exposure. It is postulated that SelW serves as a H_2O_2 signal transducer. Because SelW-deficient embryonic cerebral cortex cells exhibit increased sensitivity to H_2O_2, SelW may protect neurons against oxidative stress. Interestingly, SelW shows increased mRNA expression when cultured breast and prostate epithelial cells are supplemented with sodium selenite or high-selenium serum.

SelH is a recently discovered 14-kDa protein that contains a DNA-binding domain and resides in the nucleolus. Sequence and structural analyses demonstrate that SelH gene contains a conserved thioredoxin-like CXXU motif indicative of an antioxidant enzyme. SelH can upregulate glutathione level and GPX activity in the stress response in murine hippocampal HT22 cells. Overexpression of SelH in HT22 cells rescues cells from UVB-induced damage by reducing the superoxide formation. SelH can also protect neurons from UVB exposure by inhibiting apoptosis and mitochondrial depolarization. SelH expression is increased in the early stages of embryonic development and in LNCaP prostate cancer and LCC1 lung cancer cells. This suggests that SelH can maintain genome stability and suppress tumorigenesis by suppressing oxidative stress.

Selenoprotein M (SelM), a recently identified endoplasmic reticulum selenoprotein, is highly expressed in the brain and to a less extent in other tissues. The transgenic pCMV/GFP-hSelM mice exhibit lowered H_2O_2 level but increased antioxidative activity after 2,2′-azobiz injection. Overexpression of SelM in several neuron cell lines resulted in decreased oxidative stress and apoptotic cell death in response to H_2O_2 (Reeves et al., 2010). The transgenic mice overexpressed with a mutant human presenilin–2 showed suppression of SelM transcriptional products, indicating the possible protective functions of SelM in the Alzheimer's brain. Moreover, SelM may modulate cytosolic calcium homeostasis (Reeves et al., 2010).

4. SELENIUM REGULATES AGE-RELATED DISEASES

Chronic diseases are the leading cause of mortality in the world, accounting for 60% of all deaths. The incidence of chronic diseases increases with age and genome instability. Consumption of bioactive food components and certain nutrients at amounts higher than the nutritional needs can prevent or delay the onset of chronic diseases. Here the focus is on the role of selenium and selenoproteins in genome maintenance and age-related chronic diseases.

4.1 Tumorigenesis

Several decades after establishing the nutritional essentiality of selenium, a solid body of recent evidence indicates the efficacy of supranutritional selenium in counteracting tumorigenesis. In addition to the above-mentioned NPC Trial (Clark et al., 1996), a study conducted by Schrauzer and colleagues (1977) on subjects across 27 countries concludes that dietary intake of supplemental selenium is inversely correlated with age-adjusted cancer mortality. Animal studies also indicate a role of selenium in the suppression of tumorigenesis, and the efficacy of which depends on the formulation of selenium species. In Muc2/p21 double mutant mice, sodium selenite could reduce intestinal tumor formation through the inhibition of cell proliferation. In a mouse model of prostate adenocarcinoma, dietary methylseleninic acid or methylselenocysteine supplementation decreases prostate tumor volume and increases mouse survival.

Recent epidemiological studies in general lend support for selenium chemoprevention. The 1312 subjects in the NPC trial were randomized to placebo or 200 g selenium per day in the form of selenized yeast, which includes selenomethionine (65–80%) and 20 other selenium compounds (Clark et al., 1996). Several intervention studies constructed in Linxian, China, also demonstrate that selenium could reduce esophageal/ gastric cardia cancer (Stewart et al., 1999). In contrast, the SELECT Trial failed to prove the role of selenomethionine in the suppression of prostate cancer in healthy men (Lippman et al., 2009). The distinct selenium formulation and serum selenium baselines may explain the different conclusions drawn from the major selenium clinical trials. In particular, selenomethionine may not be an appropriate selenium form carrying anticancer activity. Animal studies conclude that methyl-selenium compounds, but not selenomethione, suppress prostate cancer (Li et al., 2008). Moreover, selenomethionine may enable the body to accumulate selenium and thus causing toxicity.

Selenium can also target tumorigenesis through selenoproteins. Analysis of mRNA levels in paired lung specimens of 33 non-small cell lung cancer patients showed downregulation of SelP, and this suppression may increase oxidative stress (Gresner et al., 2009). Knockdown of TrxR1 in the human colorectal carcinoma RKO cells exhibited enhanced cytotoxicity, promoting apoptosis in association with nitric oxide production. Moreover, TrxR levels are increased in breast cancer, prostate cancer, and colorectal

carcinoma as evidenced by immunocytochemical examination. These results support the role of TrxR in carcinogenesis and suggest potential targets for cancer treatment.

On the other hand, selenium chemoprevention may be executed by its prooxidative, rather than antioxidative properties (Drake, 2006). Consistent with this notion, recent reports suggest that selenium compounds can act as a prooxidant and mitigate tumorigenesis. Methylseleninic acid exhibits high anticarcinogenic potential in cells and in mice through its metabolite, methyl selenol. ROS are generated during the catabolism process of selenium compounds, which can subsequently damage DNA and result in senescence or apoptosis. Apoptosis is a genetically controlled path to death, providing a noninflammatory mechanism to eliminate unrepaired cells. Sodium selenite can trigger p53-dependent apoptosis or activation of p53 and p38 pathways in LNCaP prostate cancer and cervical carcinoma cells. Mitochondria release of cytochrome c contributes to the caspase-dependent apoptosis in DU-145 prostate cancer cells exposed to methylseleninic acid. Selenomethionine can protect cells from methyl methanesulfonate treatment by triggering the p53-depedent BER pathway in RKO cells. Selenium compounds at doses $\leq LD_{50}$ induces an ATM(ataxia telangiectasia mutated)- and ROS-dependent DNA damage and senescence responses in MRC-5 and CCD 841 normal fibroblasts but not in two lines of cancer cells. At lethal doses, selenium compounds target the hMLH1 protein of the MMR pathway for an ATM-dependent, G2/M checkpoint, and DNA damage responses and induce apoptotic response in colorectal cancer cells in a manner depending on ATM and ROS. Lack of hMLH1 may explain why MMR-deficient colorectal cancer cells are resistant to selenium-induced cell death.

4.2 Cardiovascular Diseases

Enhanced oxidative stress in cardiac and vascular myocytes is causative to cardiovascular diseases. It has been proposed that antioxidative selenoproteins may attenuate atherosclerosis and protect against cardiovascular diseases (Navas-Acien et al., 2008). Except for two studies, results from 25 other studies from 1966 to 2005 strongly support a link between body selenium status and coronary heart disease.

The antioxidative activity of GPX1 and GPX4 may account for much of the selenium protection against cardiovascular diseases. GPX4 may prevent the accumulation of oxidized low-density lipoproteins in the artery wall. Under selenium deficiency, a buildup of hydroperoxides inhibits the enzyme prostacyclin synthetase that is responsible for the production of vasodilatory prostacyclin in the endothelium, leading to vasoconstriction and platelet aggregation. Thus, the balance is tipped toward the proaggregatory state. In men with coronary artery disease, platelet aggregation is inversely related to selenium status. Epidemiologic studies provide sufficient evidence for the association of mild hyperhomocysteinemia and high blood homocysteine with cardiovascular diseases. Homocysteine can enhance ROS levels, partially through inhibiting GPX1 protein

synthesis. In fact, patients carrying low GPX1 expression but high homocysteine level are threefold more likely to develop cardiovascular diseases. Therefore, simultaneous assessment of GPX1 and homocysteine are useful to predict cardiovascular diseases.

Homocysteine is biosynthesized from methionine, instead of being directly obtained from foods. High level of homocysteine can result in elevated S-adenosylhomocysteine, but this can be reversed by folic acid and vitamin B_{12} that favors the formation of S-adenosylmethionine. Thus, folate deficiency could possibly increase the risk of cardiovascular diseases by silencing antioxidant enzymes. Besides, erythroblasts cultured in folate-deficient medium revealed increased uracil misincorporation into DNA. However, the results from selenite- and folate-fed weanling Fischer–344 rats revealed that folate deficiency can be partially rescued by selenium through induction of homocysteine conversion to glutathione.

4.3 Diabetes

Although taking selenium above the nutritional level can provide many health benefits, body selenium concentration is known to be higher in diabetic than in healthy individuals. A cross-sectional study involving 8876 subjects (Bleys et al., 2007) and a cohort study conducted in Northern Italy (Stranges et al., 2010) collectively suggest a positive association between high serum selenium and increased risk of diabetes. Indeed, the SELECT study does support the association between high selenium intake and type 2 diabetes (Lippman et al., 2009). Cardiac dysfunction occurring in both type 1 and type 2 diabetes may be attributed to redox imbalance. In contrast, feeding diabetic rats with 5 µmol selenite per kilogram of body weight per day prevents the antioxidant system defects induced by diabetes.

Intriguingly, selenium can function as an insulin-like molecule. Comparing diabetic and non-diabetic lean mice, the results implicate selenium in regulating serum glucose level with less liver damage, as well as activating the Akt and PI3K kinases insulin signaling pathway. Moreover, selenoproteins can also participate in glucose regulation. Increased SelP concentration could result in a dysfunctional insulin signaling pathway and glucose homeostasis. Considering their involvement in glucose metabolism, selenium and selenoproteins are potential targets in treating type 2 diabetes.

4.4 Other Age-Related Diseases

In general, plasma selenium levels tend to decrease with age and in age-related diseases (Richard and Roussel, 1999). Interestingly, body selenium in centenarians is maintained at a nutritionally adequate level, suggesting that there is a positive association between selenoprotein expression and longevity. Although selenium in the human body is broadly distributed and fluctuates in response to dietary selenium availability, the selenium level in the brain is always well maintained. Elevated ROS levels contributed to the

pathologies of Alzheimer's and Parkinson's disease, which can be suppressed by antioxidative selenoproteins. SelP is essential for the normal function of neuronal cells and protects against Alzheimer's disease as mentioned in Section 3.5. Also, reducing SelP expression in neuronal N2A cells resulted in increased apoptosis through aggregated amyloid β-induced toxicity.

5. CONCLUSION

Although there is no definitive, casual relationship between oxidative stress and aging, most age-related chronic diseases are associated with internal ROS imbalance and genomic instability. Since most selenoproteins acquire antioxidant enzymatic functions, they should in principle be able to attenuate or delay such diseases by regulating redox status. Because SelH is localized in the nucleus and senses redox change, this selenoprotein could function as a transcriptional factor that regulates genes involved in glutathione synthesis and phase II detoxification upon oxidative stress. In this case, selenoproteins not only can act directly in the antioxidant system to protect DNA but also function as transcription factors, indirectly maintaining genomic stability. In addition, a deeper insight into the relationship between selenium and certain age-related pathologies such as type II diabetes and its related cardiac and vascular dysfunction as part of the metabolic syndrome is desirable. This new insight should consider selenium and selenoproteins as potential targets for pharmacological treatment of the pathology.

Taken together, detailed mechanisms of *in vivo* functions of selenium are in a great urgent need to efficiently and precisely apply this nutrient for human benefits. Therefore, further studies focusing on the link between genotoxic stress imposed by selenium and pathways involved in maintaining genome stability or triggering DNA damage response can help understand the mechanisms underlying selenium chemopreventive and other effects. Moreover, the WRN and ATM protein, mutated in Werner syndrome and ataxia telangiectasia, respectively, play critical roles in the maintenance of telomere structure. Restoration of telomerase rescues the replicative senescence in normal somatic cells, as well as fibroblasts isolated from genome instability syndromes such as dyskeratosis congenita and Werner syndrome. These lines of evidence strongly support the critical role of genome and telomere stability in defending against aging. Therefore, it is of future interest to explore the role of selenium in replicative senescence and aging by targeting proteins that are essential to prevent premature aging syndromes.

GLOSSARY

Premature aging syndrome Hereditary diseases that cause patients to show aging phenotypes earlier than their chronological age. Many of the syndromes are due to mutations in DNA damage response genes.
Selenium An essential mineral that supports selenoprotein expression and optimal health.

Selenoproteins A group of 25 selenocysteine-containing proteins in humans. Selenoproteins mRNA contain the in-frame UGA codon and selenocysteine insertion sequence in the 3-UTR.

Senescence An aging process or stress response, in which cells are metabolically active but lose the capability of proliferation.

REFERENCES

Arthur, J.R., 2000. The glutathione peroxidases. Cellular and Molecular Life Sciences 57, 1825–1835.

Azevedo, M.F., Barra, G.B., Naves, L.A., et al., 2010. Selenoprotein-related disease in a young girl caused by nonsense mutations in the SBP2 gene. Journal of Clinical Endocrinology and Metabolism 95, 4066–4071.

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 116, 2571–2579.

Bleys, J., Navas-Acien, A., Guallar, E., 2007. Serum selenium and diabetes in US adults. Diabetes Care 30, 829–834.

Brigelius-Flohe, R., 1999. Tissue-specific functions of individual glutathione peroxidases. Free Radical Biology & Medicine 27, 951–965.

Burk, R.F., Hill, K.E., 2005. Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. Annual Review of Nutrition 25, 215–235.

Carlson, B.A., Yoo, M.H., Sano, Y., et al., 2009. Selenoproteins regulate macrophage invasiveness and extracellular matrix-related gene expression. BMC Immunology 10, 57–68.

Cheng, W.H., Ho, Y.S., Valentine, B.A., et al., 1998. Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. Journal of Nutrition 128, 1070–1076.

Clark, L.C., Combs Jr., G.F., Turnbull, B.W., et al., 1996. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. Journal of the American Medical Association 276, 1957–1963.

Colman, R.J., Anderson, R.M., Johnson, S.C., et al., 2009. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325, 201–204.

Downey, C.M., Horton, C.R., Carlson, B.A., et al., 2009. Osteo-chondroprogenitor-specific deletion of the selenocysteine tRNA gene, Trsp, leads to chondronecrosis and abnormal skeletal development: a putative model for Kashin–Beck disease. PLoS Genetics 5, e1000616.

Drake, E.N., 2006. Cancer chemoprevention: selenium as a prooxidant not an antioxidant. Medical Hypotheses 67, 318–322.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Gladyshev, V.N., Jeang, K.T., Wootton, J.C., Hatfield, D.L., 1998. A new human selenium-containing protein – purification, characterization, and cDNA sequence. Journal of Biological Chemistry 273, 8910–8915.

Gresner, P., Gromadzinska, J., Jablonska, E., Kaczmarski, J., Wasowicz, W., 2009. Expression of selenoprotein-coding genes SEPP1, SEP15 and hGPX1 in non-small cell lung cancer. Lung Cancer 65, 34–40.

Harrison, D.E., Strong, R., Sharp, Z.D., et al., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.

Irons, R., Tsuji, P.A., Carlson, B.A., et al., 2010. Deficiency in the 15-kDa selenoprotein inhibits tumorigenicity and metastasis of colon cancer cells. Cancer Prevention Research 3, 630–639.

Kryukov, G.V., Castellano, S., Novoselov, S.V., et al., 2003. Characterization of mammalian selenoproteomes. Science 300, 1439–1443.

Li, G.X., Lee, H.J., Wang, Z., et al., 2008. Superior *in vivo* inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. Carcinogenesis 29, 1005–1012.

Lippman, S.M., Klein, E.A., Goodman, P.J., et al., 2009. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Journal of the American Medical Association 301, 39–51.

Lu, J., Holmgren, A., 2009. Selenoproteins. Journal of Biological Chemistry 284, 723–727.

Navas-Acien, A., Bleys, J., Guallar, E., 2008. Selenium intake and cardiovascular risk: what is new? Current Opinion in Lipidology 19, 43–49.

Park, J.H., Kim, Y.S., Lee, H.L., et al., 2006. Expression of peroxiredoxin and thioredoxin in human lung cancer and paired normal lung. Respirology 11, 269–275.

Reeves, M.A., Bellinger, F.P., Berry, M.J., 2010. The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation. Antioxidants & Redox Signaling 12, 809–818.

Richard, M.J., Roussel, A.M., 1999. Micronutrients and ageing: intakes and requirements. Proceedings of the Nutrition Society 58, 573–578.

Shim, M.S., Kim, J.Y., Jung, H.K., et al., 2009. Elevation of glutamine level by selenophosphate synthetase 1 knockdown induces megamitochondrial formation in drosophila cells. Journal of Biological Chemistry 284, 32881–32894.

Soerensen, J., Jakupoglu, C., Beck, H., et al., 2008. The role of thioredoxin reductases in brain development. PLoS One 3, e1813.

Stewart, M.S., Spallholz, J.E., Neldner, K.H., Pence, B.C., 1999. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radical Biology & Medicine 26, 42–48.

Stranges, S., Sieri, S., Vinceti, M., et al., 2010. A prospective study of dietary selenium intake and risk of type 2 diabetes. BMC Public Health 10, 564–571.

Ursini, F., Heim, S., Kiess, M., et al., 1999. Dual function of the selenoprotein PHGPx during sperm maturation. Science 285, 1393–1396.

Vural, H., Demirin, H., Kara, Y., Eren, I., Delibas, N., 2010. Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. Journal of Trace Elements in Medicine and Biology 24, 169–173.

FURTHER READING

Andersen, J.K., 2004. Oxidative stress in neurodegeneration: cause or consequence? Nature Medicine 10, S18–S25.

Anne-Marie, R., Tasnime, A.N., 2007. Selenium and aging. Agro Food Industry Hi-Tech 18, 59–60.

Beck, M.A., Kolbeck, P.C., Shi, Q., et al., 1994. Increased virulence of a human enterovirus (coxsackievirus B3) in selenium-deficient mice. Journal of Infectious Diseases 170, 351–357.

Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J., Larsen, P.R., 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocrine Reviews 23, 38–89.

Brigelius-Flohe, R., Kipp, A., 2009. Glutathione peroxidases in different stages of carcinogenesis. Biochimica et Biophysica Acta – General Subjects 1790, 1555–1568.

Brozmanova, J., Manikova, D., Vlckova, V., Chovanec, M., 2010. Selenium: a double-edged sword for defense and offence in cancer. Archives of Toxicology 84, 919–938.

Burk, R.F., Hill, K.E., Motley, A.K., 2003. Selenoprotein metabolism and function: evidence for more than one function for selenoprotein P. Journal of Nutrition 133, 1517S–1520S.

Chen, C.M., Liu, J.L., Wu, Y.R., et al., 2009. Increased oxidative damage in peripheral blood correlates with severity of Parkinson's disease. Neurobiology of Disease 33, 429–435.

Cheng, W.H., 2009. Impact of inorganic nutrients on maintenance of genomic stability. Environmental and Molecular Mutagenesis 50, 349–360.

Conrad, M., Jakupoglu, C., Moreno, S.G., et al., 2004. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Molecular and Cellular Biology 24, 9414–9423.

Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. Journal of Hypertension 18, 655–673.

Felix, K., Gerstmeier, S., Kyriakopoulos, A., et al., 2004. Selenium deficiency abrogates inflammation-dependent plasma cell tumors in mice. Cancer Research 64, 2910–2917.

Hoffmann, P.R., Hoge, S.C., Li, P.A., et al., 2007. The selenoproteome exhibits widely varying, tissue-specific dependence on selenoprotein P for selenium supply. Nucleic Acids Research 35, 3963–3973.

Lincoln, D.T., Ali Emadi, E.M., Tonissen, K.F., Clarke, F.M., 2003. The thioredoxin–thioredoxin reductase system: over-expression in human cancer. Anticancer Research 23, 2425–2433.

Lubos, E., Loscalzo, J., Handy, D.E., 2011. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxidants & Redox Signaling 15 (7), 1957–1997.

Misu, H., Takamura, T., Takayama, H., et al., 2010. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metabolism 12, 483–495.

Papp, L.V., Lu, J., Holmgren, A., Khanna, K.K., 2007. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxidants & Redox Signaling 9, 775–806.

Qi, Y., Schoene, N.W., Lartey, F.M., Cheng, W.H., 2010. Selenium compounds activate ATM-dependent DNA damage response via the mismatch repair protein hMLH1 in colorectal cancer cells. Journal of Biological Chemistry 285, 33010–33017.

Rayman, M.P., 2009. Selenoproteins and human health: insights from epidemiological data. Biochimica et Biophysica Acta – General Subjects 1790, 1533–1540.

Schrauzer, G.N., White, D.A., Schneider, C.J., 1977. Cancer mortality correlation studies. 3. Statistical associations with dietary selenium intakes. Bioinorganic Chemistry 7, 23–34.

Shiloh, Y., 2003. ATM and related protein kinases: safeguarding genome integrity. Nature Reviews. Cancer 3, 155–168.

Vitoux, D., Chappuis, P., Arnaud, J., et al., 1996. Selenium, glutathione peroxidase, peroxides and platelet functions. Annales de Biologie Clinique 54, 181–187.

Zhang, S., Rocourt, C., Cheng, W.H., 2010. Selenoproteins and the aging brain. Mechanisms of Ageing and Development 131, 253–260.

Zhang, S., Luo, Y., Zeng, H., Wang, Q., Tian, F., Song, J., Cheng, W.H., 2011. Encapsulation of selenium in chitosan nanoparticles improves selenium availability and protects cells from selenium-induced DNA damage response. Journal of Nutritional Biochemistry 11, 1137–1142.

Zhang, J., Wang, L., Anderson, L.B., et al., 2010. Proteomic profiling of potential molecular targets of methyl-selenium compounds in the transgenic adenocarcinoma of mouse prostate model. Cancer Prevention Research (PHILA) 3, 994–1006.

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Antioxidants and Aging: From Theory to Prevention

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ABBREVIATIONS

8-oxodG 8-hydroxyguanine
AMD Age-related macular degeneration
COX-2 Cycloxygenase-2
NF-κB Nuclear factor-κB
PGE2 Prostaglandin E2
ROS Reactive oxygen species

1. INTRODUCTION

Aging is commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for the increased risk of disease and death. Aging itself is not a disease; rather, it is a normal process of any living species in the world and an extremely complex and multifactorial process that makes it difficult to study. Although many theories are proposed to provide useful and important insights to understand the physiological changes occurring with aging, different theories of aging should not be considered as mutually exclusive; rather they are complementary of each other. The major theories of aging include the free radical (or oxidative stress) theory, the mitochondria theory, the immunological theory, and the inflammation theory.

Since 1800, human life expectancy has doubled. The advances which proceeded from modern medicine reflect major improvements in the environment and nutrition. Research on aging and age-related diseases is increasingly becoming a very hot area. Animal models of aging such as flies, worms, rodents, primates, and canine are often used to understand aging, age-related diseases, and antiaging intervention. In this chapter, we discuss some of the animal and human research that reflect aging theories and talk about how antioxidants may contribute to antiaging intervention and age-related disease prevention.

2. FREE RADICAL (OXIDATIVE STRESS) THEORY OF AGING

The free radical theory of aging postulates that aging and its related diseases are the consequence of free radical-induced damage to cellular macromolecules and the inability to counterbalance these changes by endogenous antioxidant defenses. The free radical theory of aging was first formulated in the 1950s by Harman (1957) who hypothesized a single common process, modifiable by genetic and environmental factors, in which the accumulation of endogenous oxygen radicals generated in cells could be responsible for the aging and death of all living beings. It is the most popular explanation of how aging occurs at the molecular level.

Both animals and humans live in an environment that contains reactive oxygen species (ROS), which can come from ultraviolet radiation, smoke, normal physical activities, and energy metabolisms. Mitochondrial respiration, the basis of energy production in all mammals, generates ROS by leaking intermediates from the electron transport chain (Finkel and Holbrook, 2000). The increasing age-related oxidative stress is a consequence of the imbalance between the free radical production and antioxidant defenses with a higher production of the former. In fact, elevated levels of both oxidant-damaged DNA and protein have been found in aged animals. Marlin et al. (2004) reported that young foals had significantly less endogenous DNA damage than mature or aged horses and Blount et al. (2004) found that levels of DNA damage increase with age in Labrador retriever dogs.

3. MITOCHONDRIA THEORY OF AGING

The free radical theory was then revised by Harman (1972) when mitochondria were identified as responsible for the initiation of the majority of the free radical reactions related to the aging process. The mitochondria theory of aging is often considered as an extension and refinement of the free radical theory. It was suggested that the life span is determined by the rate of free radical damage to the mitochondria. Several studies have emerged to give support to this theory (Harman, 1972). The premise of the mitochondrial free radical theory of aging is that mitochondria are both producers and targets of ROS. According to the theory, oxidative stress attacks mitochondria, leading to increased oxidative damage of proteins, DNA, and lipids. Mitochondrial electron transport chain complexes I and III are the principal sites of ROS production, and oxidative modifications to the complex subunits inhibit their activity, leading to mitochondrial dysfunction. As a consequence, damaged mitochondria progressively become less efficient, losing their functional integrity and releasing more oxygen molecules, increasing oxidative damage to the mitochondria, and culminating in an accumulation of dysfunctional mitochondria with age. For example, the electron leakage from complexes causes specific damage to their subunits and increased ROS generation as oxidative damage

accumulates, leading to further mitochondrial dysfunction, a cyclical process that under-lies the progressive decline in physiological function seen in aged mouse kidney (Choksi et al., 2007). Barja and Herrero (2000) discovered that oxidative damage to mitochon-drial DNA is inversely related to maximum life span in the heart and brain of several mammal species: mice, rats, guinea pigs, rabbits, sheep, pigs, cows, and horses. They found for the first time that the steady-state concentration of the oxidative damage marker 8-hydroxyguanine (8-oxodG) in mitochondrial DNA is inversely correlated with maximum life span in the heart and brain of mammals; that is, slowly aging mammals show lower 8-oxodG levels in mitochondrial DNA than rapidly aging ones.

4. IMMUNOLOGICAL THEORY OF AGING

Aging correlates with a marked susceptibility to infectious diseases. The progressive decline with age of the immune system is also known as immunosenescence, in which the immune system represents the most powerful mechanism to face stressors. Immuno-senescence affects the functions of both innate immune cells (e.g., neutrophils, macro-phages, and dendritic cells; Panda et al., 2009) and cells involved in adaptive immunity (T and B lymphocytes), which is the primary underlying cause of increased susceptibility.

Neutrophil function declines with age, with reduced phagocytosis and superoxide production. Aberrant migration of neutrophils to a site of infection may reduce pathogen clearance. Butcher et al. (2001) reported that reduced neutrophil CD16 expression and phagocytosis contribute to human immunosenescence. The group compared neutrophil function in healthy, young (23–35 years), and elderly (>65 years) volunteers. Superoxide generation in response to formyl–Met–Leu–Phe was slightly increased in neutrophils from elderly donors, and serum from the elderly was able to opsonize *E. coli* efficiently. In contrast, phagocytic index was significantly lower in neutrophils from the elderly, compared with young donors. CD11a and CD11b expression was not affected by age, but CD16 expression and phagocytosis were significantly reduced in neutrophils from elderly donors. In elderly patients with bacterial infection, CD16 expression remained low.

The macrophage activation due to chronic stress may provide a potential explanation to the subclinical chronic inflammatory status in the elderly. Meydani and Wu (2008) reported that macrophages from old mice had significantly higher levels of prostaglandin E2 (PGE2) production compared with those from young mice, a result of increased cycloxygenase-2 (COX-2) expression and protein levels, leading to increased COX enzyme activity. The studies suggest that the age–associated increase in macrophage PGE2 production is due to ceramide-induced upregulation of nuclear factor-κB (NF-κB) activation. Such processes may also occur in cell types other than macrophages, lead-ing to further insight into potential mechanisms of age-related diseases. Moreover, the excess PGE2 induces harmful effects in other cell types such as T cells and adipocytes

through the negative crosstalk between macrophages with other cells, resulting in further increased susceptibility to diseases.

Lymphocytes are also affected by the continuous age-related antigenic stress. In both humans and animals, aging of the immune system is characterized by diminished T cell production in the thymus and a loss of naive and accumulation of effector memory T cells, leading to dysregulated maintenance of peripheral T cell homeostasis. These changes lead to weakening of the immune defense against a wide spectrum of pathogens particularly those that have not been previously encountered by the host. Greeley et al. (1996) reported that an age-related decrease in the percentage of B cells was observed simultaneously with the increases in T cell percentages in Labrador retrievers. In another study, the immune function including the antibody response to vaccine was determined in 32 young adult (3.15 ± 0.8 years of age) and 33 old dogs (12.1 ± 1.3 years of age) of various breeds. The old dogs had significantly lower lymphocyte proliferative responses to T cell mitogen concanavalin A/phytohemagglutinin and a lower percentage of $CD4^+$ T cells and $CD45R^+/CD4^+$ T cells, and a higher percentage of $CD8^+$ T cells and a higher concentration of serum and salivary IgA (HogenEsch et al., 2004).

5. INFLAMMATION THEORY OF AGING

Innate immunity and adaptive immunity are the major defense mechanisms of higher organisms against inherent and environmental threats. Interestingly, during aging, adaptive immunity significantly declines, a phenomenon called immunosenescence as previously discussed, whereas innate immunity seems to be activated which induces a characteristic proinflammatory profile. Inflammation is increasingly considered a cornerstone of the mechanisms underlying the aging process, even generating a new name, 'inflamm-aging' (Franceschi et al., 2000).

The master regulator of the innate immunity and inflammatory response is the NF-κB system, which is in the critical point linking together the pathogenic assault signals and cellular danger signals, and organizing cellular resistance. Since this signaling system integrates the intracellular regulation of immune responses in both aging and age-related diseases, it has been studied a lot lately (Colavitti and Finkel, 2005; Salminen et al., 2008). NF-κB can be activated by over 150 stimuli; in turn, this regulates transcription of over 150 different genes, including proinflammatory cytokines IL-1β, IL-6, and TNFα, as well as proinflammatory enzymes inducible nitric oxide synthase and COX-2 which are related to aging (Wu and Meydani, 2008).

6. IMPLICATIONS OF ANTIOXIDANTS

In a normal situation, a balanced equilibrium exists among the three elements: oxidants, antioxidants, and biomolecules. The ideal oxidative balance is called 'a golden triangle.'

Antioxidants are substances that inhibit or delay oxidation of a substrate while present in minute amounts. Endogenous antioxidant defense is both nonenzymatic (e.g., uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors including vitamins and phenols) and enzymatic (e.g., the superoxide dismutases, the glutathione peroxidases, and catalase). In a normal healthy animal, the endogenous antioxidant defense balances ROS production, whereas in an aged animal, the ROS production outbalances endogenous antioxidant defense. Excess generation of free radicals may overwhelm natural cellular antioxidant defenses, leading to lipid peroxidation and further contributing to cell damages and ultimately the deleterious effects of aging.

Antioxidants can maintain the integrity and function of membrane lipids, cellular proteins, and nucleic acids and the control of signal transduction of gene expression in immune cells. Not surprisingly, immune system cells usually contain higher concentrations of antioxidants than do other cells (Knight, 2000), given the high percentage of polyunsaturated fatty acids in their plasma membranes. Thus, the immune cell functions are strongly influenced by the antioxidant/oxidant balance, and therefore, the antioxidant levels play a pivotal role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress, so as to preserve their adequate functioning.

Therefore, it is hypothesized that nutritional antioxidants can help overcome the imbalance and protect against free radical damage and immune dysfunction in aged animals, which may reduce the risk of developing degenerative diseases associated with aging (e.g., sarcopenia, arthritis, cancer, cataract, neurologic and immunological dysfunction).

Nutritional antioxidants such as vitamin C, vitamin E, carotenoids, and polyphenols act through different mechanisms to help overcome the imbalance and affect aging: (1) they directly neutralize free radicals, (2) they reduce the peroxide concentrations and repair oxidized membranes, (3) they support the stimulation of antioxidant enzymes including glutathione, catalase, and superoxide dismutase, (4) they modulate NF-κB pathway to affect innate immunity and inflammation pathway, and (5) they maintain immune cell function.

Vitamin C is the major water-soluble antioxidant and acts as the first defense against free radicals in whole blood and plasma. It is a powerful inhibitor of lipid peroxidation and regenerates vitamin E in lipoproteins and membranes. A strong inverse association has been shown between plasma ascorbic acid and isoprostanes (Block et al., 2002). Isoprostanes represent a family of prostaglandin isomers which, in contrast to classic prostaglandins formed through an enzymatic action of the prostaglandin-H-synthase from arachidonic acid, result from a free radical–catalyzed mechanism. Therefore, isoprostanes provide an optimal estimate of oxidative damage to cellular lipids and represent an excellent biomarker of lipid peroxidation in studies on aging.

Vitamin E is a lipid-soluble vitamin found in cell membranes and circulating lipoproteins. It protects against oxidative damage by acting directly with a variety of oxygen

radicals. Its antioxidant function is strongly supported by regeneration promoted by vitamin C.

Vitamin C along with vitamin E supplementation increased levels of endogenous antioxidant enzymes and improved indices of oxidative stress associated with repetitive loading exercise and aging and further improved the positive work output of muscles in aged rodents (Ryan et al., 2010). Antioxidant supplementation containing vitamin E and taurine significantly reduced both endogenous and exogenous DNA damage in dogs as measured by the comet assay (Heaton et al., 2002). Wu and Meydani (2008) reported that vitamin E reverses an age-associated defect in T cells, particularly naïve T cells. This effect of vitamin E is also reflected in a reduced rate of upper respiratory tract infection in the elderly and enhanced clearance of influenza infection in a rodent model. The T cell-enhancing effect of vitamin E is accomplished via its direct effect on T cells and indirectly by inhibiting PGE2 production in macrophages.

Carotenoids are color pigments and naturally occurring antioxidants that are abundant in yellow, orange, and red color vegetables and fruits. Carotenoids in those plants protect the chlorophylls from UV damage of sunlight. β-Carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin have all been found to be associated with inflammation. Carotenoids quench free radicals, reduce damage from ROS, and appear to modulate redox-sensitive transcription factors such as NF-κB that are involved in the upregulation of IL-6 and other proinflammatory cytokines. Recent epidemiological studies (Semba et al., 2006, 2007) in community-dwelling older adults show that low serum/plasma carotenoids are independently associated with low skeletal muscle strength and the development of walking disability.

The most known and studied carotenoid is β-carotene, a potent antioxidant able to quench singlet oxygen rapidly. Massimino et al. (2003) discovered that β-carotene supplementation significantly restored immune responses including T cell/B cell proliferation and delayed type hypersensitivity response to mitogen in older dogs when compared with their age-matched controls and younger counterparts. Lutein and zeaxanthin belong to xanthophylls and one of 600 known naturally occurring carotenoids. Lutein and zeaxanthin act as a filter of the high-energy blue light. In addition, these carotenoids are strong antioxidants and neutralize light-generated free radicals. Plant foods are the exclusive dietary sources of carotenoids. Macular lutein and zeaxanthin concentrations are related to their consumption. Age-related macular degeneration (AMD) is a major cause of visual impairment and blindness in the aging population. Recent human studies report a decreased AMD risk with increased intakes of lutein/zeaxanthin, B vitamins, zinc, and docosahexaenoic acid (Johnson, 2010). These findings are consistent with previous reports (Moeller et al., 2008)

Polyphenols found in fruit and vegetable extracts such as spinach, strawberry, or blueberry extracts have been studied for their effectiveness in reducing the deleterious effects of brain aging and behavior in many studies. Research from Joseph et al. (1999) suggested

that the combinations of antioxidant/anti–inflammatory polyphenolic compounds found in fruits and vegetables may show efficacy in altering behavioral and neuronal effects with aging. Another study on rats by Balu et al. (2006) reported that grape seed extract had an inhibiting effect on the accumulation of age-related oxidative DNA damages in spinal cord and in various brain regions such as the cerebral cortex, striatum, and hippocampus.

7. CONCLUSIONS

To put all these together, theories of aging often overlap each other, suggesting interactions across different systems and mechanisms, including mitochondria respiration, ROS generation, and immune functions. The excessive amount of ROS not counteracted by the antioxidant defenses can become a potential source of tissue damage. On the other hand, intracellular ROS functioning as signaling molecules and oxidants play a central role as mediators of cellular senescence through regulating the NF-κB pathway. Therefore, it remains a challenge to define both the normal and pathologically relevant sites of ROS formation in the mitochondrial electron transport chain and immune defense process to find clinically useful agents that can minimize cellular ROS production and perhaps delay aging and prevent aging-related diseases. More research is warranted to understand more completely how nutritional antioxidants can benefit healthy aging in the greatest degree.

REFERENCES

Balu, M., Sangeetha, P., Murali, G., Panneerselvam, C., 2006. Modulatory role of grape seed extract on age-related oxidative DNA damage in central nervous system of rats. Brain Research Bulletin 68, 469–473.

Barja, G., Herrero, A., 2000. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. The FASEB Journal 14, 312–318.

Block, G., Dietrich, M., Norkus, E.P., et al., 2002. Factors associated with oxidative stress in human populations. American Journal of Epidemiology 156, 274–285.

Blount, D.G., Heaton, P.R., Pritchard, D.I., 2004. Changes to levels of DNA damage and apoptotic resistance in peripheral blood mononuclear cells and plasma antioxidant potential with age in labrador retriever dogs. Journal of Nutrition 134, 2120S–2123S.

Butcher, S.K., Chahal, H., Nayak, L., et al., 2001. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. Journal of Leukocyte Biology 70, 881–886.

Choksi, K.B., Nuss, J.E., Boylston, W.H., Rabek, J.P., Papaconstantinou, J., 2007. Age-related increases in oxidatively damaged proteins of mouse kidney mitochondrial electron transport chain complexes. Free Radical Biology and Medicine 43, 1423–1438.

Colavitti, R., Finkel, T., 2005. Reactive oxygen species as mediators of cellular senescence. IUBMB Life 57, 277–281.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of aging. Nature 408, 239–247.

Franceschi, C., Bonafĩ, M., Valensin, S., et al., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Annals of the New York Academy of Sciences 908, 244–254.

Greeley, E.H., Kealy, R.D., Ballam, J.M., Lawler, D.F., Segre, M., 1996. The influence of age on the canine immune system. Veterinary Immunology and Immunopathology 55, 1–10.

Harman, D., 1957. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology 2, 298–300.

Harman, D., 1972. The biologic clock: the mitochondria? Journal of the American Geriatrics Society 20, 145–147.

Heaton, P.R., Reed, C.F., Mann, S.J., et al., 2002. Role of dietary antioxidants to protect against DNA damage in adult dogs. Journal of Nutrition 132, 1720S–1724S.

HogenEsch, H., Thompson, S., Dunham, A., Ceddia, M., Hayek, M., 2004. Effect of age on immune parameters and the immune response of dogs to vaccines: a cross-sectional study. Veterinary Immunology and Immunopathology 97, 77–85.

Johnson, E.J., 2010. Age-related macular degeneration and antioxidant vitamins: recent findings. Current Opinion in Clinical Nutrition and Metabolic Care 13, 28–33.

Joseph, J.A., Shukitt-Hale, B., Denisova, N.A., et al., 1999. Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach or strawberry dietary supplementation. Journal of Neuroscience 19, 8114–8121.

Knight, J.A., 2000. Review: free radicals, antioxidants, and the immune system. Annals of Clinical Laboratory Science 30, 145–158.

Marlin, D.J., Johnson, L., Kingston, D.A., et al., 2004. Application of the comet assay for investigation of oxidative DNA damage in equine peripheral blood mononuclear cells. Journal of Nutrition 134, 2133S–2140S.

Massimino, S.P., Kearns, R.J., Loos, K.M., et al., 2003. Effects of age and dietary β-carotene on immunological variables in dogs. Journal of Veterinary Internal Medicine 17, 835–842.

Meydani, S.N., Wu, D., 2008. Nutrition and age-associated inflammation: implications for disease prevention. Journal of Parenteral and Enteral Nutrition 32, 626–629.

Moeller, S.M., Voland, R., Tinker, L., et al., 2008. Associations between age-related nuclear cataract and lutein and zeaxanthin in the diet and serum in the carotenoids in the age-related eye disease study, an ancillary study of the Women's Health Initiative. Archives of Ophthalmology 126, 354–364.

Panda, A., Arjona, A., Sapey, E., et al., 2009. Human innate immunosenescence: causes and consequences for immunity in old age. Trends in Immunology 30, 325–333.

Ryan, M.J., Dudash, H.J., Docherty, M., et al., 2010. Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats. Experimental Gerontology 45, 882–895.

Salminen, A., Huuskonen, J., Ojala, J., et al., 2008. Activation of innate immunity system during Aging: NF-kB signaling is the molecular culprit of inflamm-aging. Aging Research Reviews 7, 83–105.

Semba, R.D., Bartali, B., Zhou, J., et al., 2006. Low serum micronutrient concentrations predict frailty among older women living in the community. Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 61, 594–599.

Semba, R.D., Lauretani, F., Ferrucci, L., 2007. Carotenoids as protection against sarcopenia in older adults. Archives of Biochemistry and Biophysics 458, 141–145.

Wu, D., Meydani, S.N., 2008. Age-associated changes in immune and inflammatory responses: impact of vitamin E intervention. Journal of Leukocyte Biology 84, 900–914.

Diet and Brain Aging: Effects on Cell and Mitochondrial Function and Structure

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ABBREVIATIONS

GSH Glutathione
GSHPx Glutathione peroxidase
H$_2$O$_2$ Hydrogen peroxide
HNE 4-Hydroxy-2,3-*trans*-nonenal
MDA Malondialdehyde
PUFA Polyunsaturated fatty acids
ROS Reactive oxygen species
SOD Superoxide dismutase

1. INTRODUCTION

Oxidative stress is commonly defined as an imbalance between oxidants and reactive oxygen species (ROS) and the protective antioxidant system. Under low levels of anti-oxidants and accelerated production of ROS, permanent cellular damage can occur. ROS can attack proteins, lipids, and DNA, changing their structure and thus disrupting function. Unchecked oxidative stress ultimately leads to cell death. Free radical attack of deoxynucleic acids results in impaired DNA replication, single-stranded DNA breakage, and mutagenesis. Lipids particularly vulnerable to peroxidation are the polyunsaturated fatty acids (PUFAs) since the allylic hydrogen atoms adjacent to the double bonds are easily removed by ROS. The lipid carbon radical rearranges allowing for cross-linking with other fatty acids causing rigidity in the cell membrane. The lipid radical can also decompose to smaller radicals and aldehydes such as malondialdehyde (MDA), 4-hydroxy-2,3-*trans*-nonenal (HNE), or acrolein, which react with thiols in proteins disrupting function. Many amino acid residues in protein molecules such as lysine, histidine, arginine, cystine, methionine, and tyrosine are susceptible to hydroxyl radical attack. Protein oxidation results in loss of function, fragmentation, proteolysis, and cross-linking. Glycoxidation can also modify proteins resulting in protein damage. This occurs by condensation of reducing sugars with protein amino acids, particularly lysine, leading to advanced glycosylation end products.

ROS are counteracted by enzymatic and nonenzymatic antioxidants. Antioxidants offer protection on many different levels by preventing radical formation, neutralizing free radicals, repairing oxidative damage, and eliminating damaged molecules. Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H_2O_2) (20.1). There are two copper (Cu) and zinc (Zn) SODs, one localized extracellularly and the other in peroxisomes and the cytoplasm of the cell. Manganese (Mn) SOD is found exclusively in the mitochondria. Antioxidants catalase (CAT) and glutathione peroxidase (GSHPx) neutralize the H_2O_2 produced by SOD. CAT quickly turns H_2O_2 into water and O_2 (Eq. 20.2), but there is very little CAT activity in the brain (Ceballos–Picot, 1997). GSHPx works in conjunction with reduced glutathione (GSH). The most prevalent antioxidant in the brain, GSH, is found in millimolar concentrations in most cells. A thiol–containing molecule, GSH, is capable of reacting with ROS and nucleophilic compounds from lipid peroxidation such as HNE and acrolein. Reduced GSH reacts with free radicals to produce oxidized glutathione (GSSG), which can be catalyzed by the enzyme GSH peroxidase or occur independently. GSSG is recycled back to two GSHs by GSH reductase and cofactor NADPH (Figure 20.1). Glutathione–S–transferases (GSTs) are a group of enzymes that catalyze the reaction between GSH and nucleophilic
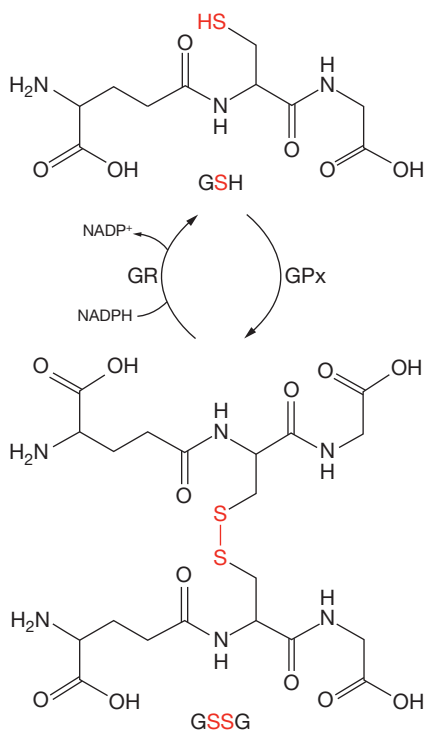
Figure 20.1 Recycling of glutathione (GSH) and oxidized glutathione (GSSG).

compounds such as HNE and acrolein. These cellular antioxidant proteins are upregulated under oxidative stress conditions to protect the cell from ROS attack.

$$O_2{}^- + O_2{}^- + 2H^+ \rightarrow H_2O_2 + O_2(\text{enzymeSOD}) \qquad [20.1]$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH \qquad [20.2]$$
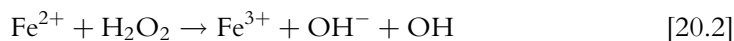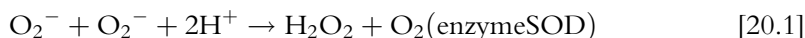
Protection against free radicals can also come from small, nonprotein, cellular antioxidants such as GSH, vitamin C, vitamin E, carotenoids, and flavonoids. Vitamin C is located in the aqueous environment of the cytosol. Active transport systems in the choroid plexus elevate concentrations of ascorbic acid in the cerebrospinal fluid (CSF) 10-fold compared to blood. Free radicals are also mitigated by proteins such as hemoglobin, transferrin, and ceruloplasmin that bind ferrous and copper ions, thus preventing their involvement in redox reactions.

The central nervous system (CNS) is particularly susceptible to attack by ROS. The brain utilizes 30% of the total oxygen intake more than any other organ in the body. The high metabolic rate leads to an increased generation of ROS. Antioxidant defense mechanisms are not elevated in the brain to counteract the increased ROS, allowing for the possibility of increased cellular oxidative damage. Indeed, several major antioxidants such as GSH, and GSHPx, are concentrated in glial cells rather than neurons. The brain is also composed of high levels of PUFA. The unsaturated bonds (double bonds) of PUFAs are extremely vulnerable to damage by ROS leading to lipid peroxidation. In addition, neuronal tissues contain high concentrations of free iron, known to generate the highly toxic hydroxyl radical through the Fenton reaction (Eq. 20.3). As mentioned, high concentrations of ascorbic acid (vitamin C) are found in the CNS. In the presence of free iron, ascorbic acid acts as an oxidant producing ROS. Finally, excitatory neurotransmitters such as glutamate generate high levels of ROS after their release, causing damage in concentrated areas of the brain such as the hippocampus (Ceballos-Picot, 1997). Neurons do not readily divide and multiply, so once damaged, neurons may die. The CNS appears to be at a disadvantage when countering an oxidative stress condition:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad [20.3]$$

Oxidative stress plays a role in many neurodegenerative diseases possibly as a cause or consequence of disease. The evidence supporting the role of oxidative stress in Alzheimer's disease (AD) is extensive. In AD, concentration and activity of many antioxidants are increased, while evidence of rampant neuronal ROS is overwhelming. Some antioxidant enzymes are redox-sensitive and easily oxidized, rendering them inactive even though protein expression level is high. End products of oxidative stress including lipid peroxidation, protein oxidation, oxidized nuclear DNA, and oxidized mitochondrial DNA are increased in AD brain and are extensively localized in neurofibrillary tangles (Sultana and Butterfield, 2010). The main cellular antioxidant GSH decreases with age and in AD (Calabrese et al., 2006). The ratio of GSSG to GSH is used as a

marker of redox thiol status and oxidative stress. In AD peripheral lymphocytes, GSH levels are decreased and GSSG levels are increased, consistent with increased oxidative stress (Calabrese et al., 2006). Indeed, with worsening of dementia in AD, GSSG and GSSG/GSH levels increase. Activities of other antioxidant enzymes SOD, CAT, GSHPx, and GSH reductase are most often elevated in specific areas of AD brain vulnerable to oxidative stress (Sultana and Butterfield, 2010). The general consensus in the literature reflects elevated antioxidant enzyme activity in AD brain regions affected by elevated oxidative stress, while redox–sensitive antioxidants display a decrease in activity even though concentrations are elevated.

2. PHENOLICS: ANTIOXIDANT POWER OF FRUIT AND VEGETABLES

Fruits and vegetables are known for 'keeping the doctor away.' This saying may be solidified by the antioxidant properties of fruits and vegetables. The antioxidant capabilities and phenolic content were measured in 25 fruits and 27 vegetables common to the American diet (e.g., Song et al., 2010). In general, berries contained higher levels of phenols, in particular anthocyanins, and displayed the greatest antioxidant activity against peroxyl radicals. Melons had the lowest phenol content and antioxidant activity consistent with other studies. The highest antioxidant activity directly correlated to the phenolic content for vegetables also. High antioxidant levels are found in spinach, beets, red peppers, and broccoli. Celery, various lettuces, and cucumbers had the lowest phenolic content and antioxidant activity. The KAME project, a large population-based prospective study of Japanese Americans from Washington State who were followed for approximately 10 years, investigated the frequency of drinking fruit and vegetable juices high in polyphenols and the risk of AD (Dai et al., 2006). Drinking fruit or vegetable juice 3 or more times a week compared to once a week decreased the hazard ratio for probable AD.

3. VITAMIN E

Vitamin E (Figure 20.2) is one of the most well–known antioxidants. A phenolic compound, vitamin E acts as an antioxidant by scavenging free radicals via the phenolic
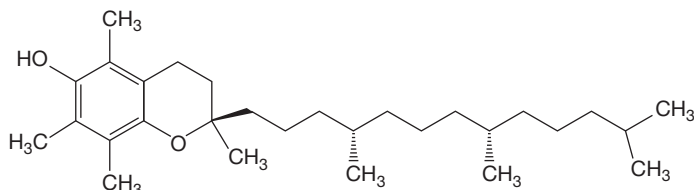
Figure 20.2 Structure of vitamin E (α-tocopherol).

hydrogen atom and is recycled through various pathways including vitamin C (ascor-bate), GSH, and thioredoxin (Figure 20.3). Vitamin E, a lipophilic compound, can insert into the lipid bilayer to protect lipid membranes from Aβ-induced oxidative stress. In neuronal cell cultures, vitamin E inhibits Aβ-induced lipid peroxidation, protein ox-idation, free radical formation, and cell death (Pocernich et al., 2011).

The effect of a diet supplemented with vitamin E in the APP/PSEN1 double trans-genic mouse model of AD compared vitamin E (high- or low-dose) and/or vitamin C. High-dose vitamin E with C was less effective at reducing lipid peroxidation than vitamin C alone or with low-dose vitamin E. The high-dose combination impaired water maze performance, while low-dose combination improved water maze performance and spa-tial memory deficits (Harrison et al., 2009). Vitamin C is water-soluble with the ability to protect against oxidative stress inside the cell and recycles vitamin E to its reduced form, although the oxidized form of vitamin C is also reactive if not reduced. Rats fed diets high in vitamin E had increases in brain vitamin E levels but to a much lesser extent than peripheral tissue (Clement et al., 1995). Thus, it appears that the brain is capable of main-taining a certain physiological range of α-tocopherol, not allowing substantial accumu-lation or depletion of this vitamin E isoform. The α-tocopherol transfer protein most likely plays an integral role in maintaining α-tocopherol levels in the brain.

Vitamin E from food sources, mainly plant oils, consists of four different forms (α-, γ-, δ-, and β-tocopherol) and four tocotrienols, but vitamin E supplements often consist only of α-tocopherol. Most studies use only α-tocopherol, which may explain the incon-sistencies reported in cognitive studies. A combination of tocopherols may have more
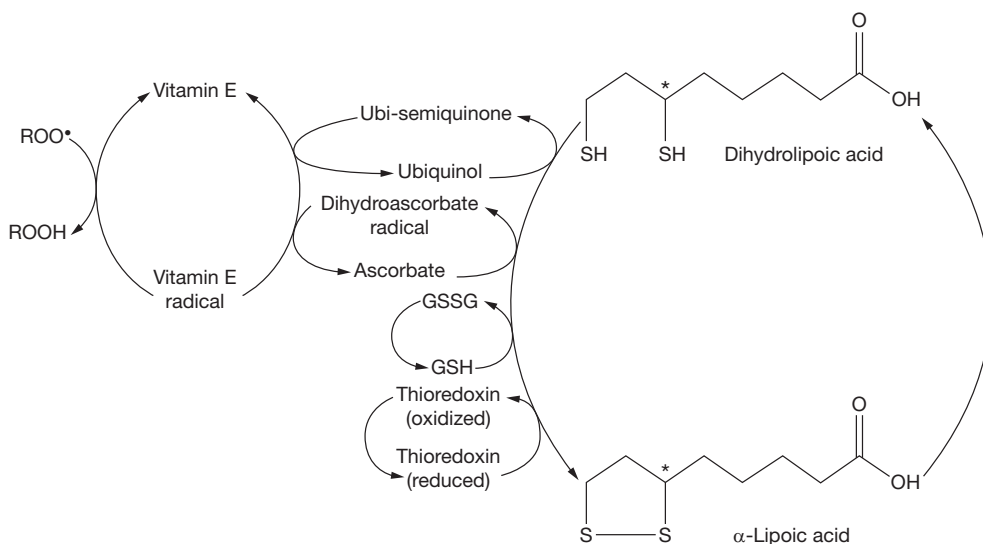
Figure 20.3 Recycling of role of antioxidants vitamin E, glutathione (GSH), vitamin C, and lipoic acid.

consistent results in slowing the decline of dementia. The Chicago Health and Aging Project followed subjects for 6 years and determined that both α-tocopherol and γ-tocopherol intake had inverse associations with AD and cognitive decline (Morris et al., 2005). Dietary supplementation with α-tocopherol decreases plasma levels of γ-tocopherol (Handelman et al., 1985), which suggests that γ-tocopherol may play an important role in decreasing oxidative stress in AD brain, while α-tocopherol supplementation alone may indeed have detrimental effects.

For decades, vitamin E and antioxidants have been investigated to determine their relationship to cognitive decline. Many studies have shown a decrease in vitamin E levels in aging and dementia with a correlation to memory loss. It is not clear whether vitamin E improves cognition. Many studies with vitamin E alone or in combination with other vitamins and minerals showed no association with dementia, while others noted a correlation with improved cognitive performance (Pocernich et al., 2011). Advanced AD patients on high-dose vitamin E had delayed entry into nursing homes and improved frailty but no clear improvement in cognition (Sano et al., 1997). A follow-up to this study concluded that there was not enough sufficient evidence to suggest vitamin E as a treatment for AD (Tabet et al., 2000).

Most recently, Lloret and colleagues studied the effect of high-dose vitamin E on redox status and cognition in AD patients. They found that half of the AD patients maintained or improved cognitive skills (respondents), while the other half showed decreased cognition compared to controls (nonrespondents). Those who responded to vitamin E supplementation also had decreased GSSG and GSSG/GSH levels and decreased MDA, a marker of lipid peroxidation (Lloret et al., 2009). High levels of GSSG and declining cognition were significantly correlated. Only AD patients who had decreased oxidative stress as measured by GSSG/GSH ratio and lipid peroxidation displayed an improvement in cognition. Vitamin E may be detrimental due to concentration in the lipid membrane. It is possible that upon oxidation, vitamin E is reactive unless recycled to the reduced form. Supplementation of vitamin E with an antioxidant that is water-soluble, such as vitamin C, capable of recycling vitamin E, and/or capable of increasing redox thiol status, such as GSH-upregulating compound *N*-acetyl–*L*-cysteine (NAC) or γ-glutamylcysteine ethyl ester (GCEE), may have more consistent positive effects on dementia by providing protection in the lipid membrane and inside the cell.

4. QUERCETIN

Flavonoids are naturally occurring polyphenolic compounds found in fruit, vegetables, nuts, tea, and wine. These polyphenols are good candidates for antioxidant therapy due to their aromatic ring structure. Quercetin (Figure 20.4) is one of the most currently researched flavonoids and is mainly found in apples, onions, and green tea (Boots et al., 2008). Quercetin is a powerful scavenger of ROS including superoxide, hydroxyl
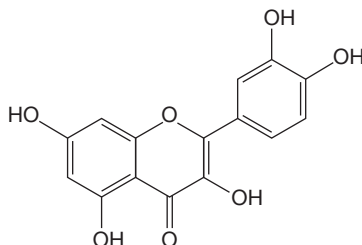
Figure 20.4 Structure of quercetin.

radicals, nitric oxide, and peroxynitrite (Boots et al., 2008). Kim and colleagues tested 39 flavonoids to determine inhibitory effects of Aβ(1–42) fibril formation, as determined by ThT fluorescence assay (Kim et al., 2005). In general, the parent-type flavone exhibited the most potent inhibitory effect, with increased number of hydroxylations increasing the ability of flavonoids to inhibit Aβ(1–42) fibril formation. Removal of a hydroxyl group from quercetin at C-5 (fisetin) or C-3 (kaempferol) greatly reduced the inhibiting effect of Aβ(1–42) fibril formation. Quercetin was more effective than other common flavonoids (+)−catechin and (−)−epicatechin (EC) at inhibiting Aβ(1–42) fibril formation and subsequent Aβ(1–42)-induced oxidative stress (Ono et al., 2003).

Discrepancies have been reported regarding cytotoxic properties of quercetin. Upon scavenging free radicals, quercetin forms possibly toxic intermediates that are highly reactive with thiols and quickly form adducts with GSH (Boots et al., 2008). The toxicity of quercetin's oxidation product must be weighed against its initial antioxidant potential. A possible solution may be to coadminister quercetin and GSH or another sulfhydryl compound to avoid potential toxicity due to oxidation metabolites.

5. RESVERATROL

Resveratrol, a naturally occurring stilbene (Figure 20.5), is a polyphenolic compound found in several plants, including grapes used for red wine. The skins and seeds of red grapes naturally synthesize resveratrol in response to fungal attack and UV light exposure. The ability of resveratrol to enter the brain seems to depend on concentration and possibly how it is administered. Resveratrol has been detected in the brain after gastric gavage and i.p. administration and is rapidly metabolized in the liver and intestinal epithelial cells (Anekonda, 2006).

Resveratrol has a wide range of biological effects including antioxidant, anti-inflammatory, and anticarcinogenic. Many reports have demonstrated resveratrol's antioxidant properties and ability to upregulate antioxidants and phase-2 enzymes, including SOD, CAT, GSH, GSH reductase, GSHPx, GST, NAD(P)H:quinone oxidoreductase-1 (NOQ1), and MnSOD (Anekonda, 2006).
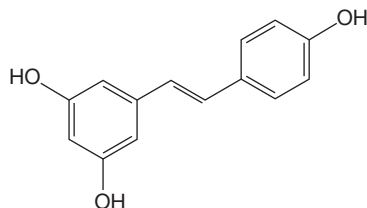
Figure 20.5 Structure of resveratrol.

Moderate consumption of red wine Cabernet Sauvignon, the equivalent of two 5-oz glasses per day for adults, conceivably may be beneficial for deterring AD. Indeed, several studies have shown a lower risk of dementia with those who drank moderate amounts of red wine compared to total abstainers (e.g., Luchsinger et al., 2004).

Antioxidant properties of resveratrol have been linked to upregulation of SIRT1. SIRT1 has been shown to be neuroprotective in models of AD. Resveratrol has been coined as a caloric restriction mimetic because of its ability to reproduce the effects of caloric restriction and induce sirtuin proteins (Anekonda, 2006). Resveratrol partially protected against Aβ-induced oxidative stress in the presence of the SIRT1 inhibitor sirtinol, suggesting a direct interaction of resveratrol with Aβ fibrils, in addition to SIRT1-involved antioxidant properties, as seen by earlier studies.

Recently, AMP-activated protein kinase (AMPK) signaling has been demonstrated to be a central event in anti-amyloidogenic effect of resveratrol (Vingtdeux et al., 2010). Resveratrol activated AMPK, and inhibition of AMPK allowed for cytotoxicity and accumulation of Aβ in the presence of resveratrol *in vitro* and *in vivo* in APP/PS1 mice. Resveratrol binds and activates SIRT1 leading to AMPK activation (Anekonda, 2006). SIRT1 and resveratrol also decrease activation of NF-κB, which is activated in the Aβ neuronal death pathway (Anekonda, 2006). The mechanism of resveratrol activation of SIRT1 and AMPK in the anti-amyloidogenic pathway is still unclear and deserves further investigation.

The protection incurred by resveratrol is multifaceted. The polyphenol resveratrol has antioxidant properties; upregulates and activates SIRT1, known to increase longevity and protect against neurodegeneration; and directly binds Aβ, reducing aggregation and cytotoxicity and increasing Aβ clearance. Treatment of AD patients with resveratrol warrants investigation.

6. CURCUMIN

The polyphenolic antioxidant curcumin (Figure 20.6) is found in turmeric, an Indian curry spice. It has long been used as a food preservative and herbal medicine in India. The prevalence of AD in patients in India aged 70–79 is 4.4-fold less than in the United States, suggesting a diet rich in curcumin may reduce the risk of AD (Kim et al., 2010).
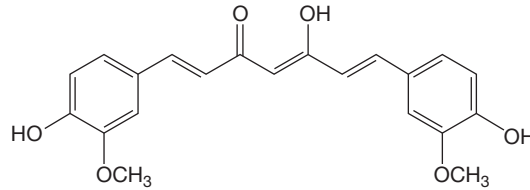
Figure 20.6 Structure of curcumin.

Curcumin has displayed antioxidant, anti–inflammatory, and anti–amyloidogenic prop–erties that could play a role in preventing AD (Kim et al., 2010).

As an antioxidant, curcumin is a stronger free radical scavenger than vitamin E (Kim et al., 2010). Curcumin scavenges NO-based radicals protecting the brain from lipid per-oxidation and scavenges hydroxyl radicals preventing DNA oxidation (Kim et al., 2010). Curcumin and its metabolites are capable of binding redox-active metals, Cu^{2+} and Fe^{2+} (Kim et al., 2010). The copper–curcumin complex has the ability to scavenge radicals and displays SOD-mimetic properties (Kim et al., 2010).

Antioxidant properties of curcumin have also been demonstrated in animal models of AD. Curcumin is a strong inducer of vitagenes HO-1, Hsp70, thioredoxin reductase, and sirtuins (Calabrese et al., 2008). Vitagenes, endogenous proteins induced by oxidative stress, counteract the NF-κB-dependent ROS/reactive nitrogen species (RNS)-mediated oxidative stress damage, thus acting as a starting point in neuroprotection. In AD animal models, curcumin counteracts oxidative stress by inducing several antioxidant pathways.

Several AD models have demonstrated curcumin's protective effects on Aβ cytotox-icity, aggregation, and accumulation *in vitro* and *in vivo*. The small polyphenolic ring structure of curcumin may allow binding to free Aβ, thereby preventing fibrilization, and binding of fibrillar Aβ may disrupt β-sheet formation found in senile plaques (Ono et al., 2004).

Although a recent *in vivo* 6-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin taken by patients with AD was unable to identify signif-icant changes in MMSE scores, serum Aβ(1–40) levels, plasma isoprostanes, or plasma antioxidant levels, with the exception of vitamin E (Baum et al., 2008). In this clinical trial, researchers found that curcumin significantly elevated the levels of vitamin E in plasma, suggesting that the antioxidant activity of curcuminoids may decrease either the need for vitamin E or prevent its depletion, at least in plasma (Baum et al., 2008). Although serum Aβ(1–40) did not significantly differ, an increasing trend over time emerged, possibly reflecting the ability of curcumin to disaggregate brain Aβ deposits, which could then be released into the peripheral blood circulation for disposal (Ono et al., 2004; Yang et al., 2005; Baum et al., 2008). Unfortunately, the lack of significant cognitive decline in patients receiving placebo would preclude the detection of protec-tive effects engendered by curcumin (Baum et al., 2008).

7. GREEN TEA POLYPHENOLS: EPIGALLOCATECHIN GALLATE

Black and green tea leaves contain high amounts of polyphenols known as catechins. Among the catechins, epigallocatechin gallate (EGCG) accounts for more than 10% of the extract dry weight followed by (−)−epigallocatechin (EGC), (−)−epicatechin (EC), and (−)−epicatechin–3–gallate (ECG) (Figure 20.7). All four catechins have demonstrated potent antioxidant properties through direct scavenging of ROS and RNS, induction of endogenous antioxidant enzymes, and the ability to chelate divalent metals, such as iron and copper (Mandel et al., 2008). EGCG elevated antioxidant enzymes SOD and CAT in mouse striatum (Mandel et al., 2008) and activated the expression of stress response genes and phase II drug metabolizing enzymes GST, HO-1, and Nrf-1 and Nrf-2 transcription factors (Mandel et al., 2008). In several studies, EGCG was a more potent radical scavenger than ECG, EC, and EGC. EGCG and other green tea catechins protect against Aβ–induced neurotoxicity through several pathways, including antioxidant capabilities and induction of the nonamyloidogenic α-secretase pathway, thus decreasing production of Aβ and its neurotoxic effects.
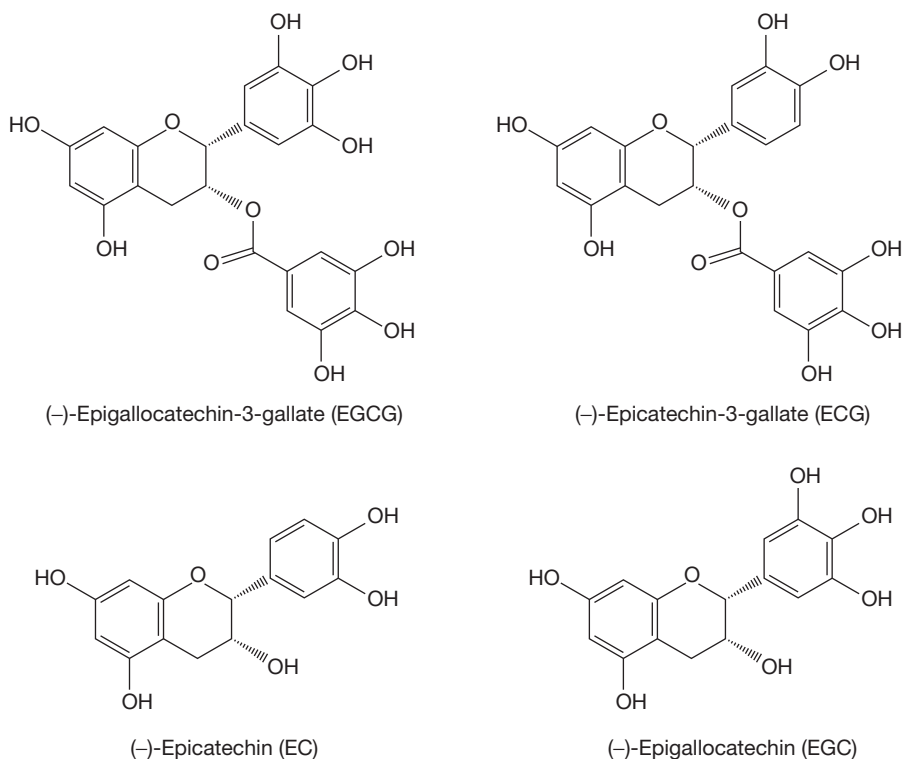
Figure 20.7 Structures of green tea polyphenols.

Unfortunately, a lack of clinical trials with tea polyphenols exists in neurodegenerative diseases, although epidemiological data suggest that tea consumption inversely correlates with incidence of dementia, AD, and Parkinson's disease. However, a few previously conducted clinical studies have demonstrated promising results. For example, higher consumption of green tea in elderly Japanese subjects has been associated with lower prevalence of cognitive impairment (Kuriyama et al., 2006). Therefore, clinical trials investigating the effect of green tea polyphenols in AD are warranted.

8. COMBINATORIAL DIETARY APPROACHES: EVIDENCE FROM A HIGHER MAMMALIAN MODEL

In humans, studies of dietary or supplemental antioxidants suggest variable cognitive or clinical benefits and appear far less robustly associated with beneficial outcomes than those reported in the rodent aging literature. Numerous possible reasons for differences between animal and human study outcomes can be identified. Variable outcomes in human antioxidant clinical trials may reflect inconsistencies in the amount of supplements provided from study to study, the form and source of the antioxidants (supplements vs. diet), the duration and regularity of antioxidant use, and the challenge of determining the exact background of dietary intake of antioxidants, particularly in studies of supplements. Not surprisingly, a panel of experts for the Duke Evidence-based Practice Center for the US Department of Health and Human Services recently reviewed the literature and reported no consistent or robust evidence to suggest that single or dual antioxidant use is protective against AD (Williams et al., 2010). In terms of preventing cognitive decline with aging, vegetable intake was only weakly associated with decreased risk of developing AD, whereas cognitive training was strongly associated with decreased risk. Thus, the role of either dietary or supplemental antioxidants and level of protection against cognitive decline or AD needs further study.

Combining antioxidants or increasing antioxidants through diet may be more beneficial, as suggested earlier in this chapter. Further, targeting mitochondrial dysfunction through the use of mitochondrial cofactors may improve efficiency and reduce ROS. Thus, we tested the hypothesis that a combination of antioxidants and mitochondrial cofactors provided in food would improve cognition in aged beagles, a model of human brain aging. Dogs are particularly useful because they naturally develop cognitive decline with age, accumulate oxidative damage and AD-like neuropathology, and absorb dietary nutrients in a similar manner as humans (Cotman and Head, 2008).

An antioxidant-enriched dog diet was formulated to include a broad spectrum of antioxidants and two mitochondrial cofactors (Milgram et al., 2005). Based on an average weight of 10 kg per animal, the daily doses for each compound were 800 IU or 210 mg day^{-1} (21 mg kg^{-1} day^{-1}) of vitamin E, 16 mg day^{-1} (1.6 mg kg^{-1} day^{-1}) of vitamin C,

52 mg day^{-1} (5.2 mg kg^{-1} day^{-1}) of carnitine, and 26 mg day^{-1} (2.6 mg kg^{-1} day^{-1}) of lipoic acid. Fruits and vegetables were also incorporated at a 1:1 exchange ratio for corn, resulting in 1% inclusions of each of the following: spinach flakes, tomato pomace, grape pomace, carrot granules, and citrus pulp. This was equivalent to raising fruits and vegetable servings from 3 to 5–6 per day. In addition to the antioxidant diet, half of the animals were provided with behavioral enrichment, which consisted of additional cognitive experience (20–30 min day^{-1}, 5 days week^{-1}), an enriched sensory environment (housing with a kennel mate, rotation of play toys in kennel once per week), and physical exercise (2 × 20 min walks per week outdoors) (Milgram et al., 2005).

To evaluate short-term and chronic treatment effects, dogs were evaluated over a 2.8-year period. Treatment with the antioxidant diet led to cognitive improvements in learning that were rapid, and within 2 weeks of beginning the diet, aged animals showed significant improvements in spatial attention (landmark task) (reviewed in Cotman and Head, 2008). Subsequent testing of animals with a more difficult complex learning task, oddity discrimination, also revealed benefits of the diet (reviewed in Cotman and Head, 2008). Improved visual discrimination and reversal (frontal function) learning ability was maintained over time with the antioxidant treatment, while untreated animals showed a progressive decline (Milgram et al., 2005).

Oxidative damage was reduced in antioxidant-fed dogs and in particular within the group of animals receiving the combination of antioxidants and behavioral enrichment (Opii et al., 2008). Endogenous antioxidant activity was also increased (Opii et al., 2008). These results suggest that cognitive benefits of antioxidants can be further enhanced with the addition of behavioral enrichment due to different yet synergistic mechanisms of action in the brain (reducing oxidative damage, maintaining neuron health).

9. SUMMARY

The aging brain shows higher levels of oxidative damage suggesting that antioxidant use would be of benefit for cognitive health. Epidemiological studies suggest a link between dietary components and healthy brain aging. Combinations of different antioxidants might, however, prove to be more beneficial than single compounds. Overall, a healthy diet rich in antioxidants may reduce the risk for age-associated neurodegenerative disease.

REFERENCES

Anekonda, T.S., 2006. Resveratrol – a boon for treating Alzheimer's disease? Brain Research Reviews 52, 316–326.
Baum, L., et al., 2008. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. Journal of Clinical Psychopharmacology 28, 110–113.
Boots, A.W., Haenen, G.R., Bast, A., 2008. Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology 585, 325–337.

Calabrese, V., Bates, T.E., Mancuso, C., et al., 2008. Curcumin and the cellular stress response in free radical–related diseases. Molecular Nutrition & Food Research 52, 1062–1073.

Calabrese, V., Sultana, R., Scapagnini, G., et al., 2006. Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. Antioxidants & Redox Signaling 8, 1975–1986.

Ceballos-Picot, I., 1997. The Role of Oxidative Stress in Neuronal Death. Chapman & Hall, Austin.

Clement, M., Dinh, L., Bourre, J.M., 1995. Uptake of dietary RRR-a- and RRR-g-tocopherol by nervous tissues, liver and muscle in vitamin-E-deficient rats. Biochimica et Biophysica Acta 1256, 175–180.

Cotman, C.W., Head, E., 2008. The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches. Journal of Alzheimer's Disease 15, 685–707.

Dai, Q., Borenstein, A.R., Wu, Y., Jackson, J.C., Larson, E.B., 2006. Fruit and vegetable juices and Alzheimer's disease: the Kame Project. The American Journal of Medicine 119, 751–759.

Handelman, G.J., Machlin, L.J., Fitch, K., Weiter, J.J., Dratz, E.A., 1985. Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. The Journal of Nutrition 115, 807–813.

Harrison, F.E., Allard, J., Bixler, R., et al., 2009. Antioxidants and cognitive training interact to affect oxidative stress and memory in APP/PSEN1 mice. Nutritional Neuroscience 12, 203–218.

Kim, H., Park, B.S., Lee, K.G., et al., 2005. Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid. Journal of Agricultural and Food Chemistry 53, 8537–8541.

Kim, J., Lee, H.J., Lee, K.W., 2010. Naturally occurring phytochemicals for the prevention of Alzheimer's disease. Journal of Neurochemistry 112, 1415–1430.

Kuriyama, S., Hozawa, A., Ohmori, K., et al., 2006. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. The American Journal of Clinical Nutrition 83, 355–361.

Lloret, A., Badia, M.C., Mora, N.J., et al., 2009. Vitamin E paradox in Alzheimer's disease: it does not prevent loss of cognition and may even be detrimental. Journal of Alzheimer's Disease 17, 143–149.

Luchsinger, J.A., Tang, M.X., Siddiqui, M., Shea, S., Mayeux, R., 2004. Alcohol intake and risk of dementia. Journal of the American Geriatrics Society 52, 540–546.

Mandel, S.A., Amit, T., Kalfon, L., et al., 2008. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). Journal of Alzheimer's Disease 15, 211–222.

Milgram, N.W., Head, E., Zicker, S.C., et al., 2005. Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: a two-year longitudinal study. Neurobiology of Aging 26, 77–90.

Morris, M.C., Evans, D.A., Tangney, C.C., et al., 2005. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. The American Journal of Clinical Nutrition 81, 508–514.

Ono, K., Hasegawa, K., Naiki, H., Yamada, M., 2004. Curcumin has potent anti-amyloidogenic effects for Alzheimer's beta-amyloid fibrils in vitro. Journal of Neuroscience Research 75, 742–750.

Ono, K., Yoshiike, Y., Takashima, A., Hasegawa, K., Naiki, H., Yamada, M., 2003. Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. Journal of Neurochemistry 87, 172–181.

Opii, W.O., Joshi, G., Head, E., et al., 2008. Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: relevance to Alzheimer's disease. Neurobiology of Aging 29, 51–70.

Pocernich, C.B., Lange, M.L.B., Sultana, R., Butterfield, D.A., 2011. Nutritional approaches to modulate oxidative stress in Alzheimer's disease. Current Alzheimer's Disease Research. 8 (5), 452–469.

Sano, M., Ernesto, C., Thomas, R.G., et al., 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. The New England Journal of Medicine 336, 1216–1222.

Song, W., Derito, C.M., Liu, M.K., He, X., Dong, M., Liu, R.H., 2010. Cellular antioxidant activity of common vegetables. Journal of Agricultural and Food Chemistry 58, 6621–6629.

Sultana, R., Butterfield, D.A., 2010. Role of oxidative stress in the progression of Alzheimer's disease. Journal of Alzheimer's Disease 19, 341–353.

Tabet, N.B.J., Evans, J.G., Orrel, M., Spector, A., 2000. Vitamin E for Alzheimer's disease. Cochrane Database of Systematic Reviews 4, CD002854.

Vingtdeux, V., Giliberto, L., Zhao, H., et al., 2010. AMPK signaling activation by resveratrol modulates amyloid–beta peptide metabolism. The Journal of Biological Chemistry 285, 9100–9113.

Williams, J. W., Plassman B. L., Burke J., Holsinger T., Benjamin S., 2010 Preventing Alzheimer's Disease and Cognitive Decline. Evidence Report/Technology Assessment No. 193 (Prepared by the Duke Evidence-based Practice Center under Contract No. HHSA 290-2007-10066-I.). In: AHRQ Publication No. 10-E005. Rockville, MD, Agency for Healthcare Research and Quality.

Yang, F., Lim, G.P., Begum, A.N., et al., 2005. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. The Journal of Biological Chemistry 280, 5892–5901.

Bioactive Prairie Plants and Aging Adults: Role in Health and Disease

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1. INTRODUCTION

Aging adults (>50 years) are a fast-growing segment of the North American population, and a fast-growing industry is that of dietary supplements and botanicals (Cavaliere et al., 2010). While not a causal association, it comes as no surprise that aging adults are significant consumers of complementary and alternative medicines (CAM), including natural products (e.g., nutraceuticals and functional foods), and traditional healing practices. Chronic health conditions are a reliable predictor of CAM use, and the incidence of chronic diseases increases markedly after age 50 years. Aging is largely due to accumulated oxidative stress with changes in cell function and gene expression leading to disease. Chronic diseases, such as diabetes mellitus, cardiovascular disease, obesity, respiratory disease, and cancer, contribute significantly to global disease burden. These and hypertension, frailty, arthritis, and osteoporosis decrease the quality of life for aging adults and increase health-care utilization and expenditures. In efforts to reverse or prevent disease, aging adults use CAM therapies to complement – or as an alternative to – allopathic medical care.

Estimates suggest that approximately 40% of surveyed U.S. adults use CAM, and ~18% of these therapies are natural products (Barnes et al., 2008; Eisenberg et al., 1998) including botanicals. The therapeutic (or toxic) properties of plants have been valued across cultures and geographic landscapes throughout the world. For example, the North American continent (herein, Canada and the United States) consists of a large bioregion (3 million km^2) of grasslands rich in biodiversity. Prairie-derived natural botanical products are appreciated by indigenous and nonindigenous peoples. Scientists in Kansas currently seek to provide evidence for the potential benefit of prairie phytomedicines and functional foods in health and disease. This paper will introduce selected prairie plants organized by family. Ethnobotanical and evidence-based information should inspire study of these plants, protection of the prairie grasslands, and aging-adult appreciation of indigenous and nonindigenous uses of botanicals in health and disease.

2. SECONDARY METABOLITES

Nonnutritive organic bioactive compounds are produced as secondary metabolites in plant metabolism, usually without primary roles in plant growth or development. Compound concentration is influenced by the plant part, developmental stage of the plant, and environment (e.g., sunlight levels). There are four major classes of bioactive secondary metabolites: terpenes, phenolics, glycosides, and alkaloids. Plants often have many bioactive compounds, across these classes, contributing to purported effects in the body. Many of these compounds are found to have antioxidant function in the body.

3. PRAIRIE BIOME

The temperate grasslands is a biome well represented within the dry interior of several continents. North America is 1/5 temperate grasslands, commonly referred to as prairie, whereas in South America, they are the pampas. In Eurasia, grasslands are known as steppes, while the veld occurs in eastern and southern Africa. The biome's characteristics are strongly influenced by climate and precipitation. Seasonal temperature extremes (45 to $-45\,°C$) and moderate precipitation ($25–100\ cm^{-y}$) favor an abundance of grasses and forbs, and a scarcity of trees. The vegetation is drought and fire resistant and contributes to a soil rich in nutrients with good water-holding capacity. In North America, this arable soil has led to agricultural modification of the native state; in some regions, almost nothing remains of the original biome. Instead, the *floor of the sky* that was once a *sea of grass* is now more often checkered with crops, to comprise the *breadbasket of the world*. Yet, many plants from the prairie bioregion continue to be used as functional foods and phytomedicines by indigenous and nonindigenous peoples (Kindscher, 1987).

4. GRASSES

The prairie plains are comprised of three east–west zones. In the moist temperate grasslands of the east is the tall grass prairie. Found covering some of Canada's prairie provinces (e.g., Manitoba), Iowa, western Minnesota, and eastern Nebraska, are grasses that grow to 2 m. These include the big bluestem (*Andropogon gerardii*), little bluestem (*Andropogon scoparius*), Indian (*Sorghastrum nutans*), and tall panic (*Panicum virgatum*) grasses. In the dry temperate grasslands of the west (western Alberta and Saskatchewan and Eastern Montana) is the short grass prairie characterized by dominant grasses that grow to 0.5 m. These grasses include buffalo (*Buchloe dactyloides*), blue grama (*Bouteloua gracilis*), and hairy grama (*Bouteloua hirsute*) grasses. Situated between these zones is the mixed grass prairie, comprised of grasses of intermediate height. These include a mixture of species from the other two zones.

4.1. Sweet Grass (Plains Cree: *wīhkaskwa*)

Sweet grass (Latin: *Hierochloe odorata*; French: foin d'odeur (Marie-Victorin, 1964)) is a member of the grass family (Poaceae) native to North America and Eurasia at latitudes above 40° and grows to 0.66 m. Sweet grass is distributed across a variety of moist terrains. It is rhizomatous – and like most perennial grasses – the majority of the sweet grass biomass is below ground. Aerial plant parts are used by indigenous North Americans in basketry, decoration, ceremony, cosmetics, and as a phytomedicine (e.g., colds and fevers). Collected leaves are usually left to dry in braids; the three braid sections signify mind, body, and spirit (Keane, 2009).

A primary means of entry of sweet grass-derived compounds into the body is inhalation of its smoke and subsequent absorption of its compounds through the epithelial lining of the respiratory track, when the grass is burned as incense (e.g., smudging). There is no scientific literature on the pharmacokinetics of sweet grass bioactive compounds through this route of entry into the body. Further research is warranted.

Another common but less popularized method of using sweet grass as a phytomedicine is through oral ingestion of infusion or decoction prepared from the leaves and stems. It is in this fashion that sweet grass has been used in the treatment of colds and fevers, for example.

In considering the potential scientific merits of this plant for use by aging adults – across the spectrum of health and disease – further work is encouraged. There are few scientific reports on sweet grass, but several indicate antioxidant compound activity for the plant. Sweet grass has 22 phenolic compounds; principal compounds identified by NMR spectroscopy and mass spectrometry are coumarins (Pukalskas et al., 2002). Coumarins are a widespread family of over 1500 compounds and have noted agricultural/ animal husbandry roles as feeding deterrents, germination inhibitors, and as antimicrobial agents.

Increased fatty acid stability has been demonstrated when sweet grass extract is added to lard and rapeseed oil emulsions (Zainuddin et al., 2002). While this indicates promise for the use of this natural antioxidant containing extraction in the food industry, work with animal/human models is needed to ascertain the capacity of sweet grass-derived compounds to scavenge free radicals *in vivo*.

Coumarin itself has been shown to have antitumor activity *in vivo*; its metabolites may abrogate Cyclin D1, a cell cycle regulator which is overexpressed in cancers (Lacy and O'Kennedy, 2004) and associated with proinflammatory cytokines (Peer et al., 2008). This knowledge has fueled the pharmaceutical industry to design novel compounds based upon the coumarin molecule structure to target oncogenesis. While coumarin is a promising bioactive compound, used as a phytomedicine, the antitumor, anti-inflammatory, and antioxidant effects of whole sweet grass have yet to be demonstrated in humans.

4.2. Wild Rice (Anishinabe: *Manomin nabob*)

The genus *Zizania* (French: zizanie, riz sauvage; Marie-Victorin, 1964) is comprised of four species of grass which occur in marshes, slow moving rivers, and small lakes of the temperate grasslands. Three occur in North America: Northern wild rice (*Zizania palustris*; annual), wild rice (*Zizania aquatica*; annual), and Texas wild rice (*Zizania texana*; perennial). One occurs in Eurasia: Manchurian wild rice (*Zizania latifolia*; perennial). The plant has a large submerged adventitious root system, and at maturity, the plant height is approximately 1 m. The seed heads are large and can reach up to 2 cm in length. These wild grains have been a staple or supplemental food source to Native Americans who harvest it, in the regions where it grows. In Eurasia, the stalks are eaten more frequently than the seed heads. In the twentieth century, paddy cultivation of wild rice hybrids contributes significantly to global wild rice production (which is about 10.5 million kg^{-y}), as these can be grown outside of its native range. Indigenous people do not consider the farmed variety to be a sacred food, as is the wild-harvested variety. Currently, Saskatchewan is the world's largest producer of wild rice.

This grain is highly nutritious. It is superior to brown rice (*Oryza sativa*) in nutritive value. Wild rice has twice the protein, less fat, and more fiber than brown rice. The Dietary Guidelines Advisory Committee 2010 Report recommends that one-half of grain products consumed should be fiber-rich whole grains. This is based on evidence from population and intervention studies demonstrating decreased chronic disease incidence with dietary patterns associated with greater dietary fiber intake. In addition to fiber, whole grains confer health benefits associated with other phytochemicals of value, such as phenolics, flavonoids, gamma-oryzanol, alkenylresorcinols, carotenoids, and vitamin E (Okarter and Liu, 2010).

The antioxidant properties of wild rice have been characterized *in vitro*. Radical scavenging capacity of wild rice is 30 times greater than that of white rice, and the antioxidants in wild rice are determined to be flavonoids (Qui et al., 2009). In a randomized controlled trial, rats fed a high-fat/cholesterol diet (with wild rice as the carbohydrate source) demonstrated improved serum triglycerides, cholesterol, and superoxide dismutase activity (endogenous antioxidant) (Zhang et al., 2009). It remains to be demonstrated in humans this exciting potential that wild rice appears to have on health markers.

5. PRAIRIE PULSES

Native prairies bring to mind images of a sea of grass. Grassland plants are dependent upon nitrogen (N) for plant growth, however. Nitrogen is provided to the soil through the action of microbes that specialize in atmospheric nitrogen fixation. Pulses (or legumes), in partnership with bacteria, incorporate atmospheric nitrogen into plant tissue. Prairie

pulses, of the family Fabaceae, also reach high densities in the native North American grasslands.

This microbial and plant interaction in pulses provides a high-protein feed for animals/humans and for the soil – through the consumption and decomposition, respectively – of pulses. Common pulse crops (prairies turned pasture) include soybeans, lentils, peas, and garbanzos. Saskatchewan is now the world's largest exporter of lentils and dry peas. Protein-rich pea crops are often used as cattle feed. With increased cost of commercial fertilizers, there is a return to the practice of grinding the nitrogen-rich plants back into the soil as green manure in Alberta. There are many reasons to appreciate pulse crops grown on the grasslands and to consider the value of native legumes.

5.1. Wild Licorice (Cheyenne: *Haht' nowasspoph*)

Wild American licorice (Latin: *Glycyrrhiza lepidota*) is a perennial plant found throughout the west/central regions of North America. Etymologically, the old French term licoresse derives from the ancient Greek name glycyrrhiza for sweet root. The species name *lepidota* means scaly and refers to the minute scales on the juvenile leaves. The aggressive rhizomatous root system can reach 4 m deep into moist soil. Birds, animals, and indigenous people have appreciated the leaves, seeds, and roots as food and medicine. The roots and leaves have been infused as traditional medicine, for a variety of ailments. More specifically, it can be used for protecting the teeth from cavities, soothing mucous membranes, and alleviating menopausal symptoms (Keane, 2009). Licorice extract is a commercial flavoring with numerous applications (e.g., tobacco flavoring).

The root of wild licorice is sweet, not due to sugars but to glycyrrhizin, the ammonium salt of glycyrrhizic acid. However, the sweetness of the root is variable and less intense than Eurasian licorice (*Glycyrrhiza glabra*) (Kindscher, 1987). Glycyrrhizin is a glycoside, the predominant triterpenoid saponin isolated from licorice with attributed pharmacological effects (Asl and Hosseinzadeh, 2008). In a systematic review of the literature, the following properties of glycyrrhizin are characterized in animals: anti-inflammatory, antioxidant, antiviral, antithrombotic, antimutagenic, antinephritic, and antihepatitic (Asl and Hosseinzadeh, 2008). These properties are of interest in the prevention and treatment of human diseases – such as cancer, immunodeficiency, HIV/AIDS, atherosclerosis – but the evidence to date is not conclusive.

The preceding discussion refers to a primary bioactive component of licorice (glycyrrhizin). Whole licorice (root or leaves taken as a phytomedicine) or its extracts have a variety of bioactive components that include glycosides (e.g., saponins such as glycyrrhizin), phenolics (e.g., flavonoids), terpenes (e.g., sterols), and alkaloids. The concentrations of these compounds vary and can affect the pharmacokinetics of glycyrrhizin and its metabolites. *In vitro* and *in vivo* findings regarding glycyrrhizin do not imply similar effects with whole licorice or licorice extracts. However, organic soluble extract from

G. lepidota leaves shows moderate anti–HIV-1 activity *in vitro* (Manfredi et al., 2001). Taken together, these findings show promise for the use of wild licorice – and/or its bioactive compounds – as phytomedicine.

5.2. Groundplum Milkvetch (Ponca: *Gansatho*)

Also known as astragale in French (Marie-Victorin, 1964), the groundplum milkvetch (Latin: *Astragalus crassicarpus*) is a short perennial forb located primarily throughout the North American central plains. The species name *crassicarpus* refers to the thick fruits that are useful as a food and medicine. The fleshy fruits can be eaten raw, boiled, or pickled (Kindscher, 1987). The fruits have been used in Native American traditional medicine for a variety of ailments. Small pieces of the root were used for toothache, to ease a baby's teething, and for sore throats (Keane, 2009).

The groundplum milkvetch is a member of a very large genus. Globally, there are over 2000 species native to temperate regions. *Astragalus membranaceus* has a history of use in Asian traditional medicine, which has piqued the curiosity of scientists. A recent meta-analysis on the use of *A. membranaceus* in the treatment of hepatocellular cancers indicates that *in vitro* immunomodulatory and antitumor properties are demonstrated (Wu et al., 2009). Another meta-analysis concludes that despite incomplete knowledge regarding the bioactive compounds of *A. membranaceus* or their mechanism of action, the botanical has shown clinical value in diabetic nephropathy (Li et al., 2010). This species has merit as a phytomedicine warranting further investigation.

6. SUNFLOWERS

Among the flowering plants, the sunflower (French: tournesol) family (Asteraceae) is one of the largest, with over 25 000 species worldwide. Many are annuals or perennials, while some are biennials. Familiar foods – such as lettuce, sunflower seeds, Jerusalem artichokes, dandelions, thistles, and safflower oils – are family members. Others produce phytochemicals used as dyes, medicines, or insecticides. In his book about the flora of the New France colony in Canada, Boucher remarked that native people used the sunflower seed to make oil with a delicate taste (Boucher, 1882).

6.1. Jerusalem Artichoke (Pawnee: *Mkisu-sit*)

Helianthus tuberosus (French: topinambour; Marie-Victorin, 1964) is an herbaceous perennial that grows widely throughout North America, especially in the central/eastern regions. The plant tubers are a carbohydrate-rich staple valued as food since before European contact. The foliage is used for animal forage. In the Algonquin language, it is called the sun root. Samuel Champlain, a seventeenth-century French explorer of the Americas, noted that Cape Cod indigenous people cultivated the plant in 1605.

He subsequently brought it to France, where it was grown for beer and wine production. The French colonists living in northeastern United States mixed topinambour with molasses and dried corn or bran to make beer, or used it to make wine (Germain, 1992).

There are several unique attributes regarding the nutritive profile of this species. It has a relatively high protein concentration, at 10%, and very little oil. The carbohydrate, although starchy, is not comprised of polymerized glucose. Rather, the tuber is ~75% inulin, a fructose polymer and possibly the most interesting bioactive compound. Most inulin that passes through the human digestive tract remains unabsorbed. In the gut, microbial digestion of inulin results in fructose release and absorption. Fructose is sweeter than glucose, and valued for its abrogated insulin response. Jerusalem artichoke as a functional food may improve glycemic control. Whole food studies of Jerusalem artichoke with diabetics are needed.

A systematic review of the literature indicates that inulin intake is associated with improved health markers (Kaur and Gupta, 2002), such as improved blood glucose control and nitrogen balance. Colon cancer risk reduction is attributed to the prebiotic effects of inulin on colonic microbes. Colonic fermentation of inulin results in short-chain fatty acid production, a preferred substrate for colonocyte metabolism. In a randomized control trial with humans, inulin intake resulted in a postprandial increase of serum short-chain fatty acids and decreased postprandial free fatty acid concentrations, which may decrease type 2 diabetes risk (Tarini and Wolever, 2010).

6.2. White Prairie Sage (Plains Cree: *Paskwāwīhkwaskwa*)

This white-wooly perennial herb (Latin: *Artemisia ludoviciana*; French: armoise (Marie-Victorin, 1964)) is renowned for its spicy fragrance and flavor and common uses as incense and infusion, popularized by Native North Americans. The *Artemisia* genus is large and found in temperate regions of both hemispheres. It grows abundantly in the central and southwest regions of North America. It is utilized in traditional medicine systems where it occurs. In Saskatchewan, indigenous Elders refer to it as women's medicine, as it can be used for smudging during their moontime (menstruation) instead of sweet grass. It can also be made into an infusion and used to calm an upset stomach and to stop diarrhea (Gendron et al., 2010).

Artemisia oils have been compositionally analyzed and both found to be high in terpenes (Lopes-Lutz et al., 2008). Oils from Canadian wild sage are shown to have antimicrobial capacity *in vitro* (Lopes-Lutz et al., 2008). In regard to radical scavenging capacity, *A. ludoviciana* demonstrates moderate capacity (Lopes-Lutz et al., 2008) *in vitro*. These findings indicate a need for studies of antioxidant capacity of *Artemisia in vivo*.

A principal bioactive compound isolated from *Artemisia* is artemisinin – a terpene lactone – known as one of the most effective antimalarial agents against drug-resistant strains. Artemisinin has also been demonstrated to have moderate activity as a cell

proliferation inhibitor in cell culture (McGovern et al., 2010) – showing promise as an anticancer agent. While *Artemisia* is used in traditional medicine, the bioactive compounds studied are mostly hydrophobic and not simple infusion extractions. Studies of the traditional preparations of the plant are needed.

7. MILKWEEDS

Milkweeds are flowering plants from the family Apocynaceae. The Greek god of healing, Asclepius, provides the motivation for the genus name *Asclepias* (French: asclépiade; Marie-Victorin, 1964). These herbaceous perennials produce a milky sap that is variably toxic across the >140 species. The healing properties of the plant are attributed to the sap, rich in secondary metabolites such as latex (isoprene polymers), cardenolides (steroid derivatives), and alkaloids. Alkaloids are a large family of chemically unrelated nitrogenous molecules that are water soluble and have high biological activity. Specialized nectar-seeking and herbivorous insects feed on milkweed, and monarch butterfly larvae are exclusively dependent upon milkweed.

7.1. Common Milkweed (Winnebago: *Mahin'tsh*)

As the name implies, this milkweed (*Asclepias syriaca*) is common, specifically throughout the eastern half of North America. The common milkweed can be a three-stage food for humans when boiled: young sprouts, young floral buds, and young green fruits. Young milkweed shoots were eaten by colonists in New France (Delage, 1992). Native Americans made a crude sweetener from the nectar. As well, the plant has many uses in indigenous traditional medicine, and some scientific experiments have recently been conducted.

A polyphenolic preparation, separated and purified from an alkaline extract of *A. syriaca* leaves, was assessed for oncostatic action in rats (Rotinberg et al., 2000). The investigators found dose-dependent antineoplastic effects. Additionally, the polyphenolic preparation was compared to several standard chemotherapeutic drugs and found to have equal or superior efficacy. The mechanism of action of this preparation has been determined to be membranotropic in an *in vitro* cancer cell line (Rotinberg et al., 2007).

7.2. Swamp Milkweed (Lakota: *Wahinheya ipi'ye*)

Asclepias incarnata grows throughout much of North America in moist and wet soils. It is 0.6–1.5 m in height. The young leaves can be eaten as a vegetable, and muskrats eat the roots.

The secondary metabolite profile of this plant is compelling. The aerial portion of the plant has 34 pregnane glycoside structures determined by spectroscopic and chemical methods (Warashina and Noro, 2000b). Seven flavonoid compounds are identified from preparations from the leaves of this species (Sikorska, 2003). The roots have been analyzed

and determined to have 32 glycosides, 2 cardenolides, and 2 pregnane glycosides (Warashina and Noro, 2000a). Assays for compound bioactivity are warranted based upon this profile.

8. ROSE FAMILY

The rose family Rosaceae is a large group of fruits and ornamentals with over 3350 species. Many of the plants have uses as phytomedicine or food. Many important fruiting plants worldwide are members of this family, and most occur in Northern temperate regions. Examples include apples, peaches, cherries, Saskatoon berries, apricots, almonds, crabapple, pears, nectarines, blackberries, plums, raspberries, prairie rose, and chokecherries.

8.1. Saskatoon Berry (Saulteaux: *Sikākominan*)

The Saskatoon serviceberry (Latin: *Amelanchier alnifolia*; French: amélanchier (Marie-Victorin, 1964)) is a deciduous shrub or tree (≤ 7 m) that grows in moist ravines of prairies, margins and interiors of aspen poplar bluffs, and forest edges. It has been called shadbush because it flowers in June, when the shad (*Amelanchier sapidissima*) run in Atlantic coastal rivers. The distribution of the native shrub is extensive, with a range from the east to the west coast of North America, and present throughout the prairie biome except in the southern regions. It is valued as an ornamental and a food source; the fruits are a berry-like pome of variable size (usually over a centimeter in diameter). They can be eaten raw, are often dried, and made into a variety of foods involving berries (e.g., pies, bannock, wines, and syrups). Indigenous Americans often incorporate the dried fruit with animal fat and dried meat into a convenient, high-energy food called pemmican. They also believe that the berries cleanse and reenergize the body (Gendron et al., 2010). An Elder from the Pasqua First Nation in Saskatchewan describes his traditional use: "I mix Saskatoon, dogwood, and prickly rose roots to make a medicine for venereal diseases, urinary tract infection, and kidney problems. The roots are picked when needed. I peel off the bark of the root, boil it, and drink it as a tea. The power is in the bark." Recently, Saskatoon berries have become a celebrity superfood, valued in the diet to impact human health.

The Saskatoon berry (not botanically a berry) contains terpenes, phenolics, glycosides, and alkaloids (Bakowska-Barczak and Kolodziejczyk, 2008; Burns Kraft et al., 2008). The total polyphenol content is higher than that found in red/black raspberries, and wild strawberries, with 100 g fresh fruit having 189.7–382.1 mg anthocyanins (Bakowska-Barczak and Kolodziejczyk, 2008). Bioassay screens of berry extracts and fractions have shown antioxidant, anti-inflammatory, glycogen accumulation, and fatty acid oxidation effects suggestive of health promotion *in vivo* (Burns Kraft et al., 2008). The wild and cultivated berries have previously been reported to not differ, and the

berries demonstrate excellent stability of antioxidant activity even after storage for 9 months at −20 °C (Bakowska-Barczak and Kolodziejczyk, 2008).

9. MINT

The mint family Lamiaceae is large and with a cosmopolitan distribution. Many familiar herbs are members: spearmint, peppermint, marjoram, oregano (not Mexican), rosemary, sage (*Salvia*), sweet basil, thyme, savory, lavender, and many others. Family members frequently have square stems, and numerous aromatic oil glands on simple or compound, usually toothed leaves. These plants have wide uses including food seasoning, companion plants in gardens, ornamental plants, and phytomedicines. Many family members have been extensively cultivated for their utility and ease of propagation. Others grow wild; in the prairie biome, there are a large number of mints, some of which are endemic to North America.

9.1. Bergamot (Blackfeet: *Ma-ne-ka-pe*)

Also known as bee balm, horsemint, Oswego tea, or by the French name monarde (Marie-Victorin, 1964), *Monarda fistulosa* is endemic to North America, preferring upland woods, thickets, and prairies. Nicolas Monardes, a sixteenth-century Spanish physician who wrote about New World medicinal plants, was the inspiration for the genus name. The genus is comprised of about 165 species of erect herbaceous annual or perennial plants that propagate by seeds or rhizomes. *M. fistulosa* is a perennial herb approximately 0.33 m tall, with creeping rhizomes and branched, hairy stems, and smokey pink flowers. With over 50 commercial cultivars, there is variation in flower color – many of which are prized as ornamentals. The plant has varied uses: a seasoning for game meat, ornamental flower, botanical medicine, and garden companion plant. Thymol, a monoterpene phenol, with a characteristic aromatic odor and strong antiseptic properties, is extracted from thyme or bee balm. It is valued as a component of mouthwash, and this compound is attributed to the many antimicrobial uses of the plant by Native North Americans. Seborrhea is a skin infection caused by yeast, and a recent study has shown essential oil of *M. fistulosa* to have antifungal properties superior to a standard medication in treatment of seborrhea (Zhilyakova et al., 2009) in animals.

9.2. Wild Mint (Nakota: *Cayágadag*)

The wild mint (Latin: *Mentha arvensis*; French: menthe; Marie-Victorin, 1964) is a 0.5-m-tall perennial herb with square stems, slightly hairy to smooth leaves that are strongly aromatic when crushed. The plant grows throughout North America and prefers prairie ravines, stream and lake margins, low woods, and backyards. It is also native to Eurasia. The species name *arvensis* means to grow in a cultivated field, attesting to its ease

of propagation. The genus name *Mentha* hails from the Greek nymph Minthe, who was changed into a mint plant by a jealous goddess. The plant (all parts) has a history of use as a phytomedicine, usually infused, for a variety of purposes. Elders in Saskatchewan use leaves and stems to make an infusion to prevent colds (Gendron et al., 2010).

In addition to infused plant parts, mint essential oils have applications in food flavoring and preservation, as fragrance, and in medicine. Essential oils are volatile products of secondary plant metabolism. A primary chemical constituent in *M. arvensis* has been determined to be menthol (Hussain et al., 2010). Mint plant preparations, such as essential oil, show very good antimicrobial and cytotoxic activities (Hussain et al., 2010). A recent study in mice exposed to ionizing radiation indicates that *M. arvensis* leaf extract confers protection against radiation-induced sickness, gastrointestinal, and bone marrow deaths (Jagetia, 2007). Ionizing radiation is an important source of mutational damage to DNA. Further testing of efficacious compounds in animal models is needed. These preliminary findings suggest that extract of *M. arvensis* may modulate DNA modifications that accelerate aging.

10. SUMMARY AND FUTURE DIRECTIONS

A burgeoning population of aging adults is associated with the search for substances with medicinal activity, and bioactive plants have always been alluring. The North American continent consists of a large bioregion of prairie grasslands rich in biodiversity and natural botanicals appreciated by indigenous and nonindigenous peoples. This chapter reviews the medicinal/cultural uses and chemical properties of selected prairie plants: *Hierochloe odorata*, *Zizania spp.*, *Glycyrrhiza lepidota*, *Astragalus crassicarpus*, *Helianthus tuberosus*, *Artemisia ludoviciana*, *Artemisia scoparia*, *Asclepias syriaca*, *Asclepias incarnata*, *Amelanchier alnifolia*, *Monarda fistulosa*, and *Mentha arvensis*. Their common names in English, French, and indigenous languages are also given. In addition to their nutritive value, a range of bioactivities have been found in these native plants including antioxidant, antitumor, anti–inflammatory, antimicrobial, and antiviral. Further study of these plants/compounds is warranted and can lead to increased value of complementary and alternative medicines/functional foods, appreciation of ethnobotany, and preservation of prairie grasslands.

GLOSSARY

Complementary and alternative medicine A group of diverse medical and health care systems (e.g., Traditional medicine), practices (e.g., sweat lodge; meditation), and products (e.g., nutraceuticals and functional foods) that are generally not evidence based and not part of allopathic (Western) medicine.

Functional food Ordinary food that has components or ingredients added to give it a purported medicinal or physiological benefit, other than nutritional benefit; any food claimed to prevent chronic disease or be health-promoting beyond the basic function of supplying nutrients.

Indigenous Native, original inhabitant of a bioregion.

Nutraceutical Any substance that may be considered a food or part of a food and provides purported medical or health benefits – beyond the basic supply of nutrients – including prevention and treatment of disease.

Phytomedicine Also called phytotherapy, the art and science of using botanical (medicinal plant) remedies to prevent or treat disease.

REFERENCES

Asl, M.N., Hosseinzadeh, H., 2008. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. Phytotherapy Research 22, 709–724.

Bakowska-Barczak, A.M., Kolodziejczyk, P., 2008. Evaluation of saskatoon berry (*Amelanchier alnifolia* Nutt.) cultivars for their polyphenol content, antioxidant properties, and storage stability. Journal of Agricultural and Food Chemistry 56, 9933–9940.

Barnes, P.M., Bloom, B., Nahin, R., 2008. CDC National Health Statistics Report #12. Complementary and alternative medicine use among adults and children: United States, 2007.

Boucher, P., 1882. Histoire véritable et naturelle des mœurs et productions du pays de la Nouvelle-France. Montreal.

Burns Kraft, T.F., Dey, M., Rogers, R.B., et al., 2008. Phytochemical composition and metabolic performance-enhancing activity of dietary berries traditionally used by Native North Americans. Journal of Agricultural and Food Chemistry 56, 654–660.

Cavaliere, C., Rea, P., Lynch, M.E., Blumenthal, M., 2010. Herbal supplement sales rise in all channels in 2009. HerbalGram 86, 62–65.

Delage, D., 1992. L'influence des Amérindiens sur les Canadiens et les Français au temps de la Nouvelle-France. Lekton 2, 103–191.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., et al., 1998. Trends in alternative medicine in the United States 1990-1997: results of a follow-up national survey. Journal of the American Medical Association 280, 1569–1575.

Gendron, F., Biden, M., Thompson, L., et al., 2010. Gitchi Mando Miyew (Creator Given): Traditional Supports for HIV/AIDS. First Nations University of Canada, Regina.

Germain, R., 1992. Boissons de nos aïeux. Cap-aux-Diamants: la revue d'histoire du Québec 28, 10–13.

Hussain, A., Anwar, F., Nigam, P.S., Ashraf, M., Gilani, N.H., 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four mentha species. Journal of the Science of Food and Agriculture 90, 1827–1836.

Jagetia, G.C., 2007. Radioprotective potential of plants and herbs against the effects of ionizing radiation. Journal of Clinical Biochemistry and Nutrition 40, 74–81.

Kaur, N., Gupta, A.K., 2002. Application of inulin and oligofructose in health and nutrition. Journal of Biosciences 27, 703–714.

Keane, K., 2009. The Standing People: Field Guide of Medicinal Plants for the Prairie Provinces, second ed. Save Our Species, Saskatoon.

Kindscher, K., 1987. Edible Wild Plants of the Prairie: An Ethnobotanical Guide. University Press of Kansas, Kansas.

Lacy, A., O'Kennedy, R., 2004. Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Current Phamaceutical Design 10, 3797–3811.

Li, M., Wang, W., Xue, J., Gu, Y., Lin, S., 2011. Meta-analysis of the clinical value of *Astragalus membranaceus* in diabetic nephropathy. Journal of Ethnopharmacology 133 (2), 412–419.

Lopes-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P., 2008. Screening of chemical composition, antimicrobial, and antioxidant activities of *Artemisia* essential oils. Phytochemistry 69, 1732–1738.

Manfredi, K.P., Vallurupalli, V., Demidova, M., Kindscher, K., Pannell, L.K., 2001. Isolation of an anti-HIV diprenylated bibenzyl from *Glycyrrhiza lepidota*. Phytochemistry 58, 153–157.

Marie-Victorin, F., 1964. Flore Laurentienne, second ed. Presses de l'Université de Montréal, Montreal.

McGovern, P.E., Christofidou-Solomidou, M., Wang, W., et al., 2010. Anticancer activity of botanical compounds in ancient, fermented beverages. International Journal of Oncology 37, 5–14.

Okarter, N., Liu, R.H., 2010. Health benefits of whole grain phytochemicals. Critical Reviews in Food Science and Nutrition 50, 193–208.

Peer, D., Park, E.J., Morishita, Y., Carman, C.V., Shimaoka, M., 2008. Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. Science 319, 627–630.

Pukalskas, A., van Beek, T.A., Venskutonis, R.P., et al., 2002. Identification of radical scavengers in sweet grass (*Hierochloe odorata*). Journal of Agricultural and Food Chemistry 50, 2914–2919.

Qui, Y., Liu, Q., Beta, T., 2009. Antioxidant activity of commercial wild rice and identification of flavonoid compounds in active fractions. Journal of Agricultural and Food Chemistry 57, 7543–7551.

Rotinberg, P.F., Gherghel, D.N., Cosmin, M.G., Neacsu, I.V., Rotinberg, H.P., 2007. Experimental modulation of HEp-2p tumoral cells reactivity to the action of some autochthonous polyphenolic cytostatics by certain membranotropic and metabolic agents. Genetica si Biologie Moleculara VIII, 45–52.

Rotinberg, P., Kelemen, S., Gramescu, M., Rotinberg, H., Nuta, V., 2000. Preclinical trial of the antitumoral therapeutic effectiveness of some natural polyphenolic biopreparations. Romanian Journal of Physiology 37, 105–118.

Sikorska, M., 2003. Flavonoids in the leaves of *Asclepias incarnata* L. Acta Poloniae Pharmaceutica 60, 471–475.

Tarini, J., Wolever, T.M., 2010. The fermentable fiber inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Applied Physiology, Nutrition, and Metabolism 35, 9–16.

Warashina, T., Noro, T., 2000a. Cardenolide and oxypregnane glycosides from the root of *Asclepias incarnata* L. Chemical and Pharmaceutical Bulletin 48, 516–524.

Warashina, T., Noro, T., 2000b. Steroidal glycosides from the aerial part of *Asclepias incarnata*. Phytochemistry 53, 485–498.

Wu, P., Dugoua, J.J., Eyawo, O., Millis, E.J., 2009. Traditional Chinese medicines in the treatment of hepatocellular cancers: a systematic review and meta-analysis. Journal of Experimental & Clinical Cancer Research 12, 112.

Zainuddin, A., Pokorny, J., Venskutonis, R., 2002. Antioxidant activity of sweet grass (*Hierochloe odorata* wahlnb.) extract in lard and rapeseed oil emulsions. Food/Nahrung 46, 15–17.

Zhang, H., Cao, P., Agellon, L.B., Zhai, C.K., 2009. Wild rice (*Zizania latifolia* (Griseb) Turcz) improves the serum lipid profile and antioxidant status of rats fed with a high fat/cholesterol diet. The British Journal of Nutrition 102, 1723–1727.

Zhilyakova, E.T., Novikov, O.O., Naumenko, E.N., et al., 2009. Study of *Monarda fistulosa* essential oil as a prospective antiseborrheic agent. Bulletin of Experimental Biology and Medicine 148, 612–614.

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Ginseng and Micronutrients for Vitality and Cognition

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1. INTRODUCTION

The substantial increases in life expectancy achieved over the previous century, combined with medical advances, escalating health and social care costs, and higher expectations for older age, have led to international interest in how to promote a healthier old age and how to age 'successfully.' 'Successful aging' can be considered as a combination of at least three factors: the avoidance of disease and disability, continued active engagement in life (including social contacts and integration in society), and the maintenance of high physical and cognitive capacity thereby ensuring independent functioning and autonomy as well as supporting psychological aspects such as self-esteem and a positive outlook and attitude (Bowling and Dieppe, 2005).

Current research indicates that nutrition plays a key role in health maintenance of older adults, that many elderly are at increased risk of inadequate nutrient intakes, and that several micronutrients are associated with decreased risk of diseases, infectious as well as chronic diseases, and frailty (Kaiser et al., 2009). Ensuring adequate intake of vitamins and minerals is one of the most efficient preventative measures, together with physical activity, against health deterioration due to aging. This prevention should start early in life, continue in older populations, and be constant throughout aging. Already mild micronutrient deficiencies can result in lack of well-being, general fatigue, and reduced resistance to infections or negatively affect mental processes (e.g., memory, concentration, attention, and mood) resulting in a reduced mental and physical capacity and vitality (Huskisson et al., 2007a,b; Maggini et al., 2008). Optimal intake of essential micronutrients is also crucial for long-term health, and suboptimal intakes of several vitamins and minerals, above levels causing classic overt deficiency, are a risk factor for chronic diseases and common in the general population, especially the elderly. Therefore, it appears prudent for all adults, but especially for vulnerable groups such as the elderly population, to take micronutrient preparations to the daily regimen (Fairfield and Fletcher, 2002; Fletcher and Fairfield, 2002) to help build a strong foundation for maintaining good health and proper nutrition.

Next to micronutrients, also, natural extracts can support health and vitality during aging. Specifically, combinations of essential micronutrients and *Panax ginseng* have shown positive effects with regard to physical and mental functions. Ginseng is probably one of the most popular herbal remedies used in many regions of the world. For more than 5000 years, the roots of this plant have been used in Chinese and modern medicine (Yun, 2001). It is cultivated in Korea, China, and Japan and exported in many countries. The name 'ginseng' is used for different species of the genus *Panax* of the plant family Araliaceae. The so-called 'true ginseng' refers only to Asian or Korean ginseng (*P. ginseng* C.A. Meyer) and American ginseng (*P. quinquefolius*). Some plants are not true ginseng deriving from different genus or family, but they have the term 'ginseng' in their common names. These include Siberian ginseng (*Eleutherococcus senticosus*) which is widely used in dietary supplements, Prince ginseng (*Pseudostellaria heterophylla*), Indian ginseng (ashwagandha), and Brazilian ginseng (*Pfaffia paniculata*) (Ocollura, 1997). *P. ginseng* is the most widely used and studied ginseng species. More than 3000 research articles have been published to date. Despite this vast amount of literature, it is notable in that there are not many publications based on rigorous and scientifically accepted methods to prove ginseng's efficacy as a medical treatment (Vogler et al., 1999).

This chapter discusses evidence related to micronutrient needs in the elderly with a strong focus on *P. ginseng* and its effects on vitality and cognition especially in combination with vitamins and minerals. Many other micronutrient and ginseng activities in other areas (e.g., immunity, diabetes, cancer, and other chronic diseases) are not discussed here.

2. MICRONUTRIENTS

A number of genetic and environmental factors, as well as socioeconomic status and availability of adequate health and social services, determine well-being, overall health, and longevity. Diet and nutrition, including appropriate amounts of essential vitamins and minerals, are among the modifiable factors that can help maintain health and prevent chronic disease and functional decline, thus extending longevity and enhancing quality of life (Maggini, 2010; Maggini et al., 2008).

2.1 Elderly Individuals Are at Risk for Deficiencies

Elderly individuals are recognized to represent a population segment with a high risk of nutritional inadequacy, and it appears that many of the signs of aging in fact may be caused by insufficient nutrient intake.

Aging is accompanied by a variety of physiological, psychological, economic, and social changes. Especially the physiological changes affect the need for several micronutrients (Trichopoulou et al., 1995). The elderly are widely considered as a target group exposed to a higher risk for micronutrient deficiencies as age-related requirements of micronutrients generally encounter mostly decreased intakes due to lower caloric

requirements (Morley, 2002; Wakimoto and Block, 2001); alterations in taste, smell, and salivary function (Morley, 2002); an ever less effective vitamin and mineral absorption; and increasingly frequent digestive tract disorders (Russell, 1992; Gillette Guyonnet et al., 2007). For example, atrophic gastritis (inflammation of the stomach lining) results in a reduced release of free vitamin B12 from food proteins (Baik and Russell, 1999; Russell, 2001). On the other hand, the micronutrient requirements are unchanged or even increased (e.g., for vitamins B6 and B12) (Institute of Medicine (IOM), 1998). The situation is further exacerbated due to frequent use of medicines with all related possibilities of interactions with vitamin and mineral absorption (High, 2001; Roe, 1989, 1992, 1993), to a reduced ability to retain vitamins, and to increased levels of loss through excretion. Oxidation is a natural constraint on lifespan (Kirkwood, 1991), and accumulating damaging effects of toxic substances in the environment through smoking and pollution (Brunekreef and Holgate, 2002; Hennessy, 2002; Hong et al., 2002) become evident as we age. Older individuals show an increased susceptibility to infection (Chandra, 1992, 2002; Chavance et al., 1989) with increased micronutrient needs and reduced micronutrient intakes (Maggini et al., 2008). Finally, degenerative diseases such as mental disorders, coronary heart disease, eye disease, and osteoporosis (Gonzalez–Gross et al., 2001; Richard and Roussel, 1999) can both be caused or exacerbated by micronutrients deficiencies but can also lead to increased micronutrient requirements.

A large number of studies have been carried out to examine the nutritional state of the older population in general and the micronutrient status in particular. Nutritional status surveys of the older population have shown a low-to-moderate prevalence of frank nutrient deficiencies but a marked increase in the risk of malnutrition and the evidence of subclinical deficiencies (Gillette Guyonnet et al., 2007; Tucker, 1995). Covering the vast literature on the topic would require a separate paper; therefore, only a few micronutrient deficiencies are discussed here.

As an example of insufficient vitamin status, Wolters et al. (2003) evaluated the dietary intake and the blood status of various B vitamins and homocysteine in younger (60–70 years old) female seniors. Indexes of vitamins B1, B6, and B12 indicated insufficient status in 35% of the women, whereas plasma homocysteine was elevated in 17.4%. An association between vitamin intake and concentration in the blood was found only for folate. The results indicated that even in younger, well-educated, female seniors, the prevalence of low B-vitamin status and elevated plasma homocysteine concentration is high. The picture is very likely similar for male seniors. Regular supplementation with vitamins B1, B6, B12, and folate should be considered in this age group according to the authors.

The prevalence of vitamin B12 deficiency increases with age, and, based on vitamin B12 serum concentrations, it is reported to be between 5% and 40% in the elderly. As discussed, gastric atrophy and the consequent malabsorption of food-bound vitamin B12 are the main causes. Because of the potential for malabsorption of vitamin B12 from food, the

IOM recommended that people over 50 years of age should consume their vitamin B12 mostly from fortified foods or from supplements containing crystalline vitamin B12 (IOM, 1998). The findings of a recent study (Campbell et al., 2003) fully support the IOM recommendation to increase consumption of crystalline vitamin B12 in the elderly.

Vitamin D can be produced in adequate quantities in the skin depending on sufficient sun [ultraviolet B (UVB)] exposure and exposed skin surface. Still, the status of vitamin D is of concern: both vitamin D deficiency and insufficiency are becoming more common in developed countries. In the United Kingdom for instance, the prevalence of vitamin D deficiency is around 14.5% and may be more than 30% in those 65 years old and as high as 94% in otherwise healthy South Asian adults (Anonymous, 2006). According to Holick (2006), vitamin D inadequacy is approximately 36% of otherwise healthy adults and up to 57% of general medicine inpatients in the United States and in even higher percentages in Europe. Also, Zadshir et al. (2005) have reported that in the United States, in large portions of the general population as well as in most minorities (i.e., Hispanics and Black or Africans), serum levels of 25(OH)D3 (a vitamin D3 metabolite) are below recommended levels, indicating an insufficient vitamin D intake.

The elderly populations of Europe, the USA, and Australia present special problems (Dawson-Hughes et al., 1997; Lips, 2001; Mosekilde, 2005). With increasing age, solar exposure is usually limited because of changes in lifestyle factors such as clothing and outdoor activity. Diet may also become less varied, with a lower natural vitamin D content. Most importantly, however, the dermal production of vitamin D following a standard exposure to UVB light decreases with age because of atrophic skin changes with a reduced amount of its precursor (Holick et al., 1989). Finally, the renal production of $1,25(OH)_2D$ decreases because of diminishing renal function with age (Lau and Baylink, 1999). These changes in vitamin D metabolism render the aging population in general at risk of vitamin D deficiency, especially in winter seasons and when living indoor and at higher latitudes (Lips, 2001). This deficiency may lead to severe consequences in terms of falls, osteoporosis, and fractures.

Dietary magnesium generally does not meet recommended intakes for adults. Results of a recent national survey in the United States indicate that a substantial proportion of women do not consume the recommended daily intake of magnesium; this problem increases among women over 50 years old. The average magnesium intake for women is 228 mg per day compared with the recommendation of 320 mg per day. The average intake estimate is derived from 1-day diet recall and thus may overestimate actual magnesium intake (Lukaski and Nielsen, 2002).

2.2 Micronutrients for Mental and Physical Capacity

Numerous factors influence cognitive development and learning abilities at different life stages (Bryan et al., 2004) and include the following: nutrition [both macronutrients and

micronutrients (Bellisle et al., 1998)], social environment and social stimuli, intellectual stimuli, parental education, nutrition status of the mother during pregnancy, embryonic cognitive development, other confounding factors (frequent infections, aggressive or a social behavior, etc.), drugs, and medications. The profound interactions among nutrition, cognitive functions, and health have been recognized already many decades ago. Micronutrient status can affect cognitive function at all ages, and an insufficient vitamin supply can impair several aspects of cognitive functions and mood (Bellisle et al., 1998; Black, 2003; Heseker et al., 1995; Huskisson et al., 2007a; Malouf and Grimley Evans, 2008; Rosenberg and Miller, 1992; Rosenthal and Goodwin, 1985; Zimmermann et al., 2006). Vitamin and mineral deficiencies can lead to irreversible effects when occurring during pregnancy (Black, 2003), to impaired learning and cognitive functions when occurring during infancy/childhood (Black, 2003; Manger et al., 2004; Zimmermann et al., 2006), and to depression and dementia in the elderly (Rosenberg and Miller, 1992; Vollset and Ueland, 2005). Dementia, a syndrome characterized by impairment of memory and other higher cognitive functions, is a major public health problem in elderly. Research indicates more and more that nutrition may play an important role to ameliorate or to buffer age-related declines in attention and psychomotor functions (Gillette Guyonnet et al., 2007; Kallus et al., 2005). Similarly, physical capacity is key for healthy aging, and while physical activity is the greatest public health intervention for the prevention of age-associated functional decline, meeting the nutritional requirements in elderly is the second big environmental factor for optimal maintenance of the physical functions and capacity (Kaiser et al., 2009).

Nutrition in general and micronutrient status in particular can influence both mental and physical capacity (Huskisson et al., 2007a,b). Even mild micronutrient deficiencies can lead to reduced mental and physical capacity and vitality (Haskell et al., 2010; Huskisson et al., 2007a,b; IOM, 1998; Kennedy et al., 2010). This is not surprising in view of the multiple roles of micronutrients (i.e., vitamin and minerals), which are essential for human life and support thousands of reactions and processes in the human body.

It is indeed very well established that vitamins and minerals are essential for optimal cognitive functions and mental acuity due to their role in a host of physiological processes that have both a direct (e.g., neurotransmitter synthesis, receptor binding, membrane ion pump function) and indirect (e.g., energy metabolism, cerebral blood supply) effect on brain function (Haller, 2005; Huskisson et al., 2007a). The same applies for optimal physical capacity and vitality that relies on efficient energy production and availability of adenosine triphosphate (ATP) to the body. The breakdown of dietary energy sources such as carbohydrates, fats, and proteins into cellular energy in the form of ATP requires B-complex vitamins, vitamin C, and minerals such as calcium, magnesium, copper, chromium, manganese, iron, and zinc (Huskisson et al., 2007b). ATP is the 'molecular unit of currency' of intracellular energy and is used for a huge number of energy-requiring processes in the human body ranging from thinking to muscle contraction.

Tables 22.1 and 22.2 give an overview of the crucial roles of selected vitamins and minerals in supporting both mental and physical capacity and vitality.

In conclusion, there is a large body of evidence showing that elderly individuals are at risk for micronutrient deficiencies for a variety of reasons including decreased intake and absorption and increased requirements. An inadequate intake of vitamins and minerals negatively impacts both mental and physical capacity and hence impairs overall vitality and diminishes quality of life. Supplementation with micronutrients is required to maintain and support both mental and physical capacity and vitality especially in elderly individuals.

The next chapter reviews the compelling evidence demonstrating the benefits of *P. ginseng* for supporting vitality during aging especially if used in combination with micronutrients.

Table 22.1 Main Functions of Selected Vitamins and Their Fundamental Roles in Energy Metabolism/ Transformation and Mental and Physical Vitality (Huskisson et al., 2007a,b; Institute of Medicine, 1998)

Vitamin	Functions and main roles in energy metabolism
Vitamin B1	• Active in the form of thiamin pyrophosphate (TPP) • As cofactor, TPP is essential to the activity of cytosolic transketolase and pyruvate dehydrogenase, as well as mitochondrial alpha-ketoglutarate dehydrogenase and branched-chain ketoacid dehydrogenase • Essential for converting carbohydrates to energy and needed for normal functioning of the brain, muscles, including the heart muscle • Needed for neurotransmitter synthesis (glutamic acid and hence GABA, gamma-amino butyric acid) • Plays a role in the conduction of nerve impulses and maintenance of membrane potentials. The brain and the peripheral nerves contain significant amounts of vitamin B1 which has numerous roles within nerve tissue • Deficiency causes fatigue, mental changes such as apathy, decrease in short-term memory, confusion and irritability, visual difficulties • Frank deficiency results in beriberi, Wernicke–Korsakoff's psychosis
Vitamin B2	• Helps in the release of energy from foods • B2 is a precursor to flavin adenine dinucleotide and flavin mononucleotide. As prosthetic groups, they are essential for the activity of flavoenzymes including oxidases, reductase, and dehydrogenases • B2 is needed for synthesis of tyrosine and hence for the neurotransmitters noradrenaline, serotonin, and benzylamine • B2 deficiency is most often accompanied by other micronutrient deficiencies • Severe B2 deficiency may impair the metabolism of vitamin B6 and the conversion of tryptophan to niacin
Vitamin B6	• Participates in the form of pyridoxal phosphate as coenzyme in numerous enzymes involved in amino acid metabolism (neurotransmitters, niacin, sulfur amino acids), carbohydrate metabolism (glycogen phosphorylase), heme and nucleic acid biosynthesis, and lipid metabolism (carnitine, phospholipids)

Continued

Table 22.1 Main Functions of Selected Vitamins and Their Fundamental Roles in Energy Metabolism/Transformation and Mental and Physical Vitality (Huskisson et al., 2007a,b; Institute of Medicine, 1998)—cont'd

Vitamin	Functions and main roles in energy metabolism
	• Through its requirements for amino acid metabolism, B6 is needed for the synthesis of the following neurotransmitters: GABA (via glutamic acid); dopamine, adrenaline, noradrenaline (via tyrosine); 5-hydroxytryptamine and serotonin (via tryptophan), and finally histamine (via histidine) • Vitamin B6 (with B12 and folic acid/folate) regulates homocysteine metabolism and can have an effect on blood supply • During exercise, pyridoxal phosphate is needed for gluconeogenesis and for glycogenolysis in which it serves as a cofactor for glycogen phosphorylase • Deficiency results in depressed mood and neurological disturbances • Frank deficiency causes peripheral neuropathy, convulsions, depression, and confusion
Vitamin B12	• Functions as a coenzyme for the methyl transfer reaction that converts homocysteine to methionine and another reaction that converts L-methyl-malonyl coenzyme A to succinyl coenzyme A • Involved in one-carbon transfer pathways. It is part of coenzymes, re-forms folate, and helps break down some fatty acids and amino acids. It is required for normal erythrocyte production • Needed for the physical integrity of neurons • Vitamin B12 is a cofactor for catechol-O-methyltransferase, important in the breakdown of catecholamines, i.e., noradrenaline and dopamine, in the synaptic cleft • Vitamin B12 (with B6 and folic acid/folate) regulates homocysteine metabolism and can have an effect on blood supply • Symptoms of deficiency include fatigue and weakness, irritability, depressed mood, loss of concentration to memory loss, mental confusion, disorientation • Frank deficiency causes peripheral neuropathy, subacute combined system degeneration, frank dementia
Folate	• Folates function as a family of cofactors that carry one-carbon units required for the synthesis of thymidylate, purines, and methionine, and are required for other methylation reactions • Folate is essential for metabolic pathways involving cell growth and replication. Thirty to fifty percent of cellular folates are located in the mitochondria • Needed for the physical integrity of neurons • Folate (or folic acid) (with vitamins B6 and B12) regulates homocysteine metabolism and can have an effect on blood supply • Deficiency symptoms include depression, insomnia, forgetfulness and difficulty in concentrating, irritability, apathy, anxiety, fatigue, and anemia
Biotin	• Prosthetic group for four cellular carboxylases and plays a role mostly on metabolism of fatty acids and utilization of B vitamins

Continued

Table 22.1 Main Functions of Selected Vitamins and Their Fundamental Roles in Energy Metabolism/ Transformation and Mental and Physical Vitality (Huskisson et al., 2007a,b; Institute of Medicine, 1998)—cont'd

Vitamin	Functions and main roles in energy metabolism
Nicotinamide	• Deficiency symptoms comprise irritability, depressed mood, central nervous system abnormalities • Promotes release of energy from foods • Niacin is a precursor to reducing groups such as nicotinamide adenine di-nucleotide and nicotinamide adenine dinucleotide phosphate. These molecules are involved in more than 500 enzymatic reactions including mitochondrial respiration, glycolysis, or even lipid oxidation • Needed for synthesis and metabolism of tryptophan and hence for the neurotransmitter 5-hydroxytryptamine • Marginal deficiency causes irritability, weakness, fatigue, mental confusion, and dizziness • Frank deficiency results in pellagra, dementia
Pantothenic acid	• Involved in acyl transfer reactions playing an essential role in energy metabolism • Pantothenic acid is the precursor of coenzyme A, a molecule essential for 4% of all known enzymatic reactions • Deficiency causes irritability and restlessness, fatigue, apathy and malaise, neurobiological symptoms such as numbness, muscle cramps, and myelin degeneration
Vitamin C	• Needed for synthesis of carnitine, which transports long-chain fatty acids into mitochondria • Facilitates the transport and uptake of nonheme iron at the mucosa, the reduction of folic acid intermediates, and the synthesis of cortisol • Vitamin C is a potent antioxidant that serves to regenerate vitamin E from its oxidized byproduct • Needed for synthesis and metabolism of tyrosine and hence for dopamine. As a member of the catecholamine family, dopamine is a precursor to norepinephrine (noradrenaline) and then epinephrine (adrenaline) in the biosynthetic pathways for these neurotransmitters • Vitamin C depletion may adversely affect various aspects of physical capacity. These detrimental effects range from nonspecific responses such as fatigue and muscle weakness to anemia • Deficiency further causes irritability and depressed mood • Frank deficiency causes scurvy

3. GINSENG

3.1 Active Compounds in *P. ginseng*

P. ginseng roots contain about 2–3% of ginsenosides consisting of steroid glycosides and triterpene saponins found exclusively in the plant genus *Panax*. They are mostly derived from tetracyclic triterpene dammarane and are subdivided into (20S)-protopanaxadiol,

Table 22.2 Main Functions of Selected Minerals and Trace Elements and Their Fundamental Roles in Energy Metabolism/Transformation and Mental and Physical Vitality (Huskisson et al., 2007a,b)

Vitamin	Functions and main roles in energy metabolism
Calcium	• Participates in numerous physiological processes and enzyme systems and is especially important for growth, maintenance and repair of bone tissue, regulation of muscle contraction, nerve conduction, and normal blood clotting • A universal messenger of extracellular signals in a variety of cells: ionized calcium is the most common signal transduction element in cells. Intracellular calcium signaling is complex and tightly regulated and is triggered by external or internal stimuli such as a hormone or neurotransmitter binding to plasma membrane receptors • Regulates several neuronal functions, such as neurotransmitter synthesis and release, neuronal excitability, phosphorylation, etc. • Interacts in many of these processes in a complex way with magnesium and vitamin B6 • Activates a series of reactions including fatty acid oxidation, tricarboxylic acid cycle, and glucose-stimulated insulin release
Magnesium	• An intracellular mineral essential for the optimal function of a diversity of life-sustaining processes • Serves as a cofactor for more than 300 enzymatic reactions in which food is catabolized and new chemical products are formed; it is required for both aerobic and anaerobic energy production, for glycolysis as part of the Mg–ATP complex, and for synthesis of fatty acids and proteins • Serves as a regulator of many physiologic functions including neuromuscular, cardiovascular, immune, and hormonal functions, as well as the maintenance of cellular membrane stability • In the central nervous system, magnesium plays an important role in the control of excitatory amino acid neurotransmission • Neuromuscular hyperexcitability is the initial problem reported in individuals who have or are developing a magnesium deficiency
Chromium	• An essential trace element involved in the metabolism of carbohydrates, lipids, and proteins mainly by increasing the efficiency of insulin • Chromium deficiency affects the maintenance of normal glucose tolerance and healthy lipid profiles • These fundamental roles of chromium and the knowledge that the general public may consume diets low in chromium have prompted physically active individuals to consider chromium as a potentially limiting nutrient for promotion of physical fitness and health
Copper	• Required for the function of over 30 proteins, including superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome *c* oxidase, tyrosinase, and dopamine–β-hydroxylase • Metabolic fates of copper and iron are intimately related. Systemic copper deficiency generates cellular iron deficiency, which in humans results in diminished work capacity, reduced intellectual capacity, diminished growth, alterations in bone mineralization, and diminished immune response

Continued

Table 22.2 Main Functions of Selected Minerals and Trace Elements and Their Fundamental Roles in Energy Metabolism/Transformation and Mental and Physical Vitality (Huskisson et al., 2007a,b)—cont'd

Vitamin	Functions and main roles in energy metabolism
Iron	• A key trace element required for the delivery of oxygen to tissues and the use of oxygen at the cellular and subcellular levels • Serves as a functional component of iron-containing proteins including hemoglobin (oxygen transport in the blood), myoglobin (transporting and storing oxygen in the muscle and releasing it when needed during contraction), cytochromes (facilitating transfer of electrons in respiratory chain and is thus important in ATP synthesis), and specific iron-containing enzymes • Necessary for red blood cell formation and function • Iron plays a critical role in energy use during physical work • Iron deficiency results in reduced physical fitness, weakness, and fatigue
Manganese	• An essential trace element found in all tissues and is required for normal amino acid, protein, and carbohydrate metabolism (e.g., as cofactor of enzymes such as pyruvate carboxylase, glucokinase, and phosphoenol pyruvate carboxykinase) • Involved in the function of numerous organ systems • Needed for regulation of blood sugar and cellular energy, among other roles
Zinc	• An important trace element in the body involved as a catalyst in more than 300 enzymes • Natural constituent of many proteins, hormones, neuropeptides, and hormone receptors • Amongst others, zinc is directly involved in the synthesis of coenzymes derived from vitamin B6 • Plays a role in growth, building and repair of muscle tissue, energy production, and immune status • Zinc is abundant in the brain, having, after iron, the highest concentration among all transition metals • Component of metalloproteins in the brain but also sequestered in the presynaptic vesicles of a specialized type of neurons called 'zinc-containing' neurons. Synaptically released zinc functions as a conventional synaptic neurotransmitter or neuromodulator • Diets low in animal protein and high in fiber and vegetarian diets, in particular, are associated with decreased zinc intake • Zinc status has been shown to directly affect thyroid hormone levels, basal metabolic rate, and protein use, which in turn can negatively affect health and physical capacity • Neuropsychological impairment is one major health consequence of zinc deficiency

(20S)-protopanaxatriol, and oleanolic acid ginsenosides. More than 30 ginsenosides have been identified, being the ginsenosides Rg, Rc, Rd, Rb the quantitatively most important (see Figure 22.1). The ginsenosides are considered as main active ingredients of the ginseng root (Wichtl, 2004). Based on preclinical and animal studies, different
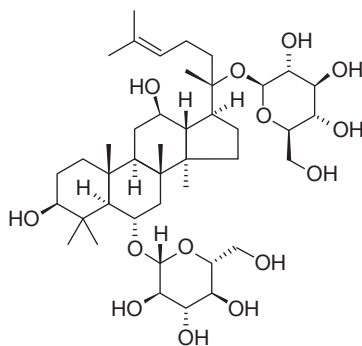
Figure 22.1 The ginsenoside Rg1 is the most abundant protopanaxatriol saponin in *Panax ginseng*.

physiological properties have also been assigned to individual ginsenosides. These compounds appear to affect multiple pathways, and their effects are complex and difficult to isolate.

The spectrum and amount of ginsenosides in ginseng can vary depending on species, location of growth, growing time before harvest, and extraction conditions. As the plant grows very slowly, the root is only harvested usually after 6 years (Hook, 1979).

Another interesting group of compounds found in ginseng are polyacetylenes, named as ginsenoynes A–K. Furthermore, *P. ginseng* contains glycans (panaxans), peptides, maltol, B vitamins, flavonoids, and volatile oil (Attele et al., 1999). Some supplement producers promote the presence of the trace element germanium in *P. ginseng*. However, its implications on health and well-being are not yet documented in the scientific literature.

3.2 The Quality of Ginseng Products

The quality of ginseng extracts, including the chemical composition and standardization, is critical for efficacy and safety of the resulting products. Commercial preparations of ginseng can vary tremendously in quality. As ginseng is a widely used and expensive product, it is liable to adulteration and falsification. Researchers and also consumer organizations analyzed a number of ginseng products and found out that the ginsenoside content can be low to negligible (Cui et al., 1994; Haller, 2005). It has also been reported that some products contain compounds that are not expected in ginseng including natural methylxanthines (Vaughan et al., 1999), pseudoephedrine (Bahrke and Morgan, 2000), phenylbutazone, and aminopyrine. Some products sold as ginseng have also contained *Mandragora officinarum* (containing hyoscine) and *Rauwolfia serpentine* (containing reserpine) (Chandler, 1988).

Also, the American ConsumerLab has found problems in many ginseng supplements over the years. In the latest review, six products failed to pass testing due to lead contamination, lack of ingredient, or inadequate labeling. One product had less than 10% of its claimed amount of ginsenosides despite its 'extra strength' label (Consumerlab, 2006).

A comprehensive ginseng evaluation program of hundreds of commercial ginseng products was initiated by the American Botanical Council (Haller, 2005; American Botanical Council, 2001).

Due to the adulteration problem, many experts recommend using standardized extracts of ginseng. One of the standardized extract of Asian ginseng, G115, contains an invariable 4% of ginsenosides (Di Geronimo et al., 2009) and has been studied extensively in clinical trials. Similar high-quality standardized extracts have also been marketed with even higher content of ginsenosides (e.g., 5%). The quality of the extracts (amount of ginsenosides and its specific pattern) depends on many parameters, including the age and part of the plant and the method of extraction. Many producers of high-quality ginseng own patents on their specific extraction process.

The quality and standardization of ginseng extracts are not only important to correctly inform both consumers and health care professionals but have of course an important impact on the outcome of scientific studies. Poor standardization of ginseng products can confound research involving ginseng as the different ginsenosides show significant different physiological properties (Kudo et al., 1998; Tachikawa et al., 1999). Only the application of standardized and well-characterized products allows replicable scientific research (Kennedy and Scholey, 2003).

3.3 Absorption, Distribution, Metabolism, and Excretion of Ginseng

Until now, there are mainly animal and *in vitro* studies related to the absorption, distribution, and excretion of ginseng saponins. It has been reported that ginsenoside Rb1 was absorbed rapidly from the upper digestive tract in rats. Peak serum and tissue concentrations were reached at 30 and 90 min, respectively. Interestingly, it was widely distributed throughout the body but was not detected in the rat brain (Toda et al., 1985). Another group confirmed the successful uptake of ginsenosides also in humans (Cui et al., 1996). Orally ingested ginsenosides pass through the stomach and small intestine without decomposition by either gastric juice or liver enzymes into the large intestine. The ginsenosides are then deglycosylated by colonic bacteria followed by transit to the circulation. The major metabolites are 20S-protopanaxadiol 20-*O*-β-D-glucopyranoside (M1) and 20S-protopanaxatriol (M4) produced by stepwise bacterial cleave of the oligosaccharide connected to the aglycone. These metabolites are further esterified with fatty acids. The resultant fatty acid conjugates are still active molecules that are sustained longer in the body than parental metabolites. It has been suggested that ginsenosides are in fact precursor of the active principle that is only built in the body by intestinal bacterial deglycosylation and fatty acid esterification (Hasegawa, 2004). Another group showed that the absorption of the final ginseng metabolites is independent of the metabolite transforming activity of intestinal microflora, but the T_{max}, C_{max}, and area under the curve of the transformed metabolites are dependent on the activity of each individual's microbial flora (Hong et al., 2002).

3.4 Traditional Indications of Ginseng

The shape of the root of Asian ginseng reminds us of a human body with stringy shoots for arms and legs, and our ancients related this look to its different kind of activities to the whole body. Used in China and Korea for thousands of years in traditional medicine, numerous scientific studies over the past 40 years evaluated the different aspects of this potent herbal ingredient. Both Oriental and Western medicines accept the invigorating property as the primary efficacy of ginseng. According to Chinese ancient medical writings, dried ginseng roots are commonly used as a tonic to energize the body (especially after physical exertion, during recovery from illness, or in old age) and restore/enhance the 'Qi' (the vital energy), which then mobilizes the blood (improvement of the blood flow). The invigorating property is usually understood to promote blood circulation and thus facilitate metabolism by warming up the body to subsequently maintain the homeostasis of human body (Bucci, 2000).

The most important indications of ginseng are obviously in the field of vitality and cognition (Kiefer and Pantuso, 2003), but also many other effects such as blood sugar lowering, antihypertensive, anticancer, sexual and immunological stimulation, memory improvement, and suppression of cough have been reported (Huang, 1999). There is also a considerable body of evidence to suggest that ginseng may have important roles in maintaining oxidative status (Kitts and Hu, 2000).

Modern guidelines acknowledge many of the traditional applications of *P. ginseng*. The German Commission E as a therapeutic guide to herbal medicine approved ginseng as a tonic for invigoration and fortification in times of fatigue and debility or declining capacity for work and concentration (EC, 1998). Ginseng was also approved for use during convalescence (EC, 2000). The World Health Organization (WHO) recommends ginseng as a prophylactic and restorative agent for enhancement of mental and physical capacities; in cases of weakness, exhaustion, tiredness, and loss of concentration; and during convalescence (WHO, 1999). Additionally, ginseng is described in various pharmacopeias, that is, in Germany, France, Austria, Switzerland, etc. Typical indications include usage as a tonic to reinforce and increase in case of tiredness and weakness, decreased concentration and effort capacity, and recovery.

3.5 Modern Clinical Research with *P. ginseng* with or without Micronutrients on Vitality and Cognition

Over the last decades, a number of clinical trials have been done with ginseng. The results have not been always unequivocal, and a possible explanation for this discrepancy may be a possible quality difference between the ginseng products applied. On the other hand, it is noteworthy that more than 60 positive clinical studies have been conducted with a ginseng extract containing at least 4% total ginsenosides. In general, the effects of ginseng supplementation on vitality and cognition have been assessed in multiple studies, and

positive results could consistently be observed when certain methodological aspects were followed in the trials.

3.5.1 Human studies with P. ginseng only

Vitality refers to the presence of energy, enthusiasm, and, in general, 'aliveness' and the absence of fatigue, weariness, and exhaustion. According to Chinese medicine, all this is directly linked with the activity of *P. ginseng*, and indeed modern methods were able to prove this ancient concept in human trials.

In an exploratory double-blind placebo study in 60 individuals, significant positive psychomotor effects after 12 weeks of 200 mg per day ginseng (G115) treatment have been reported (D'Angelo et al., 1986). Effects on physical performance tend to be more visible in mature adults as could be nicely observed for example in another study. One hundred twenty subjects (30–60 years) received for 12 weeks a daily ginseng supplementation of 200 mg or placebo. Significantly reduced reaction times were observed for subjects aged 40–60 years but not for younger participants. In addition, men in the older group did also benefit on pulmonary functions (vital capacity, forced expiratory volume, maximum expiratory flow, and breathing capacity), while those in the youngest group did not show significant effects. While the mechanisms of action remain unclear, the observed improvement in pulmonary functions can contribute to increased overall vitality and performance in the elderly (Forgo et al., 1981).

In a review paper dedicated to herbals and human performance, it was found that controlled studies of Asian ginsengs found improvements in exercise performance when most of the following conditions were true: use of standardized root extracts, study duration, and design (>8 weeks, daily dose >1 g dried root or equivalent, large number of subjects, and older subjects). Improvements in muscular strength, maximal oxygen uptake, work capacity, fuel homeostasis, serum lactate, heart rate, visual and auditory reaction times, alertness, and psychomotor skills have also been repeatedly documented (Bucci, 2000).

The acute administration of high-dosed ginseng showed also consistent effects on mood and four aspects of cognitive performance ('quality of memory,' 'speed of memory,' 'quality of attention,' and 'speed of attention') (Kennedy et al., 2001). Twenty healthy young adult volunteers received 200, 400, and 600 mg of G115 and a matching placebo, in counterbalanced order, with a 7-day wash-out period between treatments. Following a baseline cognitive assessment, further test sessions took place 1, 2.5, 4, and 6 h after the day's treatment. The most striking result was a significant improvement in 'quality of memory' and the associated 'secondary memory' factor at all time points following 400 mg of ginseng. Both the 200 and 600 mg doses were associated with a significant decrement of the 'speed of attention' factor at later testing times only. Subjective ratings of alertness were also reduced 6 h following the two lowest doses.

Data of another study measuring acute effects of ginseng in healthy young adults suggest that *P. ginseng* can improve cognitive performance and subjective feelings of mental fatigue during sustained mental activity (Reay et al., 2005).

Also, longer-term ginseng treatments resulted in improvement of some cognitive functions. The speed of performing a mental arithmetic task was increased in young healthy participants who received 200 mg of ginseng per day for 12 weeks (D'Angelo et al., 1986). In another clinical trial with 112 healthy volunteers (>40 years), a ginseng preparation or placebo was given for 8–9 weeks. The ginseng group showed a tendency to faster simple reactions and significantly better abstract thinking than the controls. However, there was no significant difference between the two groups in concentration, memory, or subjective experience (Sorensen and Sonne, 1996). In a recent pilot study (Kennedy et al., 2006), after 8 weeks treatment with 200 mg *P. ginseng* (G115), two working memory tasks and one of four scales from within the WHO 'quality of life' scale (social relations) were improved (Kennedy et al., 2006).

3.5.2 *Human studies with* P. ginseng *in combination with essential micronutrients*

An adequate micronutrient status may have an impact on the efficacy of ginseng. Several human studies have investigated the effects of supplementing ginseng extract in combination with vitamin preparations on effects of physical and mental performance.

In a double-blind, comparative study, it could be demonstrated that a combination of a multivitamin complex with ginseng is superior compared to a multivitamin complex alone. Six hundred twenty-five patients of both sexes received the treatment with the vitamin complex alone or with additional ginseng (40 mg) for 12 weeks. The resulting quality of life was assessed by a standardized 11-item questionnaire. It was shown that the ginseng combination was more effective than the multivitamin alone in improving the quality of life in a population subjected to the stress of high physical and mental activity (Caso Marasco et al., 1996).

In another study, the effects of a multivitamin/ginseng on vitality were assessed in a population with an otherwise poor diet. One hundred eighty subjects averaging 39 years of age were examined; each of them was pursuing an active career in middle management. The subjects were randomly divided into two main groups. These two groups were then split into two subgroups with the help of a questionnaire, which provided information about the subjects' eating habits. One subgroup had healthy nutritional habits, the other suffered from an unhealthy diet. Group 1 received the combination of special ingredients including a daily dose of 40 mg G115 ginseng extract and some vitamins and minerals; group 2 was merely administered a placebo. After 2 months, the results of the study showed that despite having an inadequate diet, the first group showed significant improvement in physical and mental performance. The placebo group, which likewise practiced an unhealthy diet, showed irritable reactions even in everyday stress situations.

The subjects possessed only a quarter of the inner composure shown in the first group. The conclusion was that the above-mentioned combination of special ingredients, including a ginseng extract (4% ginsenosides), increased vitality by severalfold, especially in cases of inadequate nutritional supply (Ussher et al., 1995).

A group of 232 patients aged between 25 and 60 years suffering from functional fatigue received daily a combination of a multivitamin with 80 mg of G115 ginseng extract in a placebo-controlled multicenter study setup. The daily fatigue level was assessed with a list of 20 statements before starting the treatment and after three resp. 6 weeks of treatment. Those participants taking the vitamin/ginseng combination were much improved at the end of the 6 weeks and felt that their overall levels of daily fatigue had dropped substantially in comparison with the group taking a placebo (Le Gal et al., 1996).

Another group of 50 healthy subjects (21–47 years) received the aforementioned treatment every day for 6 weeks. In this placebo-controlled trial, the participants had to perform an exercise test on a treadmill at increasing workloads. The total workload and maximal oxygen consumption during exercise were significantly greater after the ginseng preparation than after placebo. At the same workload, oxygen consumption, plasma lactate levels, ventilation, carbon dioxide production, and heart rate during exercise were significantly lower after the ginseng preparation than after placebo. The effects of ginseng were more pronounced in the subjects with maximal oxygen consumption below $60 \, \text{ml} \, \text{kg}^{-1} \, \text{min}^{-1}$ during exercise than in the subjects with levels of $60 \, \text{ml} \, \text{kg}^{-1} \, \text{min}^{-1}$ or above. The results indicate that the ginseng micronutrient preparation increased the subjects' work capacity by improving muscular oxygen utilization, thus helping to support the body's vitality (Pieralisi et al., 1991).

The vitality could also be improved in 450 healthy, employed men after treatment with the same vitamin/ginseng complex as used by Pieralisi et al. After 3 months, the vitality and attentiveness of the subjects taking the ginseng combination increased enormously. The subjects in this group felt less under time pressure, were more relaxed, and derived greater joy from life. The evaluation in this placebo-controlled trial was based on validated multiple-choice self-evaluation questionnaires (Wiklund et al., 1994).

A multivitamin/ginseng combination (40 mg per day) has also been tested on shift workers for 12 weeks (32 healthy, approx. 30-year-old male and female nurses). Before and after a three-night shift, the participants were submitted to intense examinations in order to check concentration, attention, and memory performance. The results of this placebo-controlled trial indicated that the ginseng/multivitamins combination was useful in counteracting self-reported increases in fatigue and decreases in calmness resulting from shift work. Furthermore, also the ability to store and retrieve information in memory tested with objective methods was improved (Wesnes et al., 2003).

3.6 Mode of Action

There are mainly two modes of action that could be used to explain the clinically observed benefits of ginseng especially in combination with micronutrients: the adaptogenic properties and the support of blood circulation.

3.6.1 Ginseng is an 'adaptogen'

In Chinese medicine, it is believed that the ability of ginseng to overcome fatigue is the result of having less stress on the body rather than by causing an overt stimulation. Due to this adaptogenic activity, the physical and mental performance is enhanced, vitality is promoted, and the resistance to stress and aging is increased. Ginseng allows the body to counter adverse physical, chemical, or biological stressors by raising nonspecific resistance toward such stress, thus allowing the organism to 'adapt' to the stressful circumstances. An adaptogen such as ginseng has a normalizing influence on physiology, irrespective of the direction of change from physiological norms caused by the stressor, or in other words it is a 'homeostatic metabolic regulator' (Winston and Maimes, 2007). Modern science explains the adaptogenic properties of ginseng with its effects on the hypothalamic–pituitary–adrenal axis (the hormonal stress system). Adaptogens have the ability to balance endocrine hormones and the immune system, supporting the body to maintain optimal homeostasis (Kiefer and Pantuso, 2003; WHO, 1999; Winston and Maimes, 2007).

In general, the effects of ginseng are particularly evident 'when the resistance of the organism is diminished or taxed with extra demands' (Brekhman and Dardymov, 1969). Especially for people over 50, these adaptogenic activities of ginseng support the body in counteracting against age-related changes in physiology (Figure 22.2).

3.6.2 Ginseng supports a healthy blood circulation

According to Chinese medicine, *P. ginseng* increases the blood flow and decreases fatigue. Modern research techniques revealed that ginseng extracts indeed produce vasodilatation in cerebral and coronary blood vessels, which improves brain and coronary blood flow (Huang, 1997).
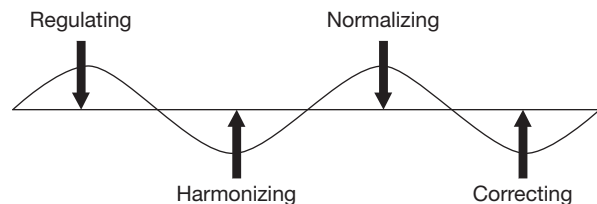
Figure 22.2 Adaptogens such as ginseng have a normalizing effect on the body and are capable of either toning down the activity of hyperfunctioning systems or strengthening the activity of hypofunctioning systems.

In a human pilot study, it was confirmed that the relative arterial oxygen levels in the blood were improved within 2 h of ingesting 200 mg of ginseng. After a period of 4 weeks, a 29% increase in the resting oxygen uptake of the organism and in the oxygen transport into the organs and tissues of the body was observed. The authors emphasized that this effect is likely to be higher in older people (von Ardenne and Klemm, 1987).

A number of further trials investigated the effects of ginseng extracts on physical performance. In this context, an increased oxygen uptake due to ginseng compared to placebo could be confirmed (Forgo, 1983; Forgo et al., 1981; Cherdrungsi and Rungroeng, 1995). When blood vessels in the muscles dilated, the blood flow is increased. The increased blood flow delivers more oxygenated blood to the working muscle. It was already postulated earlier that this oxygenation effect of ginseng was due to a vasodilatory effect at the venous end of the capillaries (von Ardenne and Klemm, 1987).

This was also confirmed in a study where the effect of ginseng on the vascular endothelial cell dysfunction in patients with hypertension was estimated after administration of 4.5 g per day of a pulverized ginseng preparation. The data revealed that ginseng could improve the vascular endothelial dysfunction in hypertensive patients. It was postulated that this effect is due to an increasing synthesis of nitric oxide (NO) (Sung et al., 2000). Only some years ago, it has been discovered that there is a link between vasodilation and NO. In 1998, the Nobel Prize was awarded to a group of researchers who found that NO is produced within the blood vessels of humans. This was a surprising discovery indicating that this small ubiquitous molecule played an important role in the maintenance of health. At low concentrations, NO can dilate the blood vessels by relaxing the smooth muscle in the walls of the arterioles thus improving the circulation (Achike and Kwan, 2003).

In another placebo-controlled trial, 200 mg per day of a ginseng extract containing 4% ginsenosides have been applied in combination with vitamins and minerals. It was shown that the total workload and maximal oxygen consumption during exercise were significantly increased in comparison to placebo. The authors concluded that the ginseng/vitamin combination improved the muscular oxygen utilization (Pieralisi et al., 1991).

Additional more research has been done in order to unequivocally prove that the vasodilatory effect and subsequent improved blood oxygenation of ginseng is caused by NO.

In a rat study, the hypotensive effect of *P. ginseng* was linked mainly to the saponin fraction and was explained by an increase in the NO production (Jeon et al., 2000a,b). Another experiment showed that the ginsenoside fraction also induces the relaxation of human bronchial smooth muscle via stimulation of NO generation (Tamaoki et al., 2000). An aqueous extract of *P. ginseng* further potentiated the relaxation induced by transmural electrical stimulation or nicotine in monkey cerebral arterial strips denuded of the endothelium and partially contracted with prostaglandin F(2 alpha). This enhancement of the neurogenic response was associated with increments in the synthesis or release of NO from the perivascular nerve (Toda et al., 2001). In one additional study, it was found that the levels of NO and NO synthase were significantly higher in the cells

treated with ginseng compared to those not treated (Friedl et al., 2001). Studies in laboratory animals have shown that *P. ginseng* enhances both libido and copulatory performance. These effects of ginseng may not be due to changes in hormone secretion but rather to direct effects of ginseng on the central nervous system and gonadal tissues. There is evidence that ginsenosides can facilitate penile erection by directly inducing the vasodilatation and relaxation of penile corpus cavernosum. The effects of ginseng on the corpus cavernosum appear to be mediated by the release and/or modification of release of NO from endothelial cells and perivascular nerves (Murphy and Lee, 2002). It was also shown that ginsenosides stimulate NO production in human aortic endothelial cells with PI3kinase/Akt and MEK/ERK pathways and androgen receptor involved in the regulation of acute eNOS activation by Rb1 (Yu et al., 2007).

In summary, the effect of *P. ginseng* on the NO production is obvious and has been confirmed in many other papers (Attele et al., 1999; Deng et al., 2010; Han et al., 2005; Kim et al., 2003, 2005; Leung et al., 2006; Lo et al., 2009; Xiang et al., 2008; Zadshir et al., 2005). In addition to the vasodilatory effect in vascular endothelial cells and arteries, ginsenosides are also credited with stimulation of NO production in immune system cells, erectile tissues, and the brain (Attele et al., 1999). All these findings show that *P. ginseng* improves the blood circulation as it was originally postulated by ancient Chinese medicine. In Figure 22.3, the effects of ginseng on the blood flow are summarized.

A possible mode of action by which *P. ginseng* promotes mental and physical well-being is linked to its ability to increase oxygen supply via its ability to promote blood circulation in the body and the brain. The ability of ginseng to promote oxygen supply and blood circulation implicates an effect on energy metabolism, since energy production in the form of ATP depends on oxygen supply.

3.7 Dosage and Safety of *P. ginseng*

The German Commission E monograph recommends a daily dosage of 1–2 g of Asian ginseng root, divided into three portions (EC, 2000). Traditional Chinese medicine prescribes 1–9 g of Asian ginseng. Study results suggest that a dosage between 40 and 200 mg standardized ginseng extract daily seems to be reasonable for most uses. In particular, the evidence presented here shows that *P. ginseng* extracts (standardized to 4% ginsenosides) administered at doses between 40 and 80 mg per day mainly in conjunction with vitamins, minerals, and trace elements support mental and physical vitality. Guidelines like the Ginseng Monograph by WHO do not mention any restriction with regard to a maximum intake period (WHO, 1999).

On the basis of ginseng's long use, and the relative infrequency of significant demonstrable side effects, it has been concluded that the use of ginseng is not associated with serious adverse effects if taken at the recommended dose (Kitts and Hu, 2000). Still, based on widespread use of ginseng supplements, this herbal ingredient was recently selected for investigation by the US National Cancer Institute. Under the conditions of these

Ginseng

⬇

Stimulates nitric oxide production

⬇

Relaxes the muscular wall of blood vessels

⬇

Widens diameter of the interior of blood vessels

⬇

Improves blood flow

⬇

Increases oxygen uptake/transport

⬇

Improves energy generation
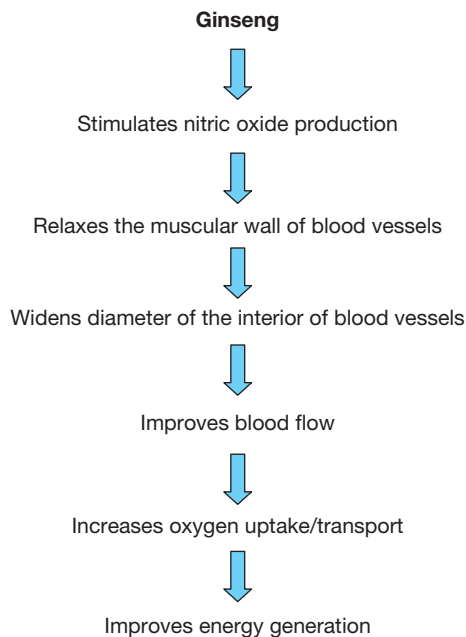
Figure 22.3 The effects of *Panax ginseng* on nitric oxide production and blood flow.

2-year gavage studies, there was no evidence of carcinogenic activity of ginseng in male or female F344/N rats or B6C3F1 mice administered 1250, 2500, or 5000 mg kg^{-1} (NIH, 2009).

Nevertheless, specific potential effects of ginseng on blood glucose and blood pressure especially for elderly have been proposed in other evaluations. Some literature indicates that *P. ginseng* may have a blood glucose lowering effect. This may be seen in general as a positive effect: especially for people over 50, blood sugar control is of high importance to prevent diabetes in the long term. However, it would potentially have practical implications on patients with already diagnosed diabetes. Single doses of 200 mg of *P. ginseng* lowered blood glucose levels in young healthy volunteers (Reay et al., 2005). Another trial showed that the same amount of an unspecified ginseng extract administered for 8 weeks reduced fasting blood glucose in newly diagnosed non-insulin-dependent diabetes dellitus (NIDDM) patients (Sotaniemi et al., 1995). In addition, high concentrations of *P. ginseng* (2214 mg per day) reduced insulin resistance and fasting plasma glucose levels in 20 diabetes type 2 patients (Ma et al., 2008). In contrast, high concentrations of Asian ginseng did not lower blood glucose in healthy subjects (Sievenpiper et al., 2003).

A potential relationship of ginseng with hypertension has been discussed in the literature. However, there is no controlled data confirming a causative relationship between ginseng and hypertension. The adverse event case reports were reported at high doses

and/or with use of multiple uncontrolled products (Hammond and Whitworth, 1981; Lukaski and Nielsen, 2002). Findings from small uncontrolled trials are difficult to interpret (Siegel, 1979, 1980), whereas findings from small controlled studies, even using higher doses, show no hypertensive effect. On the other hand, other data suggest blood pressure lowering effects through ginseng intake. In a study on patients with high blood pressure, the vasodilatory reserve with and without administration of ginseng was examined by measuring the forearm blood flow. The positive effect of ginseng on the blood flow could be confirmed again (Sung et al., 2000). Comprehensive review articles show no other indication of any major safety issues with ginseng use (Coon and Ernst, 2002; Kitts and Hu, 2000; NIH, 2009; Scaglione et al., 2005) in line with the conclusions from monographs (WHO, 1999).

4. CONCLUSIONS

The substantial increases in life expectancy achieved over the previous century, combined with medical advances, escalating health and social care costs, and higher expectations for older age, have led to international interest on how to promote a healthier old age and how to age 'successfully.' 'Successful aging' can be considered as a combination of at least three factors: the avoidance of disease and disability, continued active engagement in life, and the maintenance of high physical and cognitive capacity.

Current research indicates that nutrition plays a key role in health maintenance of older adults, that many elderly are at increased risk of inadequate nutrient intakes, and that several micronutrients are associated with decreased risk of diseases, infectious as well as chronic diseases, and frailty. Several experts agree that it is prudent for all adults, but especially for vulnerable groups such as the elderly population, to take micronutrient preparations to the daily regimen to help build a strong foundation for maintaining proper nutrition, good health, and vitality. Research further indicates that nutrition in general and micronutrient status in particular can influence both mental and physical capacity especially in older individuals. This is not surprising in view of the multiple roles of vitamins and minerals, which are essential for human life and support thousands of reactions and processes in the human body.

Next to micronutrients, also, natural extracts can support health and vitality during aging. *P. ginseng* C.A. Meyer is the most famous of all ginsengs and of all Asian medicinal plants in general. Used in China for thousands of years, numerous scientific studies have concentrated on the chemistry, pharmacology, and clinical aspects of ginseng use. The main active agents in *P. ginseng* are ginsenosides, which are triterpene saponins. *P. ginseng* is used primarily to improve psychological function and physical vitality, and such uses are endorsed by various pharmacopeias and monographs. Many of the clinical studies published in the scientific literature have been conducted on extracts of *P. ginseng* standardized to at least 4% total ginsenosides. Specifically, combinations of

essential micronutrients and *P. ginseng* at concentrations between 40 and 80 mg have consistently shown positive effects with regard to physical and mental functions.

Many mechanisms of action have been proposed for ginseng. However, there is consensus that it works through two mechanisms: as an adaptogen and by promoting blood circulation. In general, it was noted that the adaptogenic effects of ginseng were particularly evident 'when the resistance of the organism was diminished or was taxed with extra demands.' Especially for people over 50, these adaptogenic activities of ginseng support the body in counteracting against age-related changes in physiology.

In a number of studies, ginseng was indeed shown to increase resting oxygen uptake and oxygen transport and to promote blood circulation within the body including the brain. It has been postulated that these effects are due to a vasodilatory effect at the venous end of the capillaries mediated by NO production. The ability of ginseng to promote oxygen supply and blood circulation implicates an effect on energy metabolism, since energy production in the form of ATP depends on oxygen supply. Micronutrients as well play major roles in supporting energy metabolism and ATP production at various metabolic stages.

Overall, these data indicate that there is a good rationale for combining micronutrients and *P. ginseng* in supplements targeting individuals over 50 years of age with the aim of ameliorating their general health, mental and physical abilities, and overall vitality.

REFERENCES

Achike, F.I., Kwan, C.Y., 2003. Nitric oxide, human diseases and the herbal products that affect the nitric oxide signalling pathway. Clinical and Experimental Pharmacology and Physiology 30, 605–615.

American Botanical Council, 2001. Ginseng Evaluation Report. HerbalGram. American Botanical Council, Austin, TX 52.

Anonymous, 2006. Primary vitamin D deficiency in adults. Drug and Therapeutics Bulletin 44, 25–29.

Attele, A.S., Wu, J.A., Yuan, C.S., 1999. Ginseng pharmacology: multiple constituents and multiple actions. Biochemical Pharmacology 58, 1685–1693.

Bahrke, M.S., Morgan, W.R., 2000. Evaluation of the ergogenic properties of ginseng: an update. Sports Medicine 29, 113–133.

Baik, H.W., Russell, R.M., 1999. Vitamin B12 deficiency in the elderly. Annual Review of Nutrition 19, 357–377.

Bellisle, F., Blundell, J.E., Dye, L., et al., 1998. Functional food science and behaviour and psychological functions. The British Journal of Nutrition 80 (Suppl 1), S173–S193.

Black, M.M., 2003. Micronutrient deficiencies and cognitive functioning. The Journal of Nutrition 133, 3927S–3931S.

Bowling, A., Dieppe, P., 2005. What is successful ageing and who should define it? Britsh Medical Journal 331, 1548–1551.

Brekhman, Ii, Dardymov, I.V., 1969. New substances of plant origin which increase nonspecific resistance. Annual Review of Pharmacology 9, 419–430.

Brunekreef, B., Holgate, S.T., 2002. Air pollution and health. Lancet 360, 1233–1242.

Bryan, J., Osendarp, S., Hughes, D., Calvaresi, E., Baghurst, K., Van Klinken, J.W., 2004. Nutrients for cognitive development in school-aged children. Nutrition Reviews 62, 295–306.

Bucci, L.R., 2000. Selected herbals and human exercise performance. The American Journal of Clinical Nutrition 72, 624S–636S.

Campbell, A.K., Miller, J.W., Green, R., Haan, M.N., Allen, L.H., 2003. Plasma vitamin B-12 concentrations in an elderly latino population are predicted by serum gastrin concentrations and crystalline vitamin B-12 intake. The Journal of Nutrition 133, 2770–2776.

Caso Marasco, A., Vargas Ruiz, R., Salas Villagomez, A., Begona Infante, C., 1996. Double-blind study of a multivitamin complex supplemented with ginseng extract. Drugs under Experimental and Clinical Research 22, 323–329.

Chandler, R.F., 1988. Ginseng-aphrodisiac. Canadian Pharmaceutical Journal 121, 36–38.

Chandra, R.K., 1992. Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. Lancet 340, 1124–1127.

Chandra, R.K., 2002. Influence of multinutrient supplement on immune responses and infection-related illness in 50–65 year old individuals. Nutrition Research 22, 5–11.

Chavance, M., Herbeth, B., Fournier, C., Janot, C., Vernhes, G., 1989. Vitamin status, immunity and infections in an elderly population. European Journal of Clinical Nutrition 43, 827–835.

Cherdrungsi, P., Rungroeng, K., 1995. Effects of standardised ginseng extract and exercise training on aerobic and anaerobic exercise capacities in humans. Korean Journal of Ginseng Science 19, 93–100.

CONSUMERLAB, 2006. Product Review: Ginseng Supplements. CONSUMERLAB, Scarsdale, NY.

Coon, J.T., Ernst, E., 2002. Panax ginseng: a systematic review of adverse effects and drug interactions. Drug Safety 25, 323–344.

Cui, J., Garle, M., Eneroth, P., Bjorkhem, I., 1994. What do commercial ginseng preparations contain? Lancet 344, 134.

Cui, J.F., Garle, M., Bjorkhem, I., Eneroth, P., 1996. Determination of aglycones of ginsenosides in ginseng preparations sold in Sweden and in urine samples from Swedish athletes consuming ginseng. Scandinavian Journal of Clinical and Laboratory Investigation 56, 151–160.

D'angelo, L., Grimaldi, R., Caravaggi, M., et al., 1986. A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. Journal of Ethnopharmacology 16, 15–22.

Dawson-Hughes, B., Harris, S.S., Krall, E.A., Dallal, G.E., 1997. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. The New England Journal of Medicine 337, 670–676.

Deng, J., Wang, Y.W., Chen, W.M., Wu, Q., Huang, X.N., 2010. Role of nitric oxide in ginsenoside Rg (1)-induced protection against left ventricular hypertrophy produced by abdominal aorta coarctation in rats. Biological and Pharmaceutical Bulletin 33, 631–635.

Di Geronimo, G., Caccese, A.F., Caruso, L., Soldati, A., Passaretti, U., 2009. Treatment of carpal tunnel syndrome with alpha-lipoic acid. European Review for Medical and Pharmacological Sciences 13, 133–139.

EC, 1998. The Complete German Commission E Monographs, Therapeutic Guide to Herbal Medicines. Lippincott Williams & Wilkins, Philadelphia, USA.

EC, 2000. Expanded Commission E Monographs: Ginseng in Herbal Medicine: Expanded Commission E Monographs. The American Botanical Council, Austin, TX.

Fairfield, K.M., Fletcher, R.H., 2002. Vitamins for chronic disease prevention in adults: scientific review. JAMA: The Journal of the American Medical Association 287, 3116–3126.

Fletcher, R.H., Fairfield, K.M., 2002. Vitamins for chronic disease prevention in adults: clinical applications. JAMA: The Journal of the American Medical Association 287, 3127–3129.

Forgo, I., 1983. Effect of drugs on physical exertion and the hormonal system of athletes. 2. MMW, Münchener Medizinische Wochenschrift 125, 822–824.

Forgo, I., Kayasseh, L., Staub, J.J., 1981. Effect of a standardized ginseng extract on general well-being, reaction time, lung function and gonadal hormones. Die Medizinische Welt 32, 751–756.

Friedl, R., Moeslinger, T., Kopp, B., Spieckermann, P.G., 2001. Stimulation of nitric oxide synthesis by the aqueous extract of Panax ginseng root in Raw 264.7 cells. British Journal of Pharmacology 134, 1663–1670.

Gillette Guyonnet, S., Abellan Van Kan, G., Andrieu, S., et al., 2007. Iana task force on nutrition and cognitive decline with aging. The Journal of Nutrition, Health & Aging 11, 132–152.

Gonzalez-Gross, M., Marcos, A., Pietrzik, K., 2001. Nutrition and cognitive impairment in the elderly. The British Journal of Nutrition 86, 313–321.

Haller, J., 2005. Vitamins and brain function. In: Lieberman, H.R., Kanarek, R.B., Prasad, C. (Eds.), Nutritional Neuroscience. CRC Press, Boca Raton.

Hammond, T.G., Whitworth, J.A., 1981. Adverse reactions to ginseng. The Medical Journal of Australia 1, 492.

Han, K., Shin, I.C., Choi, K.J., Yun, Y.P., Hong, J.T., Oh, K.W., 2005. Korea red ginseng water extract increases nitric oxide concentrations in exhaled breath. Nitric Oxide 12, 159–162.

Hasegawa, H., 2004. Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. Journal of Pharmacological Sciences 95, 153–157.

Haskell, C.F., Robertson, B., Jones, E., et al., 2010. Effects of a multi-vitamin/mineral supplement on cognitive function and fatigue during extended multi-tasking. Human Psychopharmacology 25, 448–461.

Hennessy, E., 2002. Air pollution and short term mortality. British Medical Journal 324, 691–692.

Heseker, H., Kubler, W., Pudel, V., Westenhofer, J., 1995. Interaction of vitamins with mental performance. Bibliotheca Nutritio et Dieta 43–55.

High, K., 2001. Nutritional strategies to boost immunity and prevent infection in elderly individuals. Clinical Infectious Diseases (Aging and Infectious Diseases) 33, 1892–1900.

Holick, M.F., 2006. High prevalence of vitamin D inadequacy and implications for health. Mayo Clinic Proceedings 81, 353–373.

Holick, M.F., Matsuoka, L.Y., Wortsman, J., 1989. Age, vitamin D, and solar ultraviolet. Lancet 2, 1104–1105.

Hong, Y.C., Lee, J.T., Kim, H., Kwon, H.J., 2002. Air pollution: a new risk factor in ischemic stroke mortality. Stroke 33, 2165–2169.

Hook, I.L.I., 1979. Ginseng. Irish Pharmacy Journal 57, 421–430.

Huang, E.P., 1997. Metal ions and synaptic transmission: think zinc. Proceedings of the National Academy of Sciences of the United States of America 94, 13386–13387.

Huang, K., 1999. The Pharmacology of Chinese Herbs. CRC Press, Boca Raton.

Huskisson, E., Maggini, S., Ruf, M., 2007a. The influence of micronutrients on cognitive function and performance. The Journal of International Medical Research 35, 1–19.

Huskisson, E., Maggini, S., Ruf, M., 2007b. The role of vitamins and minerals in energy metabolism and well-being. The Journal of International Medical Research 35, 277–289.

Institute of Medicine (IOM), 1998. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. A Report of the Panel on Micronutrients, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. National Academy Press, Washington, DC.

Jeon, B.H., Kim, C.S., Kim, H.S., Park, J.B., Nam, K.Y., Chang, S.J., 2000a. Effect of Korean red ginseng on blood pressure and nitric oxide production. Acta Pharmacologica Sinica 21, 1095–1100.

Jeon, B.H., Kim, C.S., Park, K.S., et al., 2000b. Effect of Korea red ginseng on the blood pressure in conscious hypertensive rats. General Pharmacology 35, 135–141.

Kaiser, M.J., Bandinelli, S., Lunenfeld, B., 2009. The nutritional pattern of frailty – proceedings from the 5th Italian Congress of Endocrinology of Aging, Parma, Italy, 27–28 March 2009. The Aging Male 12, 87–94.

Kallus, K.W., Schmitt, J.A., Benton, D., 2005. Attention, psychomotor functions and age. European Journal of Nutrition 44, 465–484.

Kennedy, D.O., Scholey, A.B., 2003. Ginseng: potential for the enhancement of cognitive performance and mood. Pharmacology, Biochemistry, and Behavior 75, 687–700.

Kennedy, D.O., Scholey, A.B., Wesnes, K.A., 2001. Differential, dose dependent changes in cognitive performance following acute administration of a Ginkgo biloba/Panax ginseng combination to healthy young volunteers. Nutritional Neuroscience 4, 399–412.

Kennedy, D.O., Reay, J.L., Scholey, A.B., 2006. Effects of 8 weeks administration of Korean Panax ginseng extract on mood and cognitive performance in healthy individuals. In: Oh, S., Choi, T. (Eds.), Advances in Ginseng Research. Korean Society of Ginseng, Seoul, Korea.

Kennedy, D.O., Veasey, R., Watson, A., et al., 2010. Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males. Psychopharmacology 211, 55–68.

Kiefer, D., Pantuso, T., 2003. Panax ginseng. American Family Physician 68, 1539–1542.

Kim, N.D., Kim, E.M., Kang, K.W., Cho, M.K., Choi, S., Kim, S.G., 2003. Ginsenoside Rg3 inhibits phenylephrine-induced vascular contraction through induction of nitric oxide synthase. British Journal of Pharmacology 140, 661–670.

Kim, J.Y., Lee, H.J., Kim, J.S., Ryu, J.H., 2005. Induction of nitric oxide synthase by saponins of heat-processed ginseng. Bioscience, Biotechnology, and Biochemistry 69, 891–895.

Kirkwood, T., 1991. Genetic basis of limited cell proliferation. Mutation Research 256, 323–328.

Kitts, D., Hu, C., 2000. Efficacy and safety of ginseng. Public Health Nutrition 3, 473–485.

Kudo, K., Tachikawa, E., Kashimoto, T., Takahashi, E., 1998. Properties of ginseng saponin inhibition of catecholamine secretion in bovine adrenal chromaffin cells. European Journal of Pharmacology 341, 139–144.

Lau, K.H., Baylink, D.J., 1999. Vitamin D therapy of osteoporosis: plain vitamin D therapy versus active vitamin D analog (D-hormone) therapy. Calcified Tissue International 65, 295–306.

Le Gal, M., Cathebras, P., Strüby, K., 1996. Pharmaton capsules in the treatment of functional fatigue: a double-blind study versus placebo evaluated by a new methodology. Phytotherapy Research 10, 49–53.

Leung, K.W., Cheng, Y.K., Mak, N.K., Chan, K.K., Fan, T.P., Wong, R.N., 2006. Signaling pathway of ginsenoside-Rg1 leading to nitric oxide production in endothelial cells. FEBS Letters 580, 3211–3216.

Lips, P., 2001. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine Reviews 22, 477–501.

Lo, Y.T., Chang, Y.H., Lee, M.S., Wahlqvist, M.L., 2009. Health and nutrition economics: diet costs are associated with diet quality. Asia Pacific Journal of Clinical Nutrition 18, 598–604.

Lukaski, H.C., Nielsen, F.H., 2002. Dietary magnesium depletion affects metabolic responses during sub-maximal exercise in postmenopausal women. The Journal of Nutrition 132, 930–935.

Ma, S.W., Benzie, I.F., Chu, T.T., Fok, B.S., Tomlinson, B., Critchley, L.A., 2008. Effect of Panax ginseng supplementation on biomarkers of glucose tolerance, antioxidant status and oxidative stress in type 2 diabetic subjects: results of a placebo-controlled human intervention trial. Diabetes, Obesity & Metabolism 10, 1125–1127.

Maggini, S., 2010. Vitamins and Minerals: Contribution to Immune Function and Health. Springer Science+Business Media LLC, Philadelphia, PA.

Maggini, S., Beveridge, S., Sorbara, P., Senatore, G., 2008. Feeding the immune system: the role of micro-nutrients in restoring resistance to infections. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition, and Natural Resources 3, 1–21.

Malouf, R., Grimley Evans, J., 2008. Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people. Cochrane Database of Systematic Reviews Cd004514.

Manger, M.S., Winichagoon, P., Pongcharoen, T., 2004. Multiple micronutrients may lead to improved cognitive function in Ne Thai schoolchildren. Asia Pacific Journal of Clinical Nutrition 13, 46.

Morley, J.E., 2002. Nutrition in the elderly. Current Opinion in Gastroenterology 18, 240–245.

Mosekilde, L., 2005. Vitamin D and the elderly. Clinical Endocrinology 62, 265–281.

Murphy, L.L., Lee, T.J., 2002. Ginseng, sex behavior, and nitric oxide. Annals of the New York Academy of Sciences 962, 372–377.

NIH (2009) Toxicology and carcinogenesis studies of Ginseng (Cas No. 50647-08-0). In: F344/N Rats And B6C3F1 Mice (Gavage Studies) (Draft Report). National Toxicology Program. National Institutes of Health, Bethesda, MD.

Ocollura, J., 1997. Ginseng tonic of life. Vegetarian Times 235, 94.

Pieralisi, G., Ripari, P., Vecchiet, L., 1991. Effects of a standardized ginseng extract combined with dimethy-laminoethanol bitartrate, vitamins, minerals, and trace elements on physical performance during exercise. Clinical Therapeutics 13, 373–382.

Reay, J.L., Kennedy, D.O., Scholey, A.B., 2005. Single doses of Panax ginseng (G115) reduce blood glucose levels and improve cognitive performance during sustained mental activity. Journal of Psychopharmacology 19, 357–365.

Richard, M.J., Roussel, A.M., 1999. Micronutrients and ageing: intakes and requirements. The Proceedings of the Nutrition Society 58, 573–578.

Roe, D.A., 1989. Drug-nutrient interactions in the elderly. In: Munro, H.N., Danford, D.E. (Eds.), Nutrition, Aging, and the Elderly. Plenum Press, New York, London.

Roe, D., 1992. Effects of drugs on vitamin needs. Annals of the New York Academy of Sciences 669, 156–164.

Roe, D., 1993. Drug nutrient interactions. In: Garrow, J., James, W., Ralph, A. (Eds.), Human Nutrition and Dietetics. Churchill Livingstone, Edinburgh.

Rosenberg, I.H., Miller, J.W., 1992. Nutritional factors in physical and cognitive functions of elderly people. The American Journal of Clinical Nutrition 55, 1237S–1243S.

Rosenthal, M.J., Goodwin, J.S., 1985. Cognitive effects of nutritional deficiency. Advances in Nutritional Research 7, 71–100.

Russell, R., 1992. Micronutrient requirements of the elderly. Nutrition Reviews 50, 463–466.

Russell, R.M., 2001. Factors in aging that effect the bioavailability of nutrients. The Journal of Nutrition 131, 1359S–1361S.

Scaglione, F., Pannacci, M., Petrini, O., 2005. The standardised G115® Panax ginseng C.A. Meyer extract: a review of its properties and usage. Evidence Based Integrative Medicine 2, 195–206.

Siegel, R.K., 1979. Ginseng abuse syndrome. Problems with the panacea. JAMA: The Journal of the American Medical Association 241, 1614–1615.

Siegel, F.L., 1980. Calcium-binding proteins: structure and function: proceedings of an International Symposium on Calcium-Binding Proteins and Calcium Function in Health and Disease. Elsevier North-Holland, New York, NY Wisconsin Center, Madison, WI, USA, 8–12 June 1980.

Sievenpiper, J.L., Arnason, J.T., Leiter, L.A., Vuksan, V., 2003. Null and opposing effects of Asian ginseng (Panax ginseng C.A. Meyer) on acute glycemia: results of two acute dose escalation studies. Journal of the American College of Nutrition 22, 524–532.

Sorensen, H., Sonne, J., 1996. A double-masked study of the effects of ginseng on cognitive functions. Current Therapeutic Research, Clinical and Experimental 57, 959–968.

Sotaniemi, E.A., Haapakoski, E., Rautio, A., 1995. Ginseng therapy in non-insulin-dependent diabetic patients. Diabetes Care 18, 1373–1375.

Sung, J., Han, K.H., Zo, J.H., Park, H.J., Kim, C.H., Oh, B.H., 2000. Effects of red ginseng upon vascular endothelial function in patients with essential hypertension. The American Journal of Chinese Medicine 28, 205–216.

Tachikawa, E., Kudo, K., Harada, K., et al., 1999. Effects of ginseng saponins on responses induced by various receptor stimuli. European Journal of Pharmacology 369, 23–32.

Tamaoki, J., Nakata, J., Kawatani, K., Tagaya, E., Nagai, A., 2000. Ginsenoside-induced relaxation of human bronchial smooth muscle via release of nitric oxide. British Journal of Pharmacology 130, 1859–1864.

Toda, S., Miyase, T., Arichi, H., Tanizawa, H., Takino, Y., 1985. Natural antioxidants. III. Antioxidative components isolated from rhizome of Curcuma longa L. Chemical & Pharmaceutical Bulletin (Tokyo) 33, 1725–1728.

Toda, N., Ayajiki, K., Fujioka, H., Okamura, T., 2001. Ginsenoside potentiates NO-mediated neurogenic vasodilatation of monkey cerebral arteries. Journal of Ethnopharmacology 76, 109–113.

Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M.L., et al., 1995. Diet and overall survival in elderly people. British Medical Journal 311, 1457–1460.

Tucker, K., 1995. Micronutrient status and aging. Nutrition Reviews 53, S9–S15.

Ussher, J.M., Dewberry, C., Malson, H., Noakes, J., 1995. The relationship between health related quality of life and dietary supplementation in British middle managers: a double blind placebo controlled study. Psychology and Health 10, 97–111.

Vaughan, M.A., Doolittle, R.L., Gennett, T., 1999. Physiological effects of ginseng may be due to methylxanthines. Medicine and Science in Sports and Exercise 31, 121.

Vogler, B.K., Pittler, M.H., Ernst, E., 1999. The efficacy of ginseng. A systematic review of randomised clinical trials. European Journal of Clinical Pharmacology 55, 567–575.

Vollset, S.E., Ueland, P.M., 2005. B vitamins and cognitive function: do we need more and larger trials? The American Journal of Clinical Nutrition 81, 951–952.

Von Ardenne, M., Klemm, W., 1987. Measurements of the increase in the difference between the arterial and venous Hb–O2 saturation obtained with daily administration of 200 mg standardized ginseng extract G115 for four weeks. Long-term increase of the O2 transport into the organs and tissues of the organism through biologically active substances. Panminerva Medica 29, 143–150.

Wakimoto, P., Block, G., 2001. Dietary intake, dietary patterns, and changes with age: an epidemiological perspective. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 56A, 65–80.

Wesnes, K.A., Pincock, C., Richardson, D., Helm, G., Hails, S., 2003. Breakfast reduces declines in attention and memory over the morning in schoolchildren. Appetite 41, 329–331.

WHO, 1999. Radix ginseng. WHO Monographs on Selected Medicinal Plants. WHO, Geneva.

Wichtl, M., 2004. Herbal Drugs and Phytopharmaceuticals. A Handbook for Practice on a Scientific Basis. Medpharm Verlag, Stuttgart.

Wiklund, I., Karlberg, J., Lund, B., 1994. A double-blind comparison of the effect on quality of life of a combination of vital substances including standardized ginseng and placebo. Current Therapeutic Research 55, 32–42.

Winston, D., Maimes, S., 2007. Adaptogens: Herbs for Strength, Stamina, and Stress Relief. Healing Arts Press, Rochester, VT.

Wolters, M., Hermann, S., Hahn, A., 2003. B vitamin status and concentrations of homocysteine and methylmalonic acid in elderly German women. The American Journal of Clinical Nutrition 78, 765–772.

Xiang, Y.Z., Shang, H.C., Gao, X.M., Zhang, B.L., 2008. A comparison of the ancient use of ginseng in traditional Chinese medicine with modern pharmacological experiments and clinical trials. Phytotherapy Research 22, 851–858.

Yu, J., Eto, M., Akishita, M., Kaneko, A., Ouchi, Y., Okabe, T., 2007. Signaling pathway of nitric oxide production induced by ginsenoside Rb1 in human aortic endothelial cells: a possible involvement of androgen receptor. Biochemical and Biophysical Research Communications 353, 764–769.

Yun, T.K., 2001. Brief introduction of *Panax ginseng* CA Meyer. Journal of Korean Medical Science 16 (Suppl), S3–S5.

Zadshir, A., Tareen, N., Pan, D., Norris, K., Martins, D., 2005. The prevalence of hypovitaminosis D among US adults: data from the Nhanes III. Ethnicity & Disease 15, S5-97-101.

Zimmermann, M.B., Connolly, K., Bozo, M., Bridson, J., Rohner, F., Grimci, L., 2006. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study. The American Journal of Clinical Nutrition 83, 108–114.

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Asian Medicinal Remedies for Alleviating Aging Effects

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1. INTRODUCTION

Aging or senescence, fundamentally a complex process, following the reproductive phase of life, is an inevitable fact of life. Aging is characterized by a progressive decline in the efficiency of physiological processes that ensues and is manifested within an organism at genetic, molecular, cellular, biochemical, hormonal, organ, physiological, and system levels, which cumulatively results in the decrease of physical function, reduction in the fecundity, loss of vitality, etc. (Halliwell and Gutteridge, 2007). Although the basic mechanisms responsible for aging are still poorly understood, various hypotheses have been proposed. A growing body of evidence suggests that the free radical theory is the most accepted and scientific studies are emphatically substantiating this (Halliwell and Gutteridge, 2007).

Aging increases vulnerability to cancer and various metabolic and degenerative diseases. Aging impairs all the major organs and some of the most important and exclusive diseases include Parkinson's disease, Alzheimer's disease, dementia, cognitive impairment, athero-sclerosis, hearing impairment, loss of vision, cataract formation, reduced lung capacity, impairment of kidney function, loss of elasticity and wrinkling of skin, reduced immunity, and vulnerability for cancer. Additionally, exposure to xenobiotic chemicals, pollutants, carcinogens, infections, radiations, and faulty lifestyle exacerbate the aging process and increase the incidence of age-related diseases (Halliwell and Gutteridge, 2007).

2. ANTIAGING CHEMICAL COMPOUNDS

Preventing/retarding of the aging process has been a long sought goal and agents that can prevent aging, especially by improving memory, vision, and virility, and by preventing wrinkling of skin and graying of hair, are in great demand. However, the use of these agents mostly available over the counter is scientifically not validated and is based on

anecdotal evidences. Nonetheless, the market of such products is on the rise in both developed and developing countries. However, several of the antiaging remedies, especially those used in skin care, are reported to possess deleterious effects that with time may negate the beneficial effects. In this context, there is a need for safe products that are effective and devoid of any side effects.

3. PLANTS USED AS ANTIAGING COMPOUNDS

Since antiquity, plants have been an integral part of Ayurveda, Chinese, Unani, Siddha, Arabic, Sri Lankan, and Tibetan systems of traditional medicine and recently are being investigated for their effectiveness as antiaging agents according to protocols and norms suggested in the modern system of medicine. The main reasons for the increased interest in medicinal plants include their cost-effectiveness, easy availability, and safety. Scientific studies carried out in the recent past have shown that some of the plants like *Rhodiola rosea* (golden root), *Lycium barbarum* (lycii berry), *Vaccinium myrtillus* (bilberry), *Ginkgo biloba* (ginkgo), Ginseng, *Silybum marianum* (milk thistle), *Curcuma longa* (turmeric), *Gynostemma pentaphyllum* (jiaogulan), *Withania somnifera* (ashwagandha), *Vitis vinifera* (grapes), and *Centella asiatica* (gotu kola) are useful in preventing aging. In the ensuing section, the scientifically validated observations and mechanisms responsible for the prevention/amelioration of aging by these plants are addressed.

3.1 *Curcuma longa* (Turmeric)

The perennial herb *Curcuma longa* L., whose rhizome is commonly referred to as turmeric, is an important spice for Asians, especially Indians, and is one of the primary ingredients that has made the Indian curry so famous a recipe in the West. Curcuma is an important medicinal plant and, for over 4000 years, has been used in various folk and traditional Asian and African systems of medicine to treat a wide variety of ailments. The rhizome and its active principle, a group of curcuminoids, are widely used as culinary spices, preservatives, food additives, cosmetics, and as oleoresin in food and pharmaceutical industries. In the last two decades, there has been considerable interest among the biomedical scientists to explore the possible therapeutic benefits of turmeric and its active principle curcuminoids, and innumerable studies have validated the ethnomedicinal uses.

Turmeric has been used as an oral and topical agent to treat a wide variety of ailments, including infective wounds, amenorrhea, liver disease, common colds, pulmonary dysfunction, atherosclerosis, cancer, neurodegenerative diseases, pancreatitis, and rheumatoid arthritis (Aggarwal and Harikumar, 2009; Miquel et al., 2002; Salvioli et al., 2007). Preclinical studies have shown that turmeric enhances learning ability and spatial memory via the modulation of central serotoninergic system activity, and increases tolerance to stress conditions (Pyrzanowska et al., 2010). Animal studies have also shown that the application of turmeric prevents aging of the skin and also ultraviolet B

(UVB)–induced skin aging by inhibition of increase in matrix metalloproteinase-2 expression (Sumiyoshi and Kimura, 2009).

Polyphenols of turmeric are shown to induce heme oxygenase 1 and Phase II detoxification enzymes in neurons. This renders protective effects and protects the neurons against oxidative challenges and stress that may contribute to aging of brain and bring about neurodegenerative changes (Scapagnini et al., 2010). Turmeric extract is also shown to reduce glutamate levels in the hippocampus of aged rats, and this action has proposed to be responsible for the reduction of glutamate-mediated excitotoxicity, in an experimental model of neurodegenerative disease (Pyrzanowska et al., 2010). Studies have also shown that curcumin possesses antiamyloidogenic effects (Shytle et al., 2009) and improves cognitive task and locomotory activities. Administering curcumin has been shown to increase the activities of oxidative defense system and to restore the activity of mitochondrial electron transport chain in the brain cells of mice treated with D-galactose (Kumar et al., 2011). Curcumin treatment is also shown to extend the life span and modulate the expression of age-associated aging genes in *Drosophila melanogaster* (Lee et al., 2010).

3.2 Green Tea (*Camellia sinensis*)

Green tea is an antiaging herb of repute and is used by the Asian population for centuries. The aqueous soluble polysaccharides (Quan et al., 2011) and polyphenols are scientifically shown to be responsible for the antioxidant action of green tea (Khan and Mukhtar, 2007; Povichit et al., 2010). Green tea is rich in polyphenols like epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, theanine, and caffeine. These polyphenols are potent antioxidants and are far more effective than vitamin C and vitamin E (Khan and Mukhtar, 2007). In vitro assays have shown that green tea possesses free radical scavenging effects (Povichit et al., 2010), and has the ability to inhibit protein glycation (Povichit et al., 2010) and also ameliorate the metabolic syndrome (Basu et al., 2010). Additionally, the antioxidant effect of green tea was also observed in animal models of study confirming the fact that the in vitro observations translate into the animal systems (Wojciech et al., 2010).

Green tea possesses antioxidant rejuvenating potency and its role in the amelioration of senescence-mediated redox imbalance in aged rat cardiac tissue has been established (Kumaran et al., 2009). Polyphenols of green tea possess iron-chelating, neurorescue/neuroregenerative, and mitochondrial stabilization actions, and the ability to prevent deposition of amyloid proteins. It is of immense value in preventing dementia, Parkinson's disease, and Alzheimer's disease (Mandel et al., 2008). Administering green tea extract has also been shown to be effective in enhancing learning and memory in aged rats and may be useful in reversing age-related neural deficits (Kaur et al., 2008).

Prolonged consumption of green tea is also shown to protect proteins and lipids against oxidation and to reduce lipofuscin deposition in the rat hippocampal formation

as well as improving spatial memory during aging (Assuncao et al., 2010). Long-term green tea ingestion improved antioxidant systems and activated the transcription factor cyclic adenosine monophosphate (cyclic AMP) response element-binding in the aging rat's hippocampal formation, leading to neuroprotection mediated by upregulation of brain-derived neurotrophic factor (BDNF) and B-cell lymphoma-2 (Bcl-2) (Assuncao et al., 2010). Studies in animal models of carcinogenesis have shown that green tea and epigallocatechin gallate (EGCG) can inhibit tumorigenesis during the initiation, promotion, and progression stages (Lambert and Elias, 2010).

Green tea–catechin intake is reported to prevent experimental tumor metastasis in senescence-accelerated mice model via inhibition of a reduction in immune surveillance potential with age, as indicated by prevention of decrease in natural killer T cell activity (Shimizu et al., 2010). Green tea possesses antiaging effects and is known to reduce wrinkles and improve skin moisturization (Chuarienthong et al., 2010). The protective effect of green tea against skin aging is mainly attributed to the catechins (Hsu, 2005), and the polyphenols are reported to possess chemopreventive, natural healing, and antiaging effects on human skin (Hsu, 2005). Green tea-containing sunscreens also possess protective effects against ultraviolet radiation (UVR)-induced photoaging and immunosuppression (Li et al., 2009).

3.3 *Rhodiola rosea* (Golden Root)

Rhodiola rosea, also known as 'golden root,' is a member of the family Crassulaceae. Its yellow flowers smell similar to roses and, therefore, the species name is attributed as rosea. The roots of the plants are the most sought, and have been used in the traditional system of medicine in Europe and Asia most importantly as an antistress and adoptogenic agent. It is also referred to as Cosmonauts' plant, as it was carried by Russian cosmonauts in space to protect themselves against the deleterious effects of ionizing radiation in space. Studies indicate that the *R. rosea* extract possesses protective properties against free radical-mediated oxidative damage, adoptogenic effects, and potential to increase the life span of organisms (Jafari et al., 2007; Wiegant et al., 2009). Salidroside, the principal phytochemical of *R. rosea*, has also been shown to possess antioxidant effects (Guan et al., 2011; Zhang et al., 2010) to prevent apoptosis and premature senescence in cultured rat neural cells (Chen et al., 2009; Zhang et al., 2007).

3.4 *Vaccinium myrtillus* (Bilberry)

Bilberry contains several types of species and belongs to the genus Vaccinium of the family Ericaceae. *Vaccinium myrtillus* L is the most recognized and well-studied species, but there are several other closely related species and morphotypes. The plants bear edible berries that are high in nutritive value and are obviously of dietary use. The plants are of medicinal use in treating several diseases like diabetes, several ocular diseases, and

vascular disorders. The extracts of both leaf and fruits, and the phytochemicals have been shown to scavenge free radicals in vitro (Faria et al., 2005; Piljac et al., 2009; Rahman et al., 2006). The flavanoids present in the extract are also reported to possess antioxidant properties and are a major skin-rejuvenating supplement (Kahkonen, 2001).

Preclinical studies with cultured human retinal pigment epithelial (RPE) cells have shown that the anthocyanins and other phenolics present in bilberry upregulate the oxidative stress defense enzymes heme-oxygenase (HO)-1 and glutathione S-transferase–pi (GST-pi) (Milbury et al., 2007). The leaf extracts also enhanced glutamate decarboxylase gene expression in dermal fibroblasts, resulting in the stimulation of cell growth, hyaluronic acid, and glutathione synthesis. In addition, the extract also showed inhibitory activity on collagenase and elastase, enzymes responsible for the ragging and wrinkled nature of skin; it also decreased the melanin content in B16 melanoma cells and suppressed release of histamine from mast cells. Together, all these observations clearly indicate that the leaf extracts are a promising natural ingredient against aging of skin (Kenichi, 2006).

Animal studies have also shown that bilberry reduces oxidative stress and protects brain cells against oxidative damage (Sinitsyna et al., 2006; Yao and Vieira, 2007) and protects animals from senescence-induced macular degeneration and cataracts (Fursova et al., 2005). It has also been shown to ameliorate cardiotoxicity from ischemia-reperfusion injury in animals (Ziberna et al., 2010), and modulate the expression of inflammatory molecules in humans evaluated and observed to be at a higher risk for cardiovascular death (Karlsen et al., 2010).

3.5 *Ginkgo biloba* (Ginkgo)

The *Ginkgo biloba* tree is arguably one of the oldest known trees on earth, with fossil records dating back to more than 200 million years. It is a highly unusual nonflowering plant and is regarded as a 'living fossil.' The leaves are an integral component of Chinese traditional medicine for treating various ailments. Ginkgo has been reported to influence fundamental aspects of human physiology by improving blood flow to tissues including the brain, and by enhancing cellular metabolism. The plant possesses flavonoids (kaempferol, quercetin, and isorhamnetin), coumaric acid, diterpenes (called ginkgolides A, B, C, and M), sesquiterpenes (bilobalide), and the organic acids vanillic, protocatechic, and hydroxykinurenic. These phytochemicals are responsible for the various pharmacological effects that include antioxidant, anti-inflammatory, and circulation-stimulant properties.

Preclinical studies have demonstrated that Ginkgo possesses protective effects against age-related neurodegenerative changes and diseases (DeFeudis and Drieu, 2000). Experiments have also shown that the lactone of ginkgo attenuates lipid peroxidation and apoptosis of cerebral cells in aging mice (Dong et al., 2004). It is also reported to prevent age-related caspase-mediated apoptosis in rat cochlea, thereby indicating its usefulness in

preventing the age-related decrease in auditory functions (Nevado et al., 2010). Administration of Ginkgo extract prevented oxidative stress, mitochondrial DNA damage, and mitochondrial structural changes in brain and liver cells of aging rats (Sastre et al., 1998). Administering Ginkgo extract to aged mice has also been shown to restore the mitochondrial function as indicated by amelioration of decreased mitochondrial levels of cytochrome c oxidase, adenosine triphosphate (ATP), and glutathione in platelets and hippocampus (Shi et al., 2010).

A multicenter, double-blind, drug versus placebo trial of patients with cerebral disorders has also shown that ginkgo extract is effective against cerebral disorders resulting due to aging; the difference between control and treatment groups became significant at 3 months and increased during the following months (Taillandier et al., 1986). Myriad studies have shown the beneficial effects of ginkgo in neurological disorders with dementia (Leuner et al., 2007). A clinical study demonstrated that an antiwrinkle cosmetic preparation containing ginkgo increased skin moisturization and smoothness, and reduced roughness and wrinkles (Chuarienthong et al., 2010). Ginkgo is known to ameliorate oxidative stress and to prevent age-related eye diseases (Rhone and Basu, 2008). Ginkgo is also reported to improve distal left anterior descending coronary artery blood flow and endothelium-dependent brachial artery flow-mediated dilation in healthy elderly adults, thereby imparting its cardioprotective effects at least in part (Wu et al., 2008).

3.6 *Panax ginseng* (Ginseng)

Globally, among all medicinal plants, Ginseng is probably the most famous and extensively investigated plant. The generic name Panax is derived from the Greek word *panakos* meaning a panacea, a virtue ascribed to it by the Chinese, who consider it a sovereign remedy in almost all diseases for more than 2000 years. Ginseng is a slow-growing perennial plant and the fleshy root bears resemblance to the human body. Due to this morphological feature, it is also known as 'man-root.' There are 11 species of ginseng, but the most important and well-studied species are the *Panax ginseng* (Asian, Korean, or Chinese ginseng) and *Panax quinquefolius* (also called American, Canadian, or North American ginseng). The ginsenosides are reported to be responsible for myriad benefits.

In the traditional Chinese system of medicine, ginseng is referred to as the ultimate tonic that benefits the whole body. Ginseng has been used to improve the body's resistance to stress and to increase vitality, general well-being, immune function, libido, and athletic performance. Preclinical studies suggest that it possesses adaptogenic, immunomodulatory, anti-inflammatory, antineurological, hypoglycemic, antineoplastic, cardiovascular, central nervous system (CNS), endocrine, and ergogenic effects (Jia et al., 2009). Furthermore, it improves memory, learning performance, and motor activity (Jia et al., 2009).

Preclinical studies have shown ginseng to possess antioxidant (Kim et al., 2010; Liu et al., 2011; Ye et al., 2011), anti-inflammatory (Kim et al., 2010; Liu et al., 2011;

Saw et al., 2010), anti-apoptotic, antihyperglycemic, and cardioprotective effects (Jia et al., 2009). Ginkgo is shown to provide protection against neurodegeneration by multiple mechanisms. In different experimental models of Alzheimer's disease, ginseng is shown to attenuate β-amyloid and glutamate-induced toxicity, enhance clearance of β-amyloid by stimulating the phagocytic activity of microglia, promoting neuron survival, and increasing the levels of neurotrophic factor (Jia et al., 2009; Luo et al., 2011; Wollen, 2010; Xie et al., 2010). Randomized, double-blind, placebo-controlled trials have shown marginal benefits of ginseng on cognitive functions in a healthy population and patients with dementia; however, there is lack of convincing high-quality clinical evidence to show a cognitive enhancing effect of ginseng (Geng et al., 2010).

3.7 *Silybum marianum* (Milk Thistle)

Silybum marianum, also called milk thistle, is an annual or biannual plant belonging to the Asteraceae family. Milk thistle has red to purple flowers and shiny pale green leaves with white veins. Originally a native of Southern Europe through to Asia, it is now cultivated throughout the world for its medicinal and pharmaceutical value. The medicinal parts of the plant are the ripe seeds. Silymarin, an extract from this plant is a mixture of flavonolignans such as silybin, isosilybin, silydianin, and silychristin. Silymarin is a potent antioxidant, anti-inflammatory compound (Aghazadeh et al., 2010; Asghar and Masood, 2008; Wang et al., 2010) with strong hepatoprotective activity (Aghazadeh et al., 2010; Song et al., 2006; Valenzuela et al., 1989). Silymarin is also reported to mitigate oxidative stress in aging rat brain by reducing lipid peroxidation and attenuating antioxidants (Galhardi et al., 2009; Nencini et al., 2007). Preclinical studies have shown that silymarin attenuated the amyloid β-plaque burden and behavioral abnormalities in an Alzheimer's disease mouse model (Murata et al., 2010). Silymarin has also been observed to prevent skin aging, and is included as an ingredient of some cosmeceutical preparations (Singh and Agarwal, 2009).

3.8 *Lycium barbarum* (Lycii Berry)

The fruits of *Lycium barbarum* (Solanaceae), also called Fructus Lycii, have been an integral component of traditional Chinese medicine for thousands of years and are also of dietary use. *L. barbarum* is supposed to be an effective antiaging agent and has the ability to nourish the eyes, livers, and kidneys. The polysaccharides isolated from the aqueous extracts of *L. barbarum* have been identified as one of the active ingredients responsible for biological activities. *L. barbarum* is shown to possess antioxidant effects in vivo (Bucheli et al., 2011; Cheng and Kong, 2011; Reeve et al., 2010). The polysaccharides extracted from *L. barbarum* were effective in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals, superoxide anion, and hydroxyl radical in vitro (Lin et al., 2009). Animal studies have also shown that the

polysaccharides exhibited antiaging function and the ability to prevent beta amyloid peptide-induced neurotoxicity (Yu et al., 2006) and reduce age-related oxidative stress (Li et al., 2007).

3.9 *Gynostemma pentaphyllum* (Jiaogulan)

Jiaogulan is a herbaceous vine belonging to the Cucurbitaceae family. It is a herbal medicine of repute and is reported to possess powerful antioxidant and adaptogenic effects. Pharmacological studies have shown that it possesses myriad activities like antiaging, anticancer, antifatigue, antiulcer, hypolipidemic, and immunomodulatory effects. Its administration is associated with increase in life expectancy and delay in aging. The extract of the plant is also shown to be effective in ameliorating galactose-induced oxidative damage of DNA in aged rats (Sun et al., 2006).

Administering Gynostemma to patients is shown to reduce the general signs and symptoms of aging, such as fatigue, lack of energy, diarrhea, poor memory, and insomnia. It is also supposed to be useful in treating Alzheimer's (Cheng, 2005). Gypenosides, the major phytochemicals of *G. pentaphyllum*, are also known to possess antioxidant (Shang et al., 2006; Wang et al., 2010a), antidiabetic (Zhang et al., 2009), antiapoptotic (Wang et al., 2007, 2010b), and immunomodulatory (Sun and Zheng, 2005; Zhang et al., 1990) activities. Gypenosides were shown to possess neuroprotective effects due to their antioxidant and antiapoptotic actions (Shang et al., 2006; Wang et al., 2010a,b,c).

3.10 *Withania somnifera* (Ashwagandha)

Withania somnifera Dunal (Ashwagandha) is a popular medicinal plant widely used in the various folk and traditional systems of medicine in India. It is an important constituent of many polyherbal preparations and is used either alone or as a composite formulation to improve learning ability and increase energy, vigor, endurance, strength, health, as well as vital fluids, muscle fat, blood, lymph, semen, and cell production. It is also of use in counteracting chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, convalescence, and muscle tension. It is exceedingly being prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in children and elderly people (Sharma et al., 2011). The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins (sitoindoside VII and VIII), and withanolides (sitoindoside IX and X) (Mishra et al., 2000).

Preclinical studies have clearly shown that ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems (Mishra et al., 2000). Ashwagandha is also observed to

protect against ischemia–reperfusion-induced apoptosis in cardiac tissue of rats (Mohanty et al., 2008), and gentamycin-induced nephrotoxicity in mice (Jeyanthi and Subramanian, 2009). in vitro and in vivo studies have also shown that ashwagandha extract and its phytochemicals prevent/protect against neurodegerative diseases such as Parkinson's disease and Alzheimer's disease (Jayaprakasam et al., 2010; Kumar and Kumar, 2009; Rajasankar et al., 2009a,b). Ashwagandha is proposed to be a potential herbal medicine for the treatment of Alzheimer's disease (Wollen, 2010) and the withanamides isolated from the ashwagandha fruits are reported to be effective in protecting against beta-amyloid-induced neurotoxicity (Jayaprakasam et al., 2010; Kumar et al., 2010).

3.11 *Vitis vinifera* (Grapes)

For thousands of years, the fruit and the plant *Vitis vinifera*, commonly referred to as grapes have been grown and harvested for medicinal, nutritional, and economic value. Grapes are one of the richest sources of anthocyanins, which possess anti-inflammatory, antiaging, and anticarcinogenic properties (Xia et al., 2010). The major constituents of grape are epicatechin gallate, procyanidin dimers, trimers, tetramers, catechin, epicatechin, and gallic acid, procyanidin pentamers, hexamers, and heptamers, and their gallates. Grapes are among the best-known antiaging agents and have been linked to a variety of health benefits, the most important being the anticancer and cardioprotective effects. Grapes possess free radical scavenging, and antioxidant and antilipid peroxidation effects, which contribute to the observed hepatoprotective, neuroprotective, renoprotective, adaptogenic, and nootropic activities (Xia et al., 2010).

Resveratrol, a polyphenol phytoalexin present in the red wine and grapes, is shown to possess diverse biochemical and physiological properties, including estrogenic, antiplatelet, and anti-inflammatory properties as well as a wide range of health benefits that include cancer prevention, antiaging, and cardioprotection (Yang et al., 2012; Xia et al., 2010). Resveratrol has been shown to induce the expression of several longevity genes, including Sirt1, Sirt3, Sirt4, FoxO1, Foxo3a, and pre-B cell colony-enhancing factor (PBEF) and contribute to retarding aging and senescence (Das et al., 2011). Randomized clinical trials have also shown that administering grape juice improved memory in older adults with mild cognitive impairment (Krikorian et al., 2010). Additionally, the grape seed anthocyanins have been reported to increase the antioxidants and to prevent oxidative stress in aging animals (Sangeetha et al., 2005). Studies have also shown that catechins, isolated from the skin of grapes, inhibited the activation of c-Jun N-terminal kinases (JNKs) and the enzymes of the mitogen-activated protein kinase (MAPK) family involved in UV-induced carcinogenesis (Wu et al., 2006). The grape polyphenols are also shown to be effective in preventing skin aging primarily due to their antioxidant, anti-inflammatory, and DNA repair-promoting actions (Nichols and Katiyar, 2010; Ndiaye et al., 2011).

3.12 *Centella asiatica* (Gotu Kola)

Centella asiatica, commonly known as gotu kola, is a herbaceous plant belonging to the family Mackinlayaceae. It is a mild adaptogen and has been used as a medicinal herb for thousands of years in India, where it is commonly used in antiaging preparations for the skin. According to Charaka, often considered the Father of Indian Traditional System of Medicine – Ayurveda, gotu kola is a very useful medicinal plant in preventing aging. It is ranked high in the top ten herbs known for antiaging properties and this may be in part due to its antioxidative effects (Chaudhary, 2010). The plant also possesses neurotonic effects and is known to improve memory and stimulus reflex. It is also supposed to be effective in the treatment of tuberculosis, syphilis, amebic dysentery, and common cold (Ponnusamy et al., 2008).

Scientific studies have shown gotu kola to protect against neurodegerative diseases in animal models. Administration of gotu kola extract is shown to be effective in preventing oxidation of proteins, lipid peroxidation, and prooxidant processes, and to concomitantly increase the antioxidant enzymes in corpus striatum and hippocampus in rats with Parkinson's disease (Haleagrahara and Ponnusamy, 2010). Gotu kola is also known to improve the neural antioxidant status in aged rats (Subathra et al., 2005), to ameliorate 3-nitropropionic-acid-induced oxidative stress in mice brain (Shinomol et al., 2010), to decrease lead-induced neurotoxicity in mice (Ponnusamy et al., 2008), to mitigate glutamate-induced neuroexcitotoxicity in rats (Ramanathan et al., 2007), and to improve antioxidant status and cognitive skills in rats (Veerendra Kumar and Gupta, 2002). Topical treatment with gotu kola is also reported to be effective in remodeling photoaged skin and to prevent skin aging in human volunteers, thereby validating the ethnomedicinal uses (Haftek et al., 2008).

4. CONCLUSION

Pharmacological studies, with experimental systems of study, suggest that the aforementioned Asian medicinal plants are effective in preventing/retarding/ameliorating aging and aging-related ailments. However, in order for many of them to be accepted by modern systems of medicine to be relevant for clinical/pharmaceutical use, detailed investigations are required to bridge the gaps in the current understanding of knowledge and provide scientifically validated evidence. The three main lacunas are the incompleteness of the pharmacological studies, the lack of phytochemical validation, and the lack of scientifically conducted studies in humans. Detailed studies on the mechanistic aspects with different and more robust preclinical models are required especially with the active principles. Additionally, the phytochemicals which are responsible for the observed pharmacological properties are known to be varying depending on the plant age, part, and geographical and seasonal conditions. Studies should be performed with

well-characterized extracts with knowledge on the levels of different vital bioactive components as only then will the observations be reproducible and valid. Pilot studies with a small number of healthy individuals should be initially performed to understand the maximum tolerable dose as information accrued from these studies can be of use in validating preclinical observations. Nonetheless, in view of the established safety of usage of such plants for several centuries for the amelioration of overall health in the aging population, such medicinal plants will definitely find application as formulations for prophylactic use in the future.

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REFERENCES

Aggarwal, B.B., Harikumar, K.B., 2009. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. The International Journal of Biochemistry and Cell Biology 41, 40–59.

Aghazadeh, S., Amini, R., Yazdanparast, R., Ghaffari, S.H., 2010. Anti-apoptotic and anti-inflammatory effects of *Silybum marianum* in treatment of experimental steatohepatitis. Experimental and Toxicologic Pathology 63 (6), 569–574.

Asghar, Z., Masood, Z., 2008. Evaluation of antioxidant properties of silymarin and its potential to inhibit peroxyl radicals in vitro. Pakistan Journal of Pharmaceutical Sciences 21, 249–254.

Assunção, M., Santos-Marques, M.J., Carvalho, F., Andrade, J.P., 2010. Green tea averts age-dependent decline of hippocampal signaling systems related to antioxidant defenses and survival. Free Radical Biology and Medicine 15 (48), 831–838.

Basu, A., Sanchez, K., Leyva, M.J., et al., 2010. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. Journal of the American College of Nutrition 29, 31–40.

Bucheli, P., Vidal, K., Shen, L., et al., 2011. Goji berry effects on macular characteristics and plasma antioxidant levels. Optometry and Vision Science 88, 257–262.

Chaudhary, S.A., Gadhvi, K.V., Chaudhary, A.B., 2010. Comprehensive review on world herb trade and most utilized medicinal plant. International Journal of Applied Biology and Pharmaceutical Technology 1 (2), 510–517.

Chen, X., Zhang, Q., Cheng, Q., Ding, F., 2009. Protective effect of salidroside against H_2O_2-induced cell apoptosis in primary culture of rat hippocampal neurons. Molecular and Cellular Biochemistry 332, 85–93.

Cheng, D., Kong, H., 2011. The effect of Lycium barbarum polysaccharide on alcohol-induced oxidative stress in rats. Molecules 16, 2542–2550.

Cheng, Y., Shen, L.-h., Zhang, J.-t., 2005. Anti-amnestic and anti-aging effects of ginsenoside Rg1 and Rb1 and its mechanism of action. Acta Pharmacologica Sinica 26, 143–149.

Chuarienthong, P., Lourith, N., Leelapornpisid, P., 2010. Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. International Journal of Cosmetic Science 32, 99–106.

Das, D.K., Mukherjee, S., Ray, D., 2011. Erratum to: resveratrol and red wine, healthy heart and longevity. Heart Failure Reviews 16 (4), 425–435.

DeFeudis, F.V., Drieu, K., 2000. Ginkgo biloba extract (EGb 761) and CNS functions: basic studies and clinical applications. Current Drug Targets 1, 25–58.

Dong, L.Y., Fan, L., Li, G.F., et al., 2004. Anti-aging action of the total lactones of ginkgo on aging mice. Acta Pharmaceutica Sinica/Yao Xue Xue Bao 39, 176–179.

Faria, A., Oliveira, J., Neves, P., et al., 2005. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. Journal of Agricultural and Food Chemistry 53, 6896–6902.

Fursova, A.Z., Gesarevich, O.G., Gonchar, A.M., Trofimova, N.A., Kolosova, N.G., 2005. Dietary supplementation with bilberry extract prevents macular degeneration and cataracts in senesce-accelerated OXYS rats. Advances in Gerontology 16, 76–79.

Galhardi, F., Mesquita, K., Monserrat, J.M., Barros, D.M., 2009. Effect of silymarin on biochemical parameters of oxidative stress in aged and young rat brain. Food and Chemical Toxicology 47, 2655–2660.

Geng, J., Dong, J., Ni, H., et al., 2010. Ginseng for cognition. Cochrane Database of Systematic Reviews 8, CD007769.

Guan, S., Wang, W., Lu, J., et al., 2011. Salidroside attenuates hydrogen peroxide-induced cell damage through a cAMP-dependent pathway. Molecules 16, 3371–3379.

Haftek, M., Mac-Mary, S., Le Bitoux, M.A., et al., 2008. Clinical, biometric and structural evaluation of the long-term effects of a topical treatment with ascorbic acid and madecassoside in photoaged human skin. Experimental Dermatology 17, 946–952.

Haleagrahara, N., Ponnusamy, K., 2010. Neuroprotective effect of *Centella asiatica* extract (CAE) on experimentally induced parkinsonism in aged Sprague-Dawley rats. The Journal of Toxicological Sciences 35, 41–47.

Halliwell, B., Gutteridge, J.M.C., 2007. Measurement of reactive species. Free Radicals in Biology and Medicine, Fourth ed. Oxford University Press (Chapter 5).

Hsu, S., 2005. Green tea and the skin. Journal of the American Academy of Dermatology 52, 1049–1059.

Jafari, M., Felgner, J.S., Bussel, I.I., et al., 2007. Rhodiola: a promising anti-aging Chinese herb. Rejuvenation Research 10, 587–602.

Jayaprakasam, B., Padmanabhan, K., Nair, M.G., 2010. Withanamides in *Withania somnifera* fruit protect PC-12 cells from beta-amyloid responsible for Alzheimer's disease. Phytotherapy Research 24, 859–863.

Jeyanthi, T., Subramanian, P., 2009. Nephroprotective effect of *Withania somnifera*: a dose-dependent study. Renal Failure 31, 814–821.

Jia, L., Zhao, Y., Liang, X.J., 2009. Current evaluation of the millennium phytomedicine – ginseng (II): collected chemical entities, modern pharmacology, and clinical applications emanated from traditional Chinese medicine. Current Medicinal Chemistry 16, 2924–2942.

Karlsen, A., Paur, I., Bøhn, S.K., et al., 2010. Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD. European Journal of Nutrition 49, 345–355.

Kaur, T., Pathak, C.M., Pandhi, P., Khanduja, K.L., 2008. Effects of green tea extract on learning, memory, behavior and acetylcholinesterase activity in young and old male rats. Brain and Cognition 67, 25–30.

Kenichi, I., 2006. Anti aging effects of bilberry extract through up-regulation of GABA synthsiszing enzyme (GAD) in dermal fibroblasts. Fragrance Journal 34, 48–53.

Khan, N., Mukhtar, H., 2007. Tea polyphenols for health promotion. Life Sciences 81, 519–533.

Kim, K.R., Chung, T.Y., Shin, H., et al., 2010. Red ginseng saponin extract attenuates murine collagen-induced arthritis by reducing pro-inflammatory responses and matrix metalloproteinase-3 expression. Biological and Pharmaceutical Bulletin 33, 604–610.

Krikorian, R., Nash, T.A., Shidler, M.D., Shukitt-Hale, B., Joseph, J.A., 2010. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. British Journal of Nutrition 103 (5), 730–734.

Kumar, P., Kumar, A., 2009. Possible neuroprotective effect of *Withania somnifera* root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. Journal of Medicinal Food 12, 591–600.

Kumar, S., Seal, C.J., Howes, M.J., Kite, G.C., Okello, E.J., 2010. In vitro protective effects of *Withania somnifera* (L.) dunal root extract against hydrogen peroxide and β-amyloid(1-42)-induced cytotoxicity in differentiated PC12 cells. Phytotherapy Research 24, 1567–1574.

Kumar, A., Prakash, A., Dogra, S., 2011. Protective effect of curcumin (*Curcuma longa*) against D-galactose-induced senescence in mice. Journal of Asian Natural Products Research 13, 42–55.

Kumaran, V.S., Arulmathi, K., Kalaiselvi, P., 2009. Senescence mediated redox imbalance in cardiac tissue: antioxidant rejuvenating potential of green tea extract. Nutrition 25, 847–854.

Lambert, J.D., Elias, R.J., 2010. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Archives of Biochemistry and Biophysics 501 (1), 65–72.

Lee, K.S., Lee, B.S., Semnani, S., et al., 2010. Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in *Drosophila melanogaster*. Rejuvenation Research 13, 561–570.

Leuner, K., Hauptmann, S., Abdel-Kader, R., et al., 2007. Mitochondrial dysfunction: the first domino in brain aging and Alzheimer's disease?. Antioxidants and Redox Signaling 9, 1659–1675.

Li, X.M., Ma, Y.L., Liu, X.J., 2007. Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. Journal of Ethnopharmacology 111, 504–511.

Li, Y.H., Wu, Y., Wei, H.C., et al., 2009. Protective effects of green tea extracts on photoaging and photoimmunosuppression. Skin Research and Technology 15, 338–345.

Lin, C.L., Wang, C.C., Chang, S.C., Inbaraj, B.S., Chen, B.H., 2009. Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. International Journal of Biological Macromolecules 45, 146–151.

Liu, Q., Kou, J.P., Yu, B.Y., 2011. Ginsenoside Rg1 protects against hydrogen peroxide-induced cell death in PC12 cells via inhibiting NF-κB activation. Neurochemistry International 58, 119–125.

Luo, F.C., Wang, S.D., Qi, L., et al., 2011. Protective effect of panaxatriol saponins extracted from *Panax notoginseng* against MPTP-induced neurotoxicity in vivo. Journal of Ethnopharmacology 133, 448–453.

Mandel, S.A., Amit, T., Kalfon, L., et al., 2008. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). Journal of Alzheimer's Disease 15, 211–222.

Milbury, P.E., Graf, B., Curran-Celentano, J.M., Blumberg, J.B., 2007. Bilberry (*Vaccinium myrtillus*) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells. Investigative Ophthalmology and Visual Science 48, 2343–2349.

Miquel, J., Bernd, A., Sempere, J.M., Díaz-Alperi, J., Ramírez, A., 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Archives of Gerontology Geriatrics 34, 37–46.

Mishra, L.C., Singh, B.B., Dagenais, S., 2000. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): a review. Alternative Medicine Review 5, 334–346.

Mohanty, I.R., Arya, D.S., Gupta, S.K., 2008. *Withania somnifera* provides cardioprotection and attenuates ischemia-reperfusion induced apoptosis. Clinical Nutrition 27, 635–642.

Murata, N., Murakami, K., Ozawa, Y., et al., 2010. Silymarin attenuated the amyloid ß plaque burden and improved behavioral abnormalities in an Alzheimer's disease mouse model. Bioscience, Biotechnology, and Biochemistry 74, 2299–2306.

Ndiaye, M., Philippe, C., Mukhtar, H., Ahmad, N., 2011. The grape antioxidant Resveratrol for skin disorders: promise, prospects, and challenges. Archives of Biochemistry and Biophysics 508 (2), 164–170.

Nencini, C., Giorgi, G., Micheli, L., 2007. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine 14, 129–135.

Nichols, J.A., Katiyar, S.K., 2010. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Archives of Dermatological Research 302 (2), 71–83.

Nevado, J., Sanz, R., Sánchez-Rodríguez, C., et al., 2010. *Ginkgo biloba* extract (EGb761) protects against aging-related caspase-mediated apoptosis in rat cochlea. Acta Oto-Laryngologica 130, 1101–1112.

Piljac-Zegarac, J., Belscak, A., Piljac, A., 2009. Antioxidant capacity and polyphenolic content of blueberry (*Vaccinium corymbosum* L.) leaf infusions. Journal of Medicinal Food 12, 608–614.

Ponnusamy, K., Mohan, M., Nagaraja, H.S., 2008. Protective antioxidant effect of *Centella asiatica* bioflavonoids on lead acetate induced neurotoxicity. The Medical Journal of Malaysia 63 (Suppl A), 102.

Povichit, N., Phrutivorapongkul, A., Suttajit, M., Chaiyasut, C.C., Leelapornpisid, P., 2010. Phenolic content and in vitro inhibitory effects on oxidation and protein glycation of some Thai medicinal plants. Pakistan Journal of Pharmaceutical Sciences 23, 403–408.

Pyrzanowska, J., Piechal, A., Blecharz-Klin, K., et al., 2010. The influence of the long-term administration of *Curcuma longa* extract on learning and spatial memory as well as the concentration of brain neurotransmitters and level of plasma corticosterone in aged rats. Pharmacology Biochemistry and Behavior 95, 351–358.

Quan, H., Qiong-Yao, Y., Jiang, S., et al., 2011. Structural characterization and antioxidant activities of 2 water-soluble polysaccharide fractions purified from tea (*Camellia sinensis*) flower. Journal of Food Science 76, C462–C471.

Rahman, M.M., Ichiyanagi, T., Komiyama, T., Hatano, Y., Konishi, T., 2006. Superoxide radical- and peroxynitrite-scavenging activity of anthocyanins; structure–activity relationship and their synergism. Free Radical Research 40, 993–1002.

RajaSankar, S., Manivasagam, T., Sankar, V., et al., 2009a. Withania somnifera root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. Journal of Ethnopharmacology 125, 369–373.

Rajasankar, S., Manivasagam, T., Surendran, S., 2009b. Ashwagandha leaf extract: a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. Neuroscience Letters 454, 11–15.

Ramanathan, M., Sivakumar, S., Anandvijayakumar, P.R., Saravanababu, C., Pandian, P.R., 2007. Neuroprotective evaluation of standardized extract of *Centella asciatica* in monosodium glutamate treated rats. Indian Journal of Experimental Biology 5, 425–431.

Reeve, V.E., Allanson, M., Arun, S.J., Domanski, D., Painter, N., 2010. Mice drinking goji berry juice (*Lycium barbarum*) are protected from UV radiation–induced skin damage via antioxidant pathways. Photochemical and Photobiological Sciences 9, 601–607.

Rhone, M., Basu, A., 2008. Phytochemicals and age-related eye diseases. Nutrition Reviews 66, 465–472.

Salvioli, S., Sikora, E., Cooper, E.L., Franceschi, C., 2007. Curcumin in cell death processes: a challenge for CAM of age-related pathologies. Evidence-Based Complementary Alternative Medicine 4, 181–190.

Sangeetha, P., Balu, M., Haripriya, D., Panneerselvam, C., 2005. Age associated changes in erythrocyte membrane surface charge: modulatory role of grape seed proanthocyanidins. Experimental Gerontology 40, 820–828.

Sastre, J., Millam, A., Asuncion, J.G., et al., 1998. Ginkgo biloba extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. Free Radical Biology and Medicine 24 (2), 298–304.

Saw, C.L., Wu, Q., Kong, A.N., 2010. Anti-cancer and potential chemopreventive actions of ginseng by activating Nrf2 (NFE2L2) anti-oxidative stress/anti-inflammatory pathways. Chinese Medicine 5, 37.

Scapagnini, G., Caruso, C., Calabrese, V., 2010. Therapeutic potential of dietary polyphenols against brain ageing and neurodegenerative disorders. Advances in Experimental Medicine and Biology 698, 27–35.

Shang, L., Liu, J., Zhu, Q., et al., 2006. Gypenosides protect primary cultures of rat cortical cells against oxidative neurotoxicity. Brain Research 1102, 163–174.

Sharma, V., Sharma, S., Pracheta, R.P., 2011. *Withania somnifera*: a rejuvenating ayurvedic medicinal herb for the treatment of various human ailments. International Journal of PharmTech Research 3, 187–192.

Shi, C., Xiao, S., Liu, J., et al., 2010. *Ginkgo biloba* extract EGb761 protects against aging-associated mitochondrial dysfunction in platelets and hippocampi of SAMP8 mice. Platelets 21, 373–379.

Shimizu, K., Kinouchi Shimizu, N., Hakamata, W., et al., 2010. Preventive effect of green tea catechins on experimental tumor metastasis in senescence-accelerated mice. Biological and Pharmaceutical Bulletin 33, 117–121.

Shinomol, G.K., Ravikumar, H., Muralidhara, 2010. Prophylaxis with *Centella asiatica* confers protection to prepubertal mice against 3-nitropropionic-acid-induced oxidative stress in brain. Phytotherapy Research 24, 885–892.

Shytle, R.D., Bickford, P.C., Rezai-zadeh, K., et al., 2009. Optimized turmeric extracts have potent anti-amyloidogenic effects. Current Alzheimer Research 6, 564–571.

Singh, R.P., Agarwal, R., 2009. Cosmeceuticals and silibinin. Clinics in Dermatology 27, 479–484.

Sinitsyna, O., Krysanova, Z., Ishchenko, A., et al., 2006. Age-associated changes in oxidative damage and the activity of antioxidant enzymes in rats with inherited overgeneration of free radicals. Journal of Cellular and Molecular Medicine 10, 206–215.

Song, Z., Deaciuc, I., Song, M., et al., 2006. Silymarin protects against acute ethanol-induced hepatotoxicity in mice. Alcoholism, Clinical and Experimental Research 30, 407–413.

Subathra, M., Shila, S., Devi, M.A., Panneerselvam, C., 2005. Emerging role of *Centella asiatica* in improving age-related neurological antioxidant status. Experimental Gerontology 40, 707–715.

Sumiyoshi, M., Kimura, Y., 2009. Effects of a turmeric extract (*Curcuma longa*) on chronic ultraviolet B irradiation–induced skin damage in melanin-possessing hairless mice. Phytomedicine 16, 1137–1143.

Sun, H., Zheng, Q., 2005. Haemolytic activities and adjuvant effect of *Gynostemma pentaphyllum* saponins on the immune responses to ovalbumin in mice. Phytotherapy Research 19, 895–900.

Sun, Y.Y., Ma, A.G., Jia, Y.N., Zhang, X.Z., 2006. The effect of *Gynostemma pentaphyllum* extract on DNA damage of aging Rats. Carcinogenesis, Teratogenesis and Mutagenesis 18, 378–380.

Taillandier, J., Ammar, A., Rabourdin, J.P., 1986. Treatment of cerebral aging disorders with *Ginkgo biloba* extract. A longitudinal multicenter double-blind drug vs. placebo study. Presse Medicale 25, 1583–1587.

Valenzuela, A., Bustamante, J.C., Videla, C., Guerra, R., 1989. Effect of silybin dihemisuccinate on the ethanol metabolizing systems of the rat liver. Cell Biochemistry and Function 7, 173–178.

Veerendra Kumar, M.H., Gupta, Y.K., 2002. Effect of different extracts of *Centella asiatica* on cognition and markers of oxidative stress in rats. Journal of Ethnopharmacology 79, 253–260.

Wang, Q.F., Chiang, C.W., Wu, C.C., et al., 2007. Gypenosides induce apoptosis in human hepatoma Huh-7 cells through a calcium/reactive oxygen species-dependent mitochondrial pathway. Planta Medica 73, 535–544.

Wang, H.J., Jiang, Y.Y., Lu, P., Wang, Q., Ikejima, T., 2010a. An updated review at molecular pharmacological level for the mechanism of anti-tumor, antioxidant and immunoregulatory action of silibinin. Acta Pharmaceutica Sinica/Yao Xue Xue Bao 45, 413–421.

Wang, K., Zhang, H., Shen, L., Du, Q., Li, J., 2010. Rapid separation and characterization of active flavonolignans of Silybum marianum by ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 53, 1053–1057.

Wang, P., Niu, L., Gao, L., et al., 2010b. Neuroprotective effect of gypenosides against oxidative injury in the substantia nigra of a mouse model of Parkinson's disease. The Journal of International Medical Research 38, 1084–1092.

Wang, P., Niu, L., Guo, X.D., et al., 2010c. Gypenosides protects dopaminergic neurons in primary culture against MPP(+)-induced oxidative injury. Brain Research Bulletin 83, 266–271.

Wiegant, F.A., Surinova, S., Ytsma, E., et al., 2009. Plant adaptogens increase lifespan and stress resistance in *C. elegans*. Biogerontology 10, 27–42.

Wojciech, L., Ewa, Z., Elzbieta, S., 2010. Influence of green tea on erythrocytes antioxidant status of different age rats intoxicated with ethanol. Phytotherapy Research 24, 424–428.

Wollen, K.A., 2010. Alzheimer's disease: the pros and cons of pharmaceutical, nutritional, botanical, and stimulatory therapies, with a discussion of treatment strategies from the perspective of patients and practitioners. Alternative Medicine Review 15, 223–244.

Wu, W.B., Chiang, H.S., Fang, J.Y., et al., 2006. (+)-Catechin prevents ultraviolet B-induced human keratinocyte death via inhibition of JNK phosphorylation. Life Sciences 79, 801–807.

Wu, Y., Li, S., Cui, W., et al., 2008. *Ginkgo biloba* extract improves coronary blood flow in healthy elderly adults: role of endothelium-dependent vasodilation. Phytomedicine 15, 164–169.

Xia, E.-Q., Deng, D.-F., Guo, Y.-J., Li, H.-B., 2010. Biological activities of polyphenols from Grapes. International Journal of Molecular Sciences 11, 622–646.

Xie, X., Wang, H.T., Li, C.L., et al., 2010. Ginsenoside Rb1 protects PC12 cells against β-amyloid–induced cell injury. Molecular Medicine Reports 3, 635–639.

Yao, Y., Vieira, A., 2007. Protective activities of Vaccinium antioxidants with potential relevance to mitochondrial dysfunction and neurotoxicity. Neurotoxicology 28, 93–100.

Ye, R., Yang, Q., Kong, X., et al., 2011. Ginsenoside Rd attenuates early oxidative damage and sequential inflammatory response after transient focal ischemia in rats. Neurochemistry International 58, 391–398.

Yang, Y.-P., Chang, Y.-L., Huang, P.-I., et al., 2012. Resveratrol suppresses tumorigenicity and enhances radiosensitivity in primary glioblastoma tumor initiating cells by inhibiting the STAT3 axis. Journal of Cell Physiology 227, 976–993.

Yu, M.-S., Ho, Y.-S., So, K.-F., Yuen, W.-H., Chang, R.C.C., 2006. Cytoprotective effects of *Lycium barbarum* against reducing stress on endoplasmic reticulum. International Journal of Molecular Medicine 17, 1157–1161.

Zhang, C., Yang, X., Xu, L., 1990. Immunomodulatory action of the total saponin of *Gymnostemma penta-phylla*. Zhong Xi Yi Jie He Za Zhi 10 (96–8), 69–70.

Zhang, L., Yu, H., Sun, Y., et al., 2007. Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. European Journal of Pharmacology 564, 18–25.

Zhang, H.J., Ji, B.P., Chen, G., et al., 2009. A combination of grape seed-derived procyanidins and gypeno-sides alleviates insulin resistance in mice and HepG2 cells. Journal of Food Science 74, H1–H7.

Zhang, L., Yu, H., Zhao, X., et al., 2010. Neuroprotective effects of salidroside against beta-amyloid-induced oxidative stress in SH-SY5Y human neuroblastoma cells. Neurochemistry International 57, 547–555.

Ziberna, L., Lunder, M., Moze, S., et al., 2010. Acute cardioprotective and cardiotoxic effects of bilberry anthocyanins in ischemia–reperfusion injury: beyond concentration-dependent antioxidant activity. Cardiovascular Toxicology 10, 283–294.

Legumes, Genome Maintenance, and Optimal Health

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1. INTRODUCTION

Genome maintenance, including cell cycle arrest, DNA repair, and apoptosis, are crucial for cells to avoid proliferating damaged cells. A number of chronic diseases, including neurodisorders, cancer, and aging, are associated with defective genome maintenance. The notion that bioactive food components safeguard or optimize genome maintenance pathways has attracted considerable interest in recent years. Legumes are important sources of essential nutrients such as protein, carbohydrates, lipids, minerals, vitamins, and dietary fiber. Legume research has been traditionally aimed at improving the nutrient bioavailability of these foodstuffs through breeding and diverse culinary and technological treatments. However, in recent years, the focus of legume research has shifted to their beneficial health properties as functional foods. Continuous efforts are being dedicated to a better understanding of the mechanisms by which legumes act as functional foods or traditional medicines and to design new strategies to enhance their potential as important sources of phytochemicals for dietary intervention against numerous pathologies. Among the beneficial properties attributed to food legumes are their low glycemic index, their antioxidant, hypolipidemic, or anticancer activities, their role in bone health promotion and improvement of menopausal symptoms, and their prebiotic effects (Messina, 1999; Rochfort and Panozzo, 2007). Legumes exert a wide variety of beneficial effects through the specific structure and composition of their protein and carbohydrate moieties or through the action of several non–nutritional components, namely protease or carbohydrase inhibitors, polyphenols, phytic acid, saponins, and resistant starch. Interestingly, these compounds are traditionally known as antinutritional factors. Duranti (2006) has speculated that most if not all the putative nutraceutical compounds arise from the so-called antinutrients present in grain legumes. Nevertheless, the specific structure and composition of legume proteins and carbohydrates are also responsible for some of the metabolic effects of legumes. On the other hand, this author suggests that because the effect of legume seed consumption is multifactorial, legume seeds should be consumed as a whole food in order to take advantage of their beneficial and synergistic

effects. Nowadays, a renewed interest has surged regarding the potential effects of several technological treatments on the health-related properties of legumes, which may be either improved or deranged as a result of industrial or domestic processing conditions. In conclusion, legume consumption together with a healthy lifestyle will most likely contribute to healthy development and aging, ensuring a good quality of life for the population.

2. GENOMIC MAINTENANCE

The genome in the trillions of cells inside a human body constantly undergoes DNA damage events. Mammalian cells maintain genome stability mainly through DNA damage checkpoint, repair, and senescence.

2.1 DNA Damage

The genome is constantly subjected to endogenous and exogenous DNA-damaging attacks. The various types of DNA damage include base damage, adducts, strand breaks, cross-links, mismatch, and aneuploidy.

2.1.1 DNA base damages

DNA base damage, the most frequently occurring injury, can be generated by ultraviolet (UV) light, ionizing radiation (IR), alkylating agents, and reactive oxygen species (ROS). In particular, ROS can be indirectly induced by IR exposure and cause DNA base deamination, depurination, and depyrimidination. Results from the high–performance liquid chromatography–tandem mass spectrometry method characterize IR-induced base damages in cells, including thymine glycols, 5-formyluracil, 5-(hydroxymethyl) uracil, 8-oxo-7,8-dihydroguanine, and 8-oxo-7,8-dihydroadenine (Cadet et al., 2004). Upon exposure to UVB light, cells can develop 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-G) and intrastrand cross-links between two adjacent pyrimidines. Likewise, UVA exposure can induce the formation of 8-oxo-G and cyclobutane pyrimidine dimers. Bulky adducts can be induced by the exposure to benz(a)pyrene, a dietary mutagen rich in cigarette smoke and processed red meat.

2.1.2 DNA strand breaks

DNA strand breaks on the nucleotide backbones are difficult to fix. Single-strand breaks (SSBs) can be induced by defective replication and transcription. To unwind long strands of DNA during DNA replication, DNA topoisomerase 1 generates a transient nick, which, if not resealed, can cause collision with RNA or DNA polymerases or close proximity to other types of DNA lesion in favor of the formation of SSBs. In replicating cells, unrepaired SSBs block DNA replication forks, forming the more severe double-strand breaks (DSBs). In nonproliferating cells, such as neurons, SSBs can block RNA

polymerase II and stall transcription, leading to cell death (Kathe et al., 2004). Defective SSB repair is associated with cellular dysfunctions and human diseases, including spinocerebellar ataxia with axonal neuropathy 1, ataxia–oculomotor apraxia 1, and aging in neuronal cell populations (Katyal and McKinnon, 2008). DSBs can also be triggered by IR and other DNA-damaging agents such as camptothecin and etoposide, ROS attack on deoxyribose and DNA bases, and physiological immune responses. Human hereditary diseases with defective DSB response include ataxia telangiectasia and Nijmegen breakage syndrome, which are characterized by immune deficiency, sensitivity to radiations, and cancer predisposition (McKinnon and Caldecott, 2007).

2.1.3 DNA cross-links and mismatch

DNA cross-links can be induced exogenously by formaldehyde, metals, and chemotherapeutic drugs and endogenously by malondialdehyde, a lipid peroxidation end product. DNA intrastrand or interstrand cross-links are caused by covalent bonds linking DNA bases on the same or different strands, respectively. DNA can also be covalently cross-linked to proteins. The physical structures, stability, and biological consequences of DNA cross-links are dependent on the agents that induce them. An examination in peripheral blood lymphocytes of 186 subjects exposed to formaldehyde showed a positive correlation between DNA–protein cross-links and p53 mutations (Shaham et al., 2003). This may indicate the initial process of carcinogenesis in people exposed to formaldehyde. On the other hand, DNA mismatch is a postreplication error such as A–G/T–C mismatches due to DNA polymerase misincorporation and insertion/deletion due to template slippage. Moreover, uracil can be misincorporated into nascent DNA strands or by deamination of cytosine (Lindahl, 1993).

2.2 DNA Damage Checkpoints

After DNA damage, checkpoint responses are activated, which delay or arrest cell cycle progression and facilitate DNA repair. Different DNA damage checkpoints are activated in a manner depending on stages of the cell cycle (Sancar et al., 2004).

2.2.1 G1-phase checkpoint

The G1 DNA damage checkpoint prevents damaged DNA from being replicated and is the best understood checkpoint response in mammalian cells. Central to this checkpoint is the accumulation and activation of p53, a critical tumor suppressor. In normal cells, p53 levels are kept low because of its nuclear export and rapid cytoplasmic degradation. After DNA damage, p53 is phosphorylated at Ser-15 and other sites by ATM or DNA-PKcs (Canman et al., 1998). Phosphorylated p53 is disassociated from MDM2, which could otherwise target p53 for degradation. p53 acts as a transactivator for many tumor suppressor genes. One of the p53 targets is p21, which promotes G1 arrest by the inhibition of cyclin-dependent kinase 2 required for G1/S transition. ATM can also phosphorylate

MDM2 on Ser-395 to reduce its affinity to p53, thus stabilizing p53 at the posttranslational level (Maya et al., 2001). The G1 DNA damage checkpoint can also occur in a p53-independent manner. Ceramide induces dephosphorylation of retinoblastoma (Rb) protein, leading to increased p21 protein level, inhibition of Cdk2 kinase activity, and G1 arrest.

2.2.2 S-phase checkpoint

The S-phase checkpoint monitors cell cycle progression and suppresses DNA synthesis with damaged DNA. Analyses of cells from individuals with ataxia telangiectasia, which carries ATM mutations, implicate the involvement of ATM in the S-phase checkpoint response. Various forms of DNA damage, especially DSBs, trigger a cascade of ATM pathway activation (Bakkenist and Kastan, 2003). Activated ATM contributes to prevention of DNA synthesis by phosphorylating the Chk2 kinase on Thr-68, which in turn phosphorylates the cyclin-dependent kinase–25A (Cdc25A) phosphatase on Ser-123. These events couple Cdc25A to ubiquitin-mediated proteolytic degradation and hold cell cycle progression (Iliakis et al., 2003). Another pathway of IR-induced S-phase checkpoint is Cdc25A-independent. Upon IR damage, ATM phosphorylates NBS1 (Nijmegen breakage syndrome protein 1) on Ser-343, BRCA1 (breast cancer–associated protein 1) on Ser-1387, and SMC1 (structural maintenance of chromosome protein 1) on Ser-957 and Ser-966 (Yazdi et al., 2002). Mutations in any of these proteins or the indicated phosphorylation sites result in attenuated S-phase checkpoint activation. Interestingly, NBS1 and BRCA1 are required for optimal phosphorylation of SMC1 upon IR (Yazdi et al., 2002). ATM also interacts with Werner syndrome protein and modulates an S-phase checkpoint response to replication fork collapse (Cheng et al., 2008).

ATM and Rad3-related protein (ATR) is another S-phase checkpoint kinase responsible mainly for replication stress. Upon cellular exposure to UV irradiation, the MRE11–RAD50–NBS1 complex facilitates ATR activation by stalled replication forks; subsequently, ATR phosphorylates NBS1 on Ser-343 and inhibits DNA replication (Olson et al., 2007). ATR also exhibits overlapping functions with ATM after cellular exposure to IR. ATR initiates a 'slow' S-phase checkpoint response by phosphorylating Chk1 on Ser-317 and Ser-345 (Zhou et al., 2002), which in turn destabilizes Cdc25A as ATM does. In addition, SMC1 can be phosphorylated on Ser-957 and Ser-966 upon UV irradiation or hydroxyurea exposure in an ATM-independent manner. Interestingly, blocking ATR activity in colon cancer cells triggers a prolonged S-phase arrest, indicating the possible cancer target by promoting radiosensitization.

2.2.3 G2/M-phase checkpoint

The G2/M-phase checkpoint prevents S-phase cells with DNA damage from chromosome segregation. Cdc2 is a key G2 checkpoint protein that regulates the transition from the G2 phase to mitosis. In the response of G2 cells to IR or UV light, ATR plays a major

role, while ATM plays a supporting role in the checkpoint response. Upon DNA damage, the downstream kinases Chk1 and Chk2 phosphorylate the dual–specificity phosphatase Cdc25C on Ser–216, a phosphorylation event required for the G2/M checkpoint (Kaneko et al., 1999). Phosphorylation of this residue stimulates the translocation of the 14-3-3/Cdc25C protein complexes from the nucleus to the cytoplasm, thereby preventing Cdc25C from activating Cdc2. This results in the maintenance of the Cdc2/Cyclin B1 complex in its inactive state and blockage of mitosis entry.

3. BIOACTIVE EFFECTS OF LEGUME CONSUMPTION

3.1 Antioxidant, Antiproliferative, and Antigenotoxic Effects

Legume consumption may play an important role in healthy aging through its well-recognized antioxidant, antiproliferative, and antigenotoxic effects. Among the main legume-derived compounds responsible for these beneficial effects are saponins, dietary fiber, polyphenols, protease inhibitors, and phytic acid. Saponins are secondary metabolites consisting of a triterpene or steroid nucleus with mono- or disaccharides attached to its core. These compounds exhibit interesting anticancer properties and beneficial effects on hyperlipidemia. It has been reported that saponins decrease the expression of α-2,3-linked sialic acid on the cell surface, which in turn suppresses the metastatic potential of cancer cells. In addition, saponins may activate apoptotic pathways and induce the programmed cell death of neoplastic cells (Rochfort and Panozzo, 2007). Dietary fiber intake has been correlated with lower levels of DNA or 4–aminobiphenyl–hemoglobin adducts in the European Prospective Investigation into Cancer and Nutrition (Peluso et al., 2008). Such a protective effect may stem from its capacity to dilute potential food mutagens and carcinogens in the gastrointestinal tract, speeding their transit through the colon and binding carcinogenic substances. However, hardly any correlation was found between legume consumption and DNA or hemoglobin adduct formation.

The antioxidant and free radical-scavenging capacity of water or acetone extracts from whole seeds or seed coat tested in different experimental models demonstrates significant correlation with the amount of polyphenols or ascorbic acid present in the extracts (Nilsson et al., 2004). Dong et al. (2007) have isolated 24 compounds from the seed coat of black bean (*Phaseolus vulgaris*), which included 12 triterpenoids, 7 flavonoids, and other phytochemicals. The phytochemicals exhibited potent antioxidant and antiproliferative activities in three different cell culture model systems. The authors concluded that the additive and synergistic effects of phytochemicals in whole foods are responsible for their potent antioxidant and anticancer activities. Therefore, health benefits could be attributed to the complex mixtures of phytochemicals present in whole seeds. On the other hand, Jang et al. (2010) have observed that administration of anthocyanidin from black soybean to a murine model of benign prostatic hyperplasia resulted in a significant decrease in hyperplastic cells and higher apoptotic body counts when compared to

control animals. In addition, prostate weight and pathohistological changes associated with prostatic hyperplasia were significantly reduced by anthocyanidin administration. The authors enumerated the possible ways through which the reported health benefits of anthocyanins could be taking place as direct removal of ROS, augmentation of cell ability to absorb oxygen radicals, stimulation of phase II detoxificating enzyme expression, reduced synthesis of oxygen adducts in DNA, reduction of lipid peroxidation, suppression of mutagenesis by environmental toxins, and carcinogens or suppression of cell proliferation acting on various cell control proteins at different cell cycle stages. Soy isoflavones are capable of upregulating the expression of genes critical for drug transport and metabolism. Of special interest is the stimulation of several phase I (cytochromes 1A1, 3A4, and 8B1) and II (carbohydrate sulfotransferase-5 and glutathione-sulfotransferase-2) enzymes in primary human hepatocytes (Li et al., 2007). Such metabolizing enzymes may act in the chemoprevention of cancer or the activation of CYP family of enzymes with an important role in bile acid metabolism through the action of hepatocyte nuclear factor 4α.

Upon studying the molecular mechanisms involved in the antiproliferative and anticancer properties of legumes, several authors have found that different apoptotic pathways are activated by legume extracts. Fang et al. (2010) isolated six flavonoids from the stem of the traditional Chinese medicinal herb *Millettia reticulata* Benth. Genistein was the component with higher activity that induced apoptosis in SK-Hep-1 liver adenocarcinoma cells through both Fas- and mitochondrial-mediated pathways, leading to a loss of mitochondrial membrane potential; increased protein expression of Fas, FasL, and p53; regulation of Bcl-2 family members; and activation of caspases followed by cleavage of PARP. Apoptotic death is also the basis of the antiproliferative effect of *P. vulgaris* hemagglutinin reported by Lam and Ng (2011) in breast cancer MCF-7 cells. The hemagglutinin-treated cells showed cell cycle arrest in the G2/M phase, phosphatidyl serine externalization, and mitochondrial membrane depolarization. Hemagglutinin-induced apoptosis took place through the Fas death receptor pathway, which involved caspase-8 activation, BID truncation, p53 release, caspase-9 activation, and lamin A/C truncation. A different molecular pathway for legume-derived anticancer effects has been described by Wu et al. (2001) in water extracts of the traditional Chinese medical herb *Cassia tora* using the HepG2 liver carcinoma cell line experimental model. The protective action against benzo[a]pyrene-induced DNA damage was assessed using the comet assay, and the legume extract showed dose-dependent inhibition of toxicity that paralleled its inhibitory activity on certain detoxifying enzymes such as ethoxyresorufin-O-dealkylase or NADPH cytochrome P-450. In contrast, the activity of glutathione S-transferase was elevated. The beneficial activity of *C. tora* was related to specific anthraquinones that would inhibit the metabolization of benzo[a]pyrene, thus counteracting the DNA damage induced by mutagens through interference with internal activation enzymes. The enhanced

activity of glutathione S-transferase would most likely increase the hydrophilicity of reaction products, making them easier to be excreted in the urine.

Given that proteases are considered key factors in the metastatic progression of neo-plastic cells, the interference of several legume-derived protease inhibitors such as Bowman–Birk inhibitors (BBIs) or lectins can be related to the ability of these foodstuffs to block tumorigenesis (Caccialupi et al., 2010). Such an ability confers on these com-pounds great potential with regard to the prevention and dietary treatment of such pa-thologies. Phytic acid has interesting potential in the dietary prevention and treatment of cancer due to its Fe–chelating–derived antioxidant properties (Porres et al., 1999). In ad-dition, phytic acid has been described to exhibit anticancer properties through cell arrest at the G1 phase, potentially acting through the cyclin-dependent kinase pathway (Rochfort and Panozzo, 2007) and mediating the activation of natural killer cells. The degradation products of phytic acid have also proven to be efficient at suppressing the proliferation of the colorectal HCT116 cancer cell line (Ishizuka et al., 2011). Low–dose exposure to IP_3 decreased proliferation and increased the proportion of cells in S and G1 phases. At higher doses, the proportion of cells in the G0/G1 phase was reduced, whereas that of cells in G2/M and sub-G1 was increased. IP_3 administration also reduced the expression of cyclin D3 although to a lower extent when compared to IP_6, which also inhibited the expression of other cyclins and exhibited different behavior toward the different phases of the cell cycle.

3.2 Effects on Lipid and Glucose Metabolism

The benefits of legume consumption in glucose and lipid metabolism have been widely reported and may comprise numerous pathways in which several nutrient or non-nutritional compounds take part. Isoflavones, the most studied non-nutritional legume components, play an important role in lipid and glucose metabolism, although their spe-cific effects are sometimes difficult to separate from those of soybean proteins. Isoflavones are diphenolic substances with structural similarities to mammalian estradiols that exhibit affinity to both α and β isoforms of the estrogen receptor (ER). Nevertheless, binding to the ERβ receptor and activation of its estrogen response elements (EREs) is considerably more efficacious than that of ERα to its ERE. Such specificity may confer isoflavones with the potential to regulate important physiological functions (Xiao, 2008). Further-more, an important aspect of such compounds is their metabolism in the large intestine by specific bacterial populations that are rarely present in Westerners. Such a metabolism gives rise to products such as equol that are as effective as or even more potent than daid-zein, originally present in soybean (Setchell et al., 2002).

Legumes are known for their low glycemic index (Sáyado–Ayerdi et al., 2005) that may be caused by a combination of several factors such as the structure-derived higher resistance to digestion of legume starches or the presence of carbohydrase inhibitors and

phytic acid. Duranti (2006) has reviewed the potential of grain legume α-amylase inhibitors as a nutraceutical molecule in the prevention and therapy of obesity and diabetes. Some patents concern the use of food preparations containing suitable amounts of α-amylase inhibitors from various sources for obesity control and prevention and treatment of diabetes. On the other hand, phytic acid may form complexes with starch that make this nutrient less prone to carbohydrase digestion. An additional benefit of the lower starch digestibility in legumes is explained by its prebiotic effect derived from fermentation of resistant starch by the microbiota present in the large intestine. Moreover, Duranti (2006) has reviewed the hypoglycemic action of γ-conglutin from lupin and the lipid-lowering effects of a specific soybean globulin, the α'-subunit of 7S globulin, although the mechanism of action is not clearly understood. Mezei et al. (2003) observed an improvement in glucose tolerance tests in obese female Zucker rats after consumption of a high-isoflavone soybean meal. Consumption of high-isoflavone soybean also induced a significant decrease in liver weight, cholesterol, and triglycerides, as well as plasma cholesterol and triglycerides in both male and female individuals. Isoflavones improved the diabetic phenotype of male and female rats and affected peroxisome proliferator-activated receptor (PPAR) α or γ-directed gene expression.

A consistent body of literature with reference to the hypolipidemic effects of legume and, more specifically, of soybean consumption has been built during the last three decades. The current status of research is oriented toward the mechanisms behind such effects. Nagaoka et al. (1997) found lower serum cholesterol levels in rats fed soy protein peptic hydrolyzate when compared to casein tryptic hydrolyzate. These reduced levels of serum cholesterol were matched by significantly increased fecal sterol excretion and by the inhibitory effect of soybean protein hydrolyzate on cholesterol absorption by caco-2 cells. Relative liver weight was also considerably decreased in the animals fed the soy protein hydrolyzate, which exhibited decreased levels of hepatic total lipids and cholesterol. Tamuru et al. (2007) found that, compared to casein, soybean protein or hydrolyzate induced a significant reduction in the levels of serum triglycerides. Such reduction could be mediated through a suppressed triglyceride secretion from the liver to the blood circulation and the stimulation of fatty acid oxidation in the liver. There are several mechanisms that may be responsible for the hypocholesterolemic effect of soybean versus casein: (1) suppression of intestinal cholesterol absorption, (2) promotion of fecal sterol excretion (Yoshie-Stark and Wäsche, 2004), and (3) increase in the cholesterol removal rate by the LDL receptor. The ratio of Lys/Arg can also play an important role in the protective effect of soybean protein and other plant-based foods as a high lysine content lowers the liver arginase activity and spares arginine for the synthesis of arginine-rich apolipoprotein with known atherogenic effects (Kritchevsky et al., 1982). Nevertheless, Sugano et al. (1982) suggested that Lys/Arg was more effective at regulating serum triglycerides than serum cholesterol and found that serum insulin was associated with different effects of protein on serum lipids.

The molecular mechanisms through which isoflavones or soy protein may exert their beneficial effects on lipid metabolism are probably multiple. In this regard, Tachibana et al. (2005) found that hepatic gene expression in rats was significantly affected after 8 weeks of soy protein consumption when compared to casein consumption. Sixty-three genes were upregulated and 57 were downregulated, most of which were involved in lipid metabolism, antioxidant activity, transcriptional regulation, and energy metabolism. These modifications could induce the progression of steroid metabolism, suppression of fatty acid synthesis, and reduced expression of genes concerned with cell growth and/or maintenance, cytoarchitecture, and amino acid metabolism, changes that could be related to the effects on lipid metabolism and to the decreased liver and body weight of soy-fed animals. Likewise, Xiao et al. (2007) have reported the increase of hepatic retinoic acid receptor (RARβ2) as a result of soy protein isolate (SPI) consumption. The effects exerted by SPI were tissue- and isoform-specific, and no consistent effect was reported for individual isoflavones. In addition, DNA-binding activity of nuclear RARβ2 was significantly attenuated by SPI consumption, and its isoelectric points switched to a more acidic range. Such changes may have contributed to the suppression of retinol-induced hypertriglyceridemia by SPI and appeared to be mediated via posttranslational modifications such as phosphorylation of the RARβ2 protein, thus changing its structure or conformation and affecting its degradation and DNA binding. Ronis et al. (2009) described that PPARα-regulated genes involved in fatty acid degradation were upregulated by soy protein and isoflavones. This upregulation was accompanied by increased promoter binding and PPARα mRNA expression. Soy protein in combination with isoflavones was also protective against steatosis, hypercholesterolemia, or insulin resistance in a model of diet-induced hypercholesterolemia. Furthermore, nuclear sterol receptor element-binding protein-1C and mRNA as well as protein expression of enzymes involved in fatty acid synthesis were increased in casein-containing, but not soy-containing, diets. The activated LXR-α-regulated gene expression in animals fed with a diet containing soy and isoflavones could be linked to the increased bile acid excretion as a result of soybean ingestion.

3.3 Other Beneficial Effects

Legume-derived phytoestrogens can be an excellent alternative to hormone replacement therapy in the treatment of menopausal symptoms, which has been associated with increased incidence of some cancers, coronary vascular disease, or thromboembolism. In fact, Blum et al. (2003) have tested the effect of soy protein on indices of bone mass, bone density, and bone formation and resorption in a rat model of aging and menopause. Soy protein had a beneficial effect on preservation of bone density and greater periosteal bone formation rates or endocortical bone formation, as well as greater double-labeled surface and formation rates in cancellous bone. According to the authors, the ability of soy to sustain and/or improve some indices of bone formation differs from the normal action of estrogens, which suppress bone formation. Searching for a higher production of

phytoestrogens, Boué et al. (2011) treated *P. vulgaris* seeds with *Aspergillus sojae*. In response to such an elicitor treatment of *A. sojae* spore suspension applied to the cut surface of each seed, an enhanced production of phytoalexins exhibiting both estrogenic and antiestrogenic activity was achieved. Another novel effect related to the consumption of soybean isoflavones (daidzein, genistein, or equol) at high doses or at a more physiological concentration is a significant inhibition in amyloid β-peptide fibril formation *in vitro* (Henry-Vitrac et al., 2010). The aggregation of amyloid β-peptide and its accumulation in the brain have been identified as one of the major steps in the pathophysiology of Alzheimer's disease. Other isoflavones such as biochanin A may be protective against Parkinson's disease through their ability to protect dopaminergic neurons (Rochfort and Panozzo, 2007). Isoflavones can also be applied to dermatology, as reported by Huang et al. (2010) who applied a soy isoflavone extract from soybean cake that contains aglycone and acetyl glucose groups to human keratinocytes or the skin of nude mice. The authors found that soybean extract was protective against UVB-induced death of human keratinocytes and reduced the level of desquamation, transepidermal water loss, erythema, and epidermal thickness in nude mouse skin. In addition, the preparation increased the activity of catalase and suppressed that of cyclooxygenase-2 (COX-2) and proliferating cell nuclear antigen, a marker of DNA replication that could also be applied as an indicator of UVB-induced damage in the S phase of the cell cycle. Dia et al. (2008) have evaluated the anti-inflammatory properties of eight soybean bioactive compounds in the lipopolysaccharide-induced RAW264.7 macrophage. BBI and sapogenol B showed the highest potency to inhibit COX-2/prostaglandin E_2 and inducible nitric oxide synthase/nitric oxide inflammatory pathways.

3.4 Strategies to Improve the Beneficial Effects of Legumes for Human Health

Several strategies can be applied to improve the health benefits of legume consumption. In this section, specific options such as optimizing environmental factors, breeding, or technological treatments are covered. Thavarajah et al. (2008) have studied the distribution and speciation of selenium (Se) among the different parts (embryonic axis, cotyledons, or seed coat) of lentil seeds and the effect of cultivar location and genetics of mineral accumulation. In the embryonic axis and cotyledons, Se was present primarily as selenomethionine or selenocysteine, with a minor component of inorganic Se, whereas the opposite was true in the lentil seed coat. The authors indicated significant genotypic and environmental variability in total Se content in lentils, which can provide 77–122% of the recommended daily intake in 100 g of dry seeds. The Se content and chemical forms of Se could be altered by conventional breeding or optimization of agricultural production conditions.

The effects of genetic factors on the health-related properties of legumes can be exemplified by the study of Darmawan et al. (2010) who have reported differences in subunit composition and antioxidant capacity of alcalase-digested soy protein hydrolyzates

caused by genetic differences, but not by growing locations. Therefore, it would be possible to improve the protein profile and antioxidant capacity of protein hydrolyzates through the use of specific breeding programs aimed at changing the subunit composition of a particular legume, and develop new lines of protein hydrolyzates to be used as natural supplements or functional food ingredients.

Biotechnological treatments such as germination and fermentation have been shown to increase the content of specific flavonoids including resveratrol and to enhance the antioxidant capacity of legumes using different experimental models (Doblado et al., 2007). Kim et al. (2008) described multiple actions of an ethanol extract from fermented soybean, which inhibited the generation of DPPH radicals, LDL oxidation, H_2O_2-induced DNA damage, and cell death in NIH/3T3 fibroblasts. In addition, *in vivo* administration to $KBrO_3$-administered mice inhibited liver MDA formation, DNA damage, and formation of micronucleated reticulocytes. The isoflavones genistein and daidzein seemed to contribute, at least in part and together with other phenolic compounds, to the reported antioxidant and genoprotective effects. Nevertheless, the beneficial properties of legumes can be deranged by widely used thermal treatments such as conventional boiling, conventional steaming, pressure cooking, or pressure steaming (Doblado et al., 2007). Such treatments significantly decreased the total phenolic, procyanidin, saponin, and phytic acid content of cool-season food legumes, thus decreasing their cellular antioxidant and antiproliferative activities. Different processing conditions had varied effects on the parameters studied, and steaming appeared to be the best cooking method for retaining antioxidant and phenolic composition.

GLOSSARY

Bowman–Birk inhibitor (BBI) Proteinaceous compounds that are capable of inhibiting the activity of several proteases.

Glycemic index Numerical value given to carbohydrate-containing foods based on the average increase in blood glucose that takes place after food ingestion.

Lectins Glycoproteins with specific carbohydrate-binding activities that are capable of agglutinating red blood cells and producing toxic effects and growth inhibition in experimental animals.

Nutraceutical Food or food component able to produce health or medical benefits, including the prevention and treatment of disease.

Peroxisome proliferator–activated receptor (PPAR) Ligand-activated nuclear membrane-associated transcription factor of the nuclear receptor superfamily.

Phytic acid Myo-inositol hexakis phosphate. Main phosphorus storage compound present in legumes and cereals.

REFERENCES

Bakkenist, C.J., Kastan, M.B., 2003. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421 (6922), 499–506.

Blum, S.C., et al., 2003. Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. Journal of Nutrition 133 (5), 1244–1249.

Boué, S.M., et al., 2011. Estrogenic and antiestrogenic activities of phytoalexins from red kidney bean (*Phaseolus vulgaris* L). Journal of Agricultural and Food Chemistry 59 (1), 112–120.

Caccialupi, P., et al., 2010. Bowman–Birk inhibitors in lentil: heterologous expression, functional characterization and antiproliferative properties in human colon cancer cells. Food Chemistry 120 (4), 1058–1066.

Cadet, J., et al., 2004. Radiation-induced DNA damage: formation, measurement, and biochemical features. Journal of Environmental Pathology, Toxicology, and Oncology 23 (1), 33–43.

Canman, C.E., et al., 1998. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science 281 (5383), 1677–1679.

Cheng, W.H., et al., 2008. WRN is required for ATM activation and the S-phase checkpoint in response to interstrand cross-link-induced DNA double-strand breaks. Molecular Biology of the Cell 19 (9), 3923–3933.

Darmawan, R., Bringe, N.A., de Mejia, E.G., 2010. Antioxidant capacity of alcalase hydrolysates and protein profiles of two conventional and seven low glycinin soybean cultivars. Plant Foods for Human Nutrition 65 (3), 233–240.

Dia, V.P., Berhow, M.A., de Mejia, E.G., 2008. Bowman–Birk inhibitor and genistein among soy compounds that synergistically inhibit nitric oxide and prostaglandin E2 pathways in lipopolysaccharide-induced macrophages. Journal of Agricultural and Food Chemistry 56 (24), 11707–11717.

Doblado, R., Frias, J., Vidal-Valverde, C., 2007. Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. *carrilla*) seeds induced by high pressure treatment. Food Chemistry 101 (3), 918–923.

Dong, M., He, X., Liu, R.H., 2007. Phytochemicals of black bean seed coats: isolation, structure elucidation, and their antiproliferative and antioxidative activities. Journal of Agricultural and Food Chemistry 55 (15), 6044–6051.

Duranti, M., 2006. Grain legume proteins and nutraceutical properties. Fitoterapia 77 (2), 67–82.

Fang, S., et al., 2010. Anticancer effects of flavonoid derivatives isolated from *Millettia reticulata* Benth in SK-Hep-1 human hepatocellular carcinoma cells. Journal of Agricultural and Food Chemistry 58 (2), 814–820.

Henry-Vitrac, C., et al., 2010. Soy isoflavones as potential inhibitors of Alzheimer β-amyloid fibril aggregation *in vitro*. Food Research International 43 (8), 2176–2178.

Huang, C., et al., 2010. Anti-photoaging effects of soy isoflavone extract (aglycone and acetyl glucoside form) from soybean cake. International Journal of Molecular Sciences 11 (12), 4782–4795.

Iliakis, G., et al., 2003. DNA damage checkpoint control in cells exposed to ionizing radiation. Oncogene 22 (37), 5834–5847.

Ishizuka, S., et al., 2011. A partially degraded product of phytate suppresses the proliferation of HCT116 colorectal cancer cells. Food Chemistry 125 (4), 1219–1225.

Jang, H., et al., 2010. Anthocyanin extracted from black soybean reduces prostate weight and promotes apoptosis in the prostatic hyperplasia-induced rat model. Journal of Agricultural and Food Chemistry 58 (24), 12686–12691.

Kaneko, Y., et al., 1999. Cell cycle-dependent and ATM-independent expression of human Chk1 kinase. Oncogene 18 (25), 3673–3681.

Kathe, S.D., et al., 2004. Single-stranded breaks in DNA but not oxidative DNA base damages block transcriptional elongation by RNA polymerase II in HeLa cell nuclear extracts. Journal of Biological Chemistry 279 (18), 18511–18520.

Katyal, S., McKinnon, P.J., 2008. DNA strand breaks, neurodegeneration and aging in the brain. Mechanisms of Ageing and Development 129 (7–8), 483–491.

Kim, N.Y., et al., 2008. Antioxidant and antigenotoxic activities of Korean fermented soybean. Food and Chemical Toxicology 46 (3), 1184–1189.

Kritchevsky, D., et al., 1982. Atherogenicity of animal and vegetable protein. Influence of the lysine to arginine ratio. Atherosclerosis 41 (2–3), 429–431.

Lam, K.S, Ng, T.B., 2011. Apoptosis of human breast cancer cells induced by hemagglutinin from *Phaseolus vulgaris* cv. *Legumi sechi*. Food Chemistry 126 (2), 595–602.

Li, Y., Mezei, O., Shay, N.F., 2007. Human and murine hepatic sterol-12-α-hydroxylase and other xeno-biotic metabolism mRNA are upregulated by soy isoflavones. Journal of Nutrition 137 (7), 1705–1712.

Lindahl, T., 1993. Instability and decay of the primary structure of DNA. Nature 362 (6422), 709–715.

Maya, R., et al., 2001. ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. Genes & Development 15 (9), 1067–1077.

McKinnon, P.J., Caldecott, K.W., 2007. DNA strand break repair and human genetic disease. Annual Review of Genomics and Human Genetics 8, 37–55.

Messina, M.J., 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. American Journal of Clinical Nutrition 70 (Suppl 3), 439S–450S.

Mezei, O., et al., 2003. Soy isoflavones exert antidiabetic and hypolipidemic effecs through the PPAR path-ways in obese Zucker rats and murine RAW 264.7 cells. Journal of Nutrition 133 (5), 1238–1243.

Nagaoka, S., et al., 1997. Serum cholesterol reduction and cholesterol absorption inhibition in caco-2 cells by a soyprotein pectic hydrolyzate. Biosciences, Biotechnology, and Biochemistry 61 (2), 354–356.

Nilsson, J., Stegmark, R., Akesson, B., 2004. Total antioxidant capacity in different pea (*Pisum sativum*) varieties after blanching and freezing. Food Chemistry 86 (4), 501–507.

Olson, E., et al., 2007. The mre11-Rad50-Nbs1 complex acts both upstream and downstream of ataxia telangiectasia mutated and Rad3-related protein (ATR) to regulate the S-phase checkpoint following UV treatment. Journal of Biological Chemistry 282 (31), 22939–22952.

Peluso, M., et al., 2008. Bulky DNA adducts, 4-aminobiphenyl-haemoglobin adducts and diet in the European prospective investigation into cancer and nutrition (EPIC) prospective study. British Journal of Nutrition 100 (3), 489–495.

Porres, J.M., et al., 1999. Dietary intrinsic phytate protects colon from lipid peroxidation in pigs with a mod-erately high dietary iron intake. Proceedings of the Society for Experimental Biology and Medicine 221 (1), 80–86.

Rochfort, S., Panozzo, J., 2007. Phytochemicals for health, the role of pulses. Journal of Agricultural and Food Chemistry 55 (20), 7981–7994.

Ronis, M.J., et al., 2009. Dietary soy protein attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. Journal of Nutrition 139 (8), 1431–1438.

Sancar, A., et al., 2004. Molecular mechanisms of mammalian DNA repair and the DNA damage check-points. Annual Review of Biochemistry 73, 39–85.

Sáyado-Ayerdi, S.G., et al., 2005. *In vitro* starch digestibility and predicted glycemic index of corn tortilla, black beans, and tortilla–bean mixture: effect of cold storage. Journal of Agricultural and Food Chemistry 53 (4), 1281–1285.

Setchell, K.D.R., Brown, N.M., Lydeking-Olsen, E., 2002. The clinical importance of the metabolite equol – a clue to the effectiveness of soy and its isoflavones. Journal of Nutrition 132 (12), 3577–3584.

Shaham, J., et al., 2003. DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occupational and Environmental Medicine 60 (6), 403–409.

Sugano, M., et al., 1982. Effects of arginine and lysine addition to casein and soya-bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. British Journal of Nutrition 48 (2), 211–221.

Tachibana, N., et al., 2005. Intake of soy protein isolate alters hepatic gene expression in rats. Journal of Agricultural and Food Chemistry 53 (10), 4253–4257.

Tamuru, S., et al., 2007. Effects of dietary soybean peptides on hepatic production of ketone bodies and secretion of triglyceride by perfused rat liver. Bioscience, Biotechnology, and Biochemistry 71 (10), 2451–2457.

Thavarajah, D., Ruszkowski, J., Vandenberg, A., 2008. High potential for Se biofortification of lentils (*Lens culinaris*). Journal of Agricultural and Food Chemistry 56 (22), 10747–10753.

Wu, C., et al., 2001. Inhibitory effects of *Cassia tora* L. on benzo[a]pyrene-mediated DNA damage toward HepG2 cells. Journal of Agricultural and Food Chemistry 49 (5), 2579–2586.

Xiao, C.W., 2008. Health effects of soy protein and isoflavones in humans. Journal of Nutrition 138 (6), 1244S–1249S.

Xiao, C.W., et al., 2007. Dietary soy protein isolate modifies hepatic retinoic acid receptor-β proteins and inhibits their DNA binding activity in rats. Journal of Nutrition 137 (1), 1–6.

Yazdi, P.T., et al., 2002. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes & Development 16 (5), 571–582.

Yoshie-Stark, Y., Wäsche, A., 2004. *In vitro* binding of bile acids by lupin protein isolates and their hydrolysates. Food Chemistry 88 (2), 179–184.

Zhou, X.Y., et al., 2002. An ATM-independent S-phase checkpoint response involves CHK1 pathway. Cancer Research 62 (6), 1598–1603.

FURTHER READING

Amarowicz, R., et al., 2010. Free-radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). Food Chemistry 121 (3), 705–711.

Diaz-Batalla, L., et al., 2006. Chemical components with health implications in wild and cultivated mexican common bean seeds (*Phaseolus vulgaris* L). Journal of Agricultural and Food Chemistry 54 (6), 2045–2052.

Dizdaroglu, M., 1992. Oxidative damage to DNA in mammalian chromatin. Mutation Research 275 (3–6), 331–342.

Graves, P.R., et al., 2000. The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. Journal of Biological Chemistry 275 (8), 5600–5605.

Hegde, M.L., et al., 2008. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Research 18 (1), 27–47.

Hoeijmakers, J.H.J., 2001. Genome maintenance mechanisms for preventing cancer. Nature 411 (6835), 366–374.

Hurley, P.J., et al., 2007. Human cancer cells require ATR for cell cycle progression following exposure to ionizing radiation. Oncogene 26 (18), 2535–2542.

Kim, W.H., et al., 2000. Induction of p53-independent p21 during ceramide-induced G1 arrest in human hepatocarcinoma cells. Biochemistry and Cell Biology 78 (2), 127–135.

Kim, S.T., et al., 2002. Involvement of the cohesin protein, Smc1, in ATM-dependent and independent responses to DNA damage. Genes & Development 16 (5), 560–570.

Kuzminov, A., 2001. Single-strand interruptions in replicating chromosomes cause double-strand breaks. Proceedings of the National Academy of Sciences of the United States of America 98 (15), 8241–8246.

Scarafoni, A., Magni, C., Duranti, M., 2007. Molecular nutraceuticals as a mean to investigate the positive effects of legume seed proteins on human health. Trends in Food Science and Technology 18 (9), 454–463.

Shieh, S.Y., et al., 1997. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91 (3), 325–334.

Wang, K., et al., 2005. Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. Journal of Agricultural and Food Chemistry 53 (2), 242–246.

Weinberg, E.D., 1996. The role of iron in cancer. European Journal of Cancer Prevention 5 (1), 19–36.

Xu, B., Chang, S.K.C., 2009. Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. Journal of Agricultural and Food Chemistry 57 (22), 10718–10731.

Minerals and Older Adults

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1. INTRODUCTION

Sufficient dietary intake of minerals plays an important role in health maintenance and disease prevention. Many chronic diseases frequently seen in older adults are directly affected by mineral intake, including calcium and osteoporosis, iron and anemia, and magnesium and diabetes.

Older adults are at risk for mineral deficiencies due to inadequate intake, the cause of which is often multifactorial. Physical and financial restrictions often limit the ability to purchase and prepare healthful foods; reliance on easy-to-prepare and convenience items results in a decreased intake of fresh, nutrient-dense foods. Difficulty in chewing and swallowing are also common problems, as are sensory losses, including taste and smell. Polypharmacy resulting in early satiety, dry mouth, or gastrointestinal (GI) symptoms also limits the consumption of a healthy diet. Many older adults also have one or more chronic conditions that may alter the digestion, absorption, utilization, and secretion of minerals.

2. CALCIUM

Calcium is the most abundant mineral in the human body, largely found in bones and stored as hydroxyapatite, which accounts for 99% of total body calcium. The remaining calcium is located in the serum and soft tissues. Serum calcium is found in three forms: ionized, in complex with other nonprotein anions such as phosphate, or protein-bound, primarily with albumin. The ionized form of calcium is the most physiologically active, and is thus the most accurate measurement of serum calcium abnormalities. In addition to forming the structure for bones and teeth, calcium is also necessary for the adequate functioning of the endocrine, neurological, muscular, and cardiovascular systems (Clark, 2007).

Calcium is absorbed in the small intestine; absorption is reduced in the presence of low vitamin D and estrogen levels, and increased gastric pH, or hypochlorhydria (Straub, 2007). Each of these factors is prevalent in the elderly population. Vitamin D deficiency in recent years has been recognized as a widespread problem, and seniors are at particular risk, especially those who are institutionalized or who otherwise have limited exposure to sunlight (Holick et al., 2005; Lips et al., 2001). Hypochlorhydria

is also a common occurrence in the aged population (Bhutto and Morleya, 2008). Age is an important factor in calcium absorption; in young children, absorption rates can be as high as 60%, but decline to 15–20% in adulthood and continue to drop with increasing age (NIH, 2009a).

Calcium is excreted in the urine; excretion may be accelerated by increased intakes of caffeine, sodium, and protein. In a study of over 3000 subjects, Taylor and Curhan (2009) found that caffeine intake was positively associated with urinary calcium excretion. Similarly, high sodium intake has been linked with increased urinary calcium excretion (Teucher et al., 2008), estimated by Zarkadas et al. (1989) at 40 mg of calcium lost for every 2.3 g of sodium consumed. While protein intake has been widely reported to increase calcium excretion, evidence also suggests that increased protein intake also enhances calcium absorption. Hunt et al. (2009), in a study on postmenopausal women, report that the increase in calcium absorption nearly compensated for the increase in calcium excretion, when protein intake was increased from 10% to 20% of total calories.

2.1 Osteoporosis

Osteoporosis is the most common bone disease in the United States, affecting over 10 million Americans, and an additional 34 million people have reduced bone mass, putting them at significant risk for the disease (NIH, 2009a). In addition to a diet low in calcium and vitamin D, risk factors include female gender, older age, family history, smaller body frame, low levels of estrogen in women and testosterone in men, inactivity, smoking, alcohol abuse, and use of corticosteroid medications (NIH, 2009a). Osteoporosis is diagnosed via measurement of bone mineral density (BMD) or via the presence of a fragility fracture. The primary treatment goal is the prevention of fractures, which includes slowing bone loss or increasing bone density; calcium supplementation is a necessary component of treatment in those who cannot maintain adequate intake of calcium via diet alone (NAMS, 2010).

In a meta–analysis comparing postmenopausal women receiving calcium supplementation with those consuming a placebo, Shea et al. (2002) demonstrated a small but significant reduction in bone loss rates in those subjects consuming the supplement. Calcium supplements included calcium carbonate, citrate, gluconate, citrate malate, and lactate, and doses were at least 400 mg per day. Calcium supplementation's effect on fractures was also examined; the authors noted a trend toward lower rates of vertebral fractures, but concluded that more long-term studies were necessary. Increases in BMD have also been demonstrated in nonosteoporotic older men supplemented with 1200 mg per day of calcium for 2 years; the authors found no such improvements in BMD with calcium supplementation of 600 mg per day (Reid et al., 2008).

Fracture risk and calcium supplementation have also been studied. In a review of nine studies, Parikh et al. (2009) found a significant reduction in fracture risk and an increase in

BMD in those nursing home residents who were supplemented with 1200 mg calcium and 800 IU of vitamin D. However, in a Women's Health Initiative (WHI) study of over 36,000 women researchers studied calcium supplementation of 1000 mg per day and vitamin D supplementation of 400 IU per day and found a small, insignificant reduction in risk of hip fracture, although a small but significant increase in BMD was seen (Jackson et al., 2006). The authors postulated that a significant reduction in fractures was not seen because the dose of vitamin D was inadequate. Several other studies have demonstrated similar results in postmenopausal women receiving calcium and vitamin D supplementation (Grant et al., 2005; Larsen et al., 2004; Porthouse et al., 2005). While calcium supplementation results in small but significant increases in BMD, its effect on fracture risk is less clear.

2.2 Cardiovascular Health

It has been suggested that increased calcium intake may reduce blood pressure, although research results are mixed. In a meta-analysis from 1996, Allender et al. (1996) showed a positive effect of calcium intake on blood pressure, but concluded, that the effect was too small to support the recommendation of calcium supplementation to reduce blood pressure. In another meta-analysis, Bucher et al. (1996) concluded that increased calcium intake resulted in a small decrease in systolic, but not diastolic blood pressure. Similarly, authors of a systematic review in 2006 concluded that the relationship between calcium and blood pressure was weak at best, and that better-quality studies of longer duration were needed to fully elucidate the effect of calcium on blood pressure (Dickinson et al., 2006a). A more recent randomized, double-blind study from the WHI on over 36,000 women concluded that 1000 mg calcium plus 400 IU vitamin D supplementation did not reduce blood pressure or the risk of developing hypertension (Margolis et al., 2008).

Dietary calcium intake in relation to stroke has also been studied. The WHI study showed no effect on coronary or cerebrovascular risk with the supplementation of 1000 mg of calcium and 400 IU of vitamin D over a 7-year period (Hsia et al., 2007). Limitations of the study include the participants' adherence to the supplement regimen, a potentially inadequate dose of vitamin D, and the fact that the trial was designed to examine the effect of calcium supplementation on risk of bone fracture. In a review of observational, experimental, and clinical studies, Ding and Mozaffarian (2006) concluded that the data on calcium and stroke risk were too inconclusive to support the use of calcium supplements for the prevention of stroke. However, in a 13-year study on over 41,000 Japanese men aged 40–59, calcium intake was inversely associated with incidence of stroke. The authors found no relationship between calcium intake and incidence of coronary heart disease (Umesawa et al., 2008). Like hypertension, calcium's effect on cerebrovascular risk is unclear; calcium supplementation may be beneficial in some groups, but not others. Further research in this area is needed.

2.3 Cancer

In several observational studies, higher calcium intake was associated with reduced risk of colorectal cancer (Flood et al., 2005; McCullough et al., 2003; Terry et al., 2002). In an analysis of cohort studies, Cho et al. (2004) concluded that an increase in calcium intake via supplementation reduced colon cancer risk by 10–15%. In a large study of nearly 88,000 women and over 47,000 men, higher calcium intake (>1200 vs. <500 mg per day) was found to be significantly associated with a lower incidence of proximal colon cancer (Wu et al., 2002). An earlier study, however, did not show these beneficial effects (Bergsma-Kadijk et al., 1996). In the study of over 36,000 WHI subjects supplemented with calcium and vitamin D, no effect on the incidence of colorectal cancer was demonstrated. The authors speculate that it is possible that no positive results were seen because the 400 IU dose of vitamin D given was inadequate, or the study time frame of 7 years was not long enough to see positive effects (Wactawski-Wende et al., 2006). Further research is needed to elucidate the relationship between calcium intake and colorectal cancer risk.

Calcium intake has been implicated as a risk factor for prostate cancer, although research results are mixed. In one prospective study of over 29,000 men between the ages of 55 and 74, researchers discovered a modest association between calcium intake and low-fat dairy product intake and nonaggressive prostate cancer (Ahn et al., 2007). Other studies have found similar results (Kesse et al., 2006; Tseng et al., 2005). However, in a large meta-analysis examining the results from 45 observational studies, Huncharek et al. (2008) concluded that there was no association between intake of dairy products and prostate cancer risk. As yet, data remain inconclusive, and there are no recommendations at this time to limit calcium or dairy products in men to reduce the risk of prostate cancer.

2.4 Weight Management

Recent studies have indicated that calcium supplementation can perhaps help with weight control. In a retrospective study, Gonzalez et al. (2006) examined over 10,000 men and women between the ages of 53 and 57, and used linear regression to assess calcium's effect on weight changes, taking into account energy intake and physical activity. Researchers found an inverse relationship with calcium supplementation and weight gain over 10 years in women only. However, in a randomized controlled trial, Yanovski et al. (2009) found no benefit in supplementation of 1500 mg of calcium for 2 years for overweight and obese adults. In another study, Wagner et al. (2007) put overweight or obese premenopausal women on a calorie-restricted diet and physical activity regimen for 12 weeks; then subjects were randomized to receive either 800 mg of calcium phosphate, 800 mg of calcium lactate, 1% milk, or a placebo. The researchers concluded that there were no statistically significant differences in weight loss among any of the groups. While some study results are intriguing, further research is needed to

Table 25.1 RDA for Select Minerals in Men and Women over the Age of 50 years

Mineral	RDA men	RDA women
Calcium	1000 mg per day	1200 mg per day
Iron	8 mg per day	8 mg per day
Magnesium	420 mg per day	320 mg per day
Zinc	11 mg per day	8 mg per day
Selenium	55 μg per day	55 μg per day

determine which populations, if any, may benefit from calcium supplementation for weight management.

2.5 Supplementation

The recommended dietary allowance (RDA) for calcium is 1200 mg for men and women over the age of 50 (USDA, 2010) (see Table 25.1). Calcium intake declines with age; in Americans over the age of 60, calcium intake is reported to be only 80% and 55% of estimated needs for men and women, respectively (Ervin et al., 2004).

Indications for calcium supplementation include suboptimal calcium intake, presence of osteoporosis or osteopenia, chronic corticosteroid therapy, and menopause (Straub, 2007). Vegans and those with lactose intolerance are most likely to have diets deficient in calcium (Craig, 2009). It should be noted that concurrent vitamin D supplementation is also necessary in those individuals with vitamin D deficiency.

The most common form of calcium supplements are calcium carbonate and calcium citrate, although calcium lactate, gluconate, citrate malate, and phosphate are also available. Calcium lactate and gluconate are lower in elemental calcium and are thus not desirable forms for supplementation (Straub, 2007). Calcium carbonate and citrate are readily absorbed, although calcium carbonate requires an acidic environment, and thus is better taken with food (Heller et al., 2000). Medications reducing stomach acidity, such as proton-pump inhibitors, may also reduce calcium absorption, although evidence is mixed (Hansen et al., 2010). As hypochlorhydria is common in the elderly, calcium citrate may be the preferred form of calcium supplementation in these populations.

Calcium supplements come in the form of tablets, chewables, powders, capsules, and liquids. Cost is a consideration for many older adults; calcium carbonate is generally the cheapest form, and also provides the most elemental calcium by weight. Calcium carbonate is 60% elemental calcium, whereas calcium citrate is 21% elemental calcium, which therefore requires more tablets to meet supplementation dosage goals (Straub, 2007).

3. IRON

Iron is a common mineral found in cytochromes in all cells of the body, and participates in cellular energy production. Iron is also a component of red blood cells, necessary for oxygen transport. The total amount of iron in the body varies from about 2 to 4 g, with

Table 25.2 Dietary Sources of Selected Minerals

Mineral	Dietary sources
Calcium	Milk, cheese, yogurt, calcium fortified orange juice, sardines with bones, pudding, fortified cereals, instant breakfast drink, spinach, kale, turnip greens
Iron	Liver, meat, fish, oysters, clams, poultry, fortified cereals, spinach, beans, molasses, tofu
Magnesium	Nuts, seeds, beans, peas, unrefined grains, fortified cereals, spinach, potato w/skin, yogurt
Zinc	Oysters, meat, poultry, lobster, crab, fortified cereals, beans, nuts, unrefined grains
Selenium	Fish, shellfish, red meat, chicken, eggs, milk, fortified cereals

differences dependent on gender, age, body size, and nutritional status; women generally have less iron than men. Iron is stored either bound to transferrin in the blood, as ferritin in intestinal cells, or as myoglobin in muscle cells (Clark, 2007).

Iron is found in its heme form in meat, poultry, and fish, and in its nonheme form primarily in plant foods (see Table 25.2). Heme iron is more readily absorbed in the GI tract because, unlike nonheme iron, it does not require conversion to its ferrous form prior to absorption. Absorption of heme iron is about 15–35%, while nonheme iron absorption can vary from 2% to 20% (NIH, 2007). Absorption is dependent on iron status; iron deficiency anemia (IDA) results in an increase in enterocyte transferrin receptors, thus enhancing iron absorption. Acidity aids in maintaining iron in its ferrous form; ingestion of foods containing compounds such as vitamin C, and aspartic and glutamic acids increases acidity and thus helps in nonheme absorption. Poor iron absorption is seen in malabsorptive conditions such as short bowel syndrome, celiac disease, and inflammatory bowel disease (Clark, 2007; Zhu et al., 2010). Presence of hypochlorhydria, common in the aged, use of gastric acid–reducing medications such as proton–pump inhibitors, and the absence of gastric acid secretion as seen with gastrectomy patients may also limit iron absorption (Bhutto and Morleya, 2008; Mimura et al., 2008).

3.1 Iron Deficiency Anemia

The World Health Organization considers IDA the most prevalent nutritional deficiency, affecting as much as 30% of the world's population (Stoltzfus, 2001). It has been estimated that IDA accounts for about 16% of all cases of anemia in the United States (Guralnik et al., 2004). Symptoms include tachycardia, fatigue and pallor, reduced mental performance, reduced resistance to infection, and impaired thermoregulation. The etiology of IDA is often multifactorial; causes include insufficient intake, impaired absorption, or blood loss (Clark, 2007; Coban et al., 2003). Individuals with chronic kidney disease undergoing hemodialysis are at increased risk for IDA, as they are unable to produce sufficient amounts of erythropoietin, necessary for the formation of red blood cells, and because of blood lost during the hemodialysis process, which can result in a loss of up

to 3 g of iron per year (Kalantar–Zadeh et al., 2009). A higher incidence of IDA has also been associated with *Helicobacter pylori* infection (Qu et al., 2010).

IDA is a microcytic, hypochromic anemia, although in the early stages of deficiency, red blood cells may still be normocytic (Clark, 2007). Diagnosis therefore is often determined by a decrease in hemoglobin and mean corpuscular volume (MCV), as well as low serum iron, low transferrin, low ferritin, and elevated total iron-binding capacity (Zhu et al., 2010). Diagnosis of iron deficiency may be problematic in the elderly population, as serum ferritin has been shown to increase with age; Rimon et al. (2002) demonstrated that serum iron, ferritin, and transferrin saturation were poorly sensitive to capturing iron deficiency in patients over the age of 80. The transferrin receptor–ferritin index or ratio has been shown to be a more sensitive test in diagnosing iron deficiency, and should be considered when screening elderly patients (Rimon et al., 2002). An increase in the red cell distribution width has also been proposed as a sensitive indicator of IDA (Aulakh et al., 2009). The patient's medical and nutritional history should also be considered in the diagnostic process, as well as the level of inflammation; in acute inflammatory conditions, serum iron levels are low and ferritin levels are elevated.

3.2 Supplementation

The RDA for iron is 8 mg for adults over the age of 50 (USDA, 2010). Supplementation is necessary with symptomatic IDA, and should be considered if dietary intake is insufficient to meet estimated needs. Supplemental iron comes in several forms, including ferrous gluconate, sulfate, and fumarate. It should be noted that iron absorption may be inhibited by phytic acid found in grains, oxalic acid found in spinach, tea, and chocolate, and polyphenols in coffee and tea, and so should not be taken with these foods and beverages (Aulakh et al., 2009; Clark, 2007). Iron absorption can also be inhibited by other minerals, so iron supplements should be taken separately from calcium and multivitamin supplements. In fact, most manufacturers recommend taking iron on an empty stomach, at least 1 h before and 2 h after eating; however, some common GI side effects such as nausea may be reduced by taking the supplement with meals.

The recommended dose for supplementation is 150–200 mg of elemental iron per day, typically achieved via the consumption of 325 mg of ferrous sulfate or ferrous gluconate 3 times per day. The sulfate form contains more elemental iron than the gluconate form, 60 vs. 36 mg per tablet, and thus may be preferred (Aulakh et al., 2009). Iron should be given in divided doses, as absorption decreases when ingested iron increases (NIH, 2007). Common side effects include nausea and constipation.

Parenteral iron can also be given in the event that deficiency does not improve with oral supplementation; parenteral forms are iron dextran, sodium ferric gluconate, and iron sucrose, with the latter two being associated with fewer side effects than iron dextran (Clark, 2007).

Iron should be given with caution to critically ill patients as supplemented iron can contribute to microbial growth and oxidative reactions (Clark, 2007). However, in a recent randomized double-blind trial, Pieracci et al. (2009) supplemented anemic, critically ill patients with 325 mg of ferrous sulfate three times daily; there was no difference between the iron supplemented group and the placebo group in antibiotic days, rate of infection, length of stay, and mortality rate.

4. MAGNESIUM

Total body magnesium is approximately 25 g, 50–60% of which is found in the bone; the rest is primarily in the intracellular fluid, with small amounts in the blood serum. Serum magnesium is protein-bound or in an ionized form, and small amounts are complexed with phosphate, citrate, and other compounds. Magnesium is necessary for over 300 biochemical reactions, including those associated with protein, glucose, and DNA metabolism, as well as neuromuscular transmissions, muscle contraction, and cardiovascular excitability (Langley, 2007). Magnesium is also critical in the production of parathyroid hormone, and plays an important role in calcium homeostasis and vitamin D production.

Magnesium absorption occurs in the jejunum and ileum, and about 30–50% of dietary magnesium is absorbed. Absorption may be impeded in diets high in fiber, oxalate, phytate, and phosphate, as magnesium may become bound to these substances. Magnesium is excreted in the kidneys, which are the primary organs that maintain normal serum magnesium levels (Rude, 1998).

4.1 Cardiovascular Health

Some studies have indicated that diets rich in magnesium may reduce the incidence of hypertension; many of these studies investigated the DASH diet (dietary approaches to stop hypertension). While high in magnesium, this diet is also high in potassium, calcium, and fiber, and low in sodium, and thus it is difficult to determine to what extent magnesium independently played a role in the successful reduction of blood pressure in these trails (NIH, 2009b). Similarly, Song et al. (2006b) in a prospective study of over 28,000 women over the age of 45 found that dietary magnesium intake was inversely associated with the risk of developing hypertension.

The ability of magnesium supplementation to reduce blood pressure has also been studied. In a meta-analysis of 12 studies of 545 hypertensive subjects receiving magnesium supplements, Dickinson et al. (2006b) found a significant reduction in diastolic blood pressure, but not systolic blood pressure. The authors concluded that the association between magnesium and blood pressure was weak, and likely due to bias because of the poor quality and heterogeneity of the studies. However, in another meta-analysis involving 1220 normotensive and hypertensive participants, Jee et al. (2002) concluded that there was a dose-dependent decrease in blood pressure with magnesium supplementation.

Some observational research has linked higher serum magnesium levels with lower incidence of coronary heart disease, and higher magnesium intake with reduced occurrence of strokes (Ascherio et al., 1998; Ford, 1999). Several other studies on magnesium supplementation have shown encouraging results; however, these trials are small and thus definitive conclusions cannot be drawn regarding magnesium and the risk of coronary heart disease and stroke (NIH, 2009b). In the WHI randomized, double-blind, placebo-controlled trial of nearly 40,000 women aged 39–89 years, magnesium intake was estimated using food frequency questionnaires. After a median of 10 years of follow-up, researchers concluded that incidents of myocardial infarction and strokes were not significantly linked with magnesium intake (Song, 2005).

4.2 Osteoporosis

Because of magnesium's role in calcium metabolism, it is thus necessary for the maintenance of bone health. Dietary magnesium supplementation has been shown to suppress bone turnover in both postmenopausal women and young men (Aydin et al., 2010; Dimai et al., 1998). Higher intakes of magnesium have also been associated with increased BMD in the elderly (Tucker et al., 1999). While it is clear that adequate magnesium intake is an important component of bone health maintenance, further research is needed to determine magnesium's exact role in the prevention and treatment of osteoporosis.

4.3 Diabetes

Magnesium has been shown to play an important role in the control of blood glucose levels in people with diabetes, as hypomagnesemia can lead to insulin resistance. In turn, hyperglycemia may lead to magnesium deficiency, as the kidneys lose their ability to retain magnesium (NIH, 2009b). A randomized, double-blind, placebo-controlled study showed that magnesium supplementation for 16 weeks in those subjects with diabetes and hypomagnesemia resulted in improvements in blood glucose and hemoglobin A1C levels (Rodriguez-Moran and Guerrero-Romero, 2003). In a similar study, De Lourdes Lima et al. (1998) provided magnesium supplements to subjects with normal serum magnesium levels and poorly controlled diabetes; while serum magnesium levels increased, there was no corresponding improvement in blood glucose control. This suggests that magnesium supplementation may only exert a positive influence on blood glucose control in the presence of hypomagnesemia.

In addition to blood glucose control in people with diabetes, magnesium may also have an effect on the development of the disease. In a large study of over 85,000 women aged 30–55 and over 42,000 men aged 40–75, subjects' diets were analyzed and followed for 18 and 12 years, for women's and men's groups, respectively. Researchers discovered an inverse relationship between magnesium intake and risk of diabetes (Lopez-Ridaura et al., 2004). However, in a similar study of over 12,000 men and women aged

45–64 years, Kao et al. (1999) found that although there was an inverse relationship between serum magnesium levels and risk for development of diabetes in white participants, this may have been confounded by the effect of diabetes on magnesium metabolism. Additionally, there was no correlation between dietary magnesium intake and development of diabetes (Kao et al., 1999).

While diabetes and magnesium research is mixed, it does appear that correction of magnesium deficiency will help control blood glucose levels in people with diabetes. In a position statement from the American Diabetes Association, the authors do not advocate for routine magnesium supplementation in people with diabetes, although they do acknowledge that micronutrient deficiencies are common in those with poorly controlled blood glucose levels, and that these deficiencies should be treated with a healthful diet and supplementation if necessary (Bantle et al., 2008).

4.4 Supplementation

The RDA for magnesium for adults 31 years and older is 420 and 320 mg per day, for men and women respectively (NIH, 2009b). Good food sources of magnesium include green vegetables, unrefined grains, legumes, nuts, and seeds. Magnesium intake in the United States has been shown to be inadequate, especially in the elderly, African Americans, and Hispanics (Ford and Mokdad, 2003).

Hypomagnesemia can be caused by inadequate intake or absorption, increased renal excretion, or redistribution of magnesium from the extracellular to intracellular fluid. Symptoms include loss of appetite, nausea, vomiting, and weakness; as deficiency progresses, numbness and tingling, muscle cramps, cardiac arrhythmias, and seizures can occur. Because only a small amount of magnesium is found in the extracellular fluid, serum magnesium does not necessarily correlate with total body magnesium stores. Insufficient stores of magnesium may adversely affect bone health, but may not result in any acute symptoms of deficiency. Magnesium deficiency may be caused by medications, including certain diuretic, antineoplastic, and antibiotic medications, which either increase excretion or decrease absorption of magnesium. Medical conditions in which deficiency is common include GI malabsorptive disorders such as short bowel syndrome, inflammatory bowel disease, and celiac disease; alcoholism; and poorly controlled diabetes (NIH, 2009b). Individuals with these conditions and the elderly are at particular risk for magnesium deficiency.

Magnesium can be supplemented parenterally or orally. For acute hypomagnesemia in the acute healthcare setting, parenteral supplementation of magnesium sulfate, in doses of 1–3 g, is the preferred method of replacement, because of poor GI tolerance and a slow onset of action for oral doses. Oral forms of magnesium supplementation include magnesium oxide, carbonate, hydroxide, citrate, lactate, chloride, and sulfate. The amount of elemental magnesium as well as the bioavailability should be considered when choosing a magnesium supplement. Magnesium oxide has the highest amount of elemental magnesium, at 60%, while magnesium sulfate has the lowest, at 10% (NIH, 2009b) (see Table 25.3).

Table 25.3 Recommended Supplement Doses for Select Minerals

Mineral	Supplement	Dose	Duration	Comments
Calcium	Calcium citrate Calcium carbonate (gluconate, lactate, phosphate also available)	Dependent on dietary intake and type of calcium supplement; goal is 1200 mg per day total Most common forms are tablets, chewables, capsules	Indefinitely; until needs can be met with dietary intake alone	Citrate preferred for adults with hypochlorhydria Carbonate contains more elemental calcium (40% vs. 21%)
Iron	Ferrous gluconate Ferrous sulfate	325 mg tablets 3 times daily, or dosed to provide 150–200 mg per day elemental iron Ferrous gluconate contains 60 mg elemental iron, sulfate 36 mg	3 months for repletion; until needs can be met with dietary intake alone; longer in the presence of continued iron losses	Do not take with other mineral supplements or high phytic acid foods, 1 h before and 2 h after meals preferred Tablets should be taken in divided doses
Magnesium	Magnesium oxide, carbonate, hydroxide, citrate, lactate, chloride, or sulfate	Dose dependent on degree of deficiency, current intake from dietary sources, and form of supplement IV: 1–3 g per day magnesium sulfate for treatment of hypomagnesemia	Daily until serum levels are corrected; until needs can be met with dietary intake alone	Magnesium also used as an antacid, laxative, and as a treatment for migraines Use cautiously in renal dysfunction Magnesium oxide, carbonate, and hydroxide have the highest % of elemental magnesium
Zinc	Zinc sulfate Zinc gluconate	Orally: 200–220 mg per day IV: 6–10 mg per day Add 12.2 mg per l of small bowel fluid lost, 17.1 mg per kg of stool or ileostomy output	No established parameters; in the acute care setting, 10–14 days is common Indefinite supplementation for those with chronic losses	Large doses may induce copper and iron deficiency Zinc gluconate may make some antibiotics less effective Zinc sulfate contains more elemental zinc than gluconate (23% vs. 14%)

Continued

Table 25.3 Recommended Supplement Doses for Select Minerals—cont'd

Mineral	Supplement	Dose	Duration	Comments
Selenium	Selenious acid	Orally: 50–200 μg/d; available in tablets or capsules IV: 20–40 μg per day; up to 100 μg per day for deficiency	24–31 days	Oral doses should be taken with food

Oral dosage of magnesium supplements may vary based on the severity of the deficiency, presence of comorbid conditions, and the chosen form of supplement. Caution should be used in those with renal disease, as magnesium is primarily excreted by the kidneys. For the treatment of hypomagnesemia in patients with diabetes, studied doses vary widely, ranging as high as 2.5 g per day of magnesium chloride (Rodriguez-Moran and Guerrero-Romero, 2003). In a meta-analysis of nine trials, the median of the studied doses was 360 mg per day, with the duration of supplementation of 4–16 weeks (Song et al., 2006b). In De Lourdes Lima et al.'s (1998) study on 128 patients with type 2 diabetes, the studied dose which showed positive results was 1000 mg of magnesium oxide per day for 30 days. Ideal magnesium dosage for the treatment or prevention of hypertension is also unclear. In most studies, doses range from approximately 240 to 970 mg per day for 8–26 weeks (Dickinson et al., 2006b; Jee et al., 2002).

5. ZINC

Zinc is a trace mineral found primarily in the intracellular compartment, bound to proteins in virtually all cells of the body (Clark, 2007). Total body zinc is only 2–3 g; as most zinc is distributed in all cells of the body, there is no storage system for the mineral (Haase et al., 2006). Zinc plays a role in immune function, wound healing, protein and DNA synthesis, growth, and the metabolism of over 200 enzymes (Clark, 2007; NIH, 2009c).

Approximately 20–40% of dietary zinc is absorbed; zinc absorption occurs in the small intestine. After absorption, zinc is carried to the liver bound primarily to albumin, thus hypoalbuminemia may impair hepatic release of zinc. Serum zinc levels may also decrease in acute illness and infection, as zinc is redistributed from the blood to the cells, and zinc absorption decreases (Clark, 2007).

5.1 Immunity

The elderly have been shown to have an impaired immune response, and are thus more susceptible to bacterial and viral infections (Rink et al., 1998). Zinc deficiency is also a cause of immune impairment, as zinc is essential in cell proliferation and differentiation,

and the immune system is characterized by rapid cell turnover (Haase et al., 2006). Decreased zinc stores have been frequently documented in the elderly population, as zinc absorption decreases with age (Ervin et al., 2004).

Zinc supplementation's effect on immune status has been studied. Boukaiba et al. (1993) supplemented elderly institutionalized subjects with 20 mg of zinc gluconate for 8 weeks. There was a resultant increase in thymulin levels, a hormone that is a measure of T-cell function. In another study, similar results were seen in elderly subjects supplemented with 30 mg of zinc gluconate (Prasad et al., 1993). Supplementation of 45 mg elemental zinc for 12 months resulted in a decreased incidence of infection in elderly subjects, as well as improvements in parameters of cell-mediated immunity and oxidative stress markers compared to a control group of younger subjects (Prasad et al., 2007).

Zinc and selenium supplementation has also been studied. Girodon et al. (1999) administered 20 mg of zinc sulfide and 100 μg of selenium sulfide to institutionalized elderly subjects for 24 months. Antibody titers after administration of influenza vaccine were measured; results suggest that supplementation of zinc and selenium together improved humoral immunity responses. It should be noted that 79% of the subjects had selenium deficiency and 81% of subjects had zinc deficiency at the beginning of the study. Improvements in immunological parameters with supplementation may occur only in those individuals who are nutrient-deficient.

5.2 Wound Healing

Zinc is necessary for the synthesis of granulation tissue and re-epithelialization, and thus is vital for the wound-healing process. The effect of zinc supplementation on healing has been studied in various types of wounds, including pressure ulcers, burns, and chronic lower-extremity stasis ulcers. It is difficult to draw definitive conclusions because of the heterogeneity of subjects, including age, disease state, type of wound, and zinc status, as well as study size and the differences in study design, such as the type and amount of zinc being investigated. Many studies do not investigate the effects of zinc alone; zinc is often given with a host of other nutrients, including energy, protein, arginine, glutamine, and antioxidant vitamins. Although some studies have demonstrated positive results with patients supplemented with arginine, vitamin C, and zinc, it is impossible to determine to what extent zinc had a role in the improved outcomes (Cereda et al., 2009; Desneves et al., 2005; Heyman et al., 2008). As yet, there is no evidence that routine zinc supplementation facilitates wound healing except in those with zinc deficiency.

5.3 The Common Cold

Zinc is commonly found in many over-the-counter cold remedies, including throat lozenges and nasal sprays. Prasad et al. (2008) studied the effects of lozenges with 13.3 mg of zinc oxide versus a placebo administered every 2–3 h to 50 volunteers with colds while they were awake. There was a resultant decrease in both the duration and severity of cold

symptoms in the zinc lozenge group. However, in a similar study of lozenges and nasal spray containing zinc, no positive effects were seen (Eby and Halcomb, 2006). Of the randomly controlled clinical trials on zinc and the common cold, only half showed positive effects. Because of these inconsistent results, the use of zinc in treating the common cold has not been recommended (Simasek and Blandino, 2008).

5.4 Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness and visual impairment in the United States in individuals 65 years and older. It has been recognized that zinc may play a role in the treatment of AMD, perhaps through its antioxidant properties. In the Age Related Eye Disease Study (2001), researchers supplemented 3640 subjects, aged 55–80 years, with antioxidants (vitamins C and E, and beta carotene), 80 mg of zinc oxide, or both. Results demonstrated that supplementation of both zinc and antioxidants with zinc resulted in a significantly reduced risk of developing advanced AMD in high-risk groups. A reduction in rate of development of moderate visual acuity loss was also seen in the antioxidants plus zinc group. It should be noted that the groups receiving zinc had a significantly higher incidence of hospitalizations for genitourinary conditions (Age Related Eye Disease Study, 2001). In an observational study, Van Leeuwen et al. (2005) discovered that elderly subjects with high dietary intake of zinc and other antioxidants had a reduced risk of AMD, although there is no compelling evidence that supplementation of zinc and antioxidants helps in the prevention of the disease (Evans, 2008).

5.5 Supplementation

The RDA for zinc is 11 and 8 mg per day for adult men and women, respectively. Zinc is found in a wide variety of foods, including meat, poultry, nuts, seeds, beans, whole grains, and fortified cereals; oysters in particular are extremely high in zinc. It should be noted that phytates found in plant foods, such as whole grains, cereals, and legumes, inhibit the absorption of zinc; thus, animal sources of zinc are more readily absorbed (NIH, 2009c). Vegetarians are at greater risk for zinc deficiency, and may need as much as 50% more of the RDA for zinc (IOM, 2001). Other minerals may also significantly decrease zinc absorption, so zinc should not be taken with calcium or other vitamin and mineral supplements (Clark, 2007).

Zinc deficiency may cause diarrhea, fatigue, skin lesions, alopecia, alterations in immune function, taste abnormalities, and impaired wound healing. Zinc status is often difficult to assess, in part because of the changes in zinc metabolism during the acute phase response, and the unreliability of serum zinc as a marker of zinc status (Gibson et al., 2008). Groups at risk for zinc deficiency include those with malabsorptive diseases such as celiac disease, inflammatory bowel disease, and short bowel syndrome; as well as bariatric surgery, renal disease, excessive GI fluid losses such as high-output GI fistulas and diarrhea,

excessive alcohol intake, sickle cell disease, and burns (Clark, 2007; NIH, 2009c). The elderly have been shown to have suboptimal intakes of zinc (Ervin et al., 2004).

Zinc supplements, including zinc sulfate and gluconate, come in a variety of forms such as tablets, lozenges, and intravenous doses. Oral doses of zinc are usually 200–220 mg per day, as enteral zinc is not 100% bioavailable. Parenteral zinc is 100% bioavailable; therefore, doses should be limited to 40 mg per day or less. See Table 25.4 for Tolerable Upper Levels established by the Institute of Medicine. Excessive zinc administration may cause nausea and diarrhea, interfere with copper and iron absorption, and impair wound healing (Gray, 2003). There are no specific recommendations regarding an appropriate duration for zinc supplementation; common practice in the acute care setting is to supplement for 10–14 days, then reassess zinc status. It may be necessary, however, to provide long-term zinc supplementation for those who have chronic insufficient dietary intake of zinc, excessive GI fluid losses, or impaired absorption.

6. SELENIUM

Selenium is a trace element that is complexed with proteins, called selenoproteins, which play a role in immune function, thyroid function, and act as antioxidants (NIH, 2009d).

Dietary selenium is primarily in the selenomethionine form, and 90% is absorbed in the duodenum and proximal jejunum. Inorganic forms of selenium are not as readily absorbed. Selenium absorption does not appear to be linked to selenium status; rather excretion is the method in which selenium status is maintained. Selenium is excreted in both the urine and feces (Sriram and Lonchyna, 2009).

6.1 Immunity

In one small study, Broome et al. (2004) supplemented individuals with marginally low serum selenium levels with 50 or 100 µg of sodium selenite for 14 weeks. Subjects demonstrated an improved selenium status with supplementation and an increase in cellular immune response to an administered vaccine. Researchers concluded that a dose of 100 µg of sodium selenite was beneficial in optimizing immune function in individuals with suboptimal selenium status. Improvements in humoral immunity in response to a vaccine were demonstrated by Girodon et al. (1999) when elderly subjects were

Table 25.4 Tolerable Upper Levels of Select Minerals for Adults over the Age of 50

Calcium	2000 mg per day
Iron	45 mg per day
Magnesium	350 mg per day
Zinc	40 mg per day
Selenium	400 µg per day

supplemented with 20 mg of zinc sulfide and 100 μg of selenium sulfide. Most subjects had both selenium and zinc deficiency. There is no evidence that supplementation of selenium in the absence of deficiency results in immunologic benefits.

6.2 Cancer

It has been noted that people living in areas with low soil selenium levels have a higher incidence of certain kinds of cancer, including skin cancer (Fleet, 1997). Lower serum selenium levels are associated with higher risk of several cancers, including colorectal, lung, and prostate. It has been hypothesized that selenium may reduce the risk of cancer via its antioxidant protection against free radicals, and also has been shown to slow tumor growth (NIH, 2009d).

In a meta-analysis of studies examining nutritional supplementation and prostate cancer risk, Jiang et al. (2010) concluded that supplementation of selenium did not result in a lower incidence or mortality from prostate cancer. In another study, intake of dietary selenium was not associated with prostate cancer risk (Kristal et al., 2010). However, in work by Penney et al. (2010), poor selenium intake was associated with higher risk of poor-prognosis prostate cancer, but only in men with specific genes that influence the requirement for selenium. Selenium supplementation has also not been shown to reduce the risk of colon cancer (Cooper et al., 2010). Further research is needed to fully understand the role of selenium on cancer risk.

6.3 Supplementation

The RDA for selenium is 55 μg per day for adults. Selenium is found in a variety of foods, including both plant and animal sources, such as fish, shellfish, organ meats, red meat, chicken, eggs, and milk; the amount of selenium from plant sources depends on the amount of selenium in the soil in which the plant grew. Amounts in animal products also vary depending on the amount of selenium in the animal feed (Clark, 2007; NIH, 2009d). Although evidence indicates that selenium intake is marginal in some areas of the world, including parts of China, Northern Europe, New Zealand, and Russia, research suggests that most Americans obtain adequate amounts of selenium via their usual diets (Boosalis, 2008; Combs, 2001).

Individuals at risk for selenium deficiency include those with suboptimal dietary selenium intake, generally as a result of living in a geographic area with low soil selenium levels, and severe malabsorptive GI diseases (NIH, 2009d). Selenium deficiency has also been reported in individuals receiving long-term parenteral nutrition without selenium, and in patients with high-output chylous fistula losses (Clark, 2007; De Berranger et al., 2006). Selenium deficiency may lead to Keshan's disease, a form of cardiomyopathy, as well as oxidative injury and altered thyroid metabolism. Serum selenium is an indicator of short-term selenium status; depressed levels have been observed in the

acute phase response so this should be taken into consideration when assessing selenium status. Erythrocyte selenium levels may also be measured to assess long-term selenium status (Clark, 2007).

Selenium can be supplemented in its organic form, selenomethionine, or in an inorganic form, such as sodium selenite or sodium selanate; however, because of improved absorption, selenomethionine is generally recognized as the preferred form. There are no specific guidelines regarding the ideal dose or duration of selenium supplementation; however, most commonly studied doses range from 50 to 200 µg d^{-1}. Duration of supplementation should be determined on an individual basis, taking into account selenium intake from other sources and malabsorptive disorders (NIH, 2009d).

7. CONCLUSION

A healthful diet high in a variety of nutrient-dense foods is necessary for older adults to maintain an adequate intake of minerals. However, physical limitations, finances, polypharmacy, and chronic diseases often result in suboptimal intakes of these micronutrients. A thorough nutrition assessment should identify these issues, and proper nutrition treatment, via alterations in food intake or the addition of supplements, can improve mineral levels and thus help treat and prevent many chronic diseases.

REFERENCES

Age-Related Eye Disease Study Research Group, 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Archives of Ophthalmology 119, 1417–1436.

Ahn, J., Albanes, D., Peters, U., et al., 2007. Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiology, Biomarkers and Prevention 16, 2623–2630.

Allender, P.S., Cutler, J.A., Follmann, D., et al., 1996. Dietary calcium and blood pressure. Annals of Internal Medicine 124, 825–831.

Ascherio, A., Rimm, E.B., Hernan, M.A., et al., 1998. Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. Circulation 98, 1198–1204.

Aulakh, R., Sohi, I., Singh, T., Kakkar, N., 2009. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. Indian Journal of Pediatrics 76, 265–268.

Aydin, H., Deyneli, O., Yavuz, D., et al., 2010. Short-term oral magnesium supplementation suppresses bone turnover in postmenopausal osteoporotic women. Biological Trace Element Research 133, 136–143.

Bantle, J.P., Wylie-Rosett, J., Albright, A.L., et al., 2008. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. Diabetes Care 31, S61–S78.

Bergsma-Kadijk, J.A., Van't Veer, P., Kampman, E., Burema, J., 1996. Calcium does not protect against colorectal neoplasia. Epidemiology 7, 590–597.

Bhutto, A., Morleya, J.E., 2008. The clinical significance of gastrointestinal changes with aging. Current Opinion in Clinical Nutrition and Metabolic Care 11, 651–660.

Boosalis, M.G., 2008. The role of selenium in chronic disease. Nutrition in Clinical Practice 23, 152–160.

Boukaiba, N., Flament, C., Acher, S., et al., 1993. A physiological amount of zinc supplementation: effects on nutritional, lipid, and thymic status in an elderly population. American Journal of Clinical Nutrition 57, 566–572.

Broome, C.S., McArdle, F., Kyle, J.A.M., et al., 2004. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. American Journal of Clinical Nutrition 80, 154–162.

Bucher, H.C., Cook, R.J., Guyatt, G.H., et al., 1996. Effects of dietary calcium supplementation on blood pressure. Journal of the American Medical Association 275, 1016–1022.

Cereda, E., Gini, A., Pedrolli, C., Vanotti, A., 2009. Disease-specific, versus standard, nutritional support for the treatment of pressure ulcers in institutionalized older adults: a randomized controlled trial. Journal of American Geriatrics Society 57, 1395–1402.

Cho, E., Smith-Warner, S.A., Spiegelman, D., et al., 2004. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. National Cancer Institute 96, 1015–1022.

Clark, S.F., 2007. Vitamins and trace elements. In: Gottschlich, M.M. (Ed.), The ASPEN Nutrition Support Core Curriculum: A Case-Based Approach – The Adult Patient American Society for Parenteral and Enteral Nutrition. Silver Spring, MD, pp. 130–155.

Coban, E., Timuragaoglu, A., Meric, M., 2003. Iron deficiency anemia in the elderly: prevalence and endoscopic evaluation of the gastrointestinal tract in outpatients. Acta Haematologica 110, 25–28.

Combs Jr., G.F., 2001. Selenium in global food systems. British Journal of Nutrition 85, 517–547.

Cooper, K., Squires, H., Carroll, C., et al., 2010. Chemoprevention of colorectal cancer: systematic review and economic evaluation. Health Technology Assessment 14, 1–206.

Craig, W., 2009. Health effects of vegan diets. American Journal of Clinical Nutrition 89, 1627S–1633S.

De Berranger, E., Colinet, S., Michaud, L., et al., 2006. Severe selenium deficiency secondary to chylous loss. Journal of Parenteral and Enteral Nutrition 30, 173–174.

De Lourdes Lima, M., Cruz, T., Pousada, J.C., Rodrigues, L.E., Barbosa, K., Canguco, V., 1998. The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. Diabetes Care 21, 682–686.

Desneves, K.J., Todorovic, B.E., Cassar, A., Crowe, T.C., 2005. Treatment with supplementary arginine, vitamin C and zinc in patients with pressure ulcers: a randomised controlled trial. Clinical Nutrition 24, 979–987.

Dickinson, H.O., Nicolson, D.J., Cook, J.V., et al., 2006a. Calcium supplementation for the management of primary hypertension in adults. Cochrane Database of Systematic Reviews 19, CD004639.

Dickinson, H.O., Nicolson, D.J., Campbell, F., et al., 2006b. Magnesium supplementation for the management of primary hypertension in adults (review). Cochrane Database of Systematic Reviews 3, CD004640.

Dimai, H.P., Porta, S., Wirnsberger, G., et al., 1998. Daily oral magnesium supplementation suppresses bone turnover in young adult males. Journal of Clinical Endocrinology and Metabolism 83, 2742–2748.

Ding, E.L., Mozaffarian, D., 2006. Optimal dietary habits for the prevention of stroke. Seminars in Neurology 26, 11–23.

Eby, G.A., Halcomb, W.W., 2006. Ineffectiveness of zinc gluconate nasal spray and zinc orotate lozenges in common-cold treatment: a double-blind, placebo-controlled clinical trial. Alternative Therapies in Health and Medicine 12, 34–38.

Ervin, R.B., Wang, C.Y., Wright, J.D., Kennedy-Stephenson, J., 2004. Dietary intake of selected minerals for the United States population: 1999–2000. Advance Data 341, 1–5.

Evans, J., 2008. Antioxidant supplements to prevent or slow down the progression of AMD: a systematic review and meta-analysis. Eye (London, UK) 22, 751–760.

Fleet, J.C., 1997. Dietary selenium repletion may reduce cancer incidence in people at high risk who live in areas with low soil selenium. Nutrition Reviews 55, 277–279.

Flood, A., Peters, U., Chatterjee, N., Lacey Jr, J.V., Schairer, C., Schatzkin, A., 2005. Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. Cancer Epidemiology, Biomarkers and Prevention 14, 126–132.

Ford, E.S., 1999. Serum magnesium and ischaemic heart disease: findings from a national sample of US adults. International Journal of Epidemiology 28, 645–651.

Ford, E.S., Mokdad, A.H., 2003. Dietary magnesium intake in a national sample of U.S. adults. Journal of Nutrition 133, 2879–2882.

Gibson, R.S., Hess, S.Y., Hotz, C., Brown, K.H., 2008. Indicators of zinc status at the population level: a review of the evidence. British Journal of Nutrition 99, S14–S23.

Girodon, F., Galan, P., Monget, A.L., et al., 1999. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: a randomized controlled trial. Archives of Internal Medicine 159, 748–754.

Gonzalez, A.J., White, E., Kristal, A., Littman, A., 2006. Calcium intake and 10-year weight change in middle-aged adults. Journal of the American Dietetic Association 106, 1066–1073.

Grant, A.M., Avenell, A., Campbell, M.K., et al., 2005. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (randomised evaluation of calcium or vitamin D, RECORD): a randomized placebo-controlled trial. Lancet 365, 1621–1628.

Gray, M., 2003. Does oral zinc supplementation promote healing of chronic wounds? Journal of Wound, Ostomy, and Continence Nursing 30, 295–299.

Guralnik, J.M., Eisenstaedt, R.S., Ferrucci, L., Klein, H.G., Woodman, R.C., 2004. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood 104, 2263–2268.

Haase, H., Mocchegiani, E., Rink, L., 2006. Correlation between zinc status and immune function in the elderly. Biogerontology 7, 421–428.

Hansen, K.E., Jones, A.N., Lindstrom, M.J., et al., 2010. Do proton pump inhibitors decrease calcium absorption? Journal of Bone and Mineral Research 25, 2510–2519.

Heller, H.J., Greer, L.G., Haynes, S.D., Poindexter, J.R., Pak, C.Y., 2000. Pharmacokinetic and pharmacodynamic comparison of two calcium supplements in postmenopausal women. Journal of Clinical Pharmacology 40, 1237–1244.

Heyman, H., Van De Looverbosch, D.E., Meijer, E.P., Schols, J.M., 2008. Benefits of an oral nutritional supplement on pressure ulcer healing in long term care residents. Journal of Wound Care 17, 476–478 480.

Holick, M.F., Siris, E.S., Binkley, N., et al., 2005. Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. Journal of Clinical Endocrinology and Metabolism 90, 3215–3224.

Hsia, J., Heiss, G., Ren, H., et al., 2007. Women's Health Initiative Investigators. Calcium/vitamin D supplementation and cardiovascular events. Circulation 115, 846–854.

Huncharek, M., Muscat, J., Kupelnick, B., 2008. Dairy products, dietary calcium and vitamin D intake as risk factors for prostate cancer: a meta-analysis of 26,769 cases from 45 observational studies. Nutrition and Cancer 60, 421–441.

Hunt, J.R., Johnson, L.K., Roughead, Z.K.F., 2009. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. American Journal of Clinical Nutrition 89, 1357–1365.

IOM – Institute of Medicine, Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC.

Jackson, R.D., La Croix, A.Z., Gass, M., et al., 2006. Calcium plus vitamin D supplementation and the risk of fractures. The New England Journal of Medicine 354, 669–683.

Jee, S.H., Miller III, E.R., Guallar, E., Singh, V.K., Appel, L.J., Klag, M.J., 2002. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. American Journal of Hypertension 15, 691–696.

Jiang, L., Yang, K.H., Tian, J.H., et al., 2010. Efficacy of antioxidant vitamins and selenium supplement in prostate cancer prevention: a meta-analysis of randomized controlled trials. Nutrition and Cancer 62, 719–727.

Kalantar-Zadeh, K., Streja, E., Miller, J.E., Nissenson, A.R., 2009. Intravenous iron versus erythropoiesis-stimulating agents: friends or foes in treating chronic kidney disease anemia? Advances in Chronic Kidney Disease 16, 143–151.

Kao, W.H.L., Folsom, A.R., Nieto, F.J., MO, J.P., Watson, R.L., Brancati, F.L., 1999. Serum and dietary magnesium and the risk for type 2 diabetes: the atherosclerosis risk in communities study. Archives of Internal Medicine 159, 2151–2159.

Kesse, E., Bertrais, S., Astorg, P., et al., 2006. Dairy products, calcium and phosphorus intake, and the risk of prostate cancer: results of the French prospective SU.VI.MAX (supplémentation en vitamines et minéraux antioxydants study). British Journal of Nutrition 95, 539–545.

Kristal, A.R., Arnold, K.B., Neuhouser, M.L., et al., 2010. Diet, supplement use, and prostate cancer risk: results from the prostate cancer prevention trial. American Journal of Epidemiology 172, 566–577.

Langley, G., 2007. Vitamins and trace elements. In: Gottschlich, M.M. (Ed.), The ASPEN Nutrition Support Core Curriculum: A Case-Based Approach – The Adult Patient American Society for Parenteral and Enteral Nutrition. Silver Spring, MD, pp. 105–125.

Larsen, E.R., Mosekilde, L., Foldspang, A., 2004. Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. Journal of Bone and Mineral Research 19, 370–378.

Lips, P., Duong, T., Oleksik, A., et al., 2001. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. Journal of Clinical Endocrinology and Metabolism 86, 1212–1221.

Lopez-Ridaura, R., Willett, W.C., Rimm, E.B., et al., 2004. Magnesium intake and risk of type 2 diabetes in men and women. Diabetes Care 27, 134–140.

Margolis, K.L., Ray, R.M., Van Horn, L., et al., 2008. Effect of calcium and vitamin D supplementation on blood pressure: the women's health initiative randomized trial. Hypertension 52, 847–855.

McCullough, M.L., Robertson, A.S., Rodriguez, C., et al., 2003. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the cancer prevention study II nutrition cohort (United States). Cancer Causes and Control 14, 1–12.

Mimura, E.C., Breganó, J.W., Dichi, J.B., Gregório, E.P., Dichi, I., 2008. Comparison of ferrous sulfate and ferrous glycinate chelate for the treatment of iron deficiency anemia in gastrectomized patients. Nutrition 24, 663–668.

NAMS – North American Menopause Society, 2010. Position statement: management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. Menopause 17, 25–54.

NIH – National Institutes of Health, Office of Dietary Supplements, 2007. Dietary supplement fact sheet: iron. http://ods.od.nih.gov/factsheets/iron.asp#h3 (accessed 8 July 2010).

NIH – National Institutes of Health, Office of Dietary Supplements, 2009a. Dietary supplement fact sheet: Calcium. http://ods.od.nih.gov/factsheets/calcium.asp (accessed 1 July 2010).

NIH – National Institutes of Health, Office of Dietary Supplements, 2009b. Dietary supplement fact sheet: magnesium. http://ods.od.nih.gov/factsheets/magnesium.asp (accessed 12 July 2010).

NIH – National Institutes of Health, Office of Dietary Supplements, 2009c. Dietary supplement fact sheet: zinc. http://ods.od.nih.gov/factsheets/Zinc.asp#h2 (accessed 12 July 2010).

NIH – National Institutes of Health, Office of Dietary Supplements, 2009d. Dietary supplement fact sheet: selenium. http://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/ (accessed 12 July 2010).

Parikh, S., Avorn, J., Solomon, D.H., 2009. Pharmacological management of osteoporosis in nursing home populations: a systematic review. Journal of American Geriatrics Society 57, 327–334.

Penney, K.L., Schumacher, F.R., Li, H., et al., 2010. A large prospective study of SEP15 genetic variation, interaction with plasma selenium levels, and prostate cancer risk and survival. Cancer Prevention Research 3, 604–610.

Pieracci, F.M., Henderson, P., Rodney, J.R., et al., 2009. Randomized, double-blind, placebo-controlled trial of effects of enteral iron supplementation on anemia and risk of infection during surgical critical illness. Surgical Infections 10, 9–19.

Porthouse, J., Cockayne, S., King, C., et al., 2005. Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. British Medical Journal 330, 1003.

Prasad, A.S., Fitzgerald, J.T., Hess, J.W., Kaplan, J., Pelen, F., Dardenne, M., 1993. Zinc deficiency in elderly patients. Nutrition 9, 218–224.

Prasad, A.S., Beck, F.W.J., Bao, B., et al., 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. American Journal of Clinical Nutrition 85, 837–844.

Prasad, A.S., Beck, F.W.J., Bao, B., Snell, D., Fitzgerald, J.T., 2008. Duration and severity of symptoms and levels of plasma interleukin-1 receptor antagonist, soluble.tumor necrosis factor receptor, and adhesion

molecules in patients with common cold treated with zinc acetate. Journal of Infectious Diseases 197, 795–802.

Qu, X.H., Huang, X.L., Xiong, P., et al., 2010. Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis. World Journal of Gastroenterology 16, 886–896.

Reid, I.R., Ames, R., Mason, B., et al., 2008. Randomized controlled trial of calcium supplementation in healthy, non-osteoporotic, older men. Archives of Internal Medicine 168, 2276–2282.

Rimon, E., Levy, S., Sapir, A., et al., 2002. Diagnosis of iron deficiency anemia in the elderly by transferrin receptor-ferritin index. Archives of Internal Medicine 162, 445–449.

Rink, L., Cakman, I., Kirchner, H., 1998. Altered cytokine production in the elderly. Mechanisms of Ageing and Development 102, 199–209.

Rodriguez-Moran, M., Guerrero-Romero, F., 2003. Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects. Diabetes Care 26, 1147–1152.

Rude, R.K., 1998. Magnesium deficiency: a cause of heterogeneous disease in humans. Journal of Bone and Mineral Research 13, 749–758.

Shea, B., Wells, G., Cranney, A., et al., 2002. Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis, VII: meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. Endocrine Reviews 23, 552–559.

Simasek, M., Blandino, D.A., 2008. Treatment of the common cold. American Family Physician 75, 515–520.

Song, Y., He, K., Levitan, E.B., Manson, J.E., Liu, S., 2006a. Effects of oral magnesium supplementation on glycaemic control in type 2 diabetes: a meta-analysis of randomized double-blind controlled trials. Diabetic Medicine 23, 1050–1056.

Song, Y., Manson, J.E., Cook, N.R., et al., 2005. Dietary magnesium intake and risk of cardiovascular disease among women. American Journal of Cardiology 96, 1135–1141.

Song, Y., Sesso, H.D., Manson, J.E., Cook, N.R., Buring, J.E., Liu, S., 2006b. Dietary magnesium intake and risk of incident hypertension among middle-aged and older US women in a 10-year follow-up study. The American Journal of Cardiology 98, 1616–1621.

Sriram, K., Lonchyna, V.A., 2009. Micronutrient supplementation in adult nutrition therapy: practical considerations. Journal of Parenteral and Enteral Nutrition 33, 1–15.

Stoltzfus, R.J., 2001. Defining iron-deficiency anemia in public health terms: reexamining the nature and magnitude of the public health problem. Journal of Nutrition 131, 565S–567S.

Straub, D.A., 2007. Calcium supplementation in clinical practice: a review of forms, doses, and indications. Nutrition in Clinical Practice 22, 286–296.

Taylor, E.N., Curhan, G.C., 2009. Demographic, dietary, and urinary factors and 24-h urinary calcium excretion. Clinical Journal of the American Society of Nephrology 4, 1980–1987.

Terry, P., Baron, J.A., Bergkvist, L., Holmberg, L., Wolk, A., 2002. Dietary calcium–vitamin D intake and risk of colorectal cancer: a prospective cohort study in women. Nutrition and Cancer 43, 39–46.

Teucher, B., Dainty, J.R., Spinks, C.A., et al., 2008. Sodium and bone health: impact of moderately high and low salt intakes on calcium metabolism in postmenopausal women. Journal of Bone and Mineral Research 23, 1477–1485.

Tseng, M., Breslow, R.A., Graubard, B.I., Ziegler, R.G., 2005. Dairy, calcium, and vitamin D intakes and prostate cancer risk in the National Health and Nutrition Examination Epidemiologic Follow-up Study cohort. American Journal of Clinical Nutrition 81, 1147–1154.

Tucker, K.L., Hannan, M.T., Chen, H., Cupples, L.A., Wilson, P.W., Kiel, D.P., 1999. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. American Journal of Clinical Nutrition 69, 727–736.

Umesawa, M., Iso, H., Ishihara, J., et al., 2008. Dietary calcium intake and risks of stroke, its subtypes, and coronary heart disease in Japanese: The JPHC Study Cohort I. Stroke 39, 2449–2456.

USDA – United States Department of Agriculture: National Agricultural Library, 2010. Dietary reference intakes: elements. http://www.iom.edu/Object.File/Master/7/294/0.pdf (accessed 5 July 2010).

Van Leeuwen, R., Boekhoorn, S., Vingerling, J.R., et al., 2005. Dietary intake of antioxidants and risk of age-related macular degeneration. Journal of the American Medical Association 294, 3101–3107.

Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., et al., 2006. Calcium plus vitamin D supplementation and the risk of colorectal cancer. The New England Journal of Medicine 354, 684–696.

Wagner, G., Kindrick, S., Hertzler, S., DiSilvestro, R.A., 2007. Effects of various forms of calcium on body weight and bone turnover markers in women participating in a weight loss program. Journal of the American College of Nutrition 26, 456–461.

Wu, K., Willett, W.C., Fuchs, C.S., Colditz, G.A., Giovannucci, E.L., 2002. Calcium intake and risk of colon cancer in women and men. Journal of the National Cancer Institute 94, 437–446.

Yanovski, J.A., Parikh, S.J., Yanoff, L.B., et al., 2009. Effects of calcium supplementation on body weight and adiposity in overweight and obese adults: a randomized trial. Annals of Internal Medicine 150, 821–829.

Zarkadas, M., Gougeon-Reyburn, R., Marliss, E.B., Block, E., Alton-Mackey, M., 1989. Sodium chloride supplementation and urinary calcium excretion in postmenopausal women. American Journal of Clinical Nutrition 50, 1088–1094.

Zhu, A., Kaneshiro, M., Kaunitz, J.D., 2010. Evaluation and treatment of iron deficiency anemia: a gastroenterological perspective. Digestive Diseases and Sciences 55, 548–559.

Nutritional Influences on Bone Health and Overview of Methods

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1. EPIDEMIOLOGIC PERSPECTIVE: OVERVIEW OF OSTEOPOROSIS

Osteoporosis is defined as a 'systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture' (World Health Organization Scientific Group, 2003). The WHO has developed an operational definition of osteoporosis based on bone mineral density (BMD) of young adult Caucasian women (Melton, 2000). Because of insufficient data on the relationship between BMD and fracture risk in men or nonwhite women, the WHO does not offer a definition of osteoporosis for groups other than Caucasian women. Nonetheless, studies have suggested that the cutoff value used in women for spine or hip BMD can also be used to diagnose osteoporosis in men, particularly since the risk of hip or vertebral fracture for a given BMD is similar in men and women (World Health Organization Scientific Group, 2003). The WHO defines osteoporosis as a BMD less than 2.5 standard deviations (SD; also referred to as a *t*-score) below the mean and osteopenia (low bone mass) as a BMD between 1 and 2.5 SD below the mean for young women. A *t*-score of -1 SD below the mean or greater indicates normal BMD. Based on these cutoffs, epidemiologic data from the Third National Health and Nutrition Examination Survey revealed that the incidence of osteopenia was 37–50% and osteoporosis was 13–18% in American women $\geq$50 years of age (Looker et al., 1997). For each SD below the mean, woman's risk of fracture doubles. Peak bone mass is the maximum BMD achieved by early adulthood and is a key determinant of future risk of fracture. Yet, the age at which this occurs differs in various populations and differs with respect to skeletal site.

Osteoporosis is a silent epidemic and a major health threat for an estimated 44 million Americans, including 55% of those $\geq$50 years of age, with 10 million who already have osteoporosis and another 34 million who have osteopenia. Osteoporotic fractures represent a significant public health burden, accounting for 2 million new fractures each year in

the United States alone (Burge et al., 2007) and ∼9 million worldwide (Compston, 2010). Lifetime risk lies within the range of 40–50% in women and 13–22% in men. Given that life expectancy is increasing worldwide, it is estimated that the number of individuals aged 65 years and older will increase by a factor of almost five by the year 2050 (Dennison et al., 2006). The longer life expectancy of women amplifies their disease burden. During the past decade, the age-adjusted incidence of osteoporotic fractures has stabilized in some countries (i.e., Switzerland, Denmark, United States) but has continued to rise in other countries (i.e., Germany, Japan). These data primarily reflect hip fractures, since other fracture types are not well documented in most countries (Compston, 2010). The projected rise in the number of older adults will correspondingly cause the number of hip fractures worldwide to increase from an estimated 1.66 million in 1990 to a projected 6.26 million in 2050 (Cooper et al., 1992). At present, the majority of hip fractures occur in Europe and North America, but enormous increases in the number of elderly in South America, Africa, and Asia will shift this burden of disease from the developed to the developing world (Genant et al., 1999). Effective prevention strategies will need to be designed and disseminated in these parts of the world to prevent the expected increase in hip fractures. By the year 2020, osteoporosis is estimated to cost our society in excess of $100 billion (Compston, 2010).

2. ASSESSMENT METHODS FOR BONE-RELATED OUTCOMES

2.1 BMD: Dual-Energy X-Ray Absorptiometry

The gold standard and most commonly used method to assess BMD in clinical practice is dual-energy x-ray absorptiometry (DXA; Table 26.1). The three most important roles of DXA include the diagnosis of osteoporosis, the assessment of fracture risk, and monitoring the response to treatment. Hip BMD is considered the most reliable measurement site for predicting hip fracture risk, whereas spine BMD is the most useful for monitoring response to treatment. Spine and hip DXA scans for postmenopausal women and older men should be interpreted using the WHO t-score cutoffs for osteopenia, osteoporosis, and established osteoporosis, whereas DXA scans for children and adults less than 50 years should be interpreted using Z-scores. The International Society for Clinical Densitometry (2005) recommends that each DXA technologist conduct a precision assessment and calculate the least significant change for each DXA instrument used and bone site measured. The precision and least significant change values provided by the DXA manufacturer cannot be applied to bone densitometry centers because of patient (participant) population differences and variability in the expertise of the technologist performing the scans. In addition, BMD measurements from different types of machines cannot be directly compared. DXA is considered the reference method for determining axial BMD, since it allows excellent resolution with low radiation doses, has low *in vivo* precision error (0.6–1.5% depending on technician positioning and bone site measured), and

Table 26.1 Assessment Methods for Bone-Related Outcomes

Assessment of bone mineral density

Dual-energy x-ray absorptiometry (DXA) • Humans • Animals	DXA is the gold standard and most commonly used method to assess BMD and fracture risk in clinical practice. BMD is linearly related to fracture risk Regions of interest are most commonly the hip and lumbar spine BMC of the region of interest is measured. BMD is a derived measurement: BMD = BMC (g)/area (cm^2) A limitation is that areal BMD is measured whereas volumetric BMD is measured by other techniques (pQCT, μCT) Three most important roles of DXA: • Diagnose osteoporosis (humans) • Assess fracture risk (humans) • Monitor response to treatment (humans, animals)

Assessment of bone strength

Peripheral quantitative computed tomography (pQCT) • Humans Microcomputed tomography (μCT) • Rodents	pQCT and μCT provide a three-dimensional image of bone structure. Trabecular and cortical bone morphology can be evaluated at a peripheral skeletal site in humans (i.e., forearm, distal tibia) using pQCT or at any skeletal site in animals using μCT. Bone strength can be predicted using microfinite element modeling of measures obtained
Bone microindentation testing • Humans	A relatively new and direct technique in which bone tissue properties are measured by inserting a probe (reference point indentation instrument) through the skin over the tibia, displacing periosteum, and applying a known force at multiple indentation cycles that results in a small microcrack. Women with osteoporosis fractures have greater total indentation distance compared to controls
Biomechanical strength testing • Animals	A destructive test in which a loading or compressive force is applied to an excised bone until fracture occurs. Using this test, material properties are directly measured. One of the measures obtained is peak load, the amount of force required to fracture an excised bone Common sites of fragility fracture in humans that can be studied using animal models: • Individual vertebra can be compressed to mimic compression fracture • Femur neck fracture mimics a hip fracture While not a common site of fragility fracture, femur midpoint, representing a site rich in cortical bone, can also be measured
Incidence of fragility fracture • Humans	Can be measured in large clinical studies that are several years in duration to ultimately determine if risk of fragility fracture is reduced with an intervention Less than ideal than other measures of bone strength due to large sample size and the lengthy study duration (>3 years) required

Table 26.1 Assessment Methods for Bone-Related Outcomes—cont'd

Assessment of bone turnover

Biochemical markers of bone turnover • Humans • Animals	Markers of bone formation (serum bone-specific alkaline phosphatase, osteocalcin, and N-terminal propeptide of procollagen 1) and bone resorption (urinary pyridinoline and deoxypyridinoline collagen cross-links; serum N-telopeptide and C-telopeptide of type1 collagen) can be measured at more frequent intervals (weeks) than BMD scans (months, years). Enzyme-linked immunoassay and radioimmunoassay are the most common methods used to measure these markers Measurement of a marker of formation and resorption may predict the rate of bone loss in menopausal women and response to some antiresorptive therapy A limitation of these markers is their circadian rhythm, and thus usually, only large differences can be detected
Calcium kinetic studies • Humans	Dosing with stable or radioactive isotopes allows for bone formation and bone resorption rates to be quantified in mg of calcium per day A novel approach includes dosing subjects with ^{41}Ca, a rare isotope with a remarkably long half-life ($\sim 10^5$ years) that can be detected in urine using accelerator mass spectrometry Interventions can be evaluated for effectiveness in reducing bone resorption by measuring the reduction in tracer appearance in urine Alternative bone-seeking tracers include ^{3}H-tetracycline and ^{45}Ca
Histomorphometry • Humans (biopsy) • Animals	Markers of osteoblast and/or osteoclast activity can be measured Fluorescent dyes that bind calcium can be administered *in vivo* at established time intervals to determine rates of mineralization in excised bone tissue

BMC bone mineral content, *BMD* bone mineral density, *DXA* dual-energy x-ray absorptiometry, *μCT* microcomputed tomography, *pQCT* peripheral quantitative computed tomography.

permits rapid scans (2–6 min for regional sites). Some additional advantages of DXA include its ability to predict fracture, effectiveness in monitoring response to treatment, and interpretability using WHO *t*-scores. A major limitation of the DXA technique is that it is based upon two-dimensional projection measuring areal (g cm^{-2}) rather than volumetric (three dimensional, g cm^{-3}) density, complicating comparisons between individuals with different bone sizes.

The underlying concept of DXA is based on dual-energy projection (two dimensional) scanning whereby x-rays are produced by a low current x-ray tube and alternating x-ray generator voltage along with an integrating detector mounted above the scanning table. DXA uses an x-ray source mounted beneath the subject (who lies recumbent on

the scanning table) that is pulsed alternately at two different photon energies. A tightly collimated beam passes through an internal calibration disk composed of bone mineral (hydroxyapatite) and soft tissue equivalent materials. The object (specific bone or whole body) is scanned by the source and detector moving together. The transmitted intensity of the beam is related to the incident intensity, the composition of the material through which it passes, and the amount of material it traversed. Part of the attenuation is due to bone and part to soft tissue. The information is digitized, sent to the computer for analysis, and an 'image' is constructed by the computer and appears on the screen (Wahner and Fogelman, 1995).

2.2 Bone Strength

Although BMD is linearly related to fracture risk, it cannot measure volumetric BMD and does not measure material properties that also predict bone strength. In humans and animals, estimates of bone strength can be made by measuring bone dimensions and morphology using sophisticated imaging such as peripheral quantitative computed tomography (pQCT) or microcomputed tomography (μCT). Bone strength can be predicted using microfinite element modeling of measures obtained. pQCT is one method to determine cross-sectional areas and volumetric BMD, making it an ideal technique to quantify structural and material properties of the tibia, femur, and radius in humans and larger animals. In rodents, high-resolution μCT can be used to obtain these same measures in addition to specific parameters that describe trabecular and cortical bone morphology (Bouxsein et al., 2010). The American Society for Bone and Mineral Research has recently published guidelines regarding standard nomenclature that should be followed for reporting of results (Bouxsein et al., 2010). Moreover, excised bones from animals can be forced to bend or be broken, using a material testing system to directly measure the material properties of bone. An excised long bone or vertebra can be held in a customized jig and allow a loading or compressive force to be applied until fracture occurs. By knowing the material properties at different skeletal sites, that is, lumbar vertebra versus midpoint of a long bone, it is possible to more fully understand the effect of an intervention on a site rich in trabecular bone as compared to a site containing predominantly cortical bone. A relatively new and direct technique for measuring bone strength in humans is called microindentation testing. Using this technique, a small microcrack is produced with a microsized probe on the surface of a flat bone such as a tibia and allows bone material properties to be directly measured (Diez-Perez et al., 2010).

2.3 Bone Turnover

Bone turnover is the sum of bone formation rates and bone resorption rates. In the event that bone formation rates exceed bone resorption rates, calcium balance is positive and

bone mass increases. This is characteristic of growth when bone formation and bone resorption are coupled: calcium balance is zero and bone mass is maintained. When estrogen concentration declines in menopause or with aging, bone resorption rates exceed bone formation rates and bone is lost. Bone turnover is assessed with biochemical markers of bone turnover, isotope tracer kinetics, or urinary appearance of tracers from prelabeled bone.

Biochemical markers of bone serve as indices of change in bone turnover, reflecting increases or decreases in rates of resorption and formation. Several markers are found either in blood or urine and can be measured by enzyme-linked immunosorbent assay, high-pressure liquid chromatography, or radioimmunoassay procedures. The advantages of biochemical markers of bone are that the method is noninvasive, may predict (albeit imperfectly) the rate of bone loss in menopausal women, may predict the response to some antiresorptive therapies (Souberbielle et al., 1999), and may be performed more frequently than bone density scans. In a research setting, measurements of bone markers are typically made at baseline and then again at one or more times during the course of treatment. In a clinical setting, bone markers may be measured at baseline and then a few weeks after the initiation of treatment to determine whether or not a patient has experienced a therapeutic response. The primary limitation of bone markers is that circadian rhythms affect circulating concentrations, and hence, biologic variability is sufficiently great to necessitate large differences in the markers to detect a response to therapy (Souberbielle et al., 1999). Additional limitations are that some markers are not sensitive or specific (i.e., bone- vs. nonbone-derived biomarkers) enough to detect small changes over time, the renal clearance capacity of the patient greatly influences values for certain blood- and urine-derived markers, sample procurement and measurement should be standardized, and the overall metabolic status of the patient at the time of sample collection should be considered. Existing data indicate that biochemical markers can aid in determining which women are at greater risk of rapid bone loss and fracture (Garnero, 2000). The most valuable biomarkers (Souberbielle et al., 1999) for bone formation are serum bone-specific alkaline phosphatase, osteocalcin, and the N-terminal propeptide of procollagen I and for bone resorption are serum N-telopeptide and C-telopeptide of type I collagen and urinary pyridinoline and deoxypyridinoline collagen cross-links.

Calcium kinetic approaches offer advantages over biochemical markers of bone turnover because they can accurately quantitate bone formation rates and bone resorption rates in mg of calcium per day (Wastney et al., 2006). However, calcium kinetic studies require controlled feeding studies and an oral and intravenous tracer administration that is labor intensive, has a large subject burden, and requires specialized capacity. Either radioisotopes or stable isotopes can be used, but stable isotopes are radiologically benign and hence better accepted and the only appropriate calcium tracers to use during growth. However, stable isotopes are more expensive to purchase and analyze by mass spectrometry than radioisotopes (Weaver, 2006). The calcium kinetics approach was used to

determine that soy protein reduced urinary calcium excretion compared to milk proteins, but this was compensated for by increased endogenous secretion, so that there was no difference in calcium retention (Spence et al., 2005).

A novel approach to determine bone resorption directly is use of ^{41}Ca, a rare isotope with a remarkably long half-life ($\sim 10^5$ years) that can be detected in urine at very low concentrations (Jackson et al., 2001). The isotope is given to a subject and allowed a period of >100 days to deep label bone and clear the soft tissue of ^{41}Ca. Subsequent appearance of the tracer into urine is derived directly from bone. The tracer can be monitored by accelerator mass spectrometry for decades following a single small dose of 50 nCi. Interventions can be evaluated for effectiveness in reducing bone resorption by measuring the reduction in tracer appearance in urine. Alternative bone-seeking tracers to ^{41}Ca include tritiated tetracycline and ^{45}Ca.

3. NUTRITION-RELATED ALTERNATIVES OR ADJUVANT THERAPY TO HORMONE TREATMENT FOR PREVENTING OSTEOPOROSIS

3.1 Calcium and Vitamin D

Bone cells are dependent upon all nutrients for their cellular activity, and hence, nutrition plays an important role in the development, prevention, and treatment of osteoporosis. The reader is referred to two excellent overviews of dietary components that affect bone (Cashman, 2007; Ilich and Kerstetter, 2000) since an in-depth review is beyond the scope of this chapter. Calcium and vitamin D are effective as adjunctive therapies in preventing and treating osteoporosis. They assume prominent roles in conjunction with antiresorptive agents such as bisphosphonates, calcitonin, estrogen, or selective estrogen receptor modulators (SERMs). Adequate calcium intake (in the presence of adequate vitamin D status) reduces bone loss in peri- and postmenopausal women and fractures in postmenopausal women older than age 60 with low calcium intakes (North American Menopause Society, 2006). Vitamin D and calcium requirements change throughout life because of skeletal growth and age-related alterations in absorption and excretion. The North American Menopause Society (2006) consensus opinion indicated that at least 1200 mg day^{-1} of calcium is required for most postmenopausal women. A meta-analysis (Tang et al., 2007) has shown that the treatment effect is greatest with calcium doses ≥ 1200 mg day^{-1}. Also, estrogen therapy exhibits a considerably greater protective effect when coadministered with supplemental calcium than when taken alone (Nieves et al., 1998). Indeed, agents that increase bone density (i.e., fluoride, bisphosphonates, parathyroid hormone) do not achieve their full effect when calcium is limiting. Vitamin D facilitates calcium and phosphorus absorption as well as osteoclastic resorption and normal mineralization. Vitamin D supplementation is particularly important in the elderly who are often deficient and assists in lowering elevated serum parathyroid hormone that leads to bone loss. Vitamin D repletion is associated with significant annual increases in

BMD at the lumbar spine ($p \leq 0.0001$) and femoral neck ($p = 0.03$) in osteopenic patients (Adams et al., 1999). Indeed, optimal vitamin D repletion appears to be necessary to maximize the response to antiresorption therapy with respect to both BMD changes and anti-fracture efficacy. Adami et al. (2009) reported that the adjusted odds ratio for incident fractures in vitamin D-deficient versus vitamin D-repleted women was 1.77 (1.20–2.59, 95% CI; $p = 0.004$). Adequate vitamin D status, defined recently by the Institute of Medicine (IOM) as 20 ng ml^{-1} (50 nmol l^{-1}) of serum 25-hydroxyvitamin D (IOM, 2011), is required to achieve the nutritional benefits of calcium, although the optimal daily oral intake of vitamin D is still hotly debated (Cashman et al., 2008) and beyond the scope of this chapter. Supplementation of calcium and vitamin D alone is insufficient but is nevertheless a cornerstone in preventing and treating osteoporosis.

3.2 Soy Protein and Soy Isoflavones: Overview

Soybeans and their constituents have been extensively investigated for their role in preventing chronic disease, such as osteoporosis and cardiovascular disease (CVD), due to ovarian hormone deficiency (without estrogen therapy) that accompanies menopause. Isoflavones are structurally similar to estrogen, bind to estrogen receptors (ER) (Kuiper et al., 1998), and affect estrogen-regulated gene products (Dip et al., 2008). Hence, the estrogen-like effect of soy isoflavones has sparked considerable interest in their potential skeletal benefits, whereas evidence has heretofore been equivocal, albeit intriguing. Chapter 2 describes the flourish of research findings on the human skeletal effects of soy protein naturally rich in isoflavones and soy isoflavones extracted from soy protein. Moreover, Chapter 3 describes findings using animal models to understand effects of soy on bone health at distinct stages of the lifespan. Observations suggesting that soybeans may contribute to bone health include the low rates of hip fractures in Asians originating from the Pacific Rim (Ho et al., 1993; Ross et al., 1991), the *in vitro* (Markiewicz et al., 1993) and *in vivo* (Song et al., 1999) estrogenic activity of soy isoflavones, and the lower urinary calcium losses in humans who consume soy versus animal protein diets (Breslau et al., 1988). However, there are caveats to these findings that do not support the role of soy isoflavones in bone health. Although earlier studies in peri- (Alekel et al., 2000) and postmenopausal (Potter et al. 1998) women demonstrated beneficial effects of soy isoflavones on BMD, more recent studies have not substantiated these favorable skeletal effects (Alekel et al., 2010; Brink et al., 2008; Kenny et al., 2009; Wong et al., 2009) in humans.

Hormone therapy prevents bone loss and importantly, reduces the risk of hip fracture (Writing Group for the Women's Health Initiative Investigators, 2002) but is associated with increased risk of endometrial cancer (Beresford et al., 1997), invasive breast cancer, and CVD (Writing Group for the Women's Health Initiative Investigators, 2002). Thus, clinical guidelines recommend against hormone therapy as a first-line therapy to prevent postmenopausal osteoporosis (US Preventive Services Task Force, 2002). Research has

recently focused on alternatives to steroid hormones, with comparable skeletal and cardiovascular benefits but without side effects.

Dietary isoflavones are weakly estrogenic, particularly in the face of postmenopausal endogenous estrogen deficiency, and are thought to conserve bone through the ER–mediated pathway. S-equol, derived from the precursor soy isoflavone daidzein, has a high affinity for ER-β (Setchell et al., 2005), implying their action is distinctly different from that of classical steroidal estrogens that preferentially bind to ER-α. Isoflavones are weak 17 β-estradiol agonists in bone cells, but they may act as estrogen antagonists in reproductive tissues, indicating that the differential tissue response is due to the distribution of ER-α and ER-β in various cell types. Further, the stronger affinity of isoflavones for ER-β compared to ER-α may be particularly important because ER-β has been identified in bone tissue (Vidal et al., 1999). Thus, because some tissues contain predominantly ER-α or ER-β and different isoflavones exert various effects, isoflavones indeed may exert tissue-selective effects. Hence, isoflavones behave much like SERMs. *In vitro* studies indicate that isoflavones both suppress osteoclastic and enhance osteoblastic function, although *in vivo* studies have provided equivocal results.

3.3 Soy Protein and Calcium Homeostasis

While there is conflicting *in vivo* evidence that soy isoflavones protect against bone loss by involvement with calcium homeostasis, soy protein itself may protect against bone loss indirectly by mechanisms independent of the estrogenic effect of isoflavones. Animal protein is more hypercalciuric than soy protein according to human studies, perhaps due to the greater net renal acid excretion with high meat diets (Sebastian, 2003). In a two-week study (Watkins et al., 1985), subjects ($N=9$) aged 22–69 years were fed with protein (~80 g) derived primarily from either soybeans or chicken but with similar mineral content. Urinary total titratable acid increased 4% from baseline on the soy but by 46% on the meat diet. Urinary calcium excretion was 169 mg on the soy versus 203 mg on the meat diet, demonstrating that soy was less hypercalciuric than meat protein. Similarly, Breslau et al. (1988) examined calcium metabolism in 15 subjects 23–46 years who consumed each of three diets in random order (crossover) for 12 days: soy protein (vegetarian), soy+egg protein (ovo vegetarian), or animal (beef, chicken, fish, cheese) protein. Eucaloric diets were kept constant in protein (75 g), calcium (400 mg), phosphorus (1000 mg), sodium (400 mg), and fluid (3 l). They reported no difference in fractional ^{47}calcium absorption among the diets, but 24-hr urinary calcium excretion increased ($p<0.02$) from 103 ± 15 mg day^{-1} on the vegetarian to 150 ± 13 mg day^{-1} on the animal protein diet. Likewise, Pie and Paik (1986) fed young Korean women ($N=6$) a meat-based (71 g protein) followed by a soy-based (83 g protein) diet for 5 days each. Subjects who consumed the meat- versus soy-based diets, respectively, despite similar dietary calcium (525 mg) intake, had higher ($p<0.025$) daily urinary (127 vs. 88 mg)

and fecal (467 vs. 284 mg) calcium excretion. Thus, overall calcium balance was more negative ($p < 0.001$) on the meat– versus soy-based diet. In contrast, daily substitution of meat protein with soy protein (25 g) in the context of a mixed diet for 7 weeks did not improve or impair calcium retention or bone markers in healthy postmenopausal women (Roughead et al., 2005). Despite higher urinary pH and lower renal acid excretion (ammonium plus titratable acidity) in the soy protein versus control group, urinary calcium excretion did not differ in this randomized crossover controlled feeding study. A similar controlled feeding study (7 weeks) by the same research group (Hunt et al., 2009) demonstrated that an increase in protein from 10% to 20% of energy slightly improved calcium absorption with a low (675 mg) but not usual (1510 mg) calcium diet, compensating partially for the hypercalciuria. Explanations for seemingly contradictory findings in these longer-term studies demonstrate adaptation and that protein–associated hypercalciuria is due to enhanced intestinal calcium absorption (which is dependent upon many factors) rather than an increase in bone resorption (Kerstetter et al., 2001). Thus, protein intake in general appears to improve calcium absorption (especially with marginal calcium intakes), and soy protein in particular may minimize the hypercalciuria that otherwise occurs with increased protein intake. Although long-term intake of animal products is not deleterious, a beneficial effect of soyfoods (2–3 servings per day) on calcium excretion may be clinically relevant.

Studies of bone turnover have been used to determine early response to soy and mechanisms of action. ^{41}Ca was IV administered in a minute dose (100 nCi), and urinary appearance was used to measure the early response of bone resorption (Cheong et al., 2007). Soy protein with isoflavones (up to 136 mg day^{-1}) consumed by postmenopausal women ($N = 13$) did not suppress bone resorption as assessed by the urinary ^{41}Ca/^{40}Ca ratio as well as assessed by other biochemical bone turnover markers. In contrast, a recent meta-analysis on randomized controlled studies (Taku et al., 2010) designed to examine selected bone turnover markers found that the overall effect of soy isoflavones (~56 mg day^{-1} aglycone units) versus placebo (10 weeks to 12 months) was to decrease ($p = 0.0007$) deoxypyridinoline by −18.0%, whereas the increase in bone formation markers (8% for BAP, $p = 0.20$; 10.3% for osteocalcin, $p = 0.13$) was not significant. Consistent with this meta-analysis, genistein (54 mg day^{-1}) alone has been shown to inhibit bone resorption ($p < 0.001$) and stimulate bone formation ($p < 0.05$), the latter of which is different than the effect of 17β-estradiol (Morabito et al., 2002). A recent study (Weaver et al., 2009) using the ^{41}Ca methodology indicated that soy isoflavones from the soy cotyledon and soy germ decreased net bone resorption by 9% ($p = 0.0002$) and 5% ($p = 0.03$), respectively, in healthy postmenopausal women ($N = 11$). Overall, the meta-analysis (Taku et al., 2010), the Italian study (Morabito et al., 2002), and the ^{41}Ca study (Weaver et al., 2009) suggested that soy isoflavones may modestly decrease bone resorption but also may prevent a decline in bone formation. Any noted discrepancies are not only due to differences in study design that complicate comparisons and to the extreme

variability of these markers (except the sensitive ^{41}Ca method), particularly in early menopausal women, but also due to differences in the type or dose of soy isoflavones, study duration, and particular biochemical markers of bone examined. This begs the question as to whether these effects on bone turnover are sufficient to maintain BMD.

3.4 Vegetables and Fruits Other than Soy

Several epidemiological studies suggest a positive link between BMD and overall fruit and vegetable consumption or alpha-linolenic acid consumption (discussed in Chapter 4). However, despite extensive data in animal and *in vitro* models suggesting bone anabolic effects associated with consumption of onions, dried plums, blueberries, and orange juice (Ronis et al., 2011 and discussed in Chapters 4 and 5), almost no clinical trials have been performed with individual fruits or vegetables. This research area requires much further investigation.

GLOSSARY

Bone formation Process by which osteoblasts (type of bone cell) replace bone to repair microdamaged bone.

Bone mineral content (BMC, grams) Amount of mineral atoms deposited within the bone matrix.

Bone mineral density (BMD, grams/bone volume) Weight of mineral per volume of bone.

Bone resorption Process by which osteoclasts (type of bone cell) break down bone and release the minerals, which results in a transfer of calcium from bone fluid to the circulation.

Dual-energy x-ray absorptiometry (DXA) DXA measures the bone mineral content (BMC) and calculates the apparent or areal bone mineral density (BMD = bone mineral content (g)/area (cm^2)), not true volumetric BMD (g cm^{-3}) as measured by quantitative computed tomography. DXA scanners assess BMD by measuring the transmission of photons from an x-ray tube through the body or body part at two energy levels, thereby distinguishing between bone mineral and soft tissue.

Hypercalciuria Excretion of abnormally large amounts of calcium in the urine.

Lumbar spine Lumbar region of the spine (includes lumbar vertebrae, L1–L5) begins directly below the cervical and thoracic regions and ends directly above the sacrum.

Microcomputed tomography Measures cross-sectional images of a specific skeletal site using x-rays. These cross-sectional images can be combined to create a virtual model of a bone (i.e., femur, tibia, lumbar vertebra). Outcomes specific to trabecular bone or cortical bone compartments can be determined.

Osteopenia (low bone mass) Operationally defined as a BMD between 1 and 2.5 SD below the mean compared with young women.

Osteoporosis "Systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (World Health Organization Scientific Group, 2003). Operationally defined as a BMD less than 2.5 standard deviations (SD; also referred to as a *t*-score) below the mean compared with young women.

Proximal femur Hip bone (includes head, neck, and greater trochanter), where the proximal end articulates with the hip joint.

REFERENCES

Adami, S., Giannini, S., Bianchi, G., et al., 2009. Vitamin D status and response to treatment in post-menopausal osteoporosis. Osteoporosis International 20, 239–244.

Adams, J.S., Kantorovich, V., Wu, C., Javanbakht, M., Hollis, B.W., 1999. Resolution of vitamin D insufficiency in osteopenic patients results in rapid recovery of bone mineral density. Journal of Clinical Endocrinology and Metabolism 84, 2729–2730.

Alekel, D.L., St. Germain, A., Peterson, C.T., Hanson, K.B., Stewart, J.W., Toda, T., 2000. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. American Journal of Clinical Nutrition 72, 844–852.

Alekel, D.L., Van Loan, M.D., Koehler, K.J., et al., 2010. Soy isoflavones for reducing bone loss (SIRBL) study: three-year randomized controlled trial to determine efficacy and safety of soy isoflavones to reduce bone loss in postmenopausal women. American Journal of Clinical Nutrition 91, 218–230.

Beresford, S.A.A., Weiss, N.S., McKnight, B., 1997. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestogen therapy in postmenopausal women. Lancet 349, 458–461.

Bouxsein, M.L., Boyd, S.K., Christiansen, B.A., Guldberg, R.E., Jepsen, K.J., Müller, R., 2010. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. Journal of Bone and Mineral Research 25, 1468–1486.

Breslau, N.A., Brinkley, L., Hill, K.D., Pak, C.Y.C., 1988. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. Journal of Clinical Endocrinology and Metabolism 66, 140–146.

Brink, E., Coxam, V., Robins, S., Wahala, K., Cassidy, A., Branca, F., 2008. Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. American Journal of Clinical Nutrition 87, 761–770.

Burge, R., Dawson-Hughes, B., Solomon, D.H., Wong, J.B., King, A., Tosteson, A., 2007. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. Journal of Bone and Mineral Research 22, 465–475.

Cashman, K.D., 2007. Diet, nutrition, and bone health. Journal of Nutrition 137 (11 supplement), 2507S–2512S.

Cashman, K.D., Hill, T.R., Lucey, A.J., et al., 2008. Estimation of the dietary requirement for vitamin D in healthy adults. American Journal of Clinical Nutrition 88, 1535–1542.

Cheong, J.M., Martin, B.R., Jackson, G.S., et al., 2007. Soy isoflavones do not affect bone resorption in postmenopausal women: a dose–response study using a novel approach with ^{41}Ca. Journal of Clinical Endocrinology and Metabolism 92 (2), 577–582.

Compston, J., 2010. Osteoporosis: social and economic impact. Radiologic Clinics of North America 48, 477–482.

Cooper, C., Campion, G., Melton III, L.J., 1992. Hip fractures in the elderly: a worldwide projection. Osteoporosis International 2, 285–289.

Dennison, E., Mohamed, M.A., Cooper, C., 2006. Epidemiology of osteoporosis. Rheumatic Diseases Clinics of North America 32 (4), 617–629.

Diez-Perez, A., Guerre, R., Nognes, X., et al., 2010. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. Journal of Bone and Mineral Research 25 (8), 1877–1885.

Dip, R., Lenz, S., Antignac, J.P., Le Bizec, B., Gmuender, H., Naegeli, H., 2008. Global gene expression profiles induced by phytoestrogens in human breast cancer cells. Endocrine-Related Cancer 15, 161–173.

Garnero, P., 2000. Markers of bone turnover for the prediction of fracture risk. Osteoporosis International 11 (supplement 6), S55–S65.

Genant, H.K., Cooper, C., Poor, G., et al., 1999. Interim report and recommendations of the World Health Organization Task-Force for Osteoporosis. Osteoporosis International 10, 259–264.

Ho, S.C., Bacon, E., Harris, T., Looker, A., Muggi, S., 1993. Hip fracture rates in Hong Kong and the United States, 1988 through 1989. American Journal of Public Health 83, 694–697.

Hunt, J.R., Johnson, L.K., Roughead, Z.K.F., 2009. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. American Journal of Clinical Nutrition 89, 1357–1365.

Ilich, J.Z., Kerstetter, J.E., 2000. Nutrition in bone health revisited: a story beyond calcium. Journal of the American College of Nutrition 19, 715–737.

International Society for Clinical Densitometry, 2005. Official Position. Available at http://www.iscd.org/Visitors/pdfs/RadiationSafetyWhitePaperrevisedWebPosting2005-06.pdf (accessed 8 December 2010).

IOM (Institute of Medicine), 2011. Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press, Washington, DC.

Jackson, G.S., Weaver, C.M., Elmore, D., 2001. Use of accelerator mass spectrometry for studies in nutrition. Nutrition Research Reviews 14, 317–334.

Kenny, A.M., Mangano, K.M., Abourizk, R.H., et al., 2009. Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial. American Journal of Clinical Nutrition 90, 234–242.

Kerstetter, J.E., O'Brien, K., Insogna, K., 2001. Dietary protein and intestinal calcium absorption. American Journal of Clinical Nutrition 73, 990–992.

Kuiper, G.G., Lemmen, J.G., Carlsson, B., et al., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor-β. Endocrinology 139, 4252–4263.

Looker, A.C., Orwoll, E.S., Johnston Jr., C.C., et al., 1997. Prevalence of low femoral bone density in older US adults from NHANES III. Journal of Bone and Mineral Research 12, 1761–1768.

Markiewicz, L., Garey, J., Adlercreutz, H., Gurpide, E., 1993. In vitro bioassays of non-steroidal phytoestrogens. Journal of Steroid Biochemistry and Molecular Biology 45, 399–405.

Melton III, L.J., 2000. Who has osteoporosis? A conflict between clinical and public health perspectives. Journal of Bone and Mineral Research 15, 2309–2314.

Morabito, N., Crisafulli, A., Vergara, C., et al., 2002. Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. Journal of Bone and Mineral Research 17 (10), 1904–1912.

Nieves, J.W., Komar, L., Cosman, F., Lindsay, R., 1998. Calcium potentiates the effect of estrogen and calcitonin on bone mass: review and analysis. American Journal of Clinical Nutrition 67, 18–24.

North American Menopause Society, 2006. The role of calcium in peri- and postmenopausal women: 2006 position statement of The North American Menopause Society. Menopause 13 (6), 862–877.

Pie, J.-E., Paik, H.Y., 1986. The effect of meat protein and soy protein on calcium metabolism in young adult Korean women. Korean Journal of Nutrition 19, 32–40.

Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., Erdman, J.W., 1998. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. American Journal of Clinical Nutrition 68, 1375S–1379S.

Ronis, M.J., Mercer, K., Chen, J.-R., 2011. Effects of nutrition and alcohol consumption on bone loss. Current Osteoporosis Reports 9 (2), 53–59.

Ross, P.D., Norimatsu, H., Davis, J.W., et al., 1991. A comparison of hip fracture incidence among native Japanese, Japanese Americans, and American Caucasians. American Journal of Epidemiology 133, 801–809.

Roughead, Z.K., Hunt, J.R., Johnson, L.K., Badger, T.M., Lykken, G.I., 2005. Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. Journal of Clinical Endocrinology and Metabolism 90, 181–189.

Sebastian, A., 2003. Low versus high meat diets: effects on calcium metabolism. Journal of Nutrition 133, 3237–3238.

Setchell, K.D., Clerici, C., Lephart, E.D., et al., 2005. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. American Journal of Clinical Nutrition 81 (5), 1072–1079.

Song, T.T., Hendrich, S., Murphy, P.A., 1999. Estrogenic activity of glycitein, a soy isoflavone. Journal of Agricultural and Food Chemistry 47, 1607–1610.

Souberbielle, J.C., Cormier, C., Kindermans, C., 1999. Bone markers in clinical practice. Current Opinion in Rheumatology 11, 312–319.

Spence, L.A., Lipscomb, E.R., Cadogan, J., et al., 2005. The effect of soy isoflavones on calcium metabolism in postmenopausal women: a randomized crossover study. American Journal of Clinical Nutrition 81, 916–922.

Taku, K., Melby, M.K., Kurzer, M.S., Mizuno, S., Watanabe, S., Ishimi, Y., 2010. Effects of soy isoflavone supplements on bone turnover markers in menopausal women: systematic review and meta-analysis of randomized controlled trials. Bone 47 (2), 413–423.

Tang, B.M., Eslick, G.D., Nowson, C., Smith, C., Bensoussan, A., 2007. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 370, 657–666.

US Preventive Services Task Force, 2002. Postmenopausal hormone replacement therapy for primary prevention of chronic conditions: recommendations and rationale. Annals of Internal Medicine 137, 834–839.

Vidal, O., Kindblom, L.-G., Ohlsson, C., 1999. Expression and localization of estrogen-receptor-β in murine and human bone. Journal of Bone and Mineral Research 14, 923–929.

Wahner, H.W., Fogelman, I., 1995. Instrument and measurement techniques, Chapter 3. In: The Evaluation of Osteoporosis: Dual Energy X-Ray Absorptiometry in Clinical Practice. Mayo Foundation and Ignac Fogelman, Martin Dunitz Ltd, London.

Wastney, M.E., Zhao, Y., Weaver, C.M., 2006. Kinetic studies, Chapter 6. In: Weaver, C.M., Heaney, R.P. (Eds.), Calcium in Human Health. Humana Press, Totowa, NJ, pp. 83–93.

Watkins, T.R., Pandya, K., Mickelsen, O., 1985. Urinary acid and calcium excretion. Effect of soy versus meat in human diets. In: Kies, C. (Ed.), Nutritional Bioavailability of Calcium. American Chemical Society, Washington, DC.

Weaver, C.M., 2006. Clinical approaches for studying calcium metabolism and its relationship to disease, Chapter 5. In: Weaver, C.M., Heaney, R.P. (Eds.), Calcium in Human Health. Humana Press, Totowa, NJ, pp. 65–81.

Weaver, C.M., Martin, B.R., Jackson, G.S., et al., 2009. Antiresorptive effects of phytoestrogen supplements compared with estradiol or risedronate in postmenopausal women using [41]Ca methodology. Journal of Clinical Endocrinology and Metabolism 94, 3798–3805.

Wong, W.W., Lewis, R.D., Steinberg, F.M., et al., 2009. Soy isoflavone supplementation and bone mineral density in menopausal women: a 2-y multicenter clinical trial. American Journal of Clinical Nutrition 90 (5), 1433–1439.

World Health Organization Scientific Group, 2003. Prevention and management of osteoporosis. WHO Technical Report Series 921. World Health Organization, Geneva.

Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Journal of the American Medical Association 288, 321–333.

Skeletal Impact of Soy Protein and Soy Isoflavones in Humans

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1. INTRODUCTION

This chapter focuses on the naturally occurring nonsteroidal mixed isoflavones derived from soy foods in prospective human intervention trials with bone mineral density (BMD) as the primary outcome. Some background information on osteoporosis is provided, as well as observational studies on soy intake, BMD, and fractures. The focus of this chapter is on prospective studies that have been published on the effect of soy protein and its isoflavones on BMD measured by dual-energy X-ray absorptiometry (DXA) and not on circulating or urinary biochemical markers of bone turnover (published previously; Alekel, 2007).

2. OSTEOPOROSIS: EPIDEMIOLOGIC PERSPECTIVE

2.1 Caucasian Versus Asian Populations: Bone Density and Fractures

Ethnic and genetic differences in bone may make some groups more susceptible than others to osteoporotic fractures. For example, Caucasian American women are at greater risk than African and Mexican Americans (Looker et al., 1997), who have lower fracture rates (Silverman and Madison, 1988). Vertebral fracture incidence among Taiwanese (Tsai, 1997) is comparable (18%) to that among Caucasian women, whereas that of hip fracture among elderly Taiwanese (Tsai, 1997) and those from mainland China (Xu et al., 1996) is lower. Despite the 10–15% lower femoral BMD than Caucasians, Taiwanese have lower hip fracture rates, which may be due to structural differences between racial/ethnic groups. Researchers have determined a shorter hip axis length in premenopausal Chinese women living in Australia (Chin et al., 1997) and women originating from the Indian subcontinent (Alekel et al., 1999) than in their Caucasian counterparts, indicating that structural differences likely contribute to variations in hip fracture prevalence in distinct racial groups. Interestingly, black and Asian men were shown to have thicker cortices and higher trabecular volumetric BMD, thought to confer greater bone strength, than Hispanic or white men

(Marshall et al., 2008). Investigators have also examined determinants of peak bone mass in Chinese women (Ho et al., 1997), risk factors for hip fracture in Asian men and women (Lau et al., 2001), and the contribution of anthropometric and lifestyle factors to peak bone mass in a multiethnic population (Davis et al., 1999). Some differences in osteoporotic risk among ethnic groups are inexplicable, but they may be largely due to frame size differences that lead to size-related artifacts in BMD measurements (Ross et al., 1996) and to differences in hip axis length (Alekel et al., 1999). Thus, when comparing BMD across ethnic groups, it is important to correct for frame size to accurately interpret spinal BMD values (Alekel et al., 2002) and to consider hip geometry to accurately assess hip fracture risk (Nakamura et al., 1994). Differences in osteoporotic risk may also be related to culture-specific dietary and exercise-related factors.

2.2 Soy Intake, Bone Density, and Fractures: Observational Studies

The first systematic review and meta-analysis examining the relationship between protein intake and bone in adults was conducted recently (Darling et al., 2009). These researchers reported a small positive association between protein intake (all sources) and lumbar spine BMD and a reduction in bone resorption markers. However, they could not identify a separate effect of soy supplements (or of milk protein) on BMD. Furthermore, the small positive relationship between protein and lumbar spine BMD did not translate into decreased relative risk of hip fracture. Germane to this review, this meta-analysis could not substantiate the hypothesis that protein is deleterious to bone.

The low hip fracture rate among Asians has been attributed to the beneficial effect of isoflavone-containing soybeans on bone health (Ross et al., 1991). However, human studies found that isoflavone-rich soy protein ($40 \, \text{g day}^{-1}$) intake was associated with favorable effects on *spinal* (Alekel et al., 2000; Potter et al., 1998) but not on *femoral* (hip) bone. Also, the amount of isoflavones (in aglycone units) consumed by subjects in the high-isoflavone groups in these two studies (80 or $90 \, \text{mg day}^{-1}$) was greater than that typically consumed by either Chinese ($39 \, \text{mg day}^{-1}$; Chen et al., 1999) or Japanese ($23 \, \text{mg day}^{-1}$; Kimira et al., 1998) women or by women from a multiethnic population in Hawaii (ranged from $5 \, \text{mg day}^{-1}$ in Filipino to $38.2 \, \text{mg day}^{-1}$ in Chinese; Maskarinec et al., 1998). Nevertheless, it is possible that lesser amounts of soy isoflavones consumed over the course of many years could have significant bone-sparing effects. Still, differences are not apparent in the lumbar spine BMD (Ross et al., 1996) or in the spinal fracture rate (Tsai et al., 1996) of Asian women compared with Caucasian women. In contrast, higher spine and hip BMD values have been reported in US-born versus Japan-born Japanese women (Kin et al., 1993). Many factors may contribute to the lower hip fracture rate in Asians, notably the shorter hip axis length and physical activity patterns of Asians originating from the Pacific Rim (Nakamura et al., 1994). Other protective factors include the lower propensity of Asians to fall (Davis et al., 1997)

and their shorter stature (Lau et al., 2001), although these nonmodifiable factors have little practical importance in preventing hip fractures.

Observational studies have published data on the link between soy intake and BMD or fracture risk. Somekawa et al. (2001) examined the relationship between soy isoflavone intake, menopausal symptoms, lipid profiles, and spinal BMD measured by DXA in 478 postmenopausal Japanese women. After adjusting BMD for weight and years since menopause, BMD values were found to be significantly different among four isoflavone intake levels (from 35 to 65 mg day^{-1}) in both the early ($p \leq 0.001$) and late ($p \leq 0.01$) postmenopausal groups. Women who had higher soy isoflavone intakes had higher BMD values. No differences in other characteristics (i.e., height, weight, years since menopause, etc.) across isoflavone intakes were noted. Another study in midlife (40–49 years) Japanese women ($N = 995$) examined the relationship of various dietary factors (including soybean intake) to metacarpal BMD (Tsuchida et al., 1999). Women who consumed soybeans at least twice per week had greater BMD than those who had soybeans once or 0 times per week, with this tendency ($p = 0.03$) remaining after controlling for age, height, weight, and weekly calcium intake. Likewise, baseline data analysis from the Study of Women's Health Across the Nation, a US community-based cohort study in women aged 42–52 years (Greendale et al., 2002), revealed that Japanese premenopausal women in the highest versus lowest tertile of genistein intake had 7.7 and 12% greater spine and femoral neck BMD, respectively. No association between genistein intake and BMD in the Chinese women was found, likely because their median intake was lower (3511 μg day^{-1}) than that of the Japanese women (7151 μg day^{-1}).

A prospective study of soy food intake and fracture risk in ~75 000 postmenopausal Chinese women (Zhang et al., 2005) indicated a protective effect of soy protein intake. After adjusting for age, BMI, energy and calcium intake, lifestyle risk factors for osteoporosis, and socioeconomic status, the relative risk of fracture ranged from 0.63 to 1.00 in the highest to lowest quintiles of soy protein intake ($p < 0.001$), with a more pronounced inverse association among women in early menopause. The Singapore Chinese Health Study (Koh et al., 2009) examined prospectively the potential risk factors for hip fracture in 63 257 Chinese men and women. They noted a significant association of tofu, soy protein, and isoflavones with hip fracture in women but not in men. Compared with women in the lowest quartile of intake for tofu (<49.4 g day^{-1}), soy protein (<2.7 g day^{-1}), and isoflavones (<5.8 mg/1000 kcal day^{-1}), women in the second through fourth quartiles displayed 21–36% reductions ($p < 0.036$) in risk. These published studies differ with respect to site (and type) of bone measured, as well as the quantity of dietary isoflavones habitually consumed. Nonetheless, the modest evidence for an effect of soy-derived isoflavones on bone seems to be stronger for trabecular (i.e., spinal) than cortical (i.e., radial, metacarpal) bone, is likely dependent on habitual long-term soy food intake, may be more pronounced in the early rather than late postmenopausal years, and may be gender specific.

3. SOY PROTEIN AND SOY ISOFLAVONES: INTERVENTION STUDIES

3.1 Relationship to BMD and Strength

Clinical studies in midlife women worldwide have examined the impact of soy food, soy protein isolate, or isoflavone tablets on bone mineral content (BMC) and BMD or bone turnover markers. Because of heterogeneous study designs, treatment doses or types, and subject characteristics (i.e., age, time since menopause), it is understandable why mixed results have emerged, and this serves to illustrate why it is necessary to distinguish between the variety of isoflavone forms (type) used in these studies. Among the more widely cited recent studies, one used soy foods rich in isoflavones (Lydeking-Olsen et al., 2004), two used carbohydrate foods enriched with soy protein (Arjmandi et al., 2005; Brink et al., 2008), and eight used soy protein isolate (Alekel et al., 2000; Evans et al., 2007; Gallagher et al., 2004; Kenny et al., 2009; Kreijkamp-Kaspers et al., 2004; Newton et al., 2006; Potter et al., 1998; Vupadhyayula et al., 2009) as the isoflavone source. Three studies used soy isoflavones extracted from soy germ (Chen et al., 2003; Wong et al., 2009; Ye et al., 2006), one used soy isoflavones extracted from soy protein (Alekel et al., 2010), and two used genistein tablets (Marini et al., 2007; Morabito et al., 2002). Among the studies that focused on bone at clinically relevant sites, nine studies published in the past decade (Alekel et al., 2000, 2010; Chen et al., 2003; Gallagher et al., 2004; Ho et al., 2001; Kenny et al., 2009; Kreijkamp-Kaspers et al., 2004; Lydeking-Olsen et al., 2004; Ye et al., 2006) are reviewed below because they illustrate the breadth of knowledge to date about soy protein or soy isoflavones (genistein, daidzein, and glycitein with a similar profile to soy protein) and bone.

Alekel et al. (2000) randomized (double-blind) 69 perimenopausal women to treatment (dose in aglycone units): isoflavone-rich soy (SPI+, 80.4 mg day^{-1}; $n=24$), isoflavone-poor soy (SPI−, 4.4 mg day^{-1}; $n=24$), or whey (control; $n=21$) protein. No change was reported in the lumbar spine BMD and BMC values, respectively, in the SPI+ (−0.2%, $p=0.7$; +0.6%, $p=0.5$) and SPI− (−0.7%, $p=0.1$; −0.6%, $p=0.3$) groups, but a loss was observed in controls (−1.3%, −1.7%, $p=0.004$). Baseline values were taken into account in the analysis of covariance (ANCOVA) and regression analysis, as baseline BMD and BMC affected percentage change (negatively, $p \leq 0.0001$) in these outcomes. ANCOVA indicated that treatment had an effect on percentage change in BMC ($p=0.021$), but not on BMD ($p=0.25$). Contrast coding using ANCOVA with BMD or BMC as the outcome revealed that isoflavones, not soy protein, exerted a positive effect. Taking various contributing factors into account, regression analysis indicated that SPI+ had a positive effect on percentage change in both BMD (5.6%, $p=0.023$) and BMC (10.1%, $p=0.0032$). Body weight at baseline (not weight gain) was related to change in BMD, suggesting that weight gain did not confound the effect of SPI+ on bone. Soy (SPI−) or whey protein had no effect on the spine, and treatment, in general, had no effect on bone sites other than the spine.

In contrast, Gallagher et al. (2004) examined the effect of SPI+ (two doses, 96 or 52 mg day^{-1}) and SPI− (<4 mg day^{-1}) on bone loss and lipids in postmenopausal women ($N=65$; 55 years, 7.5 years since menopause) for 9 months (participants were followed off treatment for another 6 months). SPI+ (either dose) had no significant effect on BMD of the lumbar spine or femoral neck, whereas trochanteric BMD increased significantly at 9 months ($p=0.02$) and 15 months ($p<0.05$) in the SPI− group versus the other two groups. These results are difficult to explain. Kenny et al. (2009) conducted a double-blind, placebo-controlled 2×2 factorial 1-year intervention with healthy older (>60 years) women ($N=97$). Subjects were randomly assigned to soy protein (18 g day^{-1}) + isoflavone (105 mg day^{-1} aglycone equivalents) tablets, soy protein + placebo tablets, control protein + isoflavone tablets, and control protein + placebo tablets, with similar intakes of protein powder and tablets among the groups. From baseline to 1 year, there were no significant differences in BMD among the four treatment groups; equol status did not impact these results. Higher protein intakes were associated with lower bone turnover.

Another study was designed to examine habitual soy intake and BMD in premenopausal Chinese women (30–40 years) living in Hong Kong (Ho et al., 2001), with an average follow-up time of 38.1 months. On adjustment for age and body size (height, weight, and bone area), researchers reported a positive effect of soy isoflavones on spinal BMD. The mean percentage decline in spinal BMD in 116 women was greater ($p<0.05$) in the lowest (−3.5%) versus highest (−1.1%) quartile of soy isoflavone intake. Multiple regression revealed that soy isoflavone intake (along with lean body mass, physical activity, energy-adjusted calcium intake, and follow-up time) accounted for 24% of the variance in spinal BMD in these women. This 3-year study indicated that soy isoflavone intake had a positive effect on maintaining spinal BMD in premenopausal women 30–40 years of age. Chen et al. (2003) conducted a double-blind, randomized clinical trial (1 year) to examine the effect of soy germ extract of isoflavones (40 or 80 mg day^{-1}) compared with placebo (corn starch) on bone loss in postmenopausal (48–62 years) Chinese women ($N=175$) who habitually consumed soy products. Univariate and multivariate analyses indicated that women in the high-dose group lost less BMC in the trochanter and total proximal femur than either the placebo or low-dose group, with or without adjusting for potential confounding factors. The positive effect of soy isoflavone supplements was observed only among women with low baseline BMC values. Results suggest that isoflavones may have a significant effect on cortical (proximal femur) bone, or that appendicular bone responds differently compared to the axial (i.e., spine) skeleton, particularly in habitual soy food consumers. Likewise, Ye et al. (2006) reported that soy isoflavones (84 or 126 mg day^{-1}) from soy germ extract exerted a beneficial effect, at 12 but not at 24 weeks, on not only the femoral neck ($p=0.016$) but also the lumbar spine ($p=0.042$) BMD in Chinese women ($N=84$) who habitually consumed soy foods. Still, short-term (≤1 year) studies cannot answer the question of whether short-term

bone-sparing effects would be sustained over a longer period encompassing a bone-remodeling cycle, which ranges from 30 to 80 weeks. Thus, the reported bone sparing in short-term studies may be due to treatment or to filling of bone resorption cavities that may not translate into long-term benefits (Heaney, 1994). In addition, soy germ (relatively rich in daidzein and glycitein and low in genistein) used in the latter two studies is different from most other treatments (the products are relatively rich in genistein) in the literature, making comparisons difficult. In addition, these studies included soy-consuming women, producing similar results to cross-sectional studies with habitual soy consumers. Nevertheless, some studies provide support for the idea that isoflavones are the bone-bioactive component of soy.

In contrast, Kreijkamp-Kaspers et al. (2004) reported that soy protein (25.6 g day^{-1}) isolate had no effect on cognitive function, BMD, and plasma lipids for 1 year in postmenopausal women. However, this trial included women considerably older (60–75 years) than most other trials reporting an effect. Their participants were advanced in age and heterogeneous in menopausal status and they did not account statistically for critical potentially confounding factors, such as current smoking status, baseline BMD (which appeared to differ between groups by $\sim 3.5\%$ for total hip and $\sim 2.4\%$ for lumbar spine, biologically important differences), and antihypertensive medication use. As acknowledged by the authors, those who had more recently transitioned through menopause experienced better results (both hip and spine) after 1 year of soy versus placebo, although the interaction was not significant ($p = 0.07$ for total hip). This suggests that either time since menopause was essential in dictating a treatment effect and/or that the power was insufficient. Also, their assumption that 'soy isoflavones (99 mg day^{-1}) are as effective as conventional hormone therapy' is not correct, perhaps resulting in insufficient power. These methodological limitations make interpretation difficult.

In contrast to findings from other studies, Lydeking-Olsen et al. (2004) reported that postmenopausal (mean age of 58.2 years, maximum of 75 years) women ($N = 89$) in the isoflavone-rich (76 mg day^{-1}) soymilk or transdermal progesterone (25.7 mg day^{-1}) group did not lose lumbar spine BMD, whereas the placebo control (isoflavone-poor soymilk plus progesterone-free cream; -4.2%, $p = 0.01$) and combination isoflavone-rich soymilk and progesterone (-2.8%, $p = 0.01$) groups had a significant loss. Daily intake of two glasses of soymilk (76 mg isoflavones) prevented lumbar spine bone loss, but when combined with progesterone cream, lumbar spine BMD was inexplicably lost, although not as great as placebo. Equol-producer status was associated with a better bone response, but this did not reach statistical significance due to insufficient sample size.

Alekel et al. (2010) conducted the longest (36 months) intervention (randomized controlled) trial to date to examine the efficacy of isoflavones (extracted from soy protein) on BMD in healthy postmenopausal (46–65 years) women ($N = 224$). Treatments included placebo control and two isoflavone (80 and 120 mg day^{-1} in aglycone equivalents) groups; all women received 500 mg calcium and 600 IU vitamin D$_3$. Compliance

was excellent in women who remained on treatment ($N=216$), with 209 (96.8%; $n=117$ at ISU, $n=92$ at UCD) of 216 women achieving $\geq$80% compliance (cumulative) with no difference ($p=0.49$) in values across the three treatment groups. ANOVA for intent-to-treat ($N=224$) models showed no treatment effect for spine ($p=0.46$), total femur ($p=0.86$), femoral neck ($p=0.17$), or whole-body ($p=0.86$) BMD. Regression analysis (compliant models, $N=208$) indicated that age, whole-body fat mass, and bone resorption were common predictors of BMD change. After adjusting for these factors, 120 mg day^{-1} (vs. placebo) was protective for femoral neck BMD ($p=0.024$), but otherwise treatment was not significant. Treatment did not affect adverse events, endometrial thickness, or bone markers. Results did not demonstrate a bone-sparing effect of soy isoflavones, except a modest effect at the femoral neck, whereas all treatment groups experienced a decline in BMD over time (Figure 27.1). An ancillary study in these women published by Shedd–Wise et al. (2011) examined bone strength and structural parameters of the one-third midshaft femur and distal tibia using peripheral quantitative computed tomography in response to treatment. Their results indicated that the isoflavone tablets were negative predictors of femur strength-strain index, but the 80-mg dose became protective as bone turnover increased ($p=0.011$). Contrary to what was hypothesized, a treatment effect could not be documented on trabecular bone of the distal tibia. Yet, the 120-mg dose was protective of cortical volumetric BMD of the femur, as time since last menstrual period increased ($p=0.012$). However, because this study did not examine fracture (no osteoporotic fractures were documented during the 3 years), one cannot draw clinical inferences based on such modest effects.

These disparate results are likely due to differences in study design, including different bone sites measured, type of product consumed (soy foods vs. soy foods rich in isoflavones vs. soy protein isolate vs. isoflavone tablets vs. soy germ tablets), dose of soy protein and/or isoflavones provided, length of intervention, sample size (often very limiting), as well as subject-related factors. Nevertheless, taken together, the results of these human studies suggest that lifetime intake of soy protein (naturally rich in isoflavones) may attenuate bone loss from the lumbar spine in estrogen-deficient women, who may otherwise be expected to lose 2–3% yearly. Such attenuation of loss, particularly if continued throughout the postmenopausal period, could translate into a decreased risk of osteoporosis. As a bone-remodeling cycle ranges from 30 to 80 weeks, short-term ($\leq$1 year) preliminary studies cannot answer the question of whether these bone-sparing effects of soy protein (whereby resorption pits are filled in) would be sustained over a longer period, or rather that this is an artifact of the bone-remodeling transient (Heaney, 1994). The longer-term study in Asian women (Ho et al., 2001) who habitually consume soy foods suggests true bone sparing. Clinical trials that have been conducted for at least 2 years, preferably 3 years, are those that should be considered authoritative in determining whether soy isoflavones affect BMD and the remodeling balance. Although it appears that protein intake ($\sim$65 g day^{-1}) in general (not specifically soy protein) exerts a beneficial effect
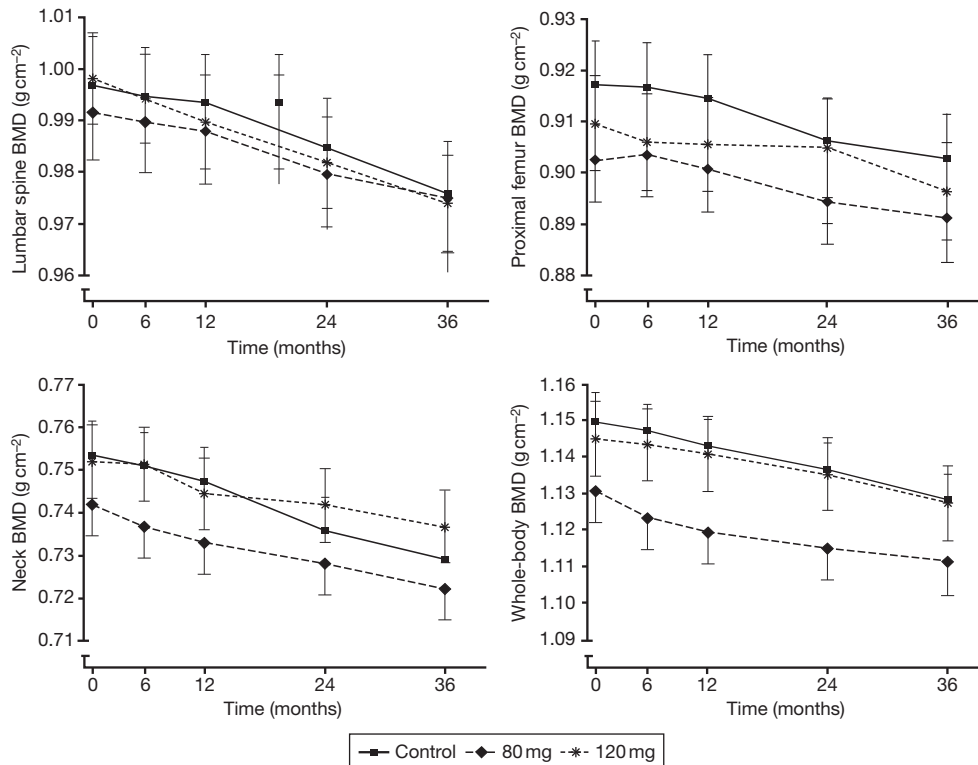
Figure 27.1 Parallel profile plot for intent-to-treat women ($N = 224$): Indicates mean (±SEM) values of lumbar spine, total proximal femur, femoral neck, and whole-body bone mineral density (BMD) at each time point (baseline, 6, 12, 24, and 36 months) for each treatment group: control (■), $n = 74$; 80 mg day^{-1} (♦), $n = 77$; 120 mg day^{-1} (∗), $n = 73$. Tests for parallel profiles for lumbar spine (Wilks' = 0.981, $p = 0.85$), neck (Wilks' = 0.951, $p = 0.21$), and whole-body (Wilks' = 0.962, $p = 0.38$) BMD indicated that there was no interaction, whereas there was an interaction between treatment and time for proximal femur BMD (Wilks' = 0.926, $p = 0.030$). *Reproduced from Alekel, D.L., Van Loan, M.D., Koehler, K.J., et al., 2010. Soy isoflavones for reducing bone loss (SIRBL) study: three-year randomized controlled trial to determine efficacy and safety of soy isoflavones to reduce bone loss in postmenopausal women.* American Journal of Clinical Nutrition *91, 218–230.*

on bone turnover in postmenopausal women (Kenny et al., 2009), particularly in those with a low calcium intake, long-term studies conducted to date do not indicate that soy isoflavones extracted from protein exert a substantive positive impact on BMD and on strength–related indices.

4. CONCLUSION

Little evidence can be had from single studies in humans that mixed isoflavones extracted from soy protein affect bone in the long term (≥ 2 years), despite a recent meta-analysis

(Taku et al., 2010b) of randomized controlled trials indicating that soy isoflavone ($\sim$82 mg day^{-1} in aglycone units) supplements for 6–12 months increased spine BMD by 2.4% ($p = 0.001$) in menopausal women. Evidence suggests that soy protein (rich in isoflavones) may favorably affect BMD, suggesting a protein-related effect; some evidence suggests that genistein alone may inhibit bone resorption and stimulate bone formation (Morabito et al., 2002). Yet, the majority of recent studies have not substantiated favorable skeletal effects using soy foods, soy protein, or extracted isoflavones, except perhaps a modest effect on bone strength. Readers are referred to previously published reviews (Alekel, 2007; Anderson and Alekel, 2002) and meta-analyses (Taku et al., 2010a,b) for further information. Because published studies do not provide definitive evidence, clinicians who practice evidence-based medicine should not recommend isoflavone supplements to treat or prevent osteoporosis. Nonetheless, health professionals should recommend soy foods because of their excellent nutrient profile and overall health benefits.

GLOSSARY

Peripheral quantitative computed tomography Minimally invasive method to assess quantitative measures of cortical and trabecular volumetric bone mineral density, cross-sectional geometry, and estimates of whole-bone strength of appendicular bone.

REFERENCES

Alekel, D.L., 2007. Skeletal effects of soy isoflavones in humans: bone mineral density and bone markers. In: Wildman, R.E.C. (Ed.), Handbook of Nutraceuticals and Functional Foods. second ed. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 247–267 Chapter 12.

Alekel, D.L., Mortillaro, E., Hussain, E.A., et al., 1999. Lifestyle and biologic contributors to proximal femur bone mineral density and hip axis length in two distinct ethnic groups of premenopausal women. Osteoporosis International 9, 327–338.

Alekel, D.L., Peterson, C.T., Werner, R.K., Mortillaro, E., Ahmed, N., Kukreja, S.C., 2002. Frame size, ethnicity, lifestyle, and biologic contributors to areal and volumetric lumbar spine bone mineral density in Indian/Pakistani and American Caucasian premenopausal women. Journal of Clinical Densitometry 5, 175–186.

Alekel, D.L., St. Germain, A., Peterson, C.T., Hanson, K.B., Stewart, J.W., Toda, T., 2000. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. American Journal of Clinical Nutrition 72, 844–852.

Alekel, D.L., Van Loan, M.D., Koehler, K.J., et al., 2010. Soy isoflavones for reducing bone loss (SIRBL) study: three-year randomized controlled trial to determine efficacy and safety of soy isoflavones to reduce bone loss in postmenopausal women. American Journal of Clinical Nutrition 91, 218–230.

Anderson, J.J.B., Alekel, D.L., 2002. Skeletal effects of phytoestrogens in humans: bone mineral density and bone markers. In: Gilani, G.S., Anderson, J.J.B. (Eds.), Phytoestrogens and Health. American Oil Chemists' Society, Champaign, IL, pp. 341–353.

Arjmandi, B.H., Lucas, E.A., Khalil, D.A., et al., 2005. One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutrition Journal 4, 8–16.

Brink, E., Coxam, V., Robins, S., Wahala, K., Cassidy, A., Branca, F., 2008. Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. American Journal of Clinical Nutrition 87, 761–770.

Chen, Y.-M., Ho, S.C., Lam, S.S.H., Ho, S.S.S., Woo, J.L.F., 2003. Soy isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. Journal of Clinical Endocrinology and Metabolism 88, 4740–4747.

Chen, Z., Zheng, W., Custer, L.J., et al., 1999. Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. Nutrition and Cancer 33, 82–87.

Chin, K., Evans, M.C., Cornish, J., Cundy, T., Reid, I.R., 1997. Differences in hip axis and femoral neck length in premenopausal women of Polynesian, Asian and European origin. Osteoporosis International 7, 344–347.

Darling, A.L., Millward, D.J., Torgerson, D.J., Hewitt, C.E., Lanham-New, S.A., 2009. Dietary protein and bone health: a systematic review and meta-analysis. American Journal of Clinical Nutrition 90 (6), 1674–1692.

Davis, J.W., Novotny, R., Wasnich, R.D., Ross, P.D., 1999. Ethnic, anthropometric, and lifestyle associations with regional variations in peak bone mass. Calcified Tissue International 65, 100–105.

Davis, J.W., Ross, P.D., Nevitt, M.C., Wasnich, R.D., 1997. Incidence rates of falls among Japanese men and women living in Hawaii. Journal of Clinical Epidemiology 50, 589–594.

Evans, E.M., Racette, S.B., Van Pelt, R.E., Peterson, L.R., Villareal, D.T., 2007. Effects of soy protein isolate and moderate exercise on bone turnover and bone mineral density in postmenopausal women. Menopause 14 (3 Pt 1), 481–488.

Gallagher, J.C., Satpathy, R., Rafferty, K., Haynatzka, V., 2004. The effect of soy protein isolate on bone metabolism. Menopause 11, 290–298.

Greendale, G.A., FitzGerald, G., Huang, M.-H., et al., 2002. Dietary soy isoflavones and bone mineral density: results from the Study of Women's Health Across the Nation. American Journal of Epidemiology 155, 746–754.

Heaney, R.P., 1994. The bone-remodeling transient: implications for the interpretation of clinical studies of bone mass change. Journal of Bone and Mineral Research 9, 1515–1523.

Ho, S.C., Chan, S.G., Yi, Q., Wong, E., Leung, P.C., 2001. Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women. Journal of Bone and Mineral Research 16, 1363–1369.

Ho, S.C., Wong, E., Chan, S.G., Lau, J., Chan, C., Leung, P.C., 1997. Determinants of peak bone mass in Chinese women aged 21–40 years. III. Physical activity and bone mineral density. Journal of Bone and Mineral Research 12, 1262–1271.

Kenny, A.M., Mangano, K.M., Abourizk, R.H., et al., 2009. Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial. American Journal of Clinical Nutrition 90, 234–242.

Kimira, M., Arai, Y., Shimoi, K., Watanabe, S., 1998. Japanese intake of flavonoids and isoflavonoids from foods. Journal of Epidemiology 8, 168–175.

Kin, K., Lee, E., Kushida, K., et al., 1993. Bone density and body composition on the Pacific Rim: a comparison between Japan-born and U.S.-born Japanese American women. Journal of Bone and Mineral Research 8, 861–869.

Koh, W.-P., Wu, A.H., Wang, R., et al., 2009. Gender-specific associations between soy and risk of hip fracture in the Singapore Chinese Health Study. American Journal of Epidemiology 170, 901–909.

Kreijkamp-Kaspers, S., Kok, L., Grobbee, D.E., et al., 2004. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women. Journal of the American Medical Association 292, 65–74.

Lau, E.M.C., Suriwongpaisal, P., Lee, J.K., et al., 2001. Risk factors for hip fracture in Asian men and women: the Asian Osteoporosis Study. Journal of Bone and Mineral Research 16, 572–580.

Looker, A.C., Orwoll, E.S., Johnston Jr., C.C., et al., 1997. Prevalence of low femoral bone density in older U.S. adults from NHANES III. Journal of Bone and Mineral Research 12, 1761–1768.

Lydeking-Olsen, E., Beck-Jensen, J.-E., Setchell, K.D.R., Holm-Jensen, T., 2004. Soymilk or progesterone for prevention of bone loss: a 2 year randomized, placebo-controlled trial. European Journal of Nutrition 43, 246–257.

Marini, H., Minutoli, L., Polito, F., et al., 2007. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. Annals of Internal Medicine 146 (12), 839–847.

Marshall, L.M., Zmuda, J.M., Chan, B.K.S., et al., 2008. Race and ethnic variation in proximal femur structure and BMD among older men. Journal of Bone and Mineral Research 23, 121–130.

Maskarinec, G., Singh, S., Meng, L., Franke, A.A., 1998. Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. Cancer Epidemiology, Biomarkers & Prevention 7, 613–619.

Morabito, N., Crisafulli, A., Vergara, C., et al., 2002. Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. Journal of Bone and Mineral Research 17 (10), 1904–1912.

Nakamura, T., Turner, C.H., Yoshikawa, T., et al., 1994. Do variations in hip geometry explain differences in hip fracture risk between Japanese and white Americans? Journal of Bone and Mineral Research 9, 1071–1076.

Newton, K.M., LaCroix, A.Z., Levy, L., et al., 2006. Soy protein and bone mineral density in older men and women: a randomized trial. Maturitas 55 (3), 270–277.

Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., Erdman Jr., J.W., 1998. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. American Journal of Clinical Nutrition 68, 1375S–1379S.

Ross, P.D., He, Y.-F., Yates, A.J., et al., 1996. Body size accounts for most differences in bone density between Asian and Caucasian women. Calcified Tissue International 59, 339–343.

Ross, P.D., Norimatsu, H., Davis, J.W., et al., 1991. A comparison of hip fracture incidence among native Japanese, Japanese Americans, and American Caucasians. American Journal of Epidemiology 133, 801–809.

Shedd-Wise, K.M., Alekel, D.L., Hofmann, H., et al., 2011. The soy isoflavones for reducing bone loss (SIRBL) study: three year effects on pQCT bone mineral density and strength measures in postmenopausal women. Journal of Clinical Densitometry: Assessment of Skeletal Health 14 (1), 47–57.

Silverman, S.L., Madison, R.E., 1988. Decreased incidence of hip fracture in Hispanics, Asians, and blacks: California Hospital Discharge Data. American Journal of Public Health 78, 1482–1483.

Somekawa, Y., Chiguchi, M., Ishibashi, T., Aso, T., 2001. Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstetrics and Gynecology 97, 109–115.

Taku, K., Melby, M.K., Kurzer, M.S., Mizuno, S., Watanabe, S., Ishimi, Y., 2010. Effects of soy isoflavone supplements on bone turnover markers in menopausal women: systematic review and meta-analysis of randomized controlled trials. Bone 47 (2), 413–423.

Taku, K., Melby, M.K., Takebayashi, J., et al., 2010. Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. Asia Pacific Journal of Clinical Nutrition 19 (1), 33–42.

Tsai, K.S., 1997. Osteoporotic fracture rate, bone mineral density, and bone metabolism in Taiwan. Journal of the Formosan Medical Association 96, 802–805.

Tsai, K., Twu, S., Chieng, P., Yang, R., Lee, T., 1996. Prevalence of vertebral fractures in Chinese men and women in urban Taiwanese communities. Calcified Tissue International 59, 249–253.

Tsuchida, K., Mizushima, S., Toba, M., Soda, K., 1999. Dietary soybeans intake and bone mineral density among 995 middle-aged women in Yokohama. Journal of Epidemiology 9, 14–19.

Vupadhyayula, P.M., Gallagher, J.C., Templin, T., Logsdon, S.M., Smith, L.M., 2009. Effects of soy protein isolate on bone mineral density and physical performance indices in postmenopausal women: a 2-year randomized, double-blind, placebo-controlled trial. Menopause 16 (2), 320–328.

Wong, W.W., Lewis, R.D., Steinberg, F.M., et al., 2009. Soy isoflavone supplementation and bone mineral density in menopausal women: a 2-y multicenter clinical trial. American Journal of Clinical Nutrition 90 (5), 1433–1439.

Xu, L., Lu, A., Zhao, X., Chen, X., Cummings, S.R., 1996. Very low rates of hip fracture in Beijing, People's Republic of China, the Beijing Osteoporosis Project. American Journal of Epidemiology 144, 901–907.

Ye, Y.B., Tang, X.Y., Verbruggen, M.A., Su, Y.X., 2006. Soy isoflavones attenuate bone loss in early postmenopausal Chinese women: a single-blind randomized, placebo-controlled trial. European Journal of Nutrition 45 (6), 327–334.

Zhang, X., Shu, X.-O., Li, H., et al., 2005. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. Archives of Internal Medicine 165, 1890–1895.

Soy: Animal Studies, Spanning the Lifespan

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1. INTRODUCTION

Animal studies, similar to human studies investigating the role of soy in bone health, have focused on the effects of soy protein or soy isoflavones on bone metabolism. To study the effect of soy protein, soy protein isolate (SPI), which is derived from defatted soybeans and contains a minimum of 90% protein, has been most commonly used. SPI can vary widely in its isoflavone content and thus allows investigators to compare the biological effects of SPIs that contain low versus high levels of isoflavones. SPI contains the glycoside (genistin, daidzin, and glycitin) and the biologically active aglycone (genistein, GEN; daidzein, DAI; and glycitein, GLY) forms of isoflavones. GEN is the predominant aglycone and this is the reason why most animal studies have focused on studying how GEN alone modulates bone health. DAI and GEN can weakly bind to both estrogen receptors-α and -β, but preferentially bind to estrogen receptor-β, which is highly expressed in the osteoblasts in trabecular bone (Onoe et al., 1997). Considering that isoflavones have estrogenic activity, it is hypothesized that they can induce the greatest biologic effects on bone when endogenous levels of sex steroids are low as occurs in early postnatal life and during aging. This chapter presents the scientific evidence from animal studies in which the effect of SPI and/or isoflavones on bone health have been investigated. Three distinct stages of the lifespan have been studied: early life, early adulthood, and aging.

2. ANIMAL MODELS USED FOR STUDYING EFFECTS OF SOY ON BONE METABOLISM

The choice of animal model depends on the scientific question and the stage of the lifespan being investigated. Practical considerations such as body size, length of time required to reach peak bone mass, and the form of the intervention (SPI, isoflavones, and soy infant formula) can dictate the choice of animal model used. Studies that investigate the effects of early life exposure to isoflavones in adulthood often use rodent models as peak

bone mass can be achieved within a relatively short period of time. However, because of their small body size compared to human infants, it is not possible to feed a sufficient quantity of soy infant formula to rodents, particularly mice, to achieve serum isoflavone levels that resemble those of human infants fed soy formula. This is one of the reasons why isoflavones, in the aglycone form, are most often administered to rodents via subcutaneous injection during early life. A few studies have administered isoflavones orally but this is technically challenging. Other investigators have used the piglet model and studied them up to ∼6 weeks of life. While it is not feasible to follow them throughout the lifespan due to their large size and the time required to reach peak bone mass (∼3 years of age), an advantage of the piglet model is that soy-based infant formula can be fed directly. Interventions at early adulthood, when endogenous concentrations of sex steroids are adequate, have primarily used the intact rodent model. Studies designed to investigate whether soy or its isoflavones attenuate deterioration of bone tissue when endogenous concentrations of sex steroids are minimal have most often used ovariectomized rodent models. The ovariectomized rat is a preclinical model approved by the Food and Drug Administration for investigating postmenopausal osteoporosis. Acute ovarian estrogen deficiency leads to a high rate of bone turnover in which the rate of bone resorption exceeds the rate of bone formation. This high rate of bone turnover post ovariectomy is followed by a slower phase of bone loss and is consistent with postmenopausal loss of bone mineral density (BMD) and structure. Moreover, both ovariectomized rodents and postmenopausal women exhibit a greater loss of trabecular than cortical bone, have reduced intestinal calcium absorption, and exhibit similar skeletal responses to commonly used drug therapies including estrogen, tamoxifen, bisphosphonate, parathyroid hormone, and calcitonin. In adult animals, it is possible to incorporate SPI directly into the diet as the main or sole source of protein. Findings obtained using animal models provide a basis for achieving a more comprehensive understanding of how SPI and isoflavones may modulate bone health at distinct stages of the lifespan.

3. EARLY LIFE

The developing fetus and the newborn are sensitive to hormonal stimuli. Thus, the biological effect of soy isoflavones, with their potential estrogenic activity at these early stages of life, has been of particular interest. Cord blood of infants born to mothers consuming significant amounts of soy does demonstrate that there is transfer of isoflavones from mother to offspring. Moreover, infants consuming soy-based infant formulas are exposed to isoflavone levels that are tenfold higher, on a body weight basis, than in adults consuming diets rich in soy. Over the past 20 years, ten animal studies investigating the effects of soy isoflavones on bone development have been published (Chen et al., 2009; De Wilde et al., 2007; Fujioka et al., 2007; Julius et al., 1982; Kaludjerovic and Ward,

2009, 2010; Peterson et al., 2008; Piekarz and Ward, 2007; Ren et al., 2001; Ward and Piekarz, 2007). These studies report either no effect or a positive effect on acquisition of bone mineral, bone strength, and changes in molecular markers of bone health. These studies can be classified according to prenatal, neonatal, and prepubertal exposure. Differences in timing and duration of exposure as well as dose likely explain why some but not all studies report changes in bone development with soy or isoflavone.

3.1 Prenatal Exposure

One study examined the effects of prenatal exposure to soy isoflavones on the long-term programming of bone metabolism (Ward and Piekarz, 2007; Table 28.1). This study found that in utero exposure to DAI, GEN, and the combination of DAI+GEN did not result in higher bone mass or greater bone strength at femurs or lumbar vertebrae in adulthood. It may be that serum estrogen levels are sufficiently high during gestation and prevent soy isoflavones from binding to estrogen receptors and thus acting like estrogen agonists. It is also possible that minimal amounts of isoflavones were transferred from the mother to the fetus during pregnancy. Alternatively, another study showed that newborn pigs whose mothers were exposed to 20.8–40 mg of DAI per day from gestation day (GD) 85–114 had reduced estrogen receptor (ER) gene expression in the hypothalamus and elevated insulin-like growth factor-I (IGF-I) receptor gene transcription in skeletal muscle (Ren et al., 2001). The long-term implications of these changes require further investigation.

3.2 Neonatal Exposure

A few studies have investigated the effects of neonatal exposure to soy isoflavones on skeletal development (Table 28.1; Chen et al., 2009; Julius et al., 1982; Kaludjerovic and Ward, 2009; Piekarz and Ward, 2007). Piglets fed soy-based infant formula for the first 21 or 35 days of life had greater bone mineral content (BMC), BMD, and trabecular number in the tibia than sow-fed piglets (Chen et al., 2009). These piglets also exhibited a greater number of osteoblasts, higher expression of bone formation genes (alkaline phosphatase and bone morphometric protein-2), and higher levels of serum bone formation markers (osteocalcin and bone-specific alkaline phosphatase) accompanied by lower levels of bone resorption markers (↓ serum CTX and ↓ expression of RANKL in tibia). Whether these benefits are sustained until adulthood is unknown. In mice, administration of isoflavones (GEN or DAI alone or in combination) during the first 5 days of postnatal life results in higher BMD, improved bone structure, and greater bone strength in female mice at adulthood (Kaludjerovic and Ward, 2009; Piekarz and Ward, 2007). These studies have administered isoflavones by subcutaneous injection and the serum levels of isoflavones resemble those in human infants fed soy infant formula. Compared to controls, mice treated with GEN had higher BMD at the lumbar spine (LV1–4) and, importantly,

Table 28.1 Effects of Early Life Exposure to Soy or Soy Isoflavones on Bone Development

Reference	Model	Timing of exposure	Studied until	Treatment	Route of administration	Findings
Prenatal exposure						
Ward and Piekarz (2007)	Mouse (CD-1) $n = 12$/group Males, females	GD 9 to GD 21	17 weeks	DAI alone (3.75 mg kg^{-1} bw day^{-1}) GEN alone (3.75 mg kg^{-1} bw day^{-1}) DAI+GEN (7.5 mg kg^{-1} bw day^{-1})	Subcutaneous injection to mothers	• Femur BMD was higher in CON and GEN group than DAI and DAI+GEN group • Treatment did not have an effect on femur peak load • Females given DAI had lower LV1–4 BMD than all other groups, but LV4 peak load did not differ between groups • In utero exposure to ISO did not result in functional benefits to bone at young adulthood Conclusion: In utero exposure to ISO did not have functional benefits to bone at adulthood
Ren et al. (2001)	Piglet $n = 6$–8/ group Males	GD 85 to GD 114	6 h post birth	DAI (8 mg kg^{-1} feed)	Oral administration to mothers	• DAI resulted in higher birth weight of males and lower levels of ER-β mRNA in the hypothalamus but not in the pituitary • DAI resulted in higher transcription of IGF-I receptor gene in skeletal muscle but had no effect on IGF-I receptor expression in the pituitary, hypothalamus, thymus, and liver

						• DAI may promote fetal growth by increasing IGF-I receptor gene expression in skeletal muscle *Conclusion*: DAI influences fetal growth and this is associated with higher expression of IGF-I receptor gene in skeletal muscle and down-regulates the expression of the ER-β gene in the hypothalamus
Neonatal exposure						
Julius et al. (1982)	Piglet $n=6$/group Males, females	PND 9–12 to PND 41–44	6 weeks	Milk protein formula[a] Soy protein isolate formula[a]	Oral	• There was no difference in body weight or growth between pigs fed SPI and milk protein formula • Pigs fed SPI had lower plasma cholesterol compared to those exposed to milk protein formula • Bone calcium, measured as percent of dry, fat-free femur or whole carcass ash, was lower in pigs fed SPI compared to those fed milk protein formula *Conclusion*: Similar growth and development were observed in pigs fed SPI or MP formula; but pigs fed SPI formulas had significantly less bone calcium

Continued

Table 28.1 Effects of Early Life Exposure to Soy or Soy Isoflavones on Bone Development—cont'd

Reference	Model	Timing of exposure	Studied until	Treatment	Route of administration	Findings
Piekarz and Ward (2007)	Mouse (CD-1) $n = 4$–14/group Males, females	PND 1 to PND 5	16 weeks	GEN (4 mg kg^{-1} bw day^{-1})	Subcutaneous injection	• Females: higher femur and LV1–4 BMD in DES and GEN groups compared to CON; there was also a higher peak load among GEN and DES groups • Males: LV BMD and peak load of LV3 were higher among GEN group compared to CON or DES groups _Conclusion_: Neonatal exposure to GEN had positive effects on femur and lumbar spine of female mice and lumbar spine of male mice at adulthood
Chen et al. (2009)	Piglet $n = 3$–5/group Males, females	PND 2 to PND 21/35	3 or 5 weeks	Cow milk-based formula – Similac advance powder[a] Enfamil Prosobee Lipil powder soy formula[a]	Oral	Compared to sow-fed piglets male and female piglets fed soy formula for 21 days had: • higher osteoblastogenesis in ex vivo bone marrow cell culture • higher serum osteocalcin, higher bone-specific ALP, and lower CTX • higher tibial BMP2 and ALP mRNA expression • higher tibial expression of extracellular kinases • lower tibial RANKL expression

						Compared to sow-fed piglets male piglets fed soy formula for 35 days had:
						• higher osteoblast number and lower osteoclast number,
						• higher BV/TV and higher trabecular bone formation rate and higher mineral apposition rate in the tibia
						Conclusion: Soy-fed piglet had the best quality bone and the anabolic effects may be mediated through enhanced BMP signaling.
Kaludjerovic and Ward (2009)	Mouse (CD-1) $n = 8\text{--}16/$ group Males, females	PND 1 to PND 5	16 weeks	DAI alone (2 mg kg^{-1} bw day^{-1}) GEN alone (5 mg kg^{-1} bw day^{-1}) DAI+GEN (7 mg kg^{-1} bw day^{-1})	Subcutaneous injection	• Females treated with ISO had improved BMD, structure, and strength at the lumbar vertebra
						• DAI+GEN did not have greater effects on bone than either treatment alone
						• In males, isoflavone exposure had neither a benefit nor an adverse effect on bone
						Conclusion: Neonatal exposure to DAI and/or GEN improved bone development of female mice at adulthood, but compared with individual treatment, DAI+GEN did not provide greater benefits in female or male mice

Continued

Table 28.1 Effects of Early Life Exposure to Soy or Soy Isoflavones on Bone Development—cont'd

Reference	Model	Timing of exposure	Studied until	Treatment	Route of administration	Findings
Kaludjerovic and Ward (2010)	Mouse (CD-1) $n = 8$–18/ group Males, females	PND 1 to PND 5	Sex organs excised at 16 weeks and mice studied to 32 weeks	DAI alone ($2\,\mathrm{mg\,kg^{-1}}$ bw $\mathrm{day^{-1}}$) GEN alone ($5\,\mathrm{mg\,kg^{-1}}$ bw $\mathrm{day^{-1}}$) DAI+GEN ($7\,\mathrm{mg\,kg^{-1}}$ bw $\mathrm{day^{-1}}$)	Subcutaneous injection	• Females treated with DAI, GEN, or DAI+GEN had higher BMD and improved trabecular connectivity at the femur neck and lumbar spine 4 months post OVX • Improvements in bone mineral and structure translated to bones that were resistant to fracture *Conclusion*: Neonatal exposure to DAI and GEN attenuated deterioration of bone tissue in ovariectomized females but not in orchidectomized males
Prepubertal exposure De Wilde et al. (2007)	Piglet $n = 8$/group Females	PND 40 to PND 84	6 weeks	Control = 6.6 mg ISO per kilogram of diet SoyLife (66% DAI+31% GEN) = 356 mg ISO per kilogram of diet or $2.8\,\mathrm{mg\,kg^{-1}}$ bw $\mathrm{day^{-1}}$	Oral (pair-fed)	• SoyLife did not alter growth rate, body weight, biomarkers of bone turnover, BMD, and strength • Stromal cells from SoyLife-fed pigs had higher ALP, OPG, RANKL, and osteocalcin, and more mineralized nodules *Conclusion*: ISO had no effect on the bone mass of growing female piglets but stimulated bone marrow osteoprogenitor cells via ERs

| Fujioka et al. (2007) | Mouse $n=8$/group Males, females | 5–9 weeks | 9 weeks | DAI alone (0.08% of diet) ($\sim$3 mg kg^{-1} bw day^{-1}) GEN alone (0.08% of diet) ($\sim$3 mg kg^{-1} bw day^{-1}) | Oral (pair-fed) | Females:
• DAI resulted in lower bone formation and BMD in whole body and femur
• GEN had no effect on female bone metabolism
Males:
• DAI resulted in higher bone formation and BMD for whole body, lumbar spine, and femur
• GEN resulted in higher bone formation and femur BMD
Conclusion: DAI has a sexually dimorphic effect on bone formation and BMD during growth in mice with positive effects in males and adverse effects in females |
| Peterson et al. (2008) | Rats (Sprague–Dawley) $n=8$–9/ group Females | 3–11 weeks | 11 weeks | Casein (200 g kg^{-1} diet) SPI containing ISO with $\sim$56% GEN: low ISO (0.11 mg g^{-1} protein), med ISO (2.16 mg g^{-1} protein), and high ISO (3.95 mg g^{-1} protein) | Oral | • High ISO consumption suppressed serum E_2 levels but had no effect on total serum estrogenicity
• Rats fed low ISO diet had lower body weight from 4 weeks of age to the end of study, and lower tibia calcium content than all other groups
• Rats fed medium or high ISO diet had higher uterine |

Table 28.1 Effects of Early Life Exposure to Soy or Soy Isoflavones on Bone Development—cont'd

Reference	Model	Timing of exposure	Studied until	Treatment	Route of administration	Findings
						weight relative to body weight compared to control
						• Soy intake had no effect on whole body or tibia BMD
						Conclusion: Bone growth and BMD were unaffected by SPI intake

ALP, alkaline phosphatase; BMD, bone mineral density; BMP2, bone morphometric protein 2; BV/TV, bone volume/total volume; CON, control; CTX, collagen cross-links; DAI, daidzein; DES, diethylstilbestrol; E2, estradiol; ER-β, estrogen receptor-β; GD, gestation day; GEN, genistein; IGF-I, insulin growth factor-I; ISO, isoflavone; LV, lumbar vertebrae; MP, milk protein; OPG, osteoprotegrin; PND, postnatal day; RANKL, receptor activator for nuclear factor β ligand; SPI, soy protein isolate.
[a] There are no published data on the ISO content or composition in the diet.

this translated to individual vertebrae that were stronger and more resistant to compression fracture as demonstrated by the significantly higher peak load of LV3 (Piekarz and Ward, 2007). Effects of GEN on bone development in females were similar to those induced by diethylstilbestrol (an environmental estrogen) while in males, GEN and diethylstilbestrol had divergent effects (Piekarz and Ward, 2007). This finding suggests that GEN enhances bone development through an estrogen–dependent mechanism in females but not males. A follow-up study used a similar experimental design that included DAI and a combination of GEN and DAI that mimicked a ratio equivalent to that occurring in soy protein formula (Kaludjerovic and Ward, 2009). The key finding from this study was that the combination of GEN+DAI does not induce greater benefits to bone than either treatment alone, suggesting that GEN and DAI could be competing for the same estrogen receptors. Female mice exposed to GEN+DAI had higher lumbar vertebra BMD, and microstructural analysis showed greater trabecular connectivity and reduced bone porosity. These structural characteristics resulted in stronger vertebrae that could withstand greater forces before compression fracture occurred. It was later shown that the higher lumbar vertebra BMD, improved bone structure, and greater strength at young adulthood were protective against the deterioration of bone tissue in ovariectomized females but not orchidectomized males. Females treated with GEN+DAI had a 1.3- and a 1.6-fold increase in lumbar spine peak load at 4 and 8 months of age, respectively, relative to controls (Kaludjerovic and Ward, 2010).

3.3 Prepubertal Exposure

Three animal studies have investigated the effects of prepubertal exposure to soy isoflavones on bone development (De Wilde et al., 2007; Fujioka et al., 2007; Peterson et al., 2008; Table 28.1). In one study, oral consumption of DAI or GEN from 5 to 9 weeks of age induced gender-specific effects on bone metabolism in a mouse model, with a higher bone formation rate and femur BMD in males and a lower bone formation rate and whole body BMD in females (Fujioka et al., 2007). These findings were unexpected and difficult to explain by what is currently known about the effects of sex steroids on bone metabolism. Estrogen is the primary sex hormone in females and has been repeatedly shown to have positive effects on female bone metabolism, so why weak dietary estrogens would induce adverse effects in females, but not in males who are more sensitive to estrogen fluctuations, is unclear. In another study, female rats were fed with SPI containing low, medium, or high levels of soy isoflavones from 3 to 11 weeks of age, and tibia BMD and histomorphometry were measured (Peterson et al., 2008). Tibial growth and BMD were unaffected by soy intake while serum estradiol concentrations were significantly lower in the high soy isoflavone group compared to the low isoflavone group. Endogenous levels of estradiol were unaltered by diet, indicating that high doses of isoflavones can suppress endogenous estrogen production and contribute to the total

estrogenic activity. The third study fed a commercial product 'SoyLIfe' containing a mixture of DAI+GEN to pigs from postnatal day (PND) 40 to PND 82 (De Wilde et al., 2007). There was no significant effect on growth rate, biochemical markers of bone formation (i.e., alkaline phosphotase and osteocalcin), tibial or metacarpal BMD, or metatarsal strength. However, analyses of bone cells isolated from the treated piglets revealed that soy isoflavones stimulate production of bone formation markers (i. e., alkaline phosphatase and osteocalcin), expression of ER-α and -β, and synthesis of molecular factors that enhance osteoblastic activity such as osteoprotegrin. The favorable changes in these factors suggest that a longer intervention, that is, greater than 6 weeks, could have resulted in measurable benefits to whole bone tissue.

In summary, while not all studies investigating effect of early life exposure suggest positive effects to bone development, some studies do demonstrate that bone tissue may be responsive to isoflavones, particularly when exposure occurs during neonatal life. These studies provide a basis for investigating the effects of soy infant formula on bone development in humans and insight into potential mechanisms by which benefits may occur. Long-term follow-up of infants who consumed soy formula will provide a definitive answer on the potential of early life exposure to isoflavones on bone development and eventual adult bone health – such prospective trials are underway.

4. EARLY ADULTHOOD

A few animal studies, all in rodent models, have examined the effects of SPI or soy isoflavones on bone health at early adulthood when endogenous sex steroid production is intact (Table 28.2). For the most part, findings from these studies suggest small, if any, benefits to bone. It may be that potential estrogenic effects of isoflavones are attenuated by the presence of endogenous sex steroids. Two studies found that exposure to SPI or soy isoflavones at 3 months of age had no significant effect on body weight, femur BMD, and uterine wet weight following 12 and 14 weeks of treatment in Sprague–Dawley and F344 rats, respectively (Nakai et al., 2005a,b). However, F344 rats exposed to SPI for 14 weeks had reduced concentrations of urinary deoxypyridinoline, a marker of bone resorption, and improved BMD at the lumbar spine compared to casein-fed rats (Nakai et al., 2005a). These findings suggest that duration of exposure and/or animal strain may affect modulation of bone tissue metabolism by soy isoflavones in adulthood. Inflammation may provide an environment in which soy isoflavones can benefit bone health. One study in mice has investigated whether isoflavones attenuate inflammation-induced changes in bone metabolism. A 6-week treatment with isoflavones in mice treated with endotoxin – which elicits a strong immune response – showed improved trabecular structure and, thus, better bone quality (Droke et al., 2007). This is an interesting area for future investigation as inflammation is not uncommon throughout the lifespan.

Table 28.2 Summary of Animal Studies: Effects of Early Adult Exposure to Soy or Soy Isoflavones on Bone Health

Reference	Model	Timing of exposure	Treatment	Route of administration	Findings
Nakai et al. (2005a)	Rats (F344) $n = 10$–14/ group Females	3.5-month-old rats were fed one of five diets for 14 weeks	• Casein = 200 g per kilogram of diet • Low soy = 100 g casein per kilogram of diet + 100 g SPI per kilogram of diet • High soy = 200 g SPI per kilogram of diet • Casein + ISO = 200 g casein per kilogram of diet + 17.2 g ISO extract per kilogram of diet • Casein + ISO = 200 g casein per kilogram of diet + 34.4 g ISO extract per kilogram of diet *Note*: SPI = 11.37 mg of ISO per gram (3.81 mg DAI, 7.09 mg GEN)	Oral (pair-fed)	• There were no differences in body weight, femur BMD, or uterine wet weight between groups • Rats fed casein + SPI had higher BMD at the lumbar spine than rats fed casein alone or low ISO • Rats fed SPI had lower urinary deoxypyridinoline compared to all other groups *Conclusion*: Exposure to SPI reduced bone turnover and improved lumbar spine BMD but had no effect on body weight, uterine weight, or femur BMD
Nakai et al. (2005b)	Rats (Sprague–Dawley) $n = 10$/group Females	3-month-old rats were fed one of five diets for 12 weeks	• Casein = 200 g per kilogram of diet • Low soy = 100 g casein per kilogram of diet + 100 g SPI per kilogram of diet • High soy = 200 g SPI per kilogram of diet • Casein + ISO = 200 g casein per kilogram of diet + 17.2 g ISO extract per kilogram of diet • Casein + ISO = 200 g casein per kilogram of diet + 34.4 g ISO extract per kilogram of diet	Oral (pair-fed)	• There were no differences in body weight, urinary deoxypyrdinoline levels, femur, and lumbar spine BMD, or uterine wet weight among groups *Conclusion*: SPI or ISO had no significant effect on body weight, uterine wet weight, or bone

Table 28.2 Summary of Animal Studies: Effects of Early Adult Exposure to Soy or Soy Isoflavones on Bone Health—cont'd

Reference	Model	Timing of exposure	Treatment	Route of administration	Findings
Droke et al. (2007)	Mouse (C57BL/6J) model of chronic inflammation $n = 12–13/$ group Females	9-week-old mice were put on one of three diets for 2 weeks and then pellets were inserted in mice so they received treatment+diet for 4 weeks	*Note*: SPI = 2.14 mg of aglycone from ISO per gram (0.84 mg DAI, 1.10 mg GEN) Mice were randomized to one of six groups: • Placebo or 1.33 μg of lipo-polysaccharide (LPS) per day in combination with 0, 126, or 504 mg aglycone ISO per kilogram of diet *Note*: lipopolysaccharide is an endotoxin that elicits a strong immune response in animals; ISO = 7.95% DAI, 16.9% GEN, or a total of 25.2% ISO expressed as aglycone equivalent	Oral	• There was no difference in final body weight or uterine weight among groups • Mice exposed to LPS had lower BV/TV, lower Tb.N, higher Tb.Sp, and lower trabecular bone strength compared to untreated mice • Exposure to high ISO diet provided protection against detrimental effects of inflammation to bone micro-architecture but not biomechanical strength properties • Exposure to ISO resulted in lower TNF-α that is elevated after administration of LPS *Conclusion*: ISO may attenuate adverse effects of chronic inflammation on bone health

BMD, bone mineral density; BV/TV, bone volume/total volume; ISO, isoflavone; LPS, lipopolysaccharide; SPI, soy protein isolate; Tb.N, trabecular number; Tb.Sp, trabecular separation; TNF-α, tumor necrosis factor-α.

5. AGING

The decline in endogenous sex steroid production during aging is responsible for loss of BMD and higher susceptibility to fragility fractures. In women, the decline in endogenous estrogen production leads to an imbalance in bone remodeling, with rates of bone resorption exceeding rates of bone formation. The volume of the resorption cavity enlarges and results in perforation of trabecular plates, a loss of trabecular connectivity, and an elevated risk of fragility fracture. In cortical bone, estrogen deficiency is associated with subendocortical cavitation that over time can cause one-third of the inner cortex to assume trabecular-like characteristics and alter the overall strength and structure of skeletal tissue. Some, but not all, studies using rodents have shown that treatment with SPI or soy isoflavones attenuates the reduction of BMD after ovariectomy when ovariectomy is performed in skeletally mature rodents (>3 months of age), which most closely mimics postmenopausal bone loss (Table 28.3).

The first animal studies conducted in rodent models examined the effects of soy milk or SPI compared to a casein diet and reported that femur BMD was higher among the soy-fed rats compared to the casein-fed rats (Arjmandi et al., 1996; Blum et al., 2003; Chen et al., 2008; Devareddy et al., 2006). Later studies showed that it is the isoflavone content itself that can protect against loss of bone tissue after ovariectomy. It was reported that oral exposure to 30 μmol of GEN per day for 30 days in ovariectomized Sprague–Dawley rats increased dry femur bone ash weight by 12% (Blair et al., 1996). Similar findings were reported in a ddY mouse model in which subcutaneous administration of 0.7 mg of GEN per day for 30 days reduced ovariectomy-induced bone loss in the femur (Ishimi et al., 2000). Female ddY mice exposed to low (0.7 mg day^{-1}) or high (5 mg day^{-1}) doses of GEN had a significantly higher BMD at the femur than sham-operated mice exposed to placebo. While there are several studies that report the protective effects of SPI or isoflavones against ovariectomy-induced deterioration of bone, it is important to note that a review of animal models used to investigate the health benefits of soy isoflavones concluded that studies in mice, rats, and monkeys do not show a consistent benefit of isoflavones in preventing bone loss (Cooke, 2006). The review also suggested that animal models may not be entirely representative of humans because ovariectomy causes a sudden drop in endogenous estrogen production whereas hormonal changes are more gradual during menopause. Several studies have reported that intervention with SPI or isoflavones does not attenuate ovariectomy-induced deterioration of bone tissue (Bahr et al., 2005; Cai et al., 2005; Ward, 2005). An 8-week isoflavone intervention to rats did not show attenuation of loss of BMD and bone strength of femur and lumbar spine (Ward, 2005). In another study, 6-month-old ovariectomized Sprague–Dawley rats were randomly assigned to one of nine treatment groups and were pair-fed soy- or casein-based diets with or without soy isoflavones for 8 weeks to characterize the effects of SPI and soy isoflavones on calcium and bone metabolism

Table 28.3 Effects of Soy or Soy Isoflavone on Preservation of Bone Tissue During Aging

Reference	Model	Age at surgery	Treatment	Route of administration	Study duration	Findings

Arjmandi et al. (1996)	OVX rat (Sprague–Dawley) $n=8$/group	95 days	• Sham
			• OVX
			• OVX+
			  ○ 17β-estradiol
			  ○ 227 g SPI per kilogram of diet[a]
				Pair-fed plus 1 g per day for growth	Right after surgery rats were treated for 30 days	• Sham-operated rats and those fed a diet fortified with 17β-estradiol or soybean had higher BMD at the femur and LV4 than OVX rats
						• OVX rats had lower serum concentration of 1,25-(OH)D than all other groups
						Conclusion: Dietary soybean protein is effective in preventing bone loss

Blum et al. (2003)	OVX rat (Sprague–Dawley) $n=8–9$/group	308 days (11 months)	• Sham+
			  ○ 140 g casein per kilogram of diet
			  ○ 140 g soy per kilogram of diet
			• OVX+
			  ○ 140 g casein per kilogram of diet
			  ○ 140 g soy per kilogram of diet
			Note: soy = 256 mg GEN+195 mg DAI per kilogram of diet
				Fed ad libitum	After surgery, diet was given for 98 days	• OVX group fed soy had higher femur BMD, higher endocortical mineral formation and apposition rate at the tibiofibular junction, and higher trabecular bone formation rate at the proximal tibial metaphysis compared to OVX+casein group
						Conclusion: Soy protein had a beneficial but not complete effect on maintaining cortical and trabecular BMD after OVX in the aged, retired breeder rats

| Devareddy et al. (2006) | OVX rat (Sprague–Dawley) $n=12$–13/group | 257 days (9 months) | • Sham+227 g casein per kilogram of diet
• OVX+227 g casein per kilogram of diet
• OVX+E$_2$+227 g casein per kilogram of diet
• 227 g SPI per kilogram of diet with
  ○ 0.01 mg ISO per gram of diet[a]
  ○ 0.81 mg ISO per gram of diet[a]
  ○ 1.61 mg ISO per gram of diet[a] | Pair-fed | 90 days post surgery, treatment was given for 125 days | • Treatment did not have an effect on vertebral BMD or bone microarchitecture
• Rats exposed to 0.81 mg ISO per gram of diet had higher tibial BMC and BMD than OVX rats but had lower BMD than sham rats
• Trabecular separation was attenuated with soy enriched with ISO
Conclusion: Soy protein does not restore bone loss but ISO may reverse OVX-induced bone loss in tibia |
| Chen et al. (2008) | OVX rat (Sprague–Dawley) $n=6$–8/group | 55 days (2 months) | • Sham/OVX+
  ○ Casein
  ○ SPI[a]
  ○ whey protein hydrolysate
  ○ rice protein isolate | Fed ad libitum | After surgery, treatment was given for 14 days | • All protein sources had positive effects on BMC or BMD relative to casein but SPI had greater effects in both intact and OVX rats
• In OVX rats, SPI resulted in higher serum osteocalcin, higher ALP, and lower levels of RatLaps
• Exposure to SPI results in higher expression of ALP and osteocalcin gene in bone tissue, as well as lower expression of RANKL, ER-α, and ER-β genes |

Continued

Table 28.3 Effects of Soy or Soy Isoflavone on Preservation of Bone Tissue During Aging—cont'd

Reference	Model	Age at surgery	Treatment	Route of administration	Study duration	Findings

Bahr et al. (2005)	OVX rat (Sprague–Dawley) $n=7$–9/ group	84 days (3 months)	• Sham
			• OVX
			• OVX+
			   ○ low SPI – 100 g per kilogram of diet
			   ○ high SPI – 200 g per kilogram of diet
			   ○ low ISO – 17.2 g per kilogram of diet
			   ○ high ISO – 100 g per kilogram of diet
			   ○ E_2
			Note: SPI = 2.14 mg of aglycone from ISO per gram (0.84 mg DAI, 1.10 mg GEN, and 0.20 mg glycerin)
				Pair-fed	A week after surgery treatment was given for 84 days	*Conclusion*: Soy has bone-sparing effects in rapidly growing animals
						• OVX resulted in higher body weight and food intake, lower uterine weight, higher deoxypyridinoline, and lower BMD
						• E_2 treatment did not alter body weight or bone outcomes but did result in lower uterine weight compared to sham.
						• SPI or low ISO diet did not abrogate the effects induce by OVX
						• Rats fed with a high ISO diet had higher bone volume and greater trabecular connectivity in the femur compared to OVX, OVX+low SPI or OVX+low ISO groups
						Conclusion: Dietary intake of ISO has minimal benefits in preserving bone tissue after ovariectomy

| Cai et al. (2005) | OVX Rat (Sprague–Dawley) n = 9/ group | 168 days (6 months) | • Sham+casein
• OVX+casein +
  o 0.3 mg ISO per gram of diet[a]
  o 0.8 mg ISO per gram of diet[a]
  o E$_2$
  o E$_2$+0.3 mg ISO per gram of diet[a]
• OVX+Soy +
  o 0.2 mg ISO per gram of diet[a]
  o 0.6 mg ISO per gram of diet[a] | Pair-fed | After surgery, treatment was given for 56 days | • Treatment did not have an effect on total Ca balance or Ca absorption but soy protein resulted in a lower urinary loss of Ca irrespective of ISO content
• ISO given as enriched SPI or supplement did not prevent trabecular or cortical bone loss of the tibia, or loss of femur BMD
Conclusion: Unlike estrogen, ISO at the levels tested do not suppress OVX-induced bone loss |
| Picherit et al. (2001) | OVX rat (Wistar) n = 9/ group | 210 days (7.5 months) | • Sham
• OVX
• OVX+
  o no ISO
  o 20 mg ISO per kilogram of bw per day
  o 40 mg ISO per kilogram of bw per day
  o 80 mg ISO per kilogram of bw per day
Note: ISO = 46% GEN, 45% DAI | Pair-fed | 80 days after surgery rats were treated for 84 days | • Exposure to ISO did not affect the body weight or femur BMD of OVX rats
• Sham-operated rats had higher femur BMD than OVX rats irrespective of ISO exposure
• There was no difference among groups in femur length, diaphyseal diameter, or strength
• Plasma osteocalcin and deoxypyridinoline levels were higher in OVX rats compared to sham or OVX rats exposed to 40 or 80 mg ISO per kilogram of bw per day |

Table 28.3 Effects of Soy or Soy Isoflavone on Preservation of Bone Tissue During Aging—cont'd

Reference	Model	Age at surgery	Treatment	Route of administration	Study duration	Findings

Picherit et al. (2000))	OVX rat (Wistar) $n=13$/ group	336 days (12 months)	• Sham
			• OVX
			• OVX+
			  o 10 µg GEN per kilogram of bw per day
			  o 10 µg DAI per kilogram of bw per day
			  o 30 µg E_2 per kilogram of bw per day
				Pair-fed	A day after surgery rats were treated for 84 days	*Conclusion*: Daily ISO intake reduces bone turnover but does not reverse established osteopenia in OVX rats
						• Exposure to DAI and E_2 restored OVX-induced BMD loss at the femur and lumbar spine but GEN had no significant effect
						• OVX rats exposed to DAI had comparable trabecular bone area to sham-operated rats
						• OVX rats fed DAI, GEN, or E_2 had higher osteocalcin and lower deoxypyridinoline compared to sham rats
						Conclusion: Consumption of E2 or DAI was more efficient than GEN in attenuating bone loss after OVX

ALP, alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; Ca, calcium; DAI, daidzein; E_2, estradiol; ER–β, estrogen receptor-β; GEN, genistein; ISO, isoflavone; OVX, ovariectomized; RANKL, receptor activator for nuclear factor ß ligand; SPI, soy protein isolate.
[a] There are no published data on the specific ISO composition in the diet.

(Cai et al., 2005). The two casein diets were enriched with isoflavones (300 or 800 mg of isoflavones per kilogram of diet) while the two soy protein–based diets contained isoflavones (200 or 400 mg of isoflavones per kilogram of diet). Isoflavone treatments did not alter total calcium balance or calcium absorption, and isoflavones did not suppress bone remodeling in trabecular or cortical bone after ovariectomy. It is possible that subtle differences were induced by soy or isoflavones but were undetected as a result of the number of group comparisons.

Several studies have examined whether combining soy isoflavones with other dietary factors can have additive or synergistic effects on preserving skeletal integrity post ovariectomy in rodents. One study showed that feeding ovariectomized rats a control diet (AIN93G) containing SPI and high calcium (2.5% of diet) for 8 weeks attenuated lumbar spine BMD, but not vertebral strength, to a greater extent than exposure to SPI or calcium alone (Ward, 2005). Similar findings were observed with isoflavone exposure in ovariectomized rats fed soy isoflavones (1600 mg kg^{-1} diet) and high calcium (2.5% of diet) for 8 weeks (Ward, 2005). In both studies, combined intervention and calcium had a more protective effect against the loss of femoral and vertebral BMD than SPI or isoflavones alone. Because equol, a metabolite of DAI, has the highest estrogenic activity of all the isoflavones, it was hypothesized that exposure to diets high in DAI may have protective effects on bone during aging. Thus, a study was undertaken to determine if combining DAI with high calcium would have greater protective effects against the loss of BMD and biomechanical bone strength in ovariectomized mice than either treatment alone (Ward, 2005). Study findings showed that combining DAI with high calcium improved cortical and trabecular bone after 12 weeks of treatment, as indicated by higher femur and lumbar spine BMD and greater bone strength, but the effects did not differ from the high calcium–alone group. Fructooligosaccharide (FOS), the indigestible sugar that increases intestinal absorption of calcium, magnesium, and iron by stimulating proliferation of beneficial intestinal bacteria, has been shown to improve bone formation and break down the isoflavone conjugates to aglycones and the sugar moieties. Thus, it was hypothesized that combining FOS with isoflavone conjugates would preserve bone tissue to a greater extent than either treatment alone. In ovariectomized mice, both isoflavone conjugates and FOS prevented femoral bone loss, and combining the two compounds had an additive effect on BMD at the proximal and distal femur (Ohta et al., 2002).

In summary, some studies using ovariectomized rats have shown that soy isoflavones can exert bone-sparing effects if exposure is maintained for several weeks or months after surgery. It is speculated that isoflavones compete with 17β-estradiol for binding to estrogen receptors so when endogenous concentrations of sex steroids are reduced, as is the case following ovariectomy, soy isoflavones have a greater probability of binding to estrogen receptors and inducing estrogen-like effects on bone tissue. Studies during aging have focused on females such that sex-specific responses to SPI and isoflavones require further investigation.

6. TRANSGENERATIONAL STUDIES

Recent advances in genomics have generated interest regarding whether isoflavones alter epigenetic regulation. Moreover, genome-wide studies have demonstrated that variations in epigenetic patterns, including chromatin restructuring and DNA methylation, are heritable. Exposure to GEN during pregnancy using heterozygous yellow agouti mice has demonstrated alterations in coat color and body weight of progeny by hypermethylating the transposable repetitive elements upstream of the transcription start site of the *Agouti* gene (Dolinoy et al., 2006). Prenatal exposure to GEN (0.8 mg of GEN per day) has also been shown to affect fetal red blood cell development, resulting in a significant increase in granulopoiesis, erythropoiesis, and macrocytosis in 12-week-old mice (Vanhees et al., 2010). This study also showed that prenatal exposure to GEN stimulates bone marrow formation. Using microarray analyses, these effects were shown to be associated with a marked down-regulation of gene expression (i.e., Cdkn 1a, Ctsd, Ccnd 1, Pcna, Apo E, C3, Igf 2, Crin 2d, Macrod 1, Vegfa, Hdac 6, Abcc 5, and Tacc 1), possibly due to hypermethylation of CpG motifs in the regulatory regions of these genes. As genome-wide studies and other approaches identify novel associations between soy isoflavones and epigenetic patterns, elucidating causal pathways and predicting long-term programming that may extend for several generations may be possible.

To our knowledge, only one transgenerational study has examined the effects of developmental and lifespan exposure to GEN in rats (Hotchkiss et al., 2005). This study treated male and female rats (F_0) from PND 42 to 120 with a diet containing 0, 5, 100, or 500 ppm of GEN. At approximately 10 weeks of age, male and female mice were mated and their progeny (F_1) were exposed to a diet containing 0, 5, 100, or 500 ppm of GEN from conception to PND 120 followed by either a control diet or a diet with continuous exposure to GEN until 2 years of age. A portion of the F_1 male and female rats were mated to obtain F_2 progeny who were also exposed to 0, 5, 100, or 500 ppm of GEN from conception to PND 120. Similarly, a portion of the F_2 rats were mated to obtain F_3 progeny who were exposed to 0, 5, 100, or 500 ppm of GEN from conception to PND 21 and were studied to PND 120. F_4 rats received only control diet, devoid of GEN. This study found no significant effects on BMD at the femur and lumbar spine across generations. However, F_1 female exposed to 500 ppm of GEN from conception to 140 days of life had significantly lower lumbar spine BMC and cross-sectional area than female rats exposed to 5 ppm of GEN at 2 years of age. Male and female rats from the F_3 generation who were exposed to treatment from conception to PND 21 and were studied to adulthood (PND 120) had greater lumbar spine BMC and area than those from the F_1 generation, but only when exposed to high doses of GEN (500 ppm). Interpretation of the intergenerational differences in bone size is challenging because each generation of rats was given GEN for a different duration and during different stages of the lifespan. Functional outcomes of bone were not reported. Studies in which rodents are treated

and then followed into subsequent generations are required to fully delineate whether changes to bone metabolism observed in a treated generation are present in offspring, and if so, for how many generations the effect persists. Maternal versus paternal influences should also be determined.

7. CONCLUSION

In conclusion, there is no clear consensus on whether exposure to soy or isoflavones benefits bone health at distinct stages of the lifespan. However, it does appear that the strongest evidence for benefits is during early life, particularly neonatal life, or during aging as studied using the ovariectomized rodent model. While studies relating epigenetic profiles to diet–disease relationships are in their infancy, ongoing improvements in molecular biology techniques will pave the way for understanding how diet can modulate bone health throughout the lifespan.

GLOSSARY

Microstructural analysis Performed using microcomputed tomography that measures cross-sectional images of a specific skeletal site using X-rays. These cross-sectional images can be combined to create a virtual model of a bone (i.e., femur, tibia, and lumbar vertebra). Outcomes specific to trabecular bone or cortical bone can be determined. Examples of measures that can be obtained include trabecular connectivity, trabecular number, and cortical thickness among many others.

Orchidectomy Surgical removal of testes to mimic deterioration of bone tissue caused by androgen deficiency in male rodents.

Ovariectomy Surgical removal of ovaries to mimic the deterioration of bone tissue caused by estrogen deficiency in female rodents.

REFERENCES

Arjmandi, B.H., Alekel, L., Hollis, B.W., et al., 1996. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. Journal of Nutrition 126, 161–167.

Bahr, J.M., Nakai, M., Rivera, A., et al., 2005. Dietary soy protein and isoflavones: minimal beneficial effects on bone and no effect on the reproductive tract of sexually mature ovariectomized Sprague–Dawley rats. Menopause 12, 165–173.

Blair, H.C., Jordan, S.E., Peterson, T.G., Barnes, S., 1996. Variable effects of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. Journal of Cellular Biochemistry 61, 629–637.

Blum, S.C., Heaton, S.N., Bowman, B.M., Hegsted, M., Miller, S.C., 2003. Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. Journal of Nutrition 133, 1244–1249.

Cai, D.J., Zhao, Y., Glasier, J., et al., 2005. Comparative effect of soy protein, soy isoflavones, and 17beta-estradiol on bone metabolism in adult ovariectomized rats. Journal of Bone and Mineral Research 20, 828–839.

Chen, J.R., Lazarenko, O.P., Blackburn, M.L., et al., 2009. Infant formula promotes bone growth in neonatal piglets by enhancing osteoblastogenesis through bone morphogenic protein signaling. Journal of Nutrition 139, 1839–1847.

Chen, J.R., Singhal, R., Lazarenko, O.P., et al., 2008. Short term effects on bone quality associated with consumption of soy protein isolate and otehr dietary protein sources in rapidly growing female rats. Experimental Biology and Medicine (Maywood, NJ) 233, 1348–1358.

Cooke, G.M., 2006. A review of the animal models used to investigate the health benefits of soy isoflavones. Journal of AOAC International 89, 1215–1227.

De Wilde, A., Rassi, C.M., Cournot, G., et al., 2007. Dietary isoflavones act on bone marrow osteoprogenitor cells and stimulate ovary development before influencing bone mass in pre-pubertal piglets. Journal of Cellular Physiology 212, 51–59.

Devareddy, L., Khalill, D.A., Smith, B.J., et al., 2006. Soy moderately improves microstructural properties without affecting bone mass in an ovariectomized rat model of osteoporosis. Bone 38, 686–693.

Dolinoy, D.C., Weidman, J.R., Waterland, R.A., Jirtle, R.L., 2006. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environmental Health Perspectives 114, 567–572.

Droke, E.A., Hager, K.A., Lerner, M.R., et al., 2007. Soy isoflavones avert chronic inflammation-induced bone loss and vascular disease. Journal of Inflammation (London) 4, 17–29.

Fujioka, M., Sudo, Y., Okumura, M., et al., 2007. Differential effects of isoflavones on bone formation in growing male and female mice. Metabolism 56, 1142–1148.

Hotchkiss, C.E., Weis, C., Blaydes, B., Newbold, R., Delclos, K.B., 2005. Multigenerational exposure to genistein does not increase bone mineral density in rats. Bone 37, 720–727.

Ishimi, Y., Arai, N., Wang, X., et al., 2000. Difference in effective dosage of genistein on bone and uterus in ovariectomized mice. Biochemical and Biophysical Research Communications 274, 697–701.

Julius, A.D., Wiggers, K.D., Richard, M.J., 1982. Effect of infant formulas on blood and tissue cholesterol, bone calcium, and body composition in weanling pigs. Journal of Nutrition 112, 2240–2249.

Kaludjerovic, J., Ward, W.E., 2009. Neonatal exposure to daidzein, genistein, or the combination modulates bone development in female CD-1 mice. Journal of Nutrition 139, 467–473.

Kaludjerovic, J., Ward, W.E., 2010. Neonatal administration of isoflavones attenuates deterioration of bone tissue in female but not male mice. Journal of Nutrition 140, 766–772.

Nakai, M., Black, M., Jeffery, E.H., Bahr, J.M., 2005. Dietary soy protein and isoflavones: no effect on the reproductive tract and minimal positive effect on bone resorption in the intact female Fischer 344 rat. Food and Chemical Toxicology 43, 945–949.

Nakai, M., Cook, L., Pyter, L.M., et al., 2005. Dietary soy protein and isoflavones have no significant effect on bone and a potentially negative effect on the uterus of sexually mature intact Sprague–Dawley female rats. Menopause 12, 291–298.

Ohta, A., Uehara, M., Sakai, K., et al., 2002. A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. Journal of Nutrition 132, 2048–2054.

Onoe, Y., Miyaura, C., Ohta, H., Nozawa, S., Suda, T., 1997. Expression of estrogen receptor beta in rat bone. Endocrinology 138, 4509–4512.

Peterson, C.A., Kubas, K.L., Hartman, S.J., et al., 2008. Lack of skeletal effect of soy isoflavones in intact growing, female rats may be explained by constant serum estrogenic activity despite reduced serum estradiol. Annals of Nutrition and Metabolism 52, 48–57.

Picherit, C., Bennetau-Pelissero, C., Chanteranne, B., et al., 2001. Soybean isoflavones dose-dependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats. Journal of Nutrition 131, 723–728.

Picherit, C., Coxam, V., Bennetau-Pelissero, C., et al., 2000. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. Journal of Nutrition 130, 1675–1681.

Piekarz, A.V., Ward, W.E., 2007. Effect of neonatal exposure to genistein on bone metabolism in mice at adulthood. Pediatric Research 61, 48–53.

Ren, M.Q., Kuhn, G., Wegner, J., et al., 2001. Feeding daidzein to late pregnant sows influences the estrogen receptor beta and type 1 insulin-like growth factor receptor mRNA expression in newborn piglets. Journal of Endocrinology 170, 129–135.

Vanhees, K., Coort, S., Ruijters, E.J., et al., 2010. Epigenetics: prenatal exposure to genistein leaves a permanent signature on the hematopoietic lineage. The FASEB Journal 25, 797–807.

Ward, W.E., 2005. Synergy of soy, flaxseed, calcium and hormone replacement therapy in osteoporosis. In: Thompson, L.U., Ward, W.E. (Eds.), Food Drug Synergy and Safety. CRC Press, Boca Raton, FL, pp. 235–253.

Ward, W.E., Piekarz, A.V., 2007. Effect of prenatal exposure to isoflavones on bone metabolism in mice at adulthood. Pediatric Research 61, 438–443.

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Skeletal Effects of Plant Products Other Than Soy

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1. INTRODUCTION

In addition to the extensive literature on the effects of soy feeding on skeletal parameters and bone turnover, there are a significant number of epidemiological studies suggesting a positive link between bone mineral density (BMD) and overall fruit and vegetable consumption. There is also evidence that consumption of flaxseed or one of its main bioactives – alpha-linolenic acid (ALA), a *n*-3 fatty acid, or its mammalian lignan precursor secoisolariciresinol diglycoside (SDG) – is associated with higher BMD. Another class of plant-derived constituents that can have benefits to bone is nondigestible fiber that can function as a prebiotic, a substrate for gut microbiota that ferment these fibers in the lower gut. This can increase mineral utilization, important to bone mineral content (BMC). The fermentation creates short-chain fatty acids, such as acetate, propionate, and butyrate, that lower the pH of the lower gut and increase the solubility of minerals. The process is also associated with increased cecal content and wall weight that could enhance mineral absorption through increased absorptive surfaces or increased exposure time as a result of increased viscosity of gut contents (Weaver et al., 2010).

2. HUMAN STUDIES

2.1 Fruit and Vegetables

Several cross-sectional studies including a recent comprehensive study using 7 days food diaries, in five age and sex cohorts by Prynne et al. (2006) demonstrated that higher fruit and vegetable intakes were positively associated with bone mineral status in adolescent boys and girls and older women, especially at the spine and femoral neck. Similarly, Chen et al. (2006) reported a positive association between total fruit and vegetable intake and BMD at the whole body, lumbar spine, and hip in a population-based cross-sectional study of 670 postmenopausal Chinese women, even after adjustment for age, years since menopause, weight, dietary energy, protein, calcium, and exercise.

2.2 Flaxseed

Flaxseed contains high levels of ALA that may mediate positive skeletal effects by altering the ratio of *n*-6 to *n*-3 fatty acids, specifically the ratio of linoleic acid (LA) to ALA. Healthy older men and postmenopausal women consuming diets with higher ratio of LA to ALA had lower BMD at the hip. Use of hormone replacement therapy (HRT) did not affect the relationship at the hip but did at the spine as lower spine BMD was also associated with a lower ratio of LA to ALA in postmenopausal women not taking HRT (reviewed in Ward et al., 2010). This finding is biologically plausible as the skeleton of older women is likely more responsive to diet in the absence of antiresorptive agents. The ratio of *n*-6 to *n*-3 fatty acids was also shown to influence bone health in younger men and women (<60 years of age) who were overweight and had hypercholesterolemia (reviewed in Ward et al., 2010). This study was a 6-week, double-blind, crossover feeding trial. Subjects receiving a diet containing an LA to ALA ratio, which was low (1.5–1) versus high (9.5–1) and represented an average American diet, had a lower *N*-telopeptide excretion, indicative of a favorably lower rate of bone resorption. Despite these favorable effects of manipulation of the LA to ALA ratio, intervention studies in which menopausal or postmenopausal women were fed 25 or 40 g of ground flaxseed for periods of 3–12 months report no changes in serum markers of bone turnover or BMD at hip or the spine (reviewed in Ward et al., 2010). Such findings may be due to the relatively healthy state of the women, none of which had a low BMD. One study has investigated the effect of supplementing older men and postmenopausal women with lignan (543 mg day^{-1} in a 4050 mg complex) in combination with a walking program and reported no change in whole body, femur, or lumbar spine BMC or BMD measured at the end of the 6-month study period (Cornish et al., 2009). The relatively short duration may have compromised the ability to observe a potential change in BMD. A study in postmenopausal women with bone health classified as normal, osteopenia, or osteoporosis did demonstrate a positive correlation between BMD at multiple skeletal sites – lumbar spine, femoral neck, and Ward's triangle – and urinary enterolactone, an established marker of lignan intake (reviewed in Ward et al., 2010). In contrast, another study in postmenopausal women reported that urinary enterolactone excretion was high among women who experienced a greater loss of bone mass over a 10-year period (reviewed in Ward et al., 2010). Thus, the relationship between lignans and skeletal health is not definitive. The discrepancy may be due to the measurement of different skeletal sites with the latter study measuring radial bone density, a site rich in cortical bone that is less metabolically active than skeletal sites containing more trabecular bone such as the spine and hip.

2.3 Fibers

The most studied fiber for its effects on bone is inulin, a fructo-oligosaccharide (FOS), isolated from hickory root or other plants. A similar fiber, galacto-oligosaccharide (GOS)

derived from milk, has also been studied for its beneficial effects on bone. Other fibers studied for their effects on bone include soluble corn fibers, polydextrose (an indigestible glucose polymer used as a bulking agent), and nondigestible starches and lactulose (Park and Weaver, 2011). The effect of fibers on mineral absorption and bone parameters depends on many factors including life stage, nature, and dose of fiber. Positive effects have been reported, but only at rather high levels of intake, typically >5% by weight in animal diets. GOS increased calcium absorption and retention, but the effects decreased with age (Perez-Conesa et al., 2006). In humans, calcium absorption was increased by a mixture of short-chain FOS and inulin in adolescent girls receiving 8 g day^{-1} and in postmenopausal women receiving 5 g day^{-1} (Abrams et al., 2005). The study period in adolescents was 1 year and changes in whole-body BMD and BMC were higher in the group receiving inulin.

3. ANIMAL AND *IN VITRO* STUDIES

3.1 Vegetables

A comprehensive survey of the effects of dietary components on bone resorption has been reported by Mühlbauer et al. (2003a) using urinary excretion of [^{3}H]-tetracycline from chronically prelabelled rats as a measure of osteoclast activity. Of the 25 out of 53 food items found to be effective at a dose of 1 g day^{-1}, several were found to be vegetables in the onion family: for example, onion, garlic, and leek. In addition, other effective vegetables included celeriac, various types of cabbage, lettuce, and green beans. It has been suggested that the effect of a high-vegetable diet on bone might be due to base excess buffering of metabolic acid normally produced by dietary animal protein. However, Mühlbauer et al. (2002) demonstrated that onion retained its antibone resorptive properties even when fed as part of a vegetarian diet with typical base excess and that a mixed vegetable/salad/herb diet retained inhibitory activity even after buffering metabolic acid by dietary supplementation with potassium citrate. It was therefore concluded that vegetables inhibit bone resorption as a result of the presence of pharmacologically active phytochemicals. The effects of onion on bone have been investigated in further detail. Addition of onion power at 3–14% (wt/wt) has been reported to dose-dependently prevent ovariectomy-induced osteopenia in 14-week-old female rats and to produce increases in bone volume/total volume (BV/TV) and trabecular number and decreased trabecular separation and osteoclast number (Huang et al., 2008). In a study in which the actions of onion extracts and extract fractions on bone resorption were examined *in vivo*, Wetli et al. (2005) demonstrated that the bone-bioactive component was hydrophilic rather than part of the hydrophobic onion components including flavonoids such as rutin. Furthermore, bioactivity-directed fractionation of hydrophilic components using an assay for the ability of osteoclasts to form resorption pits on a mineralized surface identified the compound γ-L-glutamyl-*trans*-S-1-propenyl-L-cysteine sulfoxide as the active component with a

minimal effective dose of 2 mM. However, until the bioavailability and activity of this compound can be confirmed *in vivo*, it is unclear that this represents the true bioactive component of onions responsible for inhibition of bone resorption and the mechanisms underlying inhibition of bone resorption by onion remain obscure.

3.2 Herbs and Essential Oils

Mühlbauer et al. (2003b) identified another group of plant foods with potent ability to inhibit bone resorption when added to the diet *in vivo* – herbs such as parsley, sage, rosemary, thyme, and dill. Further examination of these effects revealed similar actions of essential oils extracted from these herbs and of monoterpene components from these and other plant oils such as menthol, pinene, eucalyptol, thujone, camphor, thymol, and borneol (Mühlbauer et al., 2003b). Some of these compounds, such as camphor and thymol, were directly inhibitory of osteoclast activity *in vitro*. Others such as the nonpolar monoterpene pinene appeared to require oxidative metabolism to active metabolites such as *cis*-verbenol (Mühlbauer et al., 2003b). The mechanism of actions of the monoterpenes to inhibit bone resorption remains obscure since they have been reported to block osteoclastogenesis in bone marrow/osteoblast cocultures without effect on osteoblast proliferation, osteoblast viability, or mRNA expression of either receptor activator of NFκB ligand (RANKL) or osteoprotegerin (Dolder et al., 2006).

3.3 Fruits

Mühlbauer et al. (2003a) also identified several fruits with the ability to inhibit bone resorption. In particular, dried plums, orange juice, and freeze-dried extracts of red wine were observed to have potent effects. All three foods and their phytochemical components have subsequently been evaluated for effects on skeletal targets in more detail. Dried plums added to rodent diets at 5–25% (wt/wt) have been shown to reverse bone loss produced by ovariectomy in 90-day-old female rats (Deyhim et al., 2005), in castrated 6-month-old male rats (Bu et al., 2007; Franklin et al., 2006), and in male rats loosing bone as a result of hind limb suspension (Hooshmand and Arjmandi, 2009). In addition, high levels of dried plum have been reported to substantially increase bone quality in 6-month-old male mice and to restore BMD in 18-month-old male mice (Halloran et al., 2010). The effects of dried plum in ovariectomized and castrated rats were not associated with changes in sex steroid responsive organs (uterus or secondary sex organ weight) and were concluded not to be associated with steroid-like effects (Deyhim et al., 2005; Franklin et al., 2006). In all cases, dried plum was found to improve bone microstructure and mechanical strength with increases in BV/TV, TbN, structural model index (SMI), and connectivity and decreases in TbSp as measured by microcomputed tomography. Little or no effect of dried plum has been reported on bone formation markers *in vivo* in these animal models. However, a short-term clinical trial in

postmenopausal women did report a significant increase in serum bone-specific alkaline phosphatase activity after consumption of 100 g day^{-1} dried plum (Arjmandi et al., 2002). In addition, increases in the bone anabolic growth factor IGF-1 have been reported after dried plum consumption in both rats and humans (Arjmandi et al., 2002; Franklin et al., 2006). More consistent effects of dried plum have been observed to suppress bone resorption markers *in vivo* (Bu et al., 2007; Deyhim et al., 2005; Franklin et al., 2006; Halloran et al., 2010) and Franklin et al. (2006) reported reversal of castration-induced increases in RANKL and osteoprotegerin expression in bone after dried plum consumption. Despite consistent anabolic effects on bone, little is known of the molecular mechanisms whereby dried plum consumption acts on bone cells. Two studies have been published examining the effects of extracted dried plum polyphenol extracts on bone cells *in vitro* (Bu et al., 2008, 2009). These authors report increased differentiation of MC3T3-E1 cells and anti-inflammatory actions to prevent decreases in osteoblast differentiation, alkaline phosphatase, and Runx2 expression and to prevent increases in RANKL expression in response to TNF-alpha. In addition, they report inhibition of NO production and oxidative stress in RAW264.7 cells as a result of inhibition of iNOS and COX2 expression after cotreatment with lipopolysaccharide (LPS) or TNF-alpha and inhibition of osteoclast differentiation after RANKL treatment. However, the relevance of these observations to the actions of dried plum *in vivo* is unclear since they ignore important issues of bioavailability and metabolism. Polyphenols have extremely limited bioavailability and fruit phytochemicals undergo extensive metabolism both by gut bacteria and in the liver (Manach et al., 2005). Other foods rich in polyphenols have been examined for their effects on bone. Arjmandi et al. (2010) report improved BMD in ovariectomized female rats of 3-month-old after feeding 7.5% (wt/wt) raisin or date diets. However, other polyphenol-rich diets such as black tea, green tea, and cocoa have been reported to be ineffective at blocking bone resorption in rats *in vivo* (Mühlbauer et al., 2003b). A report using ovariectomized female rats suggested that blueberries might be effective in preventing sex-steroid-withdrawal-induced bone loss (Devareddy et al., 2008). Five percent blueberry supplementation blocked increases in expression of both alkaline phosphate and tartrate-resistant acid phosphatase mRNAs in femur bone after ovariectomy, suggesting a suppression of the increased bone turnover produced in response to estrogen removal. More recently, studies where diets of weanling rats were supplemented with 10% blueberry demonstrated dramatic increases in BMD, BV/TV, and trabecular number in both males and females before puberty and long-term protection against ovariectomy-induced bone loss in adult females 2 months after removal of blueberries from the diet (Chen et al., 2010; Zhang et al., 2011). Blueberry feeding of weanling rats for as little as 14 days increased serum markers of bone formation, osteoblast number, bone formation rate and mineral apposition rate, and *ex vivo* osteoblast differentiation. In addition, osteoclast number, *ex vivo* osteoclastogenesis, and bone resorption markers were decreased particularly after longer 45-day blueberry feeding (Chen et al., 2010). Effects on bone formation were mimicked by serum from

blueberry-fed rats *ex vivo* and were associated with activation of Wnt–β–catenin signaling. Similar stimulation of osteoblast differentiation and Wnt–β–catenin signaling were observed with an artificial mixture of phenolic acids identical to the profile of phenolic acids appearing in serum after blueberry feeding (Chen et al., 2010). These data suggest that the bone anabolic components associated with blueberry feeding are the phenolic acid metabolites of blueberry polyphenols produced by metabolism in the GI tract rather than the polyphenols themselves. With regards to studies following up on the reported skeletal effects of red wine extracts, many *in vitro* studies have examined the effects of the red wine polyphenol resveratrol on bone cells. Resveratrol has been reported to stimulate bone formation via increased bone morphogenic protein (BMP)–2 production and mesenchymal stem cell differentiation via activation of the deacetylase Sirt-1 (Backesjo et al., 2006; Su et al., 2007). In addition, resveratrol has been reported to prevent RANKL–induced osteoclastogenesis via inhibition of oxygen radical production (He et al., 2010). Unfortunately, these *in vitro* studies utilize resveratrol concentrations in the >10 μM range. Since resveratrol has very limited bioavailability and a short half life, *in vivo* concentrations of resveratrol and its conjugated metabolites following red wine consumption range from undetectable to 10–100 nM (Vitaglione et al., 2005). It is, therefore, unlikely that the effects of red wine extracts on bone reflect the bioactivity of resveratrol. In addition to dried plums, berries, and red wine, several recent studies have examined the effects of orange and its phytochemical components on bone. Morrow et al. (2009) report that orange pulp supplementation of diet prevented bone loss in 1–year–old male rats castrated for 4 months. Orange juice prevented increases in bone resorption markers, decreases in TbN, and increases in TbSp as measured by micro-CT and increased cortical thickness in a dose-dependent manner. Similar bone protective effects were reported for diets containing 0.5% hesperidin, the most abundant flavonone in citrus fruits, in ovariectomized 90–day–old female rats, accompanied by decreased bone resorption makers. These authors also report increases in bone formation markers in sham–operated controls fed with hesperidin-supplemented diets indicating bone anabolic effects (Horcajada et al., 2008). *In vitro* studies in osteoblast cultures with concentrations of the hesperidin aglycone hesperetin in the range 1–10 μM, which are attainable *in vivo* after orange juice consumption in the range of 1 l, revealed evidence for increased osteoblast differentiation as a result of stimulated signaling through BMPs (Trzeciakiewicz et al., 2010a). Small increases in BMP2, BMP4, and Smad1 mRNA accompanied increases in alkaline phosphatase and Runx2 mRNA expression. Western blotting indicated increased phosphorylation of Smad 1/5/8, which is known to be downstream of BMP signaling and alkaline phosphatase increases in response to hesperetin, were blocked by the BMP inhibitor noggin. Interestingly, in a second recent paper (Trzeciakiewicz et al., 2010b), these authors observed similar effects on BMP signaling with the major hesperetin conjugate hesperetin-7-*O*-glucuronide at similar concentrations *in vitro* with the additional effect of reducing RANKL mRNA expression 50%.

3.4 Mushrooms

Mühlbauer et al. (2003a) identified several types of mushrooms with the ability to inhibit bone resorption when fed as dried powder at levels of 1 g day^{-1}. This included the common white mushroom. Interestingly, feeding Shitake mushrooms had no effect on bone resorption. More recently, two studies have reported protective effects of extracts of the king oyster mushroom (*Pleurotus eryngii*) in rat models of ovariectomy-induced bone loss (Kim et al., 2006; Shimizu et al., 2006). *In vitro* studies with mushroom extracts suggest increased osteoblast differentiation and reduced osteoclastogenesis (Kim et al., 2006). However, these studies did not identify bioactive components or take into account bioactivity or metabolism of mushroom components *in vivo*.

3.5 Flaxseed

Flaxseed is the predominant cereal that has been studied in relation to skeletal health. Much of the interest stems from the fact that two of its major components, ALA and SDG – that is metabolized to enterodiol and enterolactone by colonic bacteria – have potential anabolic effects on bone. ALA, a *n*-3 fatty acid, can have anti-inflammatory activity while lignan may mimic the positive effect of estrogen in the skeleton. Several studies have fed developing rodents diets that differ in their ratio of ALA to LA and measured changes in serum cytokines or BMD and biomechanical breaking strength at specific skeletal sites (Lau et al., 2010; Sacco et al., 2009; reviewed in Ward et al., 2010). Studies have consistently reported that the skeleton is responsive to the type of dietary fat consumed (Lau et al., 2010; Sacco et al., 2009). In general, feeding diets rich in ground flaxseed or flaxseed oil (and thus ALA) results in higher incorporation of ALA, lower incorporation of LA, and small and sometimes significantly higher levels of longer chain fatty acids, including docosahexaenoic and eicosapentaenoic acid, in long bones and vertebra (Lau et al., 2010; Sacco et al., 2009). Benefits to bone health may be directly due to ALA as conversion to longer chain DHA and EPA is low. Specific mechanisms have not been elucidated. Much of the literature on bone health and fatty acids relates to the longer chain DHA and EPA present in fish oil and the ability to modulate cytokine and prostaglandin production.

   A 9-week intervention study in which healthy mice were fed a diet containing 10% flaxseed oil from weaning to 3 months of age resulted in similar femur and spine BMD and bone strength at these sites to control mice fed a corn-oil-based diet. Serum proinflammatory markers including interleukin-1β, interleukin-6, and tumor necrosis factor-alpha were unchanged (reviewed in Ward et al., 2010). Similarly, there was no effect of feeding rats 5 or 10% ground flaxseed or the equivalent quantity of SDG present in such diets from suckling through to adulthood (age 132 days) on femur or vertebra BMD or strength (reviewed in Ward et al., 2010). The lack of effect observed in these studies may be due to the fact that these are healthy developing animals and the level of fat consumed. Another study investigated in male rats the effect of a flaxseed oil diet in the context of a high-fat diet

(20% fat compared to standard chow containing 5.7% fat (soybean oil) by weight) (Lau et al., 2010). Interestingly, feeding a high-fat diet containing flaxseed oil to rats from 6 to 15 weeks of life resulted in greater femur BMD that were also more resistant to fracture than rats fed standard chow diet (Lau et al., 2010). With respect to aging, studies in rats have shown that 10% flaxseed diet alone does not attenuate ovariectomy-induced reductions in BMD or strength at the femur and lumbar spine (Sacco et al., 2009). These findings mirror those from the studies in which menopausal or postmenopausal women were fed ground flaxseed (reviewed in Ward et al., 2010). Positive findings have been shown in disease models. Feeding a 10% flaxseed oil diet to mice with intestinal inflammation was shown to attenuate the inflammation-associated reductions in BMD and skeletal weakening – these effects were associated with lower serum tumor necrosis factor-alpha (reviewed in Ward et al., 2010).

4. FUTURE STUDIES

While sufficient evidence exists to conclude that at least certain plant foods and constituents are beneficial to bone, there are many questions regarding which foods/constituents are most beneficial, the bioactive ingredient, and the effective dose. These questions would be prudent to address before embarking on large expensive clinical trials. Rapid screening approaches such as assessing bone turnover by measuring urinary appearance of a bone seeking tracer as described in studies by Mühlbauer et al. (2002, 2003a,b) should be applied more broadly to screen the more promising candidates. Intervention studies can be performed using (^{3}H) tetracycline or ^{45}Ca in rats. In order to determine effective doses in humans, screening studies in humans are necessary. ^{41}Ca is a useful tracer as a single dose allows bone resorption to be measured for decades, so that multiple interventions can be screened in the same individual (Jackson et al., 2001; Weaver et al., 2009).

5. CONCLUSION

Plant foods/constituents are promising interventions for bone health because they contain many compounds that target pathways that influence chronic disease and healthy aging as discussed elsewhere in this comprehensive. Plants are also readily embraced by consumers as they find natural resources more palatable than designer drugs. Nevertheless, the doses required for efficacy will determine if purified extracts are needed rather than foodstuffs and whether they have side effects.

GLOSSARY

Alpha-linoleic acid (ALA) An essential *n*-3 fatty acid.
Enterodiol and enterolactone Metabolites of a lignan precursor such as secoisolariciresinol diglycoside (SDG) that is abundant in flaxseed. Urinary levels are used as markers of lignan intake.

Inulin A fructo-oligosaccharide (FOS) isolated from chicory or other plants and used as a nondigestible bulking agent.

Prebiotic Ingested fibers that serve as a substrate for fermentation by gut microorganisms.

Rutin The putative bioactive flavonoid in onion.

Secoisolariciresinol diglycoside The mammalian lignan precursor.

REFERENCES

Abrams, S.A., Griffin, I.J., Hawthorne, K.M., et al., 2005. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. American Journal of Clinical Nutrition 82, 471–476.

Arjmandi, B.H., Johnson, C.D., Campbe, S.C., Hooshmand, S., Chai, S.C., Akhter, M.P., 2010. Combining fructooligosaccharide and dried plum has the greatest effect on restoring bone mineral density among select functional foods and bioactive compounds. Journal of Medicinal Food 13, 312–319.

Arjmandi, B.H., Khalil, D.A., Lucas, E.A., et al., 2002. Dried plums improve indices of bone formation in postmenopausal women. Journal of Women's Health & Gender-Based Medicine 11, 61–68.

Backesjo, C.M., Li, Y., Lindgren, U., Haldosen, L.A., 2006. Activation of Sirt1 decreases adipocyte formation during osteoblast differentiation of mesenchymal cells. Journal of Bone and Mineral Research 21, 993–1002.

Bu, S.Y., Hunt, T.S., Smith, B.J., 2009. Dried plum polyphenols attenuate the detrimental effects of TNFα on osteoblast function coincident with up-reguation of Runx2, Osterix and IGF-1. The Journal of Nutritional Biochemistry 20, 35–44.

Bu, S.Y., Lerner, M., Stoecker, B.J., et al., 2008. Dried plum polyphenols inhibit osteoclastogenesis by downregulating NFATc1 and inflammatory mediators. Calcified Tissue International 82, 475–488.

Bu, S.Y., Lucas, E.A., Franklin, M., et al., 2007. Comparison of dried plum supplementation and intermittent PTH in restoring bone in osteopenic orchidectomized rats. Osteoporosis International 18, 931–942.

Chen, Y.-M., Ho, S.C., Woo, J.L.F., 2006. Greater fruit and vegetable intake is associated with increased bone mass among postmenopausal Chinese women. British Journal of Nutrition 96, 745–751.

Chen, J.R., Lazarenko, O.P., Wu, X., et al., 2010. Dietary-induced serum phenolic acids promote bone growth via p38 MAPK/β-catenin canonical wnt signaling. Journal of Bone and Mineral Research 25, 2399–2411.

Cornish, S.M., Chilibeck, P.D., Paus-Jennsen, L., et al., 2009. A randomized controlled trial of the effects of flaxseed lignan complex on metabolic syndrome composite score and bone mineral in older adults. Applied Physiology, Nutrition and Metabolism 34, 89–98.

Devareddy, L., Hooshmand, S., Collins, J.K., Lucas, E.A., Chai, S.C., Arjmandi, B.H., 2008. Blueberry prevents bone loss in ovariectomized rats model of postmenopausal osteoporosis. The Journal of Nutritional Biochemistry 19, 694–699.

Deyhim, F., Stoecker, B.J., Brusewitz, G.H., Devareddy, L., Arjmandi, B.H., 2005. Dried plum reverses bone loss in an osteopenic rat model of osteoporosis. Menopause 12, 755–762.

Dolder, S., Hofstetter, W., Wetterwald, A., Mühlbauer, R.C., Felix, R., 2006. Effect of monoterpenes on the formation and activation of osteoclasts in vitro. Journal of Bone and Mineral Research 21, 647–655.

Franklin, M., Bu, S.Y., Lerner, M.R., et al., 2006. Dried plum prevents bone loss in a male osteoporosis model via IGF-1 and the RANK pathway. Bone 39, 1331–1342.

Halloran, B.P., Wronski, T.J., VonHerzen, D.C., et al., 2010. Dietary dried plum increases bone mass in adult and aged male mice. Journal of Nutrition 140, 1781–1787.

He, X., Andersson, G., Lindgren, U., Li, Y., 2010. Resveratrol prevents RANKL-induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through inhibition of ROS production. Biochemical and Biophysical Research Communications 401 (3), 356–362.

Hooshmand, S., Arjmandi, B.H., 2009. Viewpoint: Dried plum, an emerging functional food that may effectively improve bone health. Ageing Research Reviews 8, 122–127.

Horcajada, M.N., Habauzit, V., Trzeciakiewicz, A., et al., 2008. Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. Journal of Applied Physiology 104, 648–654.

Huang, T.-H., Mühlbauer, R.C., Tang, C.-H., et al., 2008. Onion decreases the ovariectomy-induced osteopenia in young adult rats. Bone 42, 1154–1163.

Jackson, G.S., Weaver, C.M., Elmore, D., 2001. Use of accelerator mass spectrometry for studies in nutrition. Nutrition Research Reviews 14, 317–334.

Kim, S.W., Kim, H.G., Lee, B.E., Hwang, H.H., Baek, D.H., Ko, S.Y., 2006. Effects of mushroom, *Pleurotus ergngii*, extracts on bone metabolism. Clinical Nutrition 25, 166–170.

Lau, B.Y., Fajardo, V.A., McMeekin, L., et al., 2010. Influence of high-fat diet from differential dietary sources on bone mineral density, bone strength, and bone fatty acid composition in rats. Applied Physiology, Nutrition and Metabolism 35, 598–606.

Manach, C., Williamson, G., Morand, C., Scalbert, A., Remesy, C., 2005. Bioavailability and bioefficiency of polyphenols in humans I: Review of 97 bioavailability studies. American Journal of Clinical Nutrition 81, 230S–242S.

Morrow, R., Deyhim, F., Patil, B.S., Stoeker, B.J., 2009. Feeding orange pulp improved bone quality in a rat model of male osteoporosis. Journal of Medicinal Food 12, 298–303.

Mühlbauer, R.C., Lozano, A., Palacio, S., Reinli, A., Felix, R., 2003a. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone 32, 372–380.

Mühlbauer, R.C., Lozano, A., Reinli, A., 2002. Onion and a mixture of vegetables, salads and herbs affect bone resorption in the rat by a mechanism independent of their base excess. Journal of Bone and Mineral Research 17, 1230–1236.

Mühlbauer, R.C., Lozano, A., Reinli, A., Wetli, H., 2003b. Various selected vegetables, fruits, mushrooms and red wine residue inhibit bone resorption in rats. Journal of Nutrition 133, 3592–3597.

Park, C.Y., Weaver, C.M., 2011. Calcium and bone health: Influence of prebiotics. Functional Food Reviews 3, 62–72.

Perez-Conesa, D., Lopez, G., Abella, P., Ros, G., 2006. Bioavailability of calcium, magnesium and phosphorus in rats fed prebiotic, prebiotic and synbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. Journal of the Science of Food and Agriculture 861, 2327–2336.

Prynne, C.J., Mishra, G.D., O'Connell, M.A., et al., 2006. Fruit and vegetable intakes and bone mineral status: A cross-sectional study in 5 age and sex cohorts. American Journal of Clinical Nutrition 83, 1420–1428.

Sacco, S.M., Jiang, J.M.Y., Reza-Lopez, S., Ma, D.W.L., Thompson, L.U., Ward, W.E., 2009. Flaxseed combined with low-dose estrogen therapy preserves bone tissue in ovariectomized rats. Menopause 16, 545–554.

Shimizu, K., Yamanaka, M., Gyokusen, M., et al., 2006. Estrogen-like activity and prevention effect of bone loss in calcium deficient ovariectomized rats by the extract of *Pleurotus ergngii*. Phytotherapy Research 20, 659–664.

Su, J.L., Yang, M., Zhao, M., Kuo, M.L., Yen, M.L., 2007. Forkhead proteins are critical for bone morphogenetic protein 2-regulation and anti-tumor activity of resveratrol. Journal of Biological Chemistry 282, 19385–19398.

Trzeciakiewicz, A., Habauzit, V., Mercier, S., et al., 2010a. Hesperetin stimulates differentiation of primary rat osteoblasts involving the BMP signaling pathway. The Journal of Nutritional Biochemistry 21, 424–431.

Trzeciakiewicz, A., Habauzit, V., Mercier, S., et al., 2010b. Molecular mechanism of hesperetin-7-O-glucuronide, the main circulating metabolite of hesperidin, involved in osteoblast differentiation. Journal of Agricultural and Food Chemistry 58, 668–675.

Vitaglione, P., Sforza, S., Galaverna, G., et al., 2005. Bioavailibility of *trans*-resveratrol from red wine in humans. Molecular Nutrition & Food Research 49, 495–504.

Ward, W.E., Lau, B., Kaludjerovic, J., Sacco, S.M., 2010. Functional foods and bone health: Where are we at? In: Smith, J., Charter, E. (Eds.), Functional Food Science and Technology Series. Wiley-Blackwell, Oxford, pp. 459–503.

Weaver, C.M., Martin, B.R., Jackson, G.S., et al., 2009. Antiresorptive effects of phytoestrogen supplements compared to estradiol or Risedronate in postmenopausal women using ^{41}Ca methodology. Journal of Clinical Endocrinology and Metabolism 94 (10), 3798–3805.

Weaver, C.M., Martin, B.R., Story, J.A., Hutchinson, I., Sanders, L., 2010. Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. Journal of Agricultural and Food Chemistry 58, 8952–8957.

Wetli, H., Brenneisen, R., Tscudi, I., et al., 2005. A γ-glutamyl peptide isolated from Onion (*Allium cepa* L.) by bioassay-directed fractionation inhibits resorption activity of osteoclasts. Journal of Agricultural and Food Chemistry 53, 3408–3414.

Zhang, J., et al., 2011. Feeding blueberry diets in early life prevent senescence of osteoblasts and bone loss in ovariectomized adult female rats. PluS ONE. 6 (9), e24486.

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Molecular Mechanisms Underlying the Actions of Dietary Factors on the Skeleton

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1. INTRODUCTION

The nutritional status and consumption of dietary components such as fruits and vegetables play a key role in the regulation of skeletal growth, attainment of peak bone mass, and determining the risk of osteoporosis. However, with the exception of certain vitamins and minerals such as vitamin D and calcium, studies of dietary factors on bone have been largely descriptive. Limited mechanistic studies conducted with fruit and vegetable extracts or isolated phytochemicals in vitro have generally overlooked the requirement for intestinal digestion and first-pass metabolism of dietary factors, ignored the potential for interaction with endogenous factors such as sex steroids, and utilized pharmacological concentrations unattainable by consumption of foodstuffs. This has resulted in potentially misleading conclusions regarding the nature of bioactive food components.

Basic studies by bone biologists using genetic approaches have revealed a central role for Wnt–β-catenin and bone morphogenic protein (BMP) signaling pathways in the regulation of bone formation (Canalis et al., 2003; Canalis, 2009) and the importance of peroxisome proliferator–activated receptor (PPARγ) in lineage commitment of mesenchymal stem cells to form osteoblasts or adipocytes (Lecka-Czernik, 2010). Likewise, the receptor activator of nuclear factor kappa–B ligand (RANKL)–RANK signaling has been determined to be essential in the differentiation of osteoclasts (Lee et al., 2009). In addition, the importance of reactive oxygen species (ROS) and of the redox status in bone cells has been shown in the regulation of bone turnover and survival of osteoblasts, osteoclasts, and osteocytes. The interaction of redox systems with sex steroids via nonnuclear mitogen–activated protein (MAP) kinase regulated pathways has now also received wide recognition (Manolagas, 2010). A number of recent in vivo feeding studies have suggested that these systems are also the molecular targets for physiologically and nutritionally relevant actions of diet on the skeleton. A proposed model for describing these effects is given in Figure 30.1.
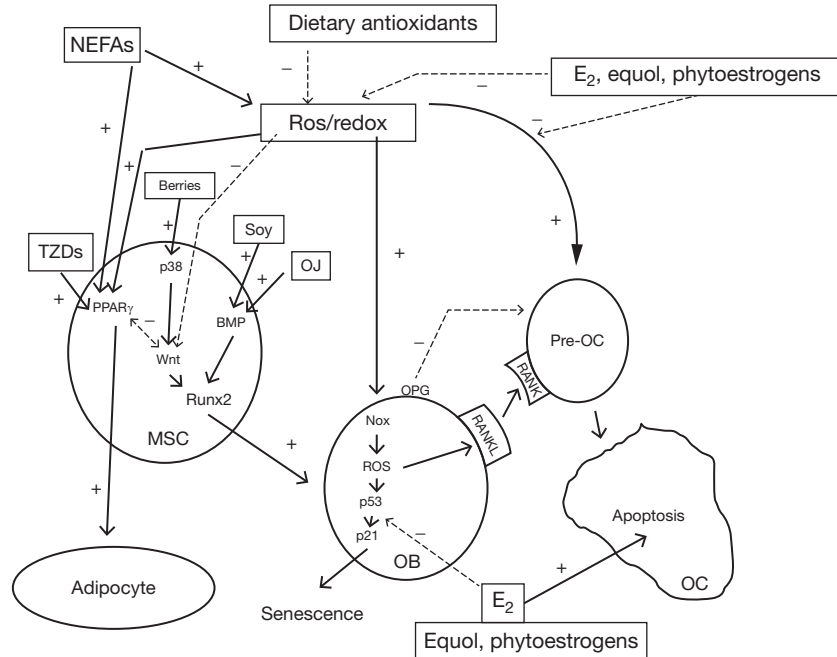
Figure 30.1 Nutritional status and dietary factors impact bone turnover and bone cell survival via common signal transduction pathways. BMP, bone morphogenic protein; E2, 17β-estradiol; MSC, mesenchymal stem cell; NEFAs, nonesterified free fatty acids; Nox, NADPH oxidase; OB, osteoblast; OC, osteoclast; OJ, orange juice; OPG, osteoprotegerin; ROS, reactive oxygen species; TZDs, thiazolidenedione antidiabetic drugs.

2. INTERACTIONS OF DIETARY FACTORS WITH ESTROGEN SIGNALING PATHWAYS IN BONE

Estrogens play a key role in the regulation of bone turnover and in the survival of bone cells (Manolagas, 2010). In general, estrogens suppress bone turnover; suppress RANKL expression in osteoblasts and osteoblast precursors, thus inhibiting osteoclast differentiation; protect osteoblasts against apoptosis and senescence; and stimulate apoptosis in osteoclasts. Some of these actions appear to involve classical estrogen receptor (ER) signaling pathways acting through nuclear receptors to alter gene expression. Others such as protection of osteoblasts against apoptosis appear to occur via nonclassical signaling, resulting from stimulation of MAP kinase pathways activated via binding to membrane-bound ER forms (Almeida et al., 2010b). MAP kinase activation, in turn, results in downstream actions on a number of cellular redox systems to increase antioxidant capacity and inhibit formation of ROS (Almeida et al., 2007, 2010a,b).

A variety of plant phytochemicals such as coumestrol, isoflavones, and lignans have weak estrogenic properties and it has been suggested that foods containing these

phytochemicals might act on bone in an estrogen–like manner (Branca and Lorenzetti, 2005). One common assumption among many investigators has been that soy diets have estrogenic properties as a result of the presence of isoflavone phytoestrogens such as genistein and daidzein (Messina, 2010), and that the effects of soy on bone are therefore the result of estrogen–like actions (Reinwald and Weaver, 2006, 2010). It is certainly true that isoflavones can activate ERs in bone marrow cells and osteoprogenitor cells in vitro. In a study using the KS483 mouse cell line, Dang and Lowik (2004) reported a dose-dependent increase in osteoblast differentiation at daidzein concentrations of $1–20$ μM, which could be blocked by the ER antagonist ICI 182 780. Similarly, Tang et al. (2011) have reported the ability of genistein at concentrations >1 μM to induce transcriptional activity through estrogen–response element sequences (EREs) and to inhibit the transcriptional activity of genes regulated by cAMP regulatory element via ER–mediated actions in osteoblastic cell lines. The problem with the interpretation of such in vitro studies is that they ignore potential interactions with endogenous estrogens and utilize concentrations of pure compounds rarely attained in vivo after consumption of soy foods. Even in studies where soy protein isolate (SPI) has been used as the sole protein source and in infants exclusively fed soy infant formulas, the plasma concentrations of genistein and daidzein aglycones, which are presumed to be the bioactive molecules, are in the range of $100–300$ nM (Gu et al., 2005, 2006). Aglycone concentrations of >1 μM are only attainable in vivo after dietary consumption of isoflavone supplements or extracts. Moreover, isoflavones, unlike estradiol, have greater affinity for ER beta than ER alpha and may behave like selective estrogen–receptor modulators (SERMs) acting like an estrogen in some tissues at some concentrations and blocking estrogen actions under other conditions (Rajah et al., 2009). In addition, genistein has other non–estrogenic actions such as inhibition of certain tyrosine kinases (Barnes et al., 2000). Comparative studies of genistein, estradiol, and the SERM raloxifene on the skeletal system have demonstrated profound differences between the three compounds. Although all three were reported to inhibit osteoclast formation in vitro as the result of inhibition of RANKL expression, only raloxifene was able to restore bone mineralization after ovariectomy (OVX) in mature female rats. Estradiol was able to fully normalize bone mechanical properties. However, genistein treatment actually augmented the deleterious effects of estrogen deficiency on bone strength (Sliwinski et al., 2009). There has been much recent interest in the skeletal actions of the daidzein metabolite equol, the most estrogenic of the isoflavones (Weaver and Legrette, 2010). Increases in estrogen–responsive genes have been reported in equol producers in comparison to nonequol producers after isoflavone treatment (Niculesc et al., 2007). Several clinical studies have also suggested that bone mineral density of equol producers is more responsive to isoflavone supplementation than that of nonequol producers (Lydeking-Olsen et al., 2004; Weaver and Legrette, 2010). However, studies using pure equol as a dietary supplement are currently limited. Animal studies in rats and mice have suggested protection against OVX-induced bone loss but also adverse estrogenic effects on uterine epithelial cell proliferation (Legette et al., 2009).

In contrast to studies with isoflavones, the effects of feeding soy diets on bone have yielded contradictory data, and evidence for estrogenic actions on bone is lacking (Cai et al., 2005; Chen et al., 2008a; Reinwald and Weaver, 2006). Most studies have utilized SPI, the protein source in soy infant formula. SPI is a complex mixture of proteins, peptides, and >100 phytochemicals, the properties of which may be significantly different from its individual components such as isoflavones (Messina, 2010). In animal studies, age also appears to be an important factor. Feeding studies in 6-month-old OVX rats with SPI demonstrated little or no effect on bone parameters (Cai et al., 2005). In contrast, recent, detailed molecular comparisons in young rapidly growing OVX female rats at age 60 days demonstrated that feeding SPI had very different actions on restoring OVX-induced bone loss compared to the classical estrogen 17β-estradiol (E2) and to the effects of a combination of SPI feeding and E2 replacement (Chen et al., 2008a). Both E2 treatment and SPI feeding significantly increased bone mass and quality. However, surprisingly, with the combination of SPI and E2 replacement, SPI feeding was not additive, but actually reduced E2 effects on tibia trabecular bone mineral density (BMD) and tibia total bone mineral content (BMC). This suggests that soy feeding actually antagonizes the actions of endogenous estrogens on bone. Consistent with the majority of previous findings, serum bone formation markers and serum bone resorption markers were both decreased in the OVX + E2 group compared with the OVX control group, indicating that E2 suppresses the high bone turnover induced by OVX. In sharp contrast, bone formation markers were increased and bone resorption markers were decreased in OVX animals fed with SPI diets. This indicates a different mechanism of action of soy diet on bone compared with the classic estrogen E2. The uncoupling effect of soy diet on bone turnover supports the hypothesis that the effect of soy diet on bone in the rapidly growing young female animal is anabolic and largely non-estrogenic. Data from our laboratory suggest that the anabolic actions of SPI on bone are mediated via activation of BMP signaling (see section "Interactions of Dietary Factors with BMP Signaling Pathways in Bone"). Interestingly, serum from both SPI-fed female rats aged 60 days and fractions of deproteinated serum from neonatal piglets fed soy formula with very low levels of isoflavone aglycones or their metabolites potently stimulate osteoblast differentiation in vitro, suggesting that the bone anabolic component of SPI is not an isoflavone (Chen et al., 2008a; Ronis et al., unpublished).

3. INTERACTIONS OF DIETARY FACTORS WITH BMP SIGNALING PATHWAYS IN BONE

BMPs are polypeptides that belong to the transforming growth factor β family. BMP-2, –4, and –6 are the most highly expressed in bone and have been shown to play an autocrine role in osteoblastogenesis and osteoblast function (Canalis et al., 2003). BMPs were originally identified due to their ability to stimulate endochondral bone formation

(Canalis, 2009) and act via type I and II receptors to stimulate signaling cascades involving signaling mothers against decapentaplegic (Smad) and MAP kinases. Serine phosphorylation of Smad-1, –5, and –8 is a known downstream target of BMP signaling and these proteins have been shown to increase transcription of the essential osteoblast differentiation factor Runx2 after nuclear translocation and heterodimerization with Smad-4 (Canalis, 2009). BMP signaling also increases phosphorylation of p38 and/or ERK depending on cell type. BMP-activated ERK has recently been shown to help stabilize Runx2 protein expression as a result of increasing p300-mediated acetylation (Jun et al., 2010).

In contrast to the relative lack of evidence linking feeding of soy products to stimulation of ER signaling in bone (see section "Interactions of Dietary Factors with Estrogen Signaling Pathways in Bone"), recent formula-feeding studies in the piglet have provided strong data to suggest that bone anabolic actions of a soy-containing diet on neonatal bone are mediated via increased BMP signaling (Chen et al., 2009a). Soy formula feeding has been reported to increase bone mineral density in neonatal piglets relative to breast-fed piglets coincident with increased serum bone formation markers, increased osteoblast number, and increased mineral apposition rate. In addition, serum from soy formula-fed piglets stimulated osteoblast differentiation ex vivo (Chen et al., 2009a). Feeding soy formula increased expression of BMP2 mRNA in bone. This was accompanied by a selective increase in the phosphorylation of ERK but not in the phosphorylation of p38 (Chen et al., 2009a). Western blots of bone from soy-fed piglets demonstrated both increased Smad-1/5/8 phosphorylation and increased expression of Runx2 protein. Moreover, the BMP inhibitor noggin was able to block ex vivo osteoblast differentiation stimulated by serum from soy formula-fed piglets (Chen et al., 2009a). What remains unclear is which soy component is responsible for activation of BMP signaling. Interestingly, recent studies with the phytochemical hesperetin-7-O-glucuronide, the major metabolite of hesperidin, a glycoside flavonoid found in high concentrations in orange juice, have reported increased osteoblast differentiation in vitro also accompanied by evidence of enhanced BMP signaling. This included increased Smad phosphorylation and Runnx2 expression(Trzeciakiewicvz et al., 2010a). Surprisingly, these authors suggested that certain conjugated phytochemicals may have biological activities on bone cells (Trzeciakiewicvz, 2010b). Distribution studies suggested little entry of the conjugate into osteoblastic cells and active export. This suggests bone anabolic actions through an as yet uncharacterized cell surface receptor (Trzeciakiewicvz et al., 2010b).

4. DIETARY BONE ANABOLIC FACTORS AND WNT-β-CATENIN SIGNALING PATHWAYS IN BONE

Like BMP signaling, the Wnt-β-catenin signaling pathway plays a key role in the regulation of osteoblastic cell differentiation of mesenchymal stem cells in bone marrow, and Wnts and BMPs have similar overlapping effects (Canalis, 2010). In bone, in the

canonical Wnt pathway, Wnt peptides bind to frizzled receptors and low-density lipo-protein receptor-related protein 5 and 6 coreceptors (Westendorf et al., 2004). Receptor binding leads to inhibition of the activity of GSK-3β mediated via disheveling and to stabilization and nuclear translocation of β-catenin (Westendorf et al., 2004). In the nucleus, β-catenin associated with T-cell factor (TCF) 4 or lymphoid enhancer-binding factor (LEF) to regulate gene transcription including that of Runx2 (Komori, 2010).

Recent animal studies have demonstrated dramatic increases in bone mass associated with feeding of artificial diets supplemented with fruits such as dried plums and berries such as blueberries (BB) (Chen et al., 2010b; Hooshmand and Arjmandi, 2009). These diets have been shown to prevent or even reverse sex-steroid defi-ciency and aging–associated bone loss (Halloran et al., 2010). The anabolic action of BB-supplemented diets to stimulate osteoblastogenesis and mineral apposition rate in vivo appears to be associated with increased expression of Runx2 in bone secondary to activation of Wnt-β-catenin signaling and increased phosphorylation of the MAP ki-nase p38 (Chen et al., 2010a, 2010b). The increase in osteoblastogenesis in vivo after BB feeding has recently been replicated in vitro in ST2 bone marrow stromal cell cultures exposed to serum from BB-fed rats. The BB serum increased p38 phosphorylation, GSK-3β phosphorylation, nuclear localization of β-catenin, as well as alkaline phospha-tase expression, an indicator of osteoblast differentiation. Nuclear localization of β-catenin and stimulation of TCF/LEF reporter gene transcription were both inhibited by the p38 inhibitor SB 239063, suggesting that p38 phosphorylation mediates the ac-tivating effects of BB on the GSK-3β–β-catenin cascade. Analysis of serum after BB feed-ing revealed substantial increases in the concentration of several phenolic acid metabolites of polyphenol pigments generated during intestinal digestion including hippuric, pheny-lacetic, and hydroxybenzoic acids. Interestingly, an artificial mixture of these phenolic acids at concentrations found in serum after BB feeding mimicked the effects of BB sup-plementation on Wnt signaling and osteoblastogenesis, indicating their potential in the prevention of bone loss (Chen et al., 2010b). It remains unclear how these phenolic acids activate p38. Moreover, it remains to be seen if the anabolic actions of other fruits such as dried plum on bone are also mediated through phenolic acid metabolites of fruit pigments.

5. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR PATHWAYS AND DIET-INDUCED BONE LOSS

PPARs have been shown to control bone turnover and regulate the differentiation of bone cells (Lecka-Czernik, 2010). These are three transcription factors (PPARα, PPARγ, and PPARδ) in the nuclear receptor family that activate gene transcription as heterodimers with the retinoid X receptor. In liver, muscle, and adipose tissue, they reg-ulate energy metabolism and control the differentiation of adipocytes (Lecka-Czernik,

2010). In bone, PPARγ also regulates lineage commitment of mesenchymal stem cells toward osteoblasts or adipocytes. Treatment with antidiabetic drugs which are PPARγ agonists, such as the thiazolidenediones (TZDs), has been shown to cause bone loss and increase bone marrow fat accumulation as a result of switching from the osteoblast to the adipocyte lineage (Lecka-Czernik, 2010). In contrast, PPARα appears not to play a role in bone and the role of PPARδ in this tissue is largely unknown.

Negative effects of high fat-driven obesity on bone mass have been shown to be accompanied by decreases in bone formation and increases in bone resorption and by increasing numbers of fat cells in the bone marrow, similar to what is observed with TZDs. This appears to be linked to the activation of PPARγ signaling. Impaired differentiation of mesenchymal stem cells into osteoblasts and increased adipogenesis has also been reported to be associated with inhibition of Wnt-β-catenin signaling both in vivo after high fat feeding and in vitro in ST2 bone marrow stromal cells exposed to serum from high fat-fed compared to low fat-diet-fed rats (Chen et al., 2010c). Reduction in β-catenin signaling was accompanied by a reciprocal increase in nuclear PPARγ protein expression and activation of PPARγ signaling (Chen et al., 2010a,c, 2011). A similar reciprocal pattern of changes in Wnt and PPARγ signaling was reported in stromal cells exposed to an artificial mixture of nonesterified free fatty acids (NEFAs) mimicking the concentration and composition of NEFA attained after high fat feeding. It is unclear if direct activation of PPARγ signaling by NEFA or fatty acid metabolites occurs in mesenchymal stem cells or if this is secondary to impaired Wnt signaling as silencing of β-catenin with siRNA in the absence of fatty acids was reported to also significantly increase PPARγ expression (Chen et al., 2010c).

6. POTENTIAL EFFECTS OF DIET ON OXIDATIVE STRESS AND INFLAMMATION IN BONE

Under conditions inducing chronic bone loss, such as aging, estrogen withdrawal during menopause, alcohol abuse, and rheumatoid arthritis, oxidative stress and proinflammatory cytokines have been implicated as key mediators of impaired bone formation and elevated bone resorption (Mundy, 2007). Oxidative stress in bone cells is associated with production of ROS from a variety of sources including lipoxygenases, NADPH oxidases (Nox), and the adaptor protein p66[shc] which generates mitochondrial ROS (Almeida et al., 2007, 2010a). ROS is counteracted by antioxidant systems including superoxide dismutase, catalase, and the glutathione system (GSH/GSSG, glutathione peroxidase, and glutathione reductase), and stimulates DNA-damage repair genes such as p53 and the Forkhead transcription factors (FoxOs) and MAP kinases such as ERK (Manolagas, 2010). In addition, ROS leads to the production of proinflammatory cytokines in bone marrow including TNFα, IL-1β, and IL-6, which have synergistic effects with ROS on bone turnover and bone cell survival (Almeida et al., 2007; Mundy, 2007). ROS production in mesenchymal stem cells has been shown to inhibit osteoblastogenesis and stimulate bone marrow

adipogenesis as a result of impaired Wnt–β–catenin signaling and reciprocal increases in PPARγ signaling (Chen et al., 2010c). This appears to be mediated in part by diversion of β-catenin from TCF to FoxO-mediated transcription and in part by sustained activation of ERK and NFκB (Almeida et al., 2007). In addition, ROS stimulates osteoblast apoptosis and senescence (Almeida et al., 2010a; Chen et al., 2009b). Sustained ERK activation by ROS in osteoblasts also results in upregulation of RANKL expression via downstream signaling through STAT3, increasing osteoclast differentiation and bone resorption (Chen et al., 2008b). ROS also stimulates osteoclast differentiation downstream of RANKL–RANK interaction in osteoclast precursors (Lee et al., 2009). The proinflammatory cytokine TNFα inhibits osteoblast differentiation through inhibits of Runx2 expression and inhibits osteoblast function as a result of induction of vitamin D resistance; inhibits of bone matrix production by blockade of collagen synthesis; and stimulates osteoblast apoptosis. In addition, TNFα and other cytokines synergize with ROS to induce RANKL expression and also enhance osteoclastogenesis by increasing the osteoclast precursor pool via increased production of macrophage colony-stimulating factor and via increased expression of RANK in osteoclast precursors (Mundy, 2007). Estrogens and other sex steroids antagonize ROS actions in bone cells via upregulation of glutathione reductase and other antioxidant systems, attenuation of p53 expression and $p66^{shc}$ phosphorylation, inhibition of Nox expression, and inhibition of cytokine production (Almeida et al., 2010a; Chen et al., 2008b, 2009b, 2011). Therefore, phytochemicals such as isoflavones which may act as estrogen–receptor agonists may have positive effects on bone post menopause, in aging, in alcohol abusers, and in arthritis secondary to ER–mediated antioxidant and anti-inflammatory effects. In addition, dietary antioxidants such as N-acetylcysteine and vitamin E derivatives and anti–inflammatory agents such as the soluble TNF antagonist sTNFR1 have been shown to be effective in preventing bone loss under these conditions (Almeida et al., 2010a; Chen et al., 2008b, 2011; Lee et al., 2009; Wahl et al., 2007, 2010). Soy products and fruits such as BB and fruit juices such as orange juice have been shown to have ER-independent antioxidant and anti–inflammatory effects in vivo in several tissues (Morrow et al., 2009; Nagarajan, 2010; Wu et al., 2010). It is therefore likely that these dietary factors exert their skeletal actions at least in part via antioxidant/anti-inflammatory effects on bone cells. Two studies have been published examining anti-inflammatory and antioxidant effects of extracted dried plum polyphenol extracts on bone cells in vitro (Bu et al., 2008, 2009). These authors report inhibition of increases in RANKL expression in osteoblast cell lines in response to TNFα; inhibition of oxidative stress in macrophage cell lines as a result of cotreatment with LPS or TNFα; and inhibition of osteoclast differentiation after RANKL treatment. However, these studies ignore important issues of polyphenol bioavailability and metabolism. The mixture of compounds bone cells, exposed to after feeding dried plums, is likely to be completely different from the polyphenol mixture in the fruit. Similarly, in vitro studies with resveratrol have reported inhibition of RANKL–RANK signaling and osteoclastogenesis in vitro as a result of inhibited ROS production

(He et al., 2010). Unfortunately, these studies were conducted at pharmacological concentrations and have little relevance to the skeletal effects of red wine consumption. As yet, no studies have examined ROS or cytokine-mediated pathways in bone cells after in vivo feeding studies or in appropriate in vitro systems.

7. VITAMIN C

In addition to vegetables, vitamin C (ascorbic acid) is an essential nutrient to maintain bone density and quality. Recent studies have utilized mouse models to study the role of ascorbate on skeletal homeostasis (Gabbay et al., 2010). Aldehyde reductase (Akr1a4) knockout mice in which endogenous ascorbate synthesis is inhibited 85% due to the inhibition of the conversion of D-glucuronate to L-gulonate develop osteopenia and have spontaneous fractures under conditions of increased ascorbate requirements such as castration and pregnancy, as the result of inhibited osteoblastogenesis and increased osteoclast number and activity (Gabbay et al., 2010). These effects could be reversed by feeding ascorbate. These data suggest a role for ascorbate as an essential cofactor in osteoblast differentiation and as an antioxidant suppressing osteoclast activity (Saito, 2009). In addition, ascorbic acid is a reductant for the iron prosthetic group of hydroxylase enzymes involved in collagen biosynthesis and collagen cross-link formation, and is therefore essential for normal bone matrix production (Francesschi, 1992).

8. FUTURE STUDIES

Despite the plethora of descriptive studies on the effects of nutritional status and diet on bone quality, to date few mechanistic studies have been performed where bioactive dietary components have been identified or molecular pathways in bone cells have been characterized. Although recent studies indicate that reciprocal regulation of Wnt-β-catenin/PPARγ signaling and the BMP pathways are the final common targets for the effects of some fruits and vegetables and of high-fat diets on the skeleton, the detailed interactions of dietary factors with these signaling pathways remain to be elucidated. In addition, the effects of dietary factors on estrogen signaling, redox status, and cytokine production in bone require further study.

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GLOSSARY

Aglycone Unconjugated form of isoflavones – genistein, daidzein, and equol. The majority of these phytochemicals are found in blood and tissues in conjugated forms with sugar (glucuronide) or sulfate.

BMP Bone morphogenic proteins. A second cell signaling pathway which stimulates osteoblast differentiation as a result of enhancing downstream regulators such as MAP kinases and Smads. Converges with the Wnt-β-catenin pathway at the level of Runx2.

Estrogen receptors Cellular receptors for the female sex steroid 17β-estradiol. There are two forms, ERα and ERβ, which can activate gene transcription via binding to estrogen-response element sequences (EREs) on gene promoters. Estrogens can also signal via a second pathway involving activation of MAP kinases.

Kinase Enzyme which catalyzes protein phosphorylation.

Mesenchymal stem cell Cells in bone marrow capable of developing into bone-forming osteoblasts or adipocytes (fat cells).

Phenolic acids Breakdown product of fruit pigments produced by the action of gut bacteria.

PPAR Peroxisome proliferator-activated receptors (PPARs) are transcription factors involved in lipid metabolism and fat cell formation. PPARγ is a master regulator with opposite expression pattern to β-catenin and which stimulates differentiation of mesenchymal stem cells into fat cells.

RANKL–RANK signaling Receptor activator of NFκB ligand (RANKL) is a member of the TNF family of proteins made by osteoblasts and osteoblast precursors which, through interaction with its receptor RANK on the surface of osteoclast precursors, controls the differentiation of osteoclasts (cells which break down bone).

Redox Intracellular balance between reducing and oxidizing states.

SERM Selective estrogen-receptor modulators. Molecules with structures similar to estrogens which can signal like estrogens in some tissues and have anti-estrogenic actions in others.

Soy protein isolate (SPI) Soy protein product used to make soy infant formula.

Wnt-β-catenin Cell signaling pathway whereby a series of soluble peptides (Wnts) regulate differentiation of bone-forming cells (osteoblasts) in bone marrow. Wnts signal via Frizzed receptors to stabilize expression of the protein β-catenin, causing it to migrate to the cell nucleus and initiate gene transcription in a complex with the transcription factors TCF/LEF. An important Wnt-β-catenin target is Runx2, a master transcription factor essential for osteoblast formation.

REFERENCES

Almeida, M., Han, L., Ambrogini, E., Bartelli, S.M., Manolagas, S.C., 2010. Oxidative stress stimulates apoptosis and activates NF-kappaB in osteoblastic cells via a PKCbeta/p66shc signaling cascade: counter regulation by estrogens or androgens. Molecular Endocrinology 24, 2030–2037.

Almeida, M., Han, L., Martin-Millan, M., et al., 2007. Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. Journal of Biological Chemistry 282, 27285–27297.

Almeida, M., Martin-Millan, M., Ambrogini, E., et al., 2010. Estrogens attenuate oxidative stress and the differentiation and apoptosis of osteoblasts by DNA-binding independent actions of ERalpha. Journal of Bone and Mineral Research 25, 769–781.

Barnes, S., Boersma, B., Patel, R., et al., 2000. Isoflavonoids and chronic disease: mechanisms of action. Biofactors 12, 209–215.

Branca, F., Lorenzetti, S., 2005. Health effects of phytoestrogens. Forum of Nutrition 57, 100–111.

Bu, S.Y., Hunt, T.S., Smith, B.J., 2009. Dried plum polyphenols attenuate the detrimental effects of TNFa on osteoblast function coincident with up-regulation of Runx2, Osterix and IGF-1. The Journal of Nutritional Biochemistry 20, 35–44.

Bu, S.Y., Lerner, M., Stoecker, B.J., et al., 2008. Dried plum polyphenols inhibit osteoclastogenesis by downregulating NFATc1 and inflammatory mediators. Calcified Tissue International 82, 475–488.

Cai, D.J., Zhao, Y., Glasier, J., et al., 2005. Comparative effect of soy protein, soy isoflavones and 17 beta estradiol on bone metabolism in adult ovariectomized rats. Journal of Bone and Mineral Research 20, 828–839.

Canalis, E., 2009. Growth factor control of bone mass. Journal of Cellular Biochemistry 108, 769–777.

Canalis, E., Economides, A.N., Gazzero, E., 2003. Bone morphogenic proteins, their antagonists, and the skeleton. Endocrine Reviews 24, 218–235.

Chen, J.-R., Badger, T.M., Nagaragian, S., Ronis, M.J.J., 2008. Inhibition of reactive oxygen species generation and downstream activation of the ERK/STAT3/RANKL-signaling cascade to osteoblasts accounts for the protective effects of estradiol on ethanol-induced bone loss. Journal of Pharmacology and Experimental Therapeutics 324, 50–59.

Chen, J.-R., Lazarenko, O.P., Blackburn, M.L., Badeaux, J., Badger, T.M., Ronis, M.J.J., 2009. Infant formula promotes bone growth in neonatal piglets by enhancing osteoblastogenesis through bone morphogenic protein signaling. Journal of Nutrition 139, 1839–1847.

Chen, J.R., Lazarenko, O.P., Blackburn, M.L., Badger, T.M., Ronis, M.J.J., 2010. A role for ethanol-induced oxidative stress in controlling lineage commitment of mesenchymal stromal cells through inhibition of Wnt/beta catenin signaling. Journal of Bone and Mineral Research 25, 1117–1127.

Chen, J.-R., Lazarenko, O.P., Haley, R.L., Blackburn, M.L., Badger, T.M., Ronis, M.J.J., 2009. Ethanol impairs estrogen receptor signaling and activates senescence pathways in osteoblasts. Protection by estradiol. Journal of Bone and Mineral Research 24, 221–230.

Chen, J.R., Lazarenko, O.P., Shankar, K., et al., 2010. Inhibition of NADPH oxidases prevents chronic ethanol-induced bone loss in female rats. Journal of Pharmacology and Experimental Therapeutics 336, 734–742.

Chen, J.-R., Lazarenko, O.P., Wu, X., et al., 2010. Dietary-induced serum phenolic acids promote bone growth via p38 MAPK/β-catenin canonical wnt signaling. Journal of Bone and Mineral Research 25, 2399–2412.

Chen, J.R., Lazarenko, O.P., Wu, X., et al., 2010. Obesity reduces bone density associated with activation of PPARγ and suppression of Wnt/β-catenin signaling in rapidly growing male rats. PLoS One e13704.

Chen, J.-R., Singhal, R., Lazarenko, O.P., et al., 2008. Shirt term effects on bone quality associated with consumption of soy protein isolate and other dietary protein sources in rapidly growing female rats. Experimental Biology and Medicine 233, 1348–1358.

Dang, Z., Lowik, C.W.G.M., 2004. The balance between concurrent activation of ERs and PPARs determines daidzein-induced osteogenesis and adipogenesis. Journal of Bone and Mineral Research 19, 853–861.

Franceschi, R.T., 1992. The role of ascorbic acid in mesenchymal differentiation. Nutrition Reviews 50, 65–70.

Gabbay, K.H., Bohren, K.M., Morello, R., Bertin, T., Liu, J., Vogel, P., 2010. Ascorbate synthesis pathway: dual role of ascorbate in bone homeostasis. Journal of Biological Chemistry 285, 19510–19520.

Gu, L., Laly, M., Chang, H.C., et al., 2005. Isoflavone conjugates are underestimated in tissues using enzymic hydrolysis. Journal of Agricultural and Food Chemistry 53, 6858–6863.

Gu, L., Prior, R.L., Fang, N., Ronis, M.J.J., Clarkson, T.M., Badger, T.M., 2006. Metabolic phenotypes of isoflavones are different in female rats, pigs, monkeys and humans. Journal of Nutrition 136, 1215–1226.

Halloran, B.P., Wronski, T.J., VonHerzen, D.C., et al., 2010. Dietary dried plum increases bone mass in adult and aged male mice. Journal of Nutrition 140, 1781–1787.

He, X., Andersson, G., Lindgren, U., Li, Y., 2010. Resveratrol prevents RANKL–induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through inhibition of ROS production. Biochemical and Biophysical Research Communications 401, 356–362.

Hooshmand, S., Arjmandi, B.H., 2009. Viewpoint: dried plum, an emerging functional food that may effectively improve bone health. Aging Research Reviews 8, 122–127.

Jun, J.H., Yoon, W.J., Seo, S.B., et al., 2010. BMP2-activated ERK/MAP kinase stabilizes Runx2 by increasing p300 levels and histone acetyltransferase activity. Journal of Biological Chemistry 285, 36410–36419.

Komori, T., 2010. Regulation of bone development and extracellular matrix protein genes by Runx2. Cell and Tissue Research 339, 189–195.

Lecka-Czernik, B., 2010. PPARs in bone: the role in bone cell differentiation and regulation of energy metabolism. Current Osteoporosis Reports 8, 84–90.

Lee, J.-L., Kim, H.-N., Yang, D., et al., 2009. Trolox prevents osteoclastogenesis by suppressing RANKL-expression and signaling. Journal of Biological Chemistry 284, 13725–13734.

Legette, L.L., Matin, B.R., Shahanazari, M., et al., 2009. Supplemental dietary racemic equol has modest effects to bone but has mild uterotrophic activity in ovariectomized rats. Journal of Nutrition 139, 1908–1913.

Lydeking-Olsen, E., Beck-Jensen, J.E., Setchell, K.D.E., Holm-Jensen, T., 2004. Soy milk or progesterone for prevention of bone loss – a 2-year randomized, placebo-controlled trial. European Journal of Nutrition 43, 246–257.

Manolagas, S.C., 2010. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocrine Revs. 31, 266–300.

Messina, M., 2010. Insights gained from 20 years of soy research. Journal of Nutrition 140, 2289S–2295S.

Morrow, R., Deyhim, F., Patil, B.S., Stoeker, B.J., 2009. Feeding orange pulp improved bone quality in a rat model of male osteoporosis. Journal of Medicinal Food 12, 298–303.

Mundy, G.R., 2007. Osteoporosis and inflammation. Nutrition Reviews 65, S147–S151.

Nagarajan, S., 2010. Mechanisms of anti-atherosclerotic functions of soy-based diets. The Journal of Nutritional Biochemistry 21, 255–260.

Niculesc, M.D., Pop, E.A., Fischer, L.M., Zeisel, S.H., 2007. Dietary isoflavones differentially induce gene expression changes in lymphocytes from postmenopausal women who form equol as compared with those who do not. The Journal of Nutritional Biochemistry 18, 380–390.

Rajah, T.T., Du, N., Drews, N., Cohn, R., 2009. Genistein in the presence of 17 beta estradiol inhibits proliferation of ER beta breast cancer cells. Pharmacology 84, 68–73.

Reinwald, S., Weaver, C.M., 2006. Soy isoflavones and bone health: a double-edged sword? Journal of Natural Products 69, 450–459.

Reinwald, S., Weaver, C.M., 2010. Soy components vs. whole soy: are we betting our bones on a long shot? Journal of Nutrition 140, 2312S–2317S.

Saito, M., 2009. Nutrition and bone health. Roles of vitamin C and vitamin B as regulators of bone mass and quality. Clinical Calcium 19, 1192–1199.

Sliwinski, L., Folwaerczna, J., Nowinska, B., et al., 2009. A comparative study of the effects of genistein, estradiol and raloxifene on the murine skeletal system. Acta Biochimica Polonica 56, 261–270.

Tang, X., Zhu, X., Liu, S., Wang, S., Ni, X., 2011. Isoflavones suppress cyclic adenosine 3'5'-monophosphate regulatory element-mediated transcription in osteoblastic cell lines. The Journal of Nutritional Biochemistry 22, 865–873.

Trzeciakiewicvz, A., Habauzit, V., Mercier, S., et al., 2010a. Hesperetin stimulates differentiation of primary rat osteoblasts involving the BMP pathway. Journal of Nutritional Biochemistry 21, 424–431.

Trzeciakiewicz, A., Habauzit, V., Mercier, S., et al., 2010b. Molecular mechanism of hesperetin-7-O-glucuronide, the main circulating metabolite of hesperidin, involved in osteoblast differentiation. Journal of Agricultural and Food Chemistry 58, 675–686.

Wahl, E.C., Aronson, J., Liu, L., et al., 2010. Restoration of regenerative osteoblastogenesis in aged mice: modulation of TNF. Journal of Bone and Mineral Research 25, 114–123.

Wahl, E.C., Aronson, J.A., Liu, L., et al., 2007. Chronic ethanol exposure inhibits distraction osteogenesis in a mouse model: role of the TNF signaling axis. Toxicology and Applied Pharmacology 220, 302–310.

Weaver, C.M., Legrette, L.L., 2010. Equol, via dietary sources or intestinal production, may ameliorate estrogen–deficiency-induced bone loss. Journal of Nutrition 140, 1377S–1379S.

Westendorf, J.J., Kahler, R.A., Schroeder, T.M., 2004. Wnt signaling in osteoblasts and bone diseases. Gene 341, 19–39.

Wu, X., Kang, J., Xie, C., et al., 2010. Dietary blueberries attenuate atherosclerosis in apolipoprotein E-deficient mice by upregulating antioxidant enzyme expression. Journal of Nutrition 140, 1628–1632.

Aging, Zinc, and Bone Health

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ABBREVIATIONS

ALP Alkaline phosphatase
BMD Bone mineral density
IGF-I Insulin-like growth factor-1
IL-1 Interleukin-1
IL-6 Interleukin-6
LPS Lipopolysaccharide
MT Metallothionein
NFκB Nuclear factor kappa B
NHANES National Health and Nutrition Examination Survey
OPG Osteoprotegerin
RANK Receptor activator for nuclear factor-κB
RANKL Receptor activator for nuclear factor-κB ligand
TNF-α Tumor necrosis factor-α

1. INTRODUCTION

Bone health is a major contributing factor to an individual's overall health and quality of life. While the skeleton has long been recognized for its critical role in mobility and protection of vital organs, it also houses the bone marrow compartment, which serves as the center for hematopoiesis and a key interface between the skeletal and immune systems. The cells primarily responsible for bone metabolism, osteoclasts, and osteoblasts act in concert to regulate bone remodeling in the adult skeleton. Osteoclasts are derived from bone marrow hematopoietic stems cells of the monocyte/macrophage lineage, whereas osteoblasts originate from mesenchymal stem cell populations within the bone marrow. Under normal conditions of bone remodeling, bone resorption by the osteoclasts is followed by new bone formation by osteoblasts, resulting in ongoing remodeling of the adult skeleton within individual bone multicellular units. Once the activity of the osteoclasts and the osteoblasts is uncoupled, such as that which occurs in the case of aging or hormone dysregulation, skeletal health is compromised.

The most common skeletal disease, osteoporosis, is characterized by a decrease in bone mass or density and deterioration of bone microstructural properties, which results

in increased risk of fracture. Osteoporotic fractures occur most often in the hip, spine, and wrist, which are regions of the skeleton rich in trabecular bone. By the year 2020, it is expected that one in two women and one in four men over the age of 50 years will experience an osteoporotic fracture based on the prevalence of the condition and the anticipated demographic shift to a more aged US population. Worldwide, the incidence of hip fracture is expected to increase by approximately threefold in men and more than twofold in women by the year 2050. The potential impact of the cost of treating these fractures on the healthcare system combined with their detrimental effects on quality of life creates an urgency to improve current prevention and treatment strategies.

Modifiable lifestyle factors such as nutrition and weight-bearing exercise contribute significantly to the accrual of bone during the first two to three decades of life and the rate of bone loss once peak bone mass is achieved. In particular, nutrition provides the fundamental components necessary to maintain bone health. Calcium and phosphorus are among the most commonly considered nutrients involved in skeletal heath due to their structural role in bone's mineralized matrix or hydroxyapatite. Other micronutrients such as vitamins D and K are considered critical for their role in the regulation of calcium homeostasis and bone mineralization. A number of trace elements are known to play important roles in bone health because of their effects on various cellular metabolic processes (e.g., antioxidant properties) and have led to renewed interest in micronutrients such as zinc.

Zinc is known to directly affect skeletal growth, bone formation, and bone resorption in rapidly growing animals. However, because of zinc's role in innate and adaptive immunity and the relationship between chronic inflammatory conditions (e.g., rheumatoid arthritis and periodontitis) and bone loss, it is reasonable to consider that compromised zinc status in older adults may play a role in age-related bone loss. In this chapter, the current knowledge related to the role of zinc in bone health and immune function will be reviewed followed by a brief discussion of how compromised zinc status in older adults may alter immune function and indirectly contribute to age-related bone loss.

2. ZINC STATUS IN OLDER ADULTS

Adequate dietary zinc intake is a concern for many population groups including older adults. The RDA for men and women, 51 years of age and older, is 11 and 8 mg day^{-1}, respectively. These recommended levels of dietary intake are the same as those for younger adults, 19–30 and 31–50 years of age. As shown in Table 31.1, zinc is found in a variety of foods. While oysters, beef, and crab meat are considered excellent sources of zinc, it is also found in whole grains, legumes, seeds, and fortified cereals. The biological availability of zinc from plant sources, however, may be less than the bioavailability from animal sources due in part to the phytate content of some plant-based foods.

Table 31.1 Dietary Zinc Sources in Selected Foods

Food	Serving size	Zinc content (mg)
Beef (ground beef)	1/4 pound raw meat	4.3
Poultry (breast, bone removed)	1/2 breast (98 g)	1.0
Poultry (thigh, bone removed)	1 thigh	1.5
Oysters	3 oz cooked	4.2
Crab	3 oz cooked	3.2
Milk (2% low fat)	1 cup	1.0
Yogurt (low fat)	1 cup	1.8
Sunflower seeds (roasted)	1 oz dry	1.5
Whole grains	1 slice of bread	1.0
Legumes (navy beans)	1 cup	7.6

USDA National Nutrient Database for Standard Reference, Release 23 (2010).

Dietary zinc intake in older adults is closely tied to food choices and total energy intake. Lower zinc intake is considered a manifestation of the lower overall calorie consumption often observed in older adults. A study based on National Health and Nutrition Examination Survey (NHANES) III data showed the mean dietary zinc intakes among nonsupplement users were 11.7 and 8.4 mg day^{-1} for men and women 51–70 years of age (Briefel et al., 2000). The mean dietary zinc intakes were 10.9 mg day^{-1} for men and 8.0 mg day^{-1} for women, 71 years of age and older. Among the supplement users, the mean intakes (i.e., combined dietary and supplemental zinc) were 14.7 and 12.1 mg day^{-1} for men and women 51–70 years of age, and 14.2 and 11.6 mg day^{-1} for men and women 71 years of age and older. Adequate zinc intake was reported for 56.8% of men and 46.1% of women in the 51–70 years age group and 43.9% of men and 41.5% of women among those 71 years of age and older. Consequently, these data suggest that a significant proportion of the older adult population in the United States is not meeting the recommended levels of daily zinc intake.

Zinc deficiency has been arbitrarily defined as a plasma or serum zinc concentration of $<10.7\ \mu\text{mol l}^{-1}$ or 70 μg dl^{-1} (Fosmire, 2006). Based on data from NHANES II, approximately 12% of men and women had plasma or serum zinc levels below 70 μg dl^{-1} (Fosmire, 2006). Low plasma or serum zinc has been reported to be more prevalent among limited income older adults and those in poor health (Fosmire, 2006). Classic clinical symptoms of zinc deficiency in older adults include an impaired immune response, delayed wound healing, dermatitis, hair loss, diminished taste acuity, anorexia, and abnormal dark adaptation (Chapman-Novakofski, 2010; Fosmire, 2006). Zinc deficiency among older adults can be caused by a combination of factors related to diet, general health status, and medication use. Some of the most common contributors include inadequate calorie intake, high dietary phytate intake, the use of iron supplements, alcoholism, surgery or physical stress, chronic diarrhea, malabsorption syndromes, and medication such as H$_2$ receptor antagonists. Controversy exists regarding efficiency of

zinc absorption among older adults in that some studies have reported older adults absorb zinc less efficiently (Turnlund et al., 1986), while others have reported zinc absorption is similar among older and younger adults (Couzy et al., 1993).

Any discussion of zinc status would be incomplete without addressing the issue that while serum and plasma zinc is often used as a reference point, a sensitive and reliable marker of zinc status is yet to be agreed upon. Plasma and serum zinc are most often used; however, other methods reported to evaluate zinc status include erythrocyte, hair, and leukocyte zinc. Plasma zinc values do not always reflect and individual's zinc reserves or recent zinc intake, and factors such as infection and physical activity can lead to a re-distribution of zinc as opposed to a true deficiency (Fosmire, 2006; Hess et al., 2007). Thus, at the present time, serum and plasma zinc have been proposed as biochemical markers for the assessment of population zinc status, but more sensitive and reliable indicators are needed to advance our understanding of the physiological effects of zinc deficiency in clinical studies.

3. ZINC AND BONE METABOLISM

In general, zinc's biological functions have been classified as catalytic, structural, and regulatory (Cousins, 2006). Zinc functions as a component of more than 50 different enzymes, a structural constituent of many proteins, and a regulator of gene expression. In humans, total body zinc ranges from ~1.5 g in women to ~2.5 g in men. An esti-mated 86% of the body's zinc is localized within skeletal muscle and bone tissue with the highest concentration found within hair (Table 31.2). Interestingly, the highest concentrations of zinc within bone tissues are found in the osteoid, or the unminera-lized protein matrix of bone secreted by osteoblasts, which supports the role of zinc in bone mineralization.

Zinc's effects on bone formation have been characterized as enhancing the differen-tiation and proliferation of osteoblasts as well as the promotion of bone mineralization. The effects of zinc on osteoblast differentiation have been attributed in part to its ability to

Table 31.2 Estimated Zinc Concentrations and Zinc Content of Major Organs in Humans

Tissues	Estimated zinc concentration (μg g^{-1})	Estimated total zinc content (g)
Hair	150	<0.01
Bone	100	0.77
Liver	58	0.13
Kidneys	55	0.02
Skeletal muscle	51	1.53
Brain	11	0.04
Plasma	1	<0.01

Source: Mills, C.F. (Ed.), 1989. Zinc in Human Biology. Springer, London.

increase the expression of runt-related transcription factor 2, a key transcription factor in osteoblastogenesis. Zinc has also been shown to stimulate the osteoblastic production of growth factors *in vitro* such as insulin-like growth factor (IGF)-I and transforming growth factor (TGF)-β that are known to promote bone formation. A study of postmenopausal women designed to examine the relationship between micronutrient intake and IGF-I demonstrated zinc was the only nutritional determinant of IGF-I concentrations in this population (Devine et al., 1998). Interestingly, in the frail elderly whey protein supplements combined with supplemental zinc resulted in the most significant increase on serum IGF-I (Rodondi et al., 2009). Biomarkers of bone resorption were also reported to decrease, but no significant changes were reported in serum IGF binding protein 3 (IGF-BP3), which can alter IGF-I bioactivity.

In addition to its effects on osteoblast differentiation and proliferation, zinc also promotes bone mineralization. Alkaline phosphatase (ALP), the zinc metalloenzyme secreted by osteoblasts, promotes mineralization by dephosphorylating inorganic pyrophosphates that inhibit the normal bone mineralization process. Peretz and colleagues (2001) showed that 12 weeks of zinc supplementation (i.e., 50 mg zinc gluconate) increased bone-specific ALP in healthy men. Zinc supplementation in osteoblast cultures has been associated with increased ALP activity and osteocalcin production, which suggests zinc is capable of promoting bone mineralization. In type 1 diabetics, a population known to develop a defect in bone formation and mineralization, zinc intake has been shown to positively correlate with serum osteocalcin (Maser et al., 2008). When considered in combination, these findings indicate that zinc plays an important role in osteoblast activity and function. Thus, it is to be expected that when zinc status is compromised (i.e., deficiency or insufficiency), skeletal growth, bone formation, and/or mineralization are stalled.

Zinc has also been shown to affect the bone resorption activity of osteoclasts. Results from the Zenith Study demonstrated a negative correlation between zinc status and markers of bone resorption (i.e., urinary pyridinoline and deoxypyridinoline) in European older adults (Hill et al., 2005). *In vitro* studies designed to understand zinc's antiresorptive effects have shown that zinc inhibits osteoclastogenesis induced by parathyroid hormone and proinflammatory mediators such as interleukin (IL)-1α, tumor necrosis factor (TNF)-α, and prostaglandin E2. The effects of zinc on osteoclast differentiation are mediated at least in part by inhibiting ligand binding of RANKL (receptor activator for nuclear factor kappa B (NF-κB) ligand) to its receptor (i.e., receptor activator for NF-κB (RANK)). Normally, formation of the RANK–RANKL complex on osteoclast precursor cells leads to osteoclast differentiation. RANK–RANKL interaction also promotes osteoclast activity. Zinc's effects on RANKL signaling are in response to its ability to upregulate the decoy receptor for RANKL, osteoprotegerin (OPG) (Yamaguchi et al., 2008). OPG binding to RANKL prevents RANK–RANKL interaction, therefore decreasing osteoclastogenesis and osteoclast activity. Thus, if adequate zinc status is

required to support a healthy ratio of OPG to RANKL, in situations of compromised zinc status, bone resorption by osteoclasts would be expected to increase.

Much of what is known about zinc's role in bone metabolism has been garnered from cell and tissue culture systems, the examination of the relationship between zinc status and clinical indicators of bone metabolism, and studies of severe zinc deficiency involving young growing animals. Studies designed to evaluate the effects of zinc in animal models have been complicated by the fact that zinc deficiency is typically produced in young growing animals, a scenario that does not represent the bone remodeling processes occurring in the adult skeleton. The severe zinc deficiency produced in these animal models is unlikely to mimic the moderate compromise in zinc status or zinc insufficiency, which is more common in elderly populations. Furthermore, in animal models of pronounced zinc deficiency, the animals' appetite is often suppressed resulting in a decrease in overall food intake and weight loss, which confounds the interpretation of the effects of zinc deficiency on bone.

Recently, the effects of a moderate zinc deficiency in adult animal models were explored in two separate studies to determine how bone metabolism is affected. Scrimgeour and colleagues (Scrimgeour et al., 2007) reported that moderate zinc deficiency (i.e., 5 ppm) in young adult Sprague Dawley rats decreased bone zinc concentration by ∼40% and led to a compromise in bone biomechanical properties of the tibia. In contrast, Erben and colleagues (Erben et al., 2009) demonstrated that marginal zinc deficiency in adult Fischer–344 rats did not alter bone mass or trabecular bone density at the proximal tibial or spine and no changes in bone formation or resorption biomarkers were observed. Although two different methods of inducing suboptimal zinc status were used in these studies, these findings raise the question as to whether or not marginal zinc deficiency alone leads to bone loss in adult animals.

4. AGE-RELATED BONE LOSS AND ZINC

4.1 Zinc Status and Bone Mineral Density

Studies evaluating the relationship between zinc and bone mineral density (BMD) in clinical studies have provided further support of the notion that zinc influences bone health in older adults. In 1983, a study examining the zinc content of bone in men showed that those with osteoporosis had significantly lower bone zinc content than their nonosteoporotic counterparts, which coincided with lower serum zinc (Atik, 1983). A similar association between serum zinc and osteoporosis was demonstrated in postmenopausal women recently and importantly serum zinc improved following treatment with an antiresorptive therapy (Gur et al., 2005). Mutlu and colleagues (2007) reported a zinc dose–response relationship with BMD in postmenopausal women in that the lowest serum zinc was reported in the osteoporotic group, second lowest levels in the osteopenic group, and the highest zinc concentration in women with a normal BMD. However, it is

important to note that not all studies have shown that a significant relationship exists between plasma or serum zinc and osteoporosis in postmenopausal women. Also, several studies have reported a strong positive correlation between osteoporosis and urinary zinc excretion. Women with osteoporosis had significantly higher urinary zinc concentrations than women without osteoporosis and a positive correlation was demonstrated between urinary zinc and hydroxyproline. These findings have led some to suggest that urinary zinc excretion may be a useful biomarker of bone resorption (Herzberg et al., 1990).

4.2 Zinc Intake and BMD

Examination of the relationship of dietary zinc intake and BMD has also been reported in the literature. The Rancho Bernardo study evaluated zinc status and BMD of community-dwelling older men, 45–92 years of age. Dietary zinc intake and plasma zinc concentrations were lower in men with BMD < -2.5 at the hip and spine compared to men without osteoporosis (Hyun et al., 2004). Higher zinc intake was positively correlated with rate of radius change in BMD (i.e., higher zinc intake associated with slower rate of bone loss) among nonsupplemented postmenopausal women (Freudenheim et al., 1986). The relationship between dietary zinc intake and BMD has also been assessed in women from 23 to 75 years of age. In premenopausal women, a weak positive correlation between dietary zinc intake and forearm bone mineral content was reported, but no correlation was observed between dietary zinc and BMD in postmenopausal women (Angus et al., 1988). Another study of premenopausal women showed that high dietary zinc intakes were significantly associated with higher lumbar spine BMD (New et al., 1997). Studies of the effects of supplemental zinc in combination with other minerals have demonstrated that calcium combined with zinc, manganese, and copper reduced the rate of postmenopausal bone loss of the spine (Strause et al., 1994). Data from these studies provide further evidence of the importance of adequate dietary zinc intake and in some cases supplemental zinc combined with other micronutrients on bone health.

4.3 Zinc Status and Fracture Risk

Studies evaluating the relationship between zinc status and fracture risk are much more limited but provide important insight into zinc's effects on bone biomechanical properties. Elmstahl and colleagues (Elmstahl et al., 1998) assessed the relationship between various dietary factors and fracture incidence in men aged 46–68 years and showed that inadequate zinc intake was associated with increased fracture risk even after adjustments for age, energy, and other nutrient intakes, and previous fracture. In fact, the group with the lowest zinc intake (i.e., 10 mg daily) had a twofold increase in risk of fracture compared with groups with higher zinc intake. Recently, biomechanical properties were compared in a younger (i.e., 48-year-old woman) and an older female (i.e., 78-year-old woman; Chan et al., 2009). Bone zinc concentrations were significantly lower in

the older subject and more susceptible to unstable brittle fracture compared to the young bone. Additionally, the porosity of the bone of the older subject was higher than the young bone, which led the authors to conclude that lower zinc concentration in the older bone may be responsible for higher porosity and lower fracture resistance.

5. ZINC AND IMMUNE FUNCTION

In addition to zinc's role in bone health, it also has a broad range of functions associated with immunity. These functions include the regulation of many aspects of lymphocyte function (e.g., mitogenesis and T-cell activation), alterations in the development and function of cells that mediate innate immunity (i.e., neutrophils and natural killer cells), and the production of cytokines.

Suboptimal zinc in young animals produces thymic atrophy (70–80%) and a significant decline in pre- and immature B-cells (King et al., 2005). Likewise, zinc deficiency reduces the $CD4^+CD8^+$ or pre-T-cell populations by 38% in the thymus (Fraker and King, 2004). These effects of zinc deficiency were similar to those that occur when bone marrow cells are treated with glucocorticoids (Fraker et al., 1995). In fact, zinc has been shown to activate the hypothalamus–pituitary–adrenocortical axis, which results in chronic production of glucocorticoids. Excess glucocorticoids increase lymphocyte precursor apoptosis and are also known to have deleterious effects on bone by promoting bone resorption and inhibiting bone formation.

Zinc deficiency has also been shown to alter cell populations that mediate the innate immune response and their function. For example, myeloid lineage cells in the bone marrow, the same cell populations from which osteoclasts originate, increased in response to zinc deficiency. Committed monocyte progenitor cells increase in number with zinc deficiency as well as activity. While adequate zinc is important for macrophage function (i.e., phagocytosis), marginal zinc deficiency has been shown to increase the risk for liver damage in acute lipopolysaccharide (LPS)-induced liver injury and mortality in a mouse model of polymicrobial sepsis.

Increased circulating levels of IL-6, TNF-α, and chemokines in the CC (i.e., MCP-1) and CXC families (i.e., IL-8) have been reported in older individuals with suppressed serum zinc (Mariani et al., 2006). *In vitro*, zinc's effects on cytokine production are cell specific, in that, zinc downregulates TNF-α, IL-1β, and IL-8 in the HL-60 monocyte–macrophage cell line, but IL-2 and IFN-γ were decreased in HUT-78 and D1.1 human malignant T lymphoblast cell lines. Zinc also represses NF-κB activity and protects against the upregulation of proinflammatory cytokines in endothelial cells. Therefore, in the case of inadequate zinc, NF-κB would be activated. Undoubtedly, increased expression of proinflammatory cytokines can lead to increased metallothionein (MT-I and MT-II) expression, which further promotes zinc sequestration and makes conditions less favorable for an effective immune response. These findings, in

conjunction with the increase in myeloid populations and their activity, suggest that the bone marrow environment created in response to zinc deficiency exhibits an increase in osteoclast progenitor cells that could potentially promote osteoclast differentiation and bone resorption.

6. AGING AND IMMUNE FUNCTION

Alterations in immune function associated with suboptimal zinc parallel some of the immunological changes associated with normal aging (i.e., immunosenescence). In contrast, centenarians seem to have a repressed inflammatory status and low MT gene expression, which permits zinc bioavailability (Mocchegiani et al., 2002). These findings suggest that low MT expression may represent a compensatory response to counteract the increase in proinflammatory cytokines associated with aging. Age-related changes in the immune system are known to reduce the body's ability to mount an effective immune response. Mechanisms involved in these age-related changes include, but are not limited to, defects in the hematopoietic bone marrow populations and peripheral lymphocyte migration, maturation, and function. Bone marrow both produces and responds to a variety of hormones and signaling molecules or cytokines. Alterations in cytokine production are considered a significant contributor to age-induced changes in bone marrow morphology and are likely to influence bone cell differentiation, activity, and apoptosis.

7. ROLE OF INFLAMMATION IN BONE LOSS

A significant body of literature has documented the relationship between osteopenia/ osteoporosis and medical conditions associated with chronic inflammation. For example, osteopenia and osteoporosis are recognized as common complications in patients with HIV, chronic periodontitis, and rheumatoid arthritis. Bone loss in these patient populations was once considered a side effect of therapeutic agents such as glucocorticoids that are known to induce bone loss; however, the underlying pathogenesis of the disease is now recognized as a component of the host response and involves components of the innate and acquired immune systems.

Chronic elevation of proinflammatory mediators such as TNF-α and IL-1β disrupts normal bone remodeling and ultimately leads to bone loss by increasing osteoclast activity, decreasing osteoblast activity, or both. These effects on osteoclast and osteoblast activity may result from increased osteoclastogenesis, delayed osteoclast apoptosis, and/or the inhibition of osteoblast development and activity. The net effect is significant bone loss.

Insights gained over the past several years have revealed the role of the TNF superfamily of transmembrane proteins (i.e., RANK, RANKL, and OPG) in osteoclastogenesis in response to inflammatory conditions. Formation of the RANK–RANKL complex on osteoclast precursor cells initiates osteoclastogenesis and activates the osteoclasts'

catabolic activity. OPG, produced by bone marrow stromal cells and osteoblasts, functions to interfere with RANK–RANKL-mediated osteoclastogenesis. Zinc's ability to enhance OPG expression suggests that zinc status is an important regulator of osteoclast differentiation and activity. Furthermore, osteoclastogenesis can be stimulated directly by inflammatory cytokines such as IL-1 and TNF-α and the activation of NF-κB in preosteoclasts by RANKL, TNF-α, IL-1, and LPS upregulates nuclear factor of activated T cells c1 (NFATc1) a key transcription factor in osteoclast differentiation. Therefore, NF-κB activation and cytokine production in response to marginal zinc deficiency and chronic inflammation provide a microenvironment within the bone marrow that favors bone resorption. Therefore, zinc deficiency could potentially exacerbate the effects of inflammation on bone in relation to both bone formation and bone resorption.

8. IMPLICATIONS

Based on NHANES data, a significant proportion of the older Americans are not meeting the recommended levels of daily zinc intake and have suboptimal zinc status. Zinc functions in the regulation of bone cell (i.e., osteoblast and osteoclast) differentiation and activity, which can directly affect bone metabolism in this population and ultimately lead to bone loss. Major advances in the study of osteoimmunology and osteoporosis over the past two decades have provided insight into the interplay between the immune and skeletal systems. Questions remain as to the influence of immunosenescence, or the host's ability to respond to an immunological challenge due to the natural aging of the immune system, on the aging skeleton. Because of zinc's role in innate and adaptive immunity, and the relationship between chronic inflammatory conditions (e.g., rheumatoid arthritis and periodontitis) and bone loss, it would seem that zinc deficiency in the elderly could create an environment that is prone to ongoing low-grade inflammation and primed to promote a catabolic state in loss. Although evidence from both clinical and *in vivo* studies supports an important relationship between zinc intake and status on BMD in older adults, further research focused on zinc's immune-modulating properties and their effects on age-related bone loss is needed.

GLOSSARY

Immunoscenescence The gradual deterioration of the immune system as a part of the natural aging process.
Osteoblastogenesis The process differentiating mesenchymal stem cells into mature osteoblasts.
Osteoclastogenesis The differentiation of hematopoietic stem cells into mature osteoclasts.
Osteoimmunology Interdisciplinary study of bone biology or osteology and its interplay with the immune system.

REFERENCES

Angus, R.M., Sambrook, P.N., Pocock, N.A., Eisman, J.A., 1988. Dietary intake and bone mineral density. Bone and Mineral 4 (3), 265–277.

Atik, O.S., 1983. Zinc and senile osteoporosis. Journal of the American Geriatric Society 31 (12), 790–791.

Briefel, R.R., Bialostosky, K., Kennedy-Stephenson, J., McDowell, M.A., Ervin, R.B., Wright, J.D., 2000. Zinc intake of the U.S. population: findings from the third National Health and Nutrition Examination Survey, 1988–1994. Journal of Nutrition 130 (Suppl. 5), 1367S–1373S.

Chan, K.S., Chan, C.K., Nicolella, D.P., 2009. Relating crack-tip deformation to mineralization and fracture resistance in human femur cortical bone. Bone 45 (3), 427–434.

Chapman-Novakofski, K.M., 2010. Mineral requirements of the older adult. In: Bernstein, M., Luggen, A.S. (Eds.), Nutrition for the Older Adult. Jones and Bartlett Publishers, Sudbury, MA.

Cousins, R.J., 2006. Zinc. In: Bowman, B.A., Russell, R.M. (Eds.), Present Knowledge in Nutrition. ninth ed. International Life Science Institute, Washington, DC.

Couzy, F., Kastenmayer, P., Mansourian, R., Guinchard, S., Munoz-Box, R., Dirren, H., 1993. Zinc absorption in healthy elderly humans and the effect of diet. American Journal of Clinical Nutrition 58 (5), 690–694.

Devine, A., Rosen, C., Mohan, S., Baylink, D., Prince, R.L., 1998. Effects of zinc and other nutritional factors on insulin-like growth factor I and insulin-like growth factor binding proteins in postmenopausal women. American Journal of Clinical Nutrition 68 (1), 200–206.

Elmstahl, S., Gullberg, B., Janzon, L., Johnell, O., Elmstahl, B., 1998. Increased incidence of fractures in middle-aged and elderly men with low intakes of phosphorus and zinc. Osteoporosis International 8 (4), 333–340.

Erben, R.G., Lausmann, K., Roschger, P., et al., 2009. Long-term marginal zinc supply is not detrimental to the skeleton of aged female rats. Journal of Nutrition 139 (4), 703–709.

Fosmire, G., 2006. Trace metal requirements. In: Chernoff, R. (Ed.), Geriatric Nutrition: The Health Professional's Handbook. Jones and Bartlett Publishers, Sudbury, MA.

Fraker, P.J., Osati-Ashtiani, F., Wagner, M.A., King, L.E., 1995. Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during zinc deficiency: a review. Journal of the American College of Nutrition 14 (1), 11–17.

Freudenheim, J.L., Johnson, N.E., Smith, E.L., 1986. Relationships between usual nutrient intake and bone-mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. American Journal of Clinical Nutrition 44 (6), 863–876.

Gur, A., Colpan, L., Cevik, R., Nas, K., Jale, S.A., 2005. Comparison of zinc excretion and biochemical markers of bone remodelling in the assessment of the effects of alendronate and calcitonin on bone in postmenopausal osteoporosis. Clinical Biochemistry 38 (1), 66–72.

Herzberg, M., Foldes, J., Steinberg, R., Menczel, J., 1990. Zinc excretion in osteoporotic women. Journal of Bone and Mineral Research 5 (3), 251–257.

Hess, S.Y., Peerson, J.M., King, J.C., Brown, K.H., 2007. Use of serum zinc concentration as an indicator of population zinc status. Food and Nutrition Bulletin 28 (Suppl. 3), S403–S429.

Hill, T., Meunier, N., Andriollo-Sanchez, M., et al., 2005. The relationship between the zinc nutritive status and biochemical markers of bone turnover in older European adults: the ZENITH study. European Journal of Clinical Nutrition 59 (Suppl. 2), S73–S78.

Hyun, T.H., Barrett-Connor, E., Milne, D.B., 2004. Zinc intakes and plasma concentrations in men with osteoporosis: the Rancho Bernardo study. American Journal of Clinical Nutrition 80 (3), 715–721.

King, L.E., Frentzel, J.W., Mann, J.J., Fraker, P.J., 2005. Chronic zinc deficiency in mice disrupted T cell lymphopoiesis and erythropoiesis while B cell lymphopoiesis and myelopoiesis were maintained. Journal of the American College of Nutrition 24 (6), 494–502.

Mariani, E., Cattini, L., Neri, S., et al., 2006. Simultaneous evaluation of circulating chemokine and cytokine profiles in elderly subjects by multiplex technology: relationship with zinc status. Biogerontology 7 (5–6), 449–459.

Maser, R.E., Stabley, J.N., Lenhard, M.J., Owusu–Griffin, P., Provost–Craig, M.A., Farquhar, W.B., 2008. Zinc intake and biochemical markers of bone turnover in type 1 diabetes. Diabetes Care 31 (12), 2279–2280.

Mocchegiani, E., Giacconi, R., Cipriano, C., et al., 2002. MtmRNA gene expression, via IL–6 and gluco-corticoids, as potential genetic marker of immunosenescence: lessons from very old mice and humans. Experimental Gerontology 37 (2–3), 349–357.

Mutlu, M., Argun, M., Kilic, E., Saraymen, R., Yazar, S., 2007. Magnesium, zinc and copper status in osteoporotic, osteopenic and normal post-menopausal women. Journal of International Medical Research 35 (5), 692–695.

New, S.A., Bolton–Smith, C., Grubb, D.A., Reid, D.M., 1997. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. American Journal of Clinical Nutrition 65 (6), 1831–1839.

Peretz, A., Papadopoulos, T., Willems, D., et al., 2001. Zinc supplementation increases bone alkaline phosphatase in healthy men. Journal of Trace Elements in Medicine and Biology 15 (2–3), 175–178.

Rodondi, A., Ammann, P., Ghilardi–Beuret, S., Rizzoli, R., 2009. Zinc increases the effects of essential amino acids-whey protein supplements in frail elderly. Journal of Nutrition, Health and Aging 13 (6), 491–497.

Scrimgeour, A.G., Stahl, C.H., McClung, J.P., Marchitelli, L.J., Young, A.J., 2007. Moderate zinc deficiency negatively affects biomechanical properties of rat tibiae independently of body composition. Journal of Nutritional Biochemistry 18 (12), 813–819.

Strause, L., Saltman, P., Smith, K.T., Bracker, M., Andon, M.B., 1994. Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. Journal of Nutrition 124, 1060–1064.

Turnlund, J.R., Durkin, N., Costa, F., Margen, S., 1986. Stable isotope studies of zinc absorption and retention in young and elderly men. Journal of Nutrition 116 (7), 1239–1247.

Yamaguchi, M., Goto, M., Uchiyama, S., Nakagawa, T., 2008. Effect of zinc on gene expression in osteoblastic MC3T3-E1 cells: enhancement of Runx2, OPG, and regucalcin mRNA expressions. Molecular and Cellular Biochemistry 312 (1–2), 157–166.

FURTHER READING

Clowes, J.A., Riggs, B.L., Khosla, S., 2005. The role of the immune system in the pathophysiology of osteoporosis. Immunological Reviews 208, 207–227.

Fraker, P.J., King, L.E., 2004. Reprogramming of the immune system during zinc deficiency. Annual Review of Nutrition 24, 277–298.

Gruver, A.L., Hudson, L.L., Sempowski, G.D., 2007. Immunosenescence of ageing. Journal of Pathology 211 (2), 144–156.

Prasad, A.S., 2009. Zinc: role in immunity, oxidative stress and chronic inflammation. Current Opinion in Clinical Nutrition and Metabolic Care 12, 646–652.

Rauner, M., Sipos, W., Pietschmann, P., 2007. Osteoimmunology. International Archives of Allergy and Immunology 143 (1), 31–48.

Relevant Websites

http://www.asbmr.org – American Society of Bone and Mineral Research.
http://www.nof.org – National Osteoporosis Foundation.
http://health.nih.gov – NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases.
http://ods.od.nih.gov/ – NIH Office of Dietary Supplements.

General Beneficial Effects of *Pongamia pinnata* (L.) Pierre on Health

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1. INTRODUCTION

1.1 Botanical Description

Pongamia pinnata (Linn) Pierre (synonyms: *Pongamia glabra* Vent., *Derris indica* (Lam.) Bennet, *Cystisus pinnatus* Lam.) family Fabaceae (Papilionaceae, Leguminosae). *P. pinnata* is a medium-sized glabrous semievergreen tree growing up to 18 m or higher, with a short bole and spreading crown. The bark is grayish–green or brown, smooth, or covered with tubercles. Leaves are alternate, shiny, young, and pinkish–red, and mature leaves are glossy and deep green. Flowers are lilac, white to pinkish, fragrant, paired along rachis in axillary, pendent, and in long racemes or panicles. Calyx cup-shaped, truncate, short dentate, the lowermost lobe sometimes longer; standard broadly suborbicular, usually with two inflexed basal ears, thinly silky haired outside; wings oblique, long, somewhat adherent to the obtuse keel. Keel petals coherent at apex, stamens ten and monadelphous, vexillary stamen free at the base but joined with others into a close tube, ovary subsessile, and stigma small and terminal. Pods are compressed, short, stalked, woody, indehiscent, yellowish–gray when ripe, varying in size and shape, elliptic to obliquely oblong, 4.0–7.5 cm long and 1.7–3.2 cm broad, with a short curved beak. Seeds are usually one, rarely two, elliptical or reniform, 1.7–2.0 cm broad, wrinkled, and with reddish–brown leathery testa (Joy et al., 1998).

1.2 Habitat

The plant comes up well in tropical areas with warm humid climates and well-distributed rainfall. Though it grows in almost all types of soils, silty soils on river banks are most ideal. It is tolerant to drought and salinity. The tree is used for afforestation, especially in watersheds in the drier parts of the country. It is propagated by seeds and vegetatively by root suckers.

1.3 Distribution

It is an Indo–Malaysian species, found almost throughout India up to an altitude of 1200 m and distributed further eastward, chiefly in the littoral regions of Southeastern Asia, Sri Lanka, Burma, Malaya, Australia, Florida, Hawaii, Malaysia, Oceania, Philippines, Polynesia, and Seychelles. The plant is distributed throughout India from the central or eastern Himalayas to Kanyakumari. The tree is considered to be a native of the Western Ghats and is chiefly found along the banks of streams and rivers or near the seacoast, in beaches and tidal forests. It is well adapted to all soil types and climatic requirements and grows in dry places far in the interior, up to an elevation of 1000 m. It resists drought well and is moderately forest hardy and highly tolerant to salinity. It is a shade bearer and is considered to be a good tree for planting in pastures, as grass grows well in its shade. The tree is suitable for afforestation especially in watershed areas and in drier parts of the country. Andhra Pradesh, Tamil Nadu, and Karnataka provide the bulk of the seeds of this tree. Large numbers of Karanja trees have been planted on the roadside, on highways and in urban areas during the last two decades (Khare, 2004).

2. PHYTOCHEMISTRY

The plant is rich in flavonoids and related compounds. The seeds and seed oil, flowers, and stem bark yield karanjin, pongapin, pongaglabrone, kanugin, desmethoxykanugin, and pinnatin. The seed and its oil also contain kanjone, isolonchocarpin, karanjachromene, isopongachromene, glabrin, glabrachalcone, glabrachromene, isopongaflavone, pongol, $2'$-methoxyfurano$[2'',3'':7,8]$-flavone, and phospholipids. The stem bark gives pongachromene, pongaflavone, tetra-O-methylfisetin, glabra I and II, lanceolatin B, gamatin, 5-methoxyfurano$[2'',3'':7,8]$-flavone, 5-methoxy-$3',4'$-methelenedioxyfurano$[2'',3''7,8]$-flavone, and β-sitosterol. The heartwood yields chromenochalcones and flavones. The flowers contain kanjone; gamatin; glabra saponin; kaempferol; g-sitosterol; quercetin glycocides; pongaglabol; isopongaglabol; 6-methoxy isopongaglabol; lanceolatin B; 5-methoxy-$3',4'$-methelenedioxyfurano$[8,7:4'',5'']$-flavone; fisetin tetramethyl ether; isolonchocarpin; ovalichromene B; pongamol; ovalitenon; two triterpenes cycloart-23-ene-3β, 25-diol and friedelin; and a dipeptide aurantiamide acetate (Joy et al., 1998).

Recently, Badole and Bodhankar (2009a,b,c) for the first time isolated the triterpene compound, namely, cycloart-23-ene-3β, 25-diol, from the stem bark of *P. pinnata*. Previously reported phytochemical analysis of the stem bark of *P. pinnata* showed the presence of alkaloids (Asalkar et al., 2000; Krishna and Grampurohit, 2006);

pongamone A–E (Li et al., 2006); flavonoids, furanoflavones (Tanaka et al., 1992); isopongaglabol, 6-methoxyisopongaglabol (Talapatra et al., 1982); 3-methoxy-(3,4-dihydro-3,4-diacetoxy)-2,2-dimethylpyrano-(7,8:5,6)-flavone; 2-methoxy-4,5-ethylene-diethylenedioxyfurano[7,8:4,5]-flavone; γ8,4–dimethoxy7–*O*γ,γ,dimethylallyl isoflavone; 3,4-methylenedioxy-10-methoxy-7-oxo[2]benzopyrano[4,3-b] benzopyran (Koysomboon et al., 2006); pongamosides A, B, C (furanoflavonoid glycosides), and D (flavonol glycoside) (Ahmad et al., 2004); lanceolatin B (Alam, 2004); pongapinones A and B (Isao et al., 1992); pongaflavone; karanjin; pongapin; pongachromene; 3,7-dimethoxy-3-,4-methylenedioxyflavone; millettocalyxin C; 3,3,4,7-tetramethoxyflavone (Yin et al., 2004); pongarotene; karanjin (Simin et al., 2002); pyranochalcones; beta sitosterol; steroids; triterpenoids; triterpenes; and volatile oils (Carcache-Blanco et al., 2003).

Rashid et al. (2008) reported isolation and crystal structure of karanjachromene from the seeds of *P. pinnata*. Ghufran et al. (2004) reported three new furanoflavonoid glucosides, pongamosides A–C, and a new flavonol glucoside, pongamoside D. The structures of these compounds were established on the basis of spectroscopic studies. This was the first time that furanoflavone glucosides were found as naturally occurring compounds. Six compounds (two sterols, three sterol derivatives, and one disaccharide) together with eight fatty acids (three saturated and five unsaturated) have been isolated from the seeds of *P. pinnata*. The metabolites, β–sitosteryl acetate and galactoside, stigmasterol, its galactoside, and sucrose were reported for the first time from this plant. Saturated and unsaturated fatty acids (two monoenoic, one dienoic, and two trienoic) were present in exactly the same amount. Oleic acid occurred in the highest amount (44.24%); stearic (29.64%) and palmitic (18.58%) acids were the next in quantity. Hiragonic and octadecatrienoic acids were present in trace amounts (0.88%). Karanjin, pongamol, pongaglabrone, and pongapin, pinnatin, and kanjone have been isolated and characterized from the seeds. Immature seeds contain the flavone derivative 'pongol.' The other flavonoid isolated from the seeds included 'glabrachalcone isopongachromene' (Shameel et al., 1996).

The flowers yielded simple flavones, hydroxyfuranoflavones, furanoflavones, a chromenoflavone, triterpenes, beta sitosterol glucosides, and aurantiamide acetate (Khare, 2004). The roots and leaves give kanugin, desmethoxykanugin, and pinnatin. The roots yielded a flavonol methyl ether-tetra-*O*-methyl fisetin. The root bark of *P. pinnata* afforded a new chalcone (karanjapin) and two known flavonoids, a pyranoflavonoid (karanjachromene) and a furanoflavonoid (karanjin) (Ghosh et al., 2009). The leaves contain triterpenoids, glabrachromenes I and II, 3′-methoxypongapin, and 4′-methoxyfurano[2″,3″:7,8]-flavone. The gum was reported to yield polysaccharides (Joy et al., 1998).

Chemical Structures of Some Phytoconstituents of *Pongamnia pinnata*
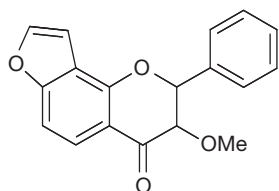
Karangin
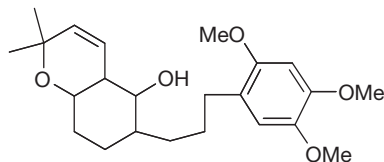
Glabrachalcone
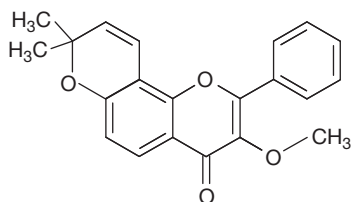
Karanjachromene
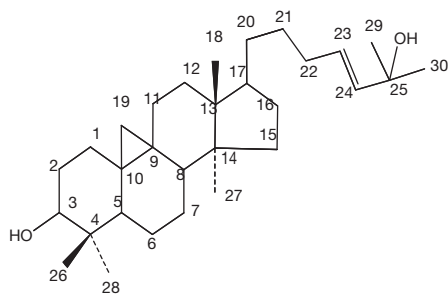
Cycloart-23-ene-3β, 25-diol

3. BENEFICIAL EFFECTS OF *PONGAMNIA PINNATA* ON HEALTH

3.1 Traditional Uses

The fruits, barks, seeds, seed oil, leaves, flowers, and roots have been used for medicinal purposes in the Ayurvedic and Unani systems.

During the sixteenth century, the fruits of karanja, kimsuka (*Butea monosperma*), and arishta (*Sapindus trifoliatus*) were used for parasitic infections, urinary diseases, and diabetes. The fruits and sprouts are used in folk remedies for abdominal tumors in India, the seeds for keloid tumors in Sri Lanka, and powder derived from the plant for tumors, in Vietnam (Buccolo and David, 1973).

Ayurvedic medicine described the bark as anthelmintic and useful in abdominal enlargement; ascites; biliousness; diseases of the eye, skin, and vagina; itch; piles; splenomegaly; tumors; ulcers; and wounds. The bark is used internally for bleeding piles and beriberi.

In the Unani system, seed ash is used to strengthen the teeth. Seeds are carminative and depurative and are used for chest complaints, chronic fevers, earache, hydrocele, and lumbago. In India, the seeds are used for skin ailments; keratitis; piles; urinary discharges; and diseases of the brain, eye, head, and skin. The juice from the plant as well as the oil is

antiseptic. It is an excellent remedy for itch and herpes. The seeds are hematinic, bitter, and acrid. Today, the oil is used as a liniment for rheumatism. The seeds and seed oil used for their carminative, antiseptic, anthelmintic and antirheumatic effects and are beneficial for biliousness, eye ailments, itch, leukoderma, rheumatism, skin disease, worms, and wounds. The powdered seeds are used as a febrifuge and tonic and in bronchitis and whopping cough. The seed oil is styptic and depurative. Karanjin is the principle responsible for the curative properties of the oil. The oil of *P. pinnata* showed antibacterial activity against *Micrococcus pyogenes* var. aureus, *M. pyogenes* var. citreus, *Bacillus subtilis*, *Corynebacterium diphtheria*, *Salmonella typhosa*, *S. paratyphi* A, *S. paratyphi* B, and *Escherichia coli*, being most active against *S. paratyphi* A. The oil is inactive against *Proteus vulgaris* and *Pseudomonas pyocyanea*. It is used as a lubricant, water paint binder, and pesticide and in the soap making and tanning industries. The oil is known to have value in folk medicine for the treatment of rheumatism as well as human and animal skin diseases. It is effective in enhancing the pigmentation of skin affected by leukoderma or scabies.

The leaves are used for their anthelmintic, digestive, and laxative effects and for inflammations, piles, and wounds. They are active against *Macrococcus*; their juice is used for cold, coughs, diarrhea, dyspepsia, flatulence, gonorrhea, and leprosy. The leaves have digestive, laxative, antidiarrheal, antigonorrheic, and antileprotic properties.

The flowers are used for diabetes and biliousness. The traditional practitioners of the Indian systems of medicine Ayurveda and Siddha boil the flowers of the plant in water, cool the water, and administer the decoction including marc for the treatment of diabetes.

Karanja root is an ingredient in Dhanvantaram Ghritam, available in the South, prescribed for diabetes and rheumatic disease. Roots are used for cleaning gums, teeth, and ulcers. Juice of the root is used for cleansing foul ulcers and closing fistulous sores. Young shoots have been recommended for rheumatism (Krishnamurthi, 1998).

3.2 Pharmacological Study

3.2.1 Antidiabetic, antihyperglycemic, and anti-lipidperoxidative activity

Recently, Badole and Bodhankar (2008, 2009a) reported the antihyperglycemic activity of petroleum and alcoholic extract of the stem bark of *P. pinnata* (L.) in alloxan–induced diabetic mice. They have also reported the concomitant administration of petroleum ether extract of the stem bark of *P. pinnata* (L.) Pierre with synthetic oral hypoglycemic drugs in alloxan–induced diabetic mice (Badole and Bodhankar, 2009b). Cycloart–23-ene-3β, 25-diol (B2) isolated from the stem bark of *P. pinnata* possesses antihyperglycemic activity in alloxan–induced diabetic mice (Badole and Bodhankar, 2009c). Later, they confirmed the antidiabetic activity of cycloart–23-ene-3β, 25-diol on specific streptozotocin–nicotinamide-induced diabetic mice models.

Cycloart–23–ene–3β, 25–diol (B2) is a promising antidiabetic compound. It also effectively improves the abnormalities of diabetic conditions in streptozotocin–nicotinamide–induced diabetic mice. The probable mechanism of action is increased serum and pancreatic insulin as well as decreased oxidative stress (Badole and Bodhankar, 2010).

Punitha et al. (2006) and Punitha and Manoharan (2006) reported the antihyperglycemic and anti-lipidperoxidative activities of aqueous as well as ethanolic extracts of *P. pinnata* flowers. Karanjin has been found to display hypoglycemic activity in normal and in alloxan–induced diabetic rats. Pongamol and karanjin isolated from fruits of *P. pinnata* were reported to have antihyperglycemic activity (Ahmad et al., 2006; Tamrakar et al., 2008).

3.2.2 Antimicrobial activity

The antimicrobial activity of three compounds, namely, 3–methoxy–(3,4–dihydro–3, 4–diacetoxy)–2,2–dimethylpyrano–(7,8:5,6)–flavon; 8,4–dimethoxy–7–*O*–dimethylallyliso flavone; and 3,4–methylenedioxy–10–methoxy–7–oxo[2] benzopyrano[4,3–b] benzopyran, has been reported by Koysomboon et al. (2006). Flavonoid lanceolatin B has been reported by Alam (2004) to have antimicrobial action and alkaloids by Krishna and Grampurohit (2006).

3.2.3 Antifungal and antibacterial activity

Petroleum ether, chloroform, ethyl acetate, and methanol extracts of the leaves of *P. pinnata* Linn. were reported to have antibacterial activity using disk diffusion methods against certain enteric bacterial pathogens such as *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *B. subtilis*, *Enterobacter aerogenes*, *P. aeruginosa*, *S. typhimurium*, *S. typhi*, *St. epidermidis*, and *P. vulgaris*. The methanolic extract had wide range of antibacterial activity on these bacterial pathogens than the petroleum ether extract. Ethyl acetate extract showed slightly higher antibacterial activity against bacterial pathogens than chloroform extract (Arote et al., 2009). Pongarotene and karanjin showed antifungal and antibacterial activity (Simin et al., 2002).

3.2.4 Antiviral activity

Antimicrobial activity of the ethanolic extract of the leaves of *P. pinnata* against selected bacteria (*Vibrio* sp., *Pseudomonas* sp., and *Streptococcus* sp.) and the virus White Spot Syndrome Virus (WSSV) was reported. The isolated compound bis(2-methylheptyl)phthalate from the leaves of the plant *P. pinnata* has been shown to exhibit antiviral activity against the WSSV (Rameshthangam and Ramasamy, 2007). Elanchezhiyan et al. (1993) reported the antiviral effect of an extract of *P. pinnata* seeds on herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) in Vero cells. A crude aqueous seed extract of *P. pinnata* completely inhibited the growth of HSV-1 and HSV-2 at concentrations

of 1 and 20 mg ml^{-1} (w/v), respectively, as shown by the complete absence of cytopathic effects.

3.2.5 In vitro *screening of antilice activity*

Samuel et al. (2009) reported *P. pinnata* leaf extracts as potent antilice agents. Extracts of *P. pinnata* succeeded in delaying the emergence of nymphs, and its oily nature may help to detach nits from the hair before hatching. Petroleum ether exhibited the maximum pediculicidal effects and completely inhibited nymph emergence at two different concentrations (10 and 20%). *P. pinnata* leaf extract (PPET) is an effective alternative for treating human head lice.

3.2.6 Antifilarial activity

Fruits and leaves extract of *P. pinnata* showed antifilarial potential on cattle filarial parasite *Setaria cervi* (Uddin et al., 2003).

3.2.7 Anti-inflammatory and analgesic activity

The ethanol extract of *P. glabra* Vent. leaf gall was investigated for anti-inflammatory and analgesic activity at the doses of 100, 200, and 400 mg kg^{-1} body weight (Ganesh et al., 2008), while different extracts of the roots and seeds (ethanol, petroleum ether, benzene extracts, and others) of *P. pinnata* have been reported to have anti-inflammatory activity (Singh and Pandey, 1996; Singh et al., 1996). Anti-inflammatory activity of 70% ethanolic extract of *P. pinnata* leaves in acute, subacute, and chronic models of inflammation was assessed in rats (Srinivasan et al., 2001). *P. pinnata* leaves reported antinociceptive and antipyretic activity (Srinivasan et al., 1993). Pongapinone A was shown to inhibit interleukin-1 production (Isao et al., 1992).

3.2.8 Antioxidant and antihyperammonemic activity

PPET was investigated for circulatory lipid peroxidation and antioxidant status in ammonium chloride-induced hyperammonium rats. It not only enhanced lipid peroxidation in the circulation of ammonium chloride-treated rats but also significantly decreased the levels of vitamin A, vitamin C, and vitamin E and reduced glutathione, glutathione peroxidase, superoxidase dismutase, and catalase. These findings showed that PPET modulates these changes by reversing the oxidant–antioxidant imbalance during ammonium chloride-induced hyperammonemia, and this could be due to its antihyperammonemic effect by detoxifying excess ammonia, urea, and creatinine and its antioxidant property (Essa and Subramanian, 2006). Karanjapin and karanjachromene were found to possess significant antioxidant activity (Ghosh et al., 2009).

3.2.9 Antiplasmodial activity

P. pinnata showed antiplasmodial activity against *Plasmodium falciparum* (Simonesen et al., 2001).

3.2.10 Antidiarrheal activity

It has been reported that the decoction of *P. pinnata* has selective antidiarrheal action with efficacy against cholera and enteroinvasive bacterial strains causing bloody diarrheal episodes (Brijesh et al., 2006).

3.2.11 Antiulcer activity

Methanolic extract of *P. pinnata* Linn. seed (PPSM) showed dose-dependent (12.5–50 mg kg^{-1}, p.o. for 5 days) ulcer-protective effects against gastric ulcer induced by 2 h cold resistance stress. PPSM (251 mg kg^{-1}) showed antiulcerogenic activity against acute gastric ulcer induced by pylorus ligation and aspirin and duodenal ulcer induced by cystamine but not ethanol–induced gastric ulcer (Prabha et al., 2009). It has been reported that methanolic extract of *P. pinnata* roots (PPRM) provided significant protection against aspirin but not against ethanol–induced ulceration. It showed a tendency to decrease acetic acid–induced ulcer after a 10-day treatment. The ulcer-protective effects of PPRM were due to augmentation of mucosal defensive factors such as mucin secretion, life span of mucosal cells, mucosal cell glycoproteins, cell proliferation, and prevention of lipid per oxidation rather than the offensive acid–pepsin secretion (Prabha et al., 2003).

3.2.12 Antidyslipidemic activity

Bhatia et al. (2008) evaluated the antidyslipidemic activity of different solvent fractions of *P. pinnata* fruits in a triton and high–fat–diet–fed hamster model. The chloroform fraction (C) of *P. pinnata* exerted lipid-lowering activity *in vivo*. This suggests that extracts of *P. pinnata*, as well as other derivatives that undergo biotransformation through the hepatic drug-metabolizing cascade, produce common active molecules that may be responsible for lipid–lowering activity *in vivo*.

3.2.13 Central nervous system activity

Ethanolic extract of *P. pinnata* leaves showed significant anticonvulsant activity comparable to the reference drug diazepam, and such activity was accompanied with reduction in locomotion and increase in brain GABA concentration (Logade et al., 2009). Ethanolic root extract of *P. pinnata* provides a protective role in ischemia–reperfusion injury and cerebrovascular insufficiency state (Raghavendra et al., 2007). Anticonvulsant activity (Basu et al., 1994), central nervous system (CNS)-depressant activity, and increased sensitivity to sound and touch (Mahli et al., 1989) by pongamol were reported.

3.2.14 Toxicological studies

The results of subacute toxicity studies have shown no abnormalities on body weight, hematological and biochemical parameters of blood, and on histopathological slides. The biological effect of pongamol and its present toxicological studies suggest that pon–gamol can be safely subjected to chronic toxicological studies and clinical trial (Baki et al., 2007).

4. SUMMARY POINTS

- *P. pinnata* (L.) Pierre (Fabaceae) is popularly known as Indian beech.
- All parts of the plant are traditionally used for treatment of diabetes mellitus.
- In the Ayurvedic and Unani systems, the plant has been used for various ailments.
- *P. pinnata* possessed antidiabetic, antihyperglycemic, anti-lipidperoxidative, anti-microbial, antifungal, antibacterial activity, as well asantiviral, antilice, antifilarial, anti-inflammatory, analgesic, antioxidant, antihyperammonemic, antiplasmodial, antidiarrheal, antiulcer, antidyslipidemic, and CNS-depressant activity.

REFERENCES

Ahmad, G., Yadav, P.P., Maurya, R., 2004. Furanoflavonoid glycosides from *Pongamia pinnata* fruits. Phytochemistry 65, 921–924.

Ahmad, G., Mishra, P.K., Gupta, P., et al., 2006. Synthesis of novel benzofuran isoxazolines as protein tyrosine phosphatase 1B inhibitors. Bioorganic & Medicinal Chemistry Letters 16, 2139–2141.

Alam, S., 2004. Synthesis and studies of antimicrobial activity of lanceolatin B. Acta Chimica Slovenica 51, 447–452.

Arote, S.R., Dahikar, S.B., Yeole, P.G., 2009. Phytochemical screening and antibacterial properties of leaves of *Pongamia pinnata* Linn. (Fabaceae) from India. African Journal of Biotechnology 8, 6393–6396.

Asalkar, L.V., Kakkar, K.K., Chakre, O.J., 2000. *Second Supplement to Glossary of Indian Medicinal Plants with Active Principles*. Part I (A–K) (1965–81). National Institute of Science Communication Publishing, New Delhi pp. 265–266.

Badole, S.L., Bodhankar, S.L., 2008. Investigation of antihyperglycaemic activity of alcoholic extract of stem bark of *Pongamia pinnata* on serum glucose in diabetic mice. Pharmaceutical Biology 46, 900–905.

Badole, S.L., Bodhankar, S.L., 2009a. Investigation of antihyperglycaemic activity of aqueous and petroleum ether extract of stem bark of *Pongamia pinnata* on serum glucose in diabetic mice. Journal of Ethnopharmacology 123, 115–120.

Badole, S.L., Bodhankar, S.L., 2009b. Concomitant administration of petroleum ether extract of stem bark of *Pongamia pinnata* (L.) Pierre with synthetic oral hypoglycaemic drugs in alloxan induced diabetic mice. European Journal of Integrative Medicine 1, 73–79.

Badole, S.L., Bodhankar, S.L., 2009c. Antihyperglycaemic activity of cycloart-23-ene-3β, 25-diol isolated from stem bark of *Pongamia pinnata* in alloxan induced diabetic mice. Research Journal of Phytochemistry 18–24 10.3923/rjphyto.2009.18.24.

Badole, S.L., Bodhankar, S.L., 2010. Antidiabetic activity of cycloart-23-ene-3β, 25-diol (B2) isolated from stem bark of *Pongamia pinnata* (L. Pierre) in streptozotocin-nicotinamide induced diabetic mice. European Journal of Pharmacology 632, 103–109.

Baki, M.A., Khan, A., Al-Bari, M.A.A., Mosaddik, A., Sadik, G., Mondal, K.A.M.S.H., 2007. Sub-acute toxicological studies of pongamol isolated from *Pongamia pinnata*. Research Journal of Medicine and Medical Sciences 2, 53–57.

Basu, S.P., Mandal, J.K., Mehdi, N.S., 1994. Anticonvulsant effect of pongamol. Indian Journal of Pharmaceutical Sciences 56, 163–167.

Bhatia, G., Puri, A., Maurya, R., et al., 2008. Medicinal Chemistry Research 17, 281–289.

Brijesh, S., Daswani, P.G., Tetali, P., 2006. Studies on *Pongamia pinnata* (L.) Pierre leaves: understanding the mechanism(s) action in infectious diarrhea. Journal of Zhejiang University Science B 7, 665–675.

Buccolo, G., David, H., 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry 19, 476–480.

Carcache-Blanco, E.J., Kang, Y.H., Park, E.J., et al., 2003. Constituents of the stem bark of *Pongamia pinnata* with the potential to induce quinone reductase. Journal of Natural Products 66, 1197–1202.

Elanchezhiyan, M., Rajarajan, S., Rajendran, P., Subramanian, S., Thyagarajan, S.P., 1993. Antiviral properties of the seed extract of an Indian medicinal plant, Pongarnia pinnata, Linn. against herpes simplex viruses: *in-vitro* studies on Vero cells. Journal of Medical Microbiology 38, 262–264.

Essa, M.M., Subramanian, P., 2006. *Pongamia pinnata* modulates oxidant and antioxidant imbalance in ammonium chloride induced hyperammonium. Fundamental and Clinical Phamacology 20, 299–303.

Ganesh, M., Vasudevan, M., Kamalakannan, K., et al., 2008. Anti–inflammatory and analgesic effects of *Pongamia glabra* leaf gall extract. Pharmacologyonline 1, 497–512.

Ghosh, A., Mandal, S., Banerji, A., Kar, M., Banerji, J., 2009. A new chalcone from *Pongamia pinnata* and its antioxidant properties. Natural Product Communications 4, 209–210.

Ghufran, A., Prem, P., Maurya, R., 2004. Furanoflavonoid glycosides from *Pongamia pinnata* fruits. Phytochemistry 65, 921–924.

Isao, K., Ru-song, Z., Kazuyuki, H., Katsutoshi, T., Hirotaka, S., 1992. Indonesian medicinal plants II: chemical structures of pongapinones A and B, two new phenylpropanoids from the bark of *Pongamia pinnata* (papillionaceae). Chemical and Pharmaceutical Bulletin 40, 2041–2043.

Joy, P.P., Thomos, J., Mathew, S., Skaria, B.P., 1998. Medicinal Plants. Kerala Agriculture University, Aromatic and Medicinal Plant Research Station, Kerala, India pp. 265–266.

Khare, C.P., 2004. Encyclopedia of Indian Medicinal Plants. Springer-Verlag Editor, New York pp. 378–379.

Koysomboon, S., Altena, I.V., Kato, S., Chantrapromma, K., 2006. Antimicrobacterial flavonoids from *Derris indica*. Phytochemistry 67, 1034–1040.

Krishna, V.N., Grampurohit, N.D., 2006. Studies on the alkaloids of stembark of *Pongamia pinnata* Linn. Investigational Drugs 43, 383–387.

Krishnamurthi, A., 1998. The Wealth of India-Raw Materials. vol. 8. Ph-Re.Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi, India pp. 206–211.

Li, L., Li, X., Shi, C., et al., 2006. Pongamone A–E, five flavonoids from the stems of a mangrove plant *Pongamia pinnata*. Phytochemistry 67, 1347–1352.

Logade, N.A., Shrivastava, N.M., Qureshi Naseem, N., 2009. An insight into the possible anticonvulsant activity of ethanolic extract of *Pongamia pinnata* leaves in mice. Saudi Pharmaceutical Journal 17, 85–89.

Mahli, S.S., Basu, S.P., Sinha, K.P., Banerjee, N.C., 1989. Pharmacological effects of karanjin and pongamol [from seed oil of *Pongamia pinnata*]. Indian Journal of Animal Sciences 59, 657–660.

Prabha, T., Dora, M., Priyambada, S., 2003. Evaluation of *Pongamia pinnata* root extract on gastric ulcer and mucosal offensive and defensive factor in rats. Indian Journal of Experimental Biology 41, 304–310.

Prabha, T., Dorababu, M., Goel, S., et al., 2009. Effect of extract of *Pongamia pinnata* Linn on gastro-duodenal ulceration and mucosal offensive and defensive factor in rats. Indian Journal of Experimental Biology 47, 649–659.

Punitha, R., Manoharan, S., 2006. Antihyperglycaemic and antilipidperoxidative effects of *Pongamia pinnata* (Linn.) *Pierre* flowers in alloxan induced diabetic rats. Journal of Ethnopharmacology 105, 39–46.

Punitha, R., Vasudevan, K., Manoharan, S., 2006. Effect of *Pongamia pinnata* flowers on blood glucose and oxidative stress in alloxan induced diabetic rats. Indian Journal of Pharmacology 38, 62–63.

Raghavendra, M., Trigunayat, A., Singh, R.K., Mtra, S., Goel, R.K., Acharya, S.B., 2007. Effect of ethanolic extract of *Pongamia pinnata* (L.) pierre on oxidative stress, behavioural and histopathological alternation induced by cerebral ischemia-reperfusion and hypoperfusion in rats. Indian Journal of Experimental Biology 45, 868–876.

Rameshthangam, P., Ramasamy, P., 2007. Antiviral activity of bis(2-methyl heptyl)phthalate isolated from *Pongamia pinnata* leaves against white spot syndrome virus of *Penaeus monodon* Fabricius. Virus Res 126 (1–2), 38–44. http://dx.doi.org/10.1016/j.virusres.2007.01.014.

Rashid, N., Abbasi, M.S.A., Tahir, M.K., Yusof, N.M., Yamin, B.M., 2008. Isolation and crystal structure of karanjachromene. Analytical Sciences 24, 21–22.

Samuel, A.J.S.J., Radhamani, S., Gopinath, R., Kalusalingam, A., Vimala, A.G.K.A., Husain, H.A., 2009. *In vitro* screening of anti-lice activity of *Pongamia pinnata* leaves. The Korean Journal of Parasitology 47, 377–380.

Shameel, S., Usmaghani, K., Ali, M.S., 1996. Chemical constituents from seeds of *Pongamia pinnata* (L.) Pierre. Pakistan Journal of Pharmaceutical Sciences 9, 11–20.

Simin, K., Ali, Z., Khaliq-Uz-Zaman, S.M., Ahmad, V.U., 2002. Structure and biological activity of a new rotenoid from *Pongamia pinnata*. Natural Product Letters 16, 351–357.

Simonesen, H.T., Nordksjold, J.B., Smitt, U.W., 2001. *In vitro* screening for Indian medicinal plants for antispamodial activity. Journal of Ethnopharmaocology 74, 195–204.

Singh, R.K., Pandey, B.L., 1996. Anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rats. Indian Journal of Physiology and Pharmacology 40, 355–358.

Singh, R.K., Joshi, V.K., Goel, R.K., Acharya, S.B., 1996. Pharmacological actions of *Pongamia pinnata* seeds—a preliminary report. Indian Journal of Experimental Biology 34, 1204–1207.

Srinivasan, K., Muruganadhan, S., Lal, J., Chandra, S., Tandon, S.K., Raviprakash, V., 1993. Antinociceptive and antipyretic activities of *Pongamia pinnata* leaves. Journal of Medical Microbiology 38, 262–264.

Srinivasan, K., Muruganadhan, S., Lal, J., et al., 2001. Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. Journal of Ethanopharamacology 78, 151–157.

Talapatra, S.K., Malik, A.K., Talapatra, B., 1982. Isopongaglabol and 6-methoxyisopongaglabol, two new hydroxyfuranoflavones from *Pongamia glabra*. Phytochemistry 21, 761–766.

Tamrakar, A.K., Yadav, P.P., Tiwari, P., Maurya, R., Srivastava, A.K., 2008. Identification of pongamol and karanjin as lead compounds with antihyperglycemic activity from *Pongamia pinnata* fruits. Journal of Ethnopharmacology 118, 435–439.

Tanaka, T., Iinuma, M., Fujii, Y., Yuki, K., Mizuno, M., 1992. Flavonoids in root bark of *Pongamia pinnata*. Phytochemistry 31, 993–998.

Uddin, Q., Parveen, N., Khan, N.U., Singhal, K.C., 2003. Antifilarial potential of the fruits and leaves extract of *Pongamia pinnata* on cattle filarial parasite *Setaria cervi*. Phytotherapy Research 17, 1104–1107.

Yin, H., Zang, S., Wu, J., 2004. Study of flavonoids stem bark of *Pongamia pinnata*. Zong Yao Cai 27, 493–495.

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Nutrition, Aging, and Sirtuin 1

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ABBREVIATIONS

ABCA1 ATP-binding cassette transporter A1
AD Alzheimer's disease
ALS Amyotrophic lateral sclerosis
ATP Adenosine triphosphate
BAT Brown adipose tissue
C/EBPα CAAT/enhancer binding protein α
CPT-1a Carnitine palmitoyltransferase 1a
CVD Cardiovascular diseases
CYP7A1 Cytochrome P450 subfamily 7A polypeptide 1
DBC-1 Deleted in breast cancer 1
eNOS Endothelial nitric oxide synthase
FOXO1 Fork head boxO1
G6Pase Glucose-6-phosphatase
HDL High-density lipoprotein
HIC-1 Hypermethylated in Cancer 1
LDL Low-density lipoprotein
LXR Liver X receptor
MCAD Medium chain acyl CoA dehydrogenase
NAM Nicotinamide
NCoR Nuclear receptor corepressor
NSC Neural stem cell
PEPCK Phosphoenolpyruvate carboxykinase
PGC1α Peroxisome-proliferators-activated receptor γ coactivator 1α
POMC Pro-opiomelanocortin
PPAR γ Peroxisome-proliferators-activated receptor γ
PTP1B Protein tyrosine phosphatase 1B
RSV Resveratrol
RXR β Retinoid acid receptor β
SLE Systemic lupus erythematosus
SMRT Silencing mediator of retinoid and thyroid hormone receptors
SR-B1 Scavenger receptor B1
SREBP Sterol-regulatory-element-binding protein 1 and 2
UCP2 Uncoupling protein 2
WAT White adipose tissue

1. NUTRITION AND AGING

Aging is seen as the functional decline at a systemic level, often associated with a heightened risk for diseases related to memory, cognition, and organ function. Laboratory data combined with population studies suggest that aging is a consequence of both extrinsic and intrinsic factors at work. The extrinsic factors are under our control and include healthy habits such as nutrition and lifestyle, whereas the intrinsic factor, genetics, is less under our control and may play a more significant causative role in aging.

1.1 Diet and Longevity

Population studies indicate dietary connection with longevity. In a recent study sponsored by the US National Institute of Aging, scientists found commonality in the diet and lifestyles of communities around the world that typically live longer than rest of the world. These communities followed a diet pattern that was high on vegetables, fruits, nuts, and whole grains and low on meat/red meat and fat. Their lifestyle involved moderate to hard physical work in their daily activities and promoted a low-stress, relaxed social environment. Interestingly, all these nutritional and social habits demonstrate good correlation with the experimental evidence for cellular processes that promote health and longevity. For example, experimental data suggest that chronic exposure to high levels of fat in the diet causes multisystemic deterioration including impaired metabolic function and systemic inflammation (Huang, 2009), leading to age-associated diseases such as cancer, metabolic, cardiovascular, and neurodegenerative disease. Similarly, a low-stress lifestyle correlates with experimental evidence that indicate prosurvival effects of proteins promoting cellular stress responses.

Another robust evidence for nutritional connection to aging comes from research related to 'Dietary/Calorie Restriction' (DR or CR), a dietary regime that restricts calorie intake while maintaining nutrition. CR promotes healthy lifespan in almost all species tested from yeast to primates. CR not only restores youthful physiological features but also alleviates many age-associated diseases such as diabetes, sarcopenia, osteoporosis, cancer, stroke, cardiovascular disease, and kidney disease. The popular theory explaining the beneficial effects of CR toward longevity is that CR triggers a mild stress-responsive state in an organism, thereby encouraging its resources for damage repair and prevention of diseases, leading to health and longevity (Sinclair, 2005).

Research in the past decade has provided the genetic connection to the longevity effects of diet and environment. Genetic screening in simple organisms has identified single gene mutations that dramatically enhance lifespan. Interestingly, extrinsic factors such as dietary restriction and mild biological stresses could activate these longevity genes, indicating the possibility of externally manipulating the intrinsic factors for enhancing longevity.

1.2 Sirtuins

The *Silent Information Regulator* (*Sir2*) was first discovered as a longevity gene in budding yeast, as it could extend the replicative lifespan by preventing genomic instability (Sinclair and Guarente, 1997). Since then, *Sir2* homologs have been discovered in various species including plants, microbes, worms, flies, and mammals. Collectively called as *Sirtuins*, these genes are evolutionarily conserved and encode a protein deacetylase enzyme that requires nicotinamide adenine dinucleotide (NAD^+) as a cofactor. NAD^+ functions as an enzyme substrate for adding or removing chemical groups from proteins and also as a coenzyme in redox metabolic reactions. The ratio of the oxidized and reduced states of NAD (NAD^+/NADH) reflects the redox state of a cell and controls the activity of several key enzymes and metabolic reactions. There are seven mammalian sirtuins, SIRT1-7. Sirtuins bind NAD^+ through their conserved catalytic domain to catalyze the deacetylation reaction. The reaction involves amide cleavage of the NAD^+, producing the deacetylated protein and two by-products: nicotinamide (NAM) and *O*-acetyl-ADP-ribose. NAM acts as a noncompetitive inhibitor of SIRT1 activity by binding to a pocket adjacent to NAD^+ binding site and reacting with the reaction intermediates to recycle back NAD^+ (Figure 33.1).

SIRT1 is the mammalian homolog closest to the yeast SIR2 protein. Myriad of reports have implicated SIRT1 in regulating a wide range of cellular processes such as nutrient sensing, energy adaptation, oxidative/genotoxic stress response, and regulation of cell growth and differentiation. Because of dependence of SIRT1on NAD^+, a redox metabolite, the role of SIRT1 in CR–mediated longevity has been intensely scrutinized.
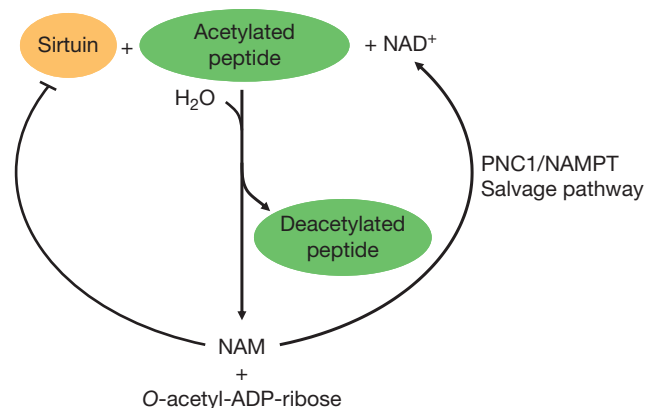
Figure 33.1 Sirtuins bind acetylated proteins and use NAD^+ as a cofactor to catalyze the deacetylation reaction. This involves amide cleavage of NAD^+ and produces deacetylated peptide, nicotinamide (NAM), and *O*-acetyl-ADP-ribose. NAM competitively inhibits this catalytic activity of SIRT1 but can also be used as a precursor for NAD^+ in the salvage pathway, thereby promoting SIRT1 activity.

Interestingly, SIRT1 is upregulated by CR, and the beneficial effects of CR have been shown to depend on SIRT1 in some species such as yeast, flies, and mice. CR was shown to regulate longevity independent of SIRT1 in certain cases, such as in *Caenorhabditis elegans* and under severe CR diet (0.05%, as oppose to 0.5% glucose) in yeast. However, the broader conclusion from studies in yeast, flies, rodents, and mice suggests that many of the beneficial effects of CR on health and lifespan either depend on or overlap with the effects of increased SIRT1 activity (Sinclair, 2005).

2. SIRT1 INTEGRATES METABOLISM AND HEALTHY LIFESPAN

Aging is often associated with the inability to adapt to changes in food intake, storage, and utilization, resulting in homeostatic imbalance and increased risk for metabolic diseases. The proper balance of energy intake and expenditure (energy homeostasis) plays an important role in ensuring correct functioning of organs and well-being at a systemic level. One of the most common conditions associated with aging is *metabolic syndrome*, which consists of increased risk for coronary diseases and type 2 diabetes. In this regard, SIRT1 plays a protective role through its regulation on multiple biochemical and endocrine pathways that are involved in adaptation to nutritional availability.

2.1 SIRT1, A Nutrient Sensor

SIRT1 functions as a nutrient sensor, as its activity is modulated by NAD^+ and its metabolites. NAD^+ is synthesized through two metabolic pathways: (1) *de novo* synthesis from amino acids tryptophan and aspartic acid and (2) *salvage pathway* that uses preformed components from food, such as the vitamin niacin, or other reaction by-products, such as NAM. Interestingly, enzymes driving the salvage pathway for NAD^+ synthesis, such as PNC1 (in yeast and flies) and nicotinamide phosphoribosyltransferase (Nampt, in mammals), are induced by nutrition deprivation/stress conditions. CR and exercise also induce SIRT1, indicating its association with stress-induced survival response. Exercise induces an energy-deprived state by increasing AMP/ATP ratio, which activates AMP activated kinase (AMPK), thereby increasing the $NAD^+/NADH$ ratio through increased mitochondrial oxidation, resulting in activation of SIRT1 (Haigis and Sinclair, 2010). Another redox sensor, the *C*-terminal binding protein (CtBP), has been shown to bind with hypermethylated in cancer (HIC1), thereby activating SIRT1 expression (Ghosh, 2008). The nutrient-sensitive regulation of SIRT1 is also evident by its fine regulation in metabolically active tissues such as liver, muscle, brain, and the white adipose tissue (WAT). SIRT1 expression is repressed by glucose and lactate, whereas the fasting metabolite, pyruvate, activates it. Furthermore, *in vivo* studies suggest that the effect of SIRT1 on critical metabolic processes is more apparent in the fasting state, supporting its function as a responder to nutritional status (Rodgers and Puigserver, 2007). SIRT1 also regulates the expression of neurons and hormones critical to appetite control.

By deacetylating FOXO1, SIRT1 represses AgRP (orexigenic neuron) expression and upregulates POMC (pro-opiomelanocortin) (anorexigenic neurons) genes (Sasaki and Kitamura, 2010). POMC-specific SIRT1 knockout mice show that SIRT1 mediates proper functioning of the appetite controlling hormone leptin through PI3K signaling. Under low food availability, SIRT1 increases neural activity in the hypothalamic nuclei by upregulating orexin type 2 receptor expression, thereby promoting physical activity to help maintain higher body temperature (Ramadori et al., 2010).

The role of SIRT1 as an energy sensor is further asserted by its active participation in energy-dependent cellular processes such as cell growth and autophagy. *In vitro* studies using telomerase-immortalized human cells indicate that SIRT1 suppresses cell growth in response to low nutrient conditions (Narala et al., 2008). Consistently, SIRT1 was also shown to negatively regulate *mammalian Target of Rapamycin* (mTOR) signaling, critical to nutrient/stress-sensitive cell growth (Ghosh et al., 2010). Under starvation, nutrients derived from degradation of nonvital components are reallocated for ensuring functioning of essential processes and survival through the catabolic process of *autophagy*. Recently, SIRT1 was shown to be required for autophagy through deacetylation of essential autophagy proteins ATG5/7/8 (Lee et al., 2008). The autophagy-mutant mice show marked resemblance with SIRT1 $-/-$ mice, such as imbalance in energy homeostasis, accumulation of damaged organelles, and early mortality.

2.2 SIRT1 Regulates Energy Homeostasis

2.2.1 Glucose metabolism

Glucose is the most important carbohydrate whose blood levels are tightly controlled by the central metabolic hormone, *insulin*. Genetic screens for longevity genes have identified the insulin/IGF-1 pathway to be an evolutionarily conserved player in lifespan extension in a variety of organisms. Glucose is broken down to produce the cellular energy currency ATP through *glycolysis*. Under low blood glucose, hormonal adaptation promotes *gluconeogenesis*, the *de novo* synthesis of glucose molecules from simple organic compounds such as amino acids.

SIRT1 facilitates different processes in a tissue-specific manner to help adapt to nutritional deprivation. Under fasting conditions, SIRT1 promotes gluconeogenesis in the liver by deacetylating transcription factors FOXO1 and PGC1α (peroxisome-proliferators-activated receptor γ coactivator 1α) and activating gluconeogenic genes PEPCK (phosphoenolpyruvate carboxykinase) and G6Pase. Knocking out SIRT1 in liver results in impaired response to fasting and defective hepatic glucose production, which could be reversed by SIRT1 overexpression. In skeletal muscles, however, SIRT1 activates PGC1α and upregulates fatty acid oxidation genes such as MCAD and CPT-1a (carnitine palmitoyltransferase 1a) in order to shift oxidation from glucose to fatty acids and preserve glucose under fasting conditions (Rodgers and Puigserver, 2007). In a separate study, fasting conditions over CR diet showed a decline in SIRT1 activity in the

liver without affecting PGC1α activity, suggesting that subtle differences in diet can influence SIRT1 activity.

SIRT1 also controls insulin secretion and signaling through regulation of UCP2 (uncoupling protein 2) and PTP1B (protein tyrosine phosphatase 1B), respectively. UCP2 uncouples mitochondrial respiration from ATP production by allowing proton leakage, thereby rendering pancreatic β-cells unresponsive to glucose levels. SIRT1 directly binds to UCP2 promotor and represses its transcription, thereby blocking UCP2 action and promoting insulin secretion. PTP1B is key insulin receptor phosphatase that functions as a negative regulator of insulin signaling. Mice lacking PTP1B gene show increased insulin sensitivity and resistance to obesity. Neuron-specific PTP1B knockout studies implicate PTP1B in neural regulation of body weight and adiposity through leptin action. SIRT1 directly represses PTP1B expression through histone (H3) decetylation at its promoter. Notably, this effect was specifically observed under insulin-resistant conditions indicating the context-dependent function of SIRT1 (Ghosh, 2008).

2.2.2 Fat metabolism

Evidence suggests that increased fat accumulation is associated with elevated risk for insulin resistance and cardiovascular diseases. At a molecular level, higher body weight correlates with shortening of telomeres, a feature of cellular aging. Interestingly, the long-lived calorie-restricted animals show minimal fat storage due to increased fat mobilization from their white adipose tissue (WAT) (Guarente, 2005).

Research has established a prominent role for SIRT1 in fat metabolism and WAT remodeling. SIRT1 promotes leanness by decreasing both fat cell (adipocyte) numbers and size. While SIRT1 deletion results in higher weight gain under high-fat diet and exhibits higher lipids after fasting, SIRT1 overexpression under high-fat diet protects from liver steatosis (Pfluger et al., 2008; Purushotham et al., 2009). The steroid hormone receptor PPARγ (peroxisome-proliferators-activated receptor γ) is an insulin-responsive transcriptional regulator in WAT, where it activates genes promoting adipogenesis and fat storage. SIRT1 represses PPARγ by binding its cofactors NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors), thereby inhibiting fat cell synthesis (adipogenesis). SIRT1 also promotes loss of fat by triggering its breakdown (lipolysis) in differentiated fat cells. Consistently, Sirt1$^{\pm}$ mice demonstrate compromised mobilization of fatty acids from WAT on fasting (Picard et al., 2004).

WAT also functions as an endocrine organ by secreting hormones such as *adiponectin,* which promotes insulin sensitivity and fatty acid oxidation while suppressing hepatic gluconeogenesis. Plasma level of adiponectin inversely correlates with adiposity. SIRT1 deacetylates FOXO1 and promotes FOXO1–C/EBPα complex formation, a positive regulator of adiponectin transcription, thereby upregulating adiponectin expression. High-fat-diet-induced obese and diabetic *db/db* mice show significantly lower SIRT1

and FOXO1 protein levels, concomitant with a dramatic reduction in serum adiponectin concentrations and high adiposity (Qiao and Shao, 2006).

SIRT1 has recently been shown to remodel WAT to BAT (brown adipose tissue). Earlier thought to be present only in infants, BAT is now known to be functionally active in adult. BAT dissipates energy through thermogenesis by burning calories from WAT, showing promise as a weight loss mechanism. SIRT1 in the pro–opiomelanocortin (POMC) neurons is required for remodeling perigonadal WAT to BAT. In addition, SIRT1 also increases BAT-specific gene expression by activating the sympathetic nerve activity (SNA) and inducing leptin function in WAT to reduce food intake. Interestingly, in contrast to muscle and WAT, CR diet was shown to reduce SIRT1 activity in liver, thereby repressing the nuclear receptor LXR (a positive regulator for fat synthesis in liver) and reducing fat synthesis (Ramadori et al., 2010).

2.2.3 Cholesterol management

Age-associated defect in cholesterol metabolism causing imbalance in 'good' versus 'bad' cholesterol is a leading cause of atherosclerotic diseases in the elderly. Cholesterol is transported in blood circulation packed within lipoprotein of varying density; the more cholesterol and less protein a lipoprotein has, the less dense it is. Low-density lipoproteins (LDL) are the major carriers of cholesterol in the blood. SREBP (sterol–regulatory-element-binding protein 1 and 2) genes regulate LDL receptor and cholesterol synthesis by sensing the levels of cholesterol already present. Under adequate cholesterol condition, LDL receptor synthesis is blocked to inhibit uptake of new LDL in the cell. Conversely, when cells are cholesterol deficient, more LDL uptake is promoted through upregulation of LDL receptor synthesis. Disruption in this balance leads to increased LDL (bad cholesterol) molecules in the blood, contributing to artherosclerotic plaque formation, the main cause of heart attacks and strokes. The high-density lipoproteins (HDL), on the other hand, also known as 'good cholesterol,' transport cholesterol back from peripheral tissues to the liver through reverse cholesterol transport (RCT) for its subsequent elimination via bile or hormone synthesis.

Studies suggest that SIRT1 plays a role in cholesterol management by regulating expression of genes critical for cholesterol sensing, synthesis, and transport. *In vivo* studies using SIRT1 knockout mice show impaired cholesterol and triglyceride homeostasis due to decreased HDL levels and RCT. The nuclear receptor proteins LXR α and β function as sterol sensors to regulate cholesterol and lipid homeostasis by inducing expression of genes involved in RCT and fat metabolism. SIRT1 activates LXR through deacetylation (Li et al., 2007). Loss of SIRT1 reduces expression of LXR targets such as the ABCA1 (ATP-binding cassette transporter A1), critical for HDL biogenesis. SIRT1 also induces genes involved in cholesterol export and degradation, such as SR–B1 (scavenger receptor B1) and bile acid synthesis enzyme CYP7A1 (cytochrome P450 subfamily 7A polypeptide 1), while inhibiting the expression of the LDL receptor (Rodgers and Puigserver,

2007). Consistently, SIRT1 knockout mice showed an increased accumulation of hepatic cholesterol, reversed by SIRT1 overexpression. Interestingly, CR, which increases SIRT1 expression in a variety of tissues, also improves cholesterol homeostasis and reduces atherosclerosis risk in humans (Fontana et al., 2004). Using liver-specific SIRT1 knockdown, it was further shown that SIRT1 has a differential impact on LXR targets depending on the type of diet. Under high-fat diet, when LXR is considered to be maximally active, SIRT1 depletion results in inefficient activation of LXR target genes ABCA1, SREBP1c, and FAS (fatty acid synthesis). Conversely, under restricted calorie diet, the already low expression of these genes remains unaltered by SIRT1 depletion (Picard et al., 2004).

3. SIRT1 AND DISEASES OF AGING

3.1 SIRT1 in Cancer

Expression levels of SIRT1 in various types of tumors project a complex correlation between SIRT1 and tumorigenesis. A number of independent studies analyzing primary tumors and cell lines show increased SIRT1 expression in colon cancer, breast and prostrate cancer, nonmelanoma skin cancer, and leukemia (AML). However, analysis of publicly available database on human tumor specimens showed decreased SIRT1 levels in prostrate, bladder, ovarian, hepatic, and BRCA1 breast cancer and glioblastoma. The modulation of SIRT1 levels in cancer could be either the cause or an effect of transformation. Since SIRT1 has been shown to affect both pro- and antisurvival processes in a context- and tissue-dependent manner, it is important to scrutinize the biology influenced by SIRT1 in tumorigenesis.

3.1.1 SIRT1, p53, and other tumor suppressors

p53 is a master tumor suppressor gene mutated in nearly all types of cancers. *p53* is activated by DNA damaging agents, radiation, and oxidative stress and functions to maintain genomic sanctity. In response to DNA damage, p53 either halts cell cycle to enable DNA repair or initiates apoptosis if cells are irreparable. The activity of p53 is regulated through phosphorylation and acetylation at specific residues. SIRT1 deacetylates the lysine 382 residue of p53, thereby reducing its activity and allowing cells to bypass p53-mediated apoptosis (Vaziri et al., 2001). It is due to this reason that SIRT1 was first suspected to have a cancer-promoting role. Subsequent *in vitro* studies showed inhibition of several other tumor suppressor genes by SIRT1 such as *Rb, p73, WRN, NBS1,* and *MLH1.* Furthermore, two tumor suppressors, HIC-1 (hypermethylated in cancer 1) and DBC-1 (deleted in breast cancer 1), have been shown to repress SIRT1 expression and activity (Deng, 2009).

Recent *in vivo* studies, using genetically engineered mice, however, do not support the *in vitro* results for the role of SIRT1 in cancer. Analysis of embryonic tissues from

SIRT1$^{-/-}$ mice showed no impact on p53 downstream genes. Furthermore, SIRT1$^{-/-}$ MEFs showed increased proliferative capacity in response to chronic sublethal doses of oxidative stress despite having hyperacetylated p53, indicating that inhibition of SIRT1 on p53 does not necessarily inhibit biological functions of p53 (Kamel et al., 2006). Since SIRT1 inactivated p53 in cell culture experiments, it was expected that SIRT1 depletion in p53 heterozygous (p53$^{\pm}$) tumor model will reduce tumor incidence. However, SIRT1 depletion in p53$^{\pm}$ mice accelerated tumorigenesis, showing increased frequency and tumor types with increasing age. Analysis of these primary tumors showed increased chromosomal aberration and aneuploidy, indicating increase in genomic instability as a result of SIRT1 depletion (Deng, 2009). These results were further supported by data from multiple mouse cancer models overexpressing SIRT1, which showed a tumor reduction after SIRT1 overexpression. Furthermore, in the p53$^{\pm}$ tumor model, overexpression of SIRT1 in the thymocytes actually increased survival following γ-irradiation and reduced the occurrence of fatal thymic lymphoma. Thus, contrary to the cell culture results, SIRT1 overexpression rescued the p53 phenotype and increased survival. Consistent with a protective role for SIRT1 against cancer, resveratrol, an activator of SIRT1, was shown to increase survival and reduce thymic lymphoma in p53$^{\pm}$ model. A separate study showed that the protective role of resveratrol in skin cancer models is significantly reduced in the absence of SIRT1, suggesting SIRT1 to be, at least, partly responsible for resveratrol's anticancer role (Haigis and Sinclair, 2010).

The discrepancy between the cell culture results and cancer mouse models can be attributed to a variety of reasons. Both SIRT1 and p53 expression and activity are very finely regulated in a highly tissue- and context-dependent manner. To make things more complex, these two proteins, in turn, regulate each other via complex feedback loops. While being a target substrate for SIRT1, p53 can also repress SIRT1 expression by directly binding SIRT1 promoter elements. Thus, increased levels of SIRT1 in many tumors could be a consequence, rather than a cause, of p53 loss.

3.1.2 SIRT1 inhibits protooncogenes

SIRT1 was recently shown to deactivate two protooncogenes, suggesting its protective role in cancer. c-Myc promotes cell proliferation, growth, and stem cell self-renewal. While c-Myc induces SIRT1 expression, SIRT1 destabilizes c-Myc through deacetylation (Haigis and Sinclair, 2010). Thus, by negatively regulating c-Myc, SIRT1 can prevent cellular transformation.

ApcMin (*Min*, multiple intestinal neoplasia) is a point mutation in the murine homolog of the APC gene. Min/+ mice develop multiple intestinal adenomas, as do humans carrying germline mutations in APC. In the APC min/+ model, loss of the remaining *min* allele leads to localization of β-catenin to the nucleus, upregulating myc and cyclinD1 and promoting adenomas. Consistent with a tumor suppressive role, overexpression of SIRT1 in the APC min/+ model reduced tumor development by reducing

cell proliferation and increasing apoptosis. In support of a protective role for SIRT1 in β–catenin–driven tumors, increased nuclear SIRT1 correlated with decreased oncogenic β–catenin in 81 human colon cancer specimens analyzed. Restoration of SIRT1 in BRCA1 mutant cancer cells inhibited their proliferation *in vitro* and prevented tumor formation when implanted in nude mice, suggesting a tumor suppressive role for SIRT1. It was further shown that BRCA1 activates SIRT1 expression, which then decetylates H3K9 at the promoter and inactivates the antiapoptotic gene Survivin, thereby promoting apoptosis (Deng, 2009).

3.1.3 SIRT1 in DNA repair and genomic stability

Genomic instability is a major cause of cancer. DNA repair mechanisms play an important role in maintaining the sanctity of DNA against a variety of cellular stressors. The role of SIRT1 in cancer has been redefined by recent evidence showing its potential for DNA repair. SIRT1 was shown to physically localize at the DNA break sites and facilitates DNA repair complex MRN (MRE-RAD50-NBS1) formation by recruiting RAD51 and NBS1 through NBS1 deacetylation (Haigis and Sinclair, 2010). Consistently, SIRT1-deficient mice are more prone to DNA–damage–induced aneuploidy and show impaired DNA break repair capacity.

SIRT1 also maintains genomic integrity through gene silencing. SIRT1 represses a functionally diverse set of genes that are derepressed under oxidative stress. Interestingly, majority of these normally repressed genes are overexpressed in older compared to younger mice, possibly due to loss of SIRT1 action. SIRT1 also silences major satellite DNA, an effect reduced in aged mice (Oberdoerffer et al., 2008). It was shown that relocalization of SIRT1 in response to DNA damage promotes altered expression of genes similar to that observed in aging, emphasizing the role of SIRT1–mediated maintenance of genomic integrity in aging.

3.2 SIRT1 in Neurodegenerative Disorder

Neurodegeneration leading to cognitive and behavioral decline is the most common age-associated phenomenon. SIRT1 has been shown to play a neuroprotective role in a number of neurodegenerative diseases including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), axonal degeneration and axonopathy, and macular degeneration (MD).

Accumulation of β–amyloid (Aβ) peptides in the brain is a hallmark of Alzheimer's disease (AD). Aβ peptides are produced from the neuronal membrane amyloid precursor protein (APP) through sequential cleavage by two enzymes: the β- and γ-secretases. The formation of these peptides can be avoided if APP is sequentially cleaved by α– and γ-secretases. SIRT1 prevents the accumulation of Aβ by diverting APP cleavage from β–secretase to the α-secretase pathway through the activation of the α-secretase gene ADAM10 by activating the RXR β (retinoid acid receptor β) and inhibiting ROCK1, an upstream negative regulator of the α-secretase pathway (Haigis and Sinclair, 2010).

SIRT1 also protects neurons from apoptosis caused by injury or genetic mutation. SIRT1 is essential for neuroprotection in the Wallerian Degeneration mutant (Wld^s) mice that are resistant to injury-induced death of neurons. By preventing apoptosis through p53 and FOXO3 deacetylation, SIRT1 stops destruction of myelin sheath, glial cells, and neurons in mouse models for AD and ALS (Kim et al., 2007). SIRT1 confers protection from MD, a leading cause for loss of vision with age, by repressing the complement factor H (CFH) gene, a genetic risk factor associated with the disease.

SIRT1 has recently been shown to also play a role in the process of neurogenesis, production of neural cells from the neural stem cell (NSC) through differentiation. Notch-Hes1 signaling plays a critical role in neurogenesis in the developing and adult brain. While inhibition of Notch is important to prevent premature neural differentiation in the developing brain, in the adult brain, it is required to promote neurogenesis and maintain brain function. SIRT1 was shown to translocate transiently to the nucleus and suppresses Hes1 expression, thereby inhibiting Notch signaling and promoting neurogenesis of NSC under differentiating conditions. In a separate study, activation of the α-secretase pathway by SIRT1 was shown to promote Notch-signaling-mediated neurogenesis in the brain of mice. In addition, under oxidative conditions, SIRT1 actually functions to inhibit neurogenesis by repressing the activator gene MASH1 through TLE1 corepressor complex (Haigis and Sinclair, 2010).

3.3 SIRT1 in Cardiovascular Disorder

Cardiovascular diseases (CVD) are a common age-associated cause of morbidity and mortality. The risk factors for CVD include cholesterol deposition in the vascular endothelium, vascular injury, and increased inflammation. Endothelial cells line the inner wall of the vasculature and are critical for new blood vessel formation and control of vascular tone. SIRT1 is highly expressed in the endothelial cells and plays a role in damage repair and cardiac function. SIRT1 upregulates antioxidant enzymes MnSOD and catalase and prevents p53-mediated apoptosis and cellular senescence, thereby protecting cardiomyocytes against oxidative stress. Endothelial nitric oxide synthase (eNOS) plays an atheroprotective role by promoting blood vessel relaxation through nitric oxide (NO) production. SIRT1 activates eNOS and aids in vasodilation. Consistently, in the apolipoprotein-E-deficient mouse model, endothelial-cell-specific SIRT1 overexpression shows vasorelaxation and reduction in atherosclerotic plaques (Potente and Dimmeler, 2008).

Formation of new blood vessels from existing ones (angiogenesis) is critical to damage repair in the cardiovascular system. SIRT1 activates angiogenesis under ischemic stress and postnatal vascular growth. Using both gain-of-function and loss-of-function studies, SIRT1 has been shown to promote angiogenesis by repressing FOXO1. Accumulation of oxidized LDL and cholesterol is common in CVD. SIRT1 plays a protective role by promoting cholesterol transport from the peripheral tissue and inhibiting cholesterol

biosynthesis. Resveratrol is particularly protective in CVD due to its anti-inflammatory and vasoprotective properties. Notably, the effect of RSV is abolished in the absence of SIRT1. Although overexpression studies with 2.5- to 7.5-fold increase in SIRT1 expression is cardioprotective, too much SIRT1 (12.5 fold) was shown to be toxic leading to apoptosis, hypertrophy, and cardiomyopathy (Alcendor et al., 2007). Thus, while targeting SIRT1 for CVD is an attractive goal, further investigation is needed to better understand the dose-specific effect of SIRT1.

3.4 SIRT1 in Autoimmunity

Age is recognized as a risk factor for autoimmune disease, a condition whereby the immune system mounts response against self-proteins resulting in accumulation of autoantibodies and lymphocyte infiltration, causing diseases such as systemic lupus erythematosus (SLE) and type 1 diabetes. A comparison of healthy centenarians and 60- to 70-year-old individuals indicated resistance to autoimmunity in the centenarians, suggesting an inverse relationship between autoimmunity and longevity.

The role of SIRT1 in autoimmunity was first detected in SIRT1-deficient mice that showed signs of SLE and occasional incidence of disease resembling diabetes inspidus-1 in older animals. Subsequently, a role for SIRT1 in maintenance of T cell tolerance, critical in preventing autoimmunity, was elucidated. Peripheral tolerance is an integral part of T cell tolerance and consists of deletion of self-recognizing T cells by apoptosis, T cell anergy (functional unresponsiveness), and suppressive action of T regulatory cells (Tregs). SIRT1 was shown to promote T cell anergy through c-Jun deacetylation and AP-1 inhibition (Zhang et al., 2009). $SIRT1^{-/-}$ mice showed an increased susceptibility for experimental allergic encephalitis (EAE), higher autoantibodies, accumulation of immune complexes, and increased lymphocyte infiltration in organs such as liver, kidney, and the lung. However, in a separate study, SIRT1 deletion was shown to enhance the suppressive function of T-regulatory (Treg) cells by upregulating Foxp3, prolonging allograft survival (Beier et al., 2011). While the previous report studied whole body SIRT1 deletion in an outbred background (an essential condition for survival of SIRT1 null mice), the later study used Treg-specific SIRT1 deletion. Both T cell anergy and Treg suppressive function are important for prevention of autoimmunity, and it is possible that SIRT1 engages in both of these phenomena in a context-dependent manner. While further investigation is required to gain deeper insight into the full spectrum of the role of SIRT1 in autoimmunity, these studies once again point out the complex nature of SIRT1 regulation (Figure 33.2).

4. MODULATING SIRT1 FOR EXTENDING HEALTH SPAN

Participation of SIRT1 in a wide range of processes influencing aging and associated diseases makes it an attractive drug target. SIRT1 overexpression in animal models has
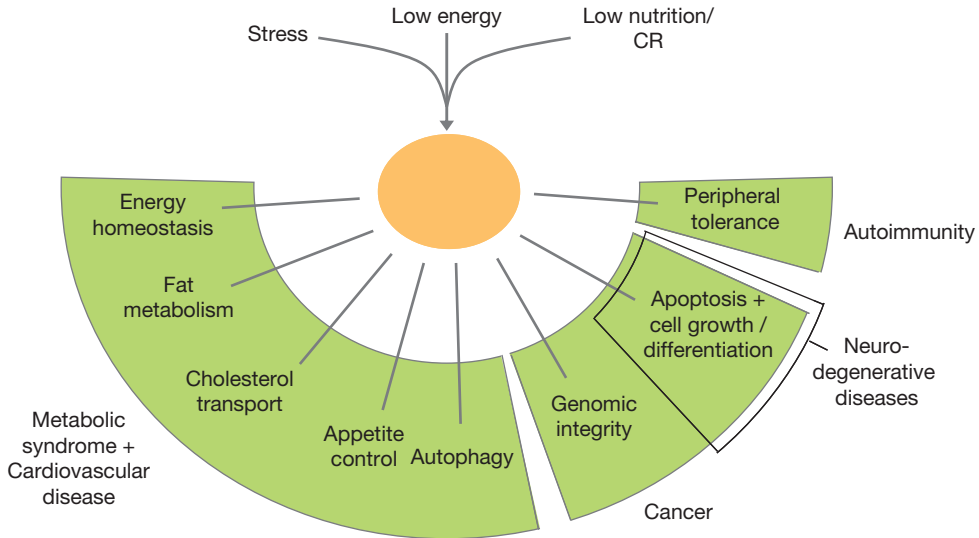
Figure 33.2 SIRT1 is induced by stress conditions caused by CR, low nutrition, oxidative/genotoxic stress, and heat shock. The deacetylase activity of SIRT1 modulates a range of processes, as shown in the figure, influencing diseases of aging such as autoimmunity, neurodegeneration, cancer, CVD, and metabolic syndrome.

shown promising results in the treatment of type 2 diabetes, age- and diet-associated atherosclerotic diseases, AD, ALS and Huntington disease, and health-span promoting CR phenotype. Compared to the rather assertive role for SIRT1 activation in metabolic syndrome and neurodegenerative diseases, its function in cancer seems to be more nuanced and complicated and may require critical assessment of both activators and inhibitors on a case-specific manner.

Several plant molecules have been discovered as SIRT1 activators, with RSV being the most prominent one. RSV is a polyphenol phytolexin found in grape skin, berries, peanut gnetum, jackfruit, and red wine in varying concentrations. Interestingly, RSV is naturally produced in response to injury, infection, and cellular stress and has been shown to mimic the longevity effects of CR. However, studies suggest that the actions of RSV involve activation of other pathways in addition to SIRT1 (Baur and Sinclair, 2006). Compounds in the similar group as RSV, such as tyrosol and hydroxytyrosol, found in white wine, also possess SIRT1-activating properties. Vitamin B3, especially in its 'niacin' form, has been shown to preferentially increase NAD^+ levels and augment SIRT1 activity. Unlike nicotinamide (NAM), which acts both as an inhibitor of SIRT1 and a precursor for SIRT1-activating NAD^+, niacin does not have the SIRT1 inhibitory property. More recently, several chemical activators of SIRT1 that show up to 1000-fold more potency for activating SIRT1 have been identified. These compounds function by

lowering the Michaelis constant for the acetylated substrate, thereby speeding up the deacetylation reaction. Initial results from mouse models for metabolic syndrome show a promising effect of these compounds for treatment of type 2 diabetes (Milne et al., 2007).

The expression and activity of SIRT1 is a highly regulated process. It is influenced by subtle changes in the levels of endogenous modulators, cell/tissue-specific localization, and even the circadian clock. The very fine regulation of SIRT1 may explain the existing conflicts in the SIRT1 literature. Although further investigation is needed to ascertain if the activation of SIRT1 at a constitutive level can be beneficial for increasing health and lifespan, current evidence suggest that tissue-specific targeting of SIRT1 is a feasible strategy for treating specific age-related diseases.

GLOSSARY

Adipocyte Fat cell
Angiogenesis Formation of new blood vessels
Anorexigenic neurons Neurons that inhibit intake of food
Catabolize Breaking down
Gluconeogenesis Synthesize glucose molecules from simple organic compounds
Leptin Hormone that inhibits appetite
Lipolysis Breakdown of fat
Neurogenesis Production of neural cells from the neural stem cell
Orexigenic neuron Neurons that promote food intake
Replicative lifespan Number of times a cell divides to produce daughter cell before dying

REFERENCES

Alcendor, R.R., Gao, S., Zhai, P., et al., 2007. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circulation Research 100, 1512–1521.
Baur, J.A., Sinclair, D.A., 2006. Therapeutic potential of resveratrol: the *in vivo* evidence. Nature Reviews Drug Discovery 5, 493–506.
Beier, U.H., Wang, L., Bhatti, T.R., et al., 2011. Sirtuin-1 targeting promotes Foxp3+ T-regulatory cell function and prolongs allograft survival. Molecular and Cellular Biology 31, 1022–1029.
Deng, C.X., 2009. SIRT1, is it a tumor promoter or tumor suppressor? International Journal of Biological Sciences 5, 147–152.
Fontana, L., Meyer, T.E., Klein, S., Holloszy, J.O., 2004. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proceedings of the National Academy of Sciences of the United States of America 101, 6659–6663.
Ghosh, H.S., 2008. The anti-aging, metabolism potential of SIRT1. Current Opinion in Investigational Drugs 9, 1095–1102.
Ghosh, H.S., McBurney, M., Robbins, P.D., 2010. SIRT1 negatively regulates the mammalian target of rapamycin. PLoS One 5, e9199.
Guarente, L., 2005. Calorie restriction and SIR2 genes–towards a mechanism. Mechanisms of Ageing and Development 126, 923–928.
Haigis, M.C., Sinclair, D.A., 2010. Mammalian sirtuins: biological insights and disease relevance. Annual Review of Pathology 5, 253–295.
Huang, P.L., 2009. A comprehensive definition for metabolic syndrome. Disease Models & Mechanisms 2, 231–237.

Kamel, C., Abrol, M., Jardine, K., He, X., McBurney, M.W., 2006. SirT1 fails to affect p53-mediated biological functions. Aging Cell 5, 81–88.

Kim, D., Nguyen, M.D., Dobbin, M.M., et al., 2007. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. The EMBO Journal 26, 3169–3179.

Lee, I.H., Cao, L., Mostoslavsky, R., et al., 2008. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proceedings of the National Academy of Sciences of the United States of America 105, 3374–3379.

Li, X., Zhang, S., Blander, G., Tse, J.G., Krieger, M., Guarente, L., 2007. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Molecular Cell 28, 91–106.

Milne, J.C., Lambert, P.D., Schenk, S., et al., 2007. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 450, 712–716.

Narala, S.R., Allsopp, R.C., Wells, T.B., et al., 2008. SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity. Molecular Biology of the Cell 19, 1210–1219.

Oberdoerffer, P., Michan, S., McVay, M., et al., 2008. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell 135, 907–918.

Pfluger, P.T., Herranz, D., Velasco-Miguel, S., Serrano, M., Tschop, M.H., 2008. Sirt1 protects against high-fat diet-induced metabolic damage. Proceedings of the National Academy of Sciences of the United States of America 105, 9793–9798.

Picard, F., Kurtev, M., Chung, N., et al., 2004. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 429, 771–776.

Potente, M., Dimmeler, S., 2008. Emerging roles of SIRT1 in vascular endothelial homeostasis. Cell Cycle 7, 2117–2122.

Purushotham, A., Schug, T.T., Xu, Q., Surapureddi, S., Guo, X., Li, X., 2009. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metabolism 9, 327–338.

Qiao, L., Shao, J., 2006. SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. Journal of Biological Chemistry 281, 39915–39924.

Ramadori, G., Fujikawa, T., Fukuda, M., et al., 2010. SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. Cell Metabolism 12, 78–87.

Rodgers, J.T., Puigserver, P., 2007. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proceedings of the National Academy of Sciences of the United States of America 104, 12861–12866.

Sasaki, T., Kitamura, T., 2010. Roles of FoxO1 and Sirt1 in the central regulation of food intake. Endocrine Journal 57, 939–946.

Sinclair, D.A., 2005. Toward a unified theory of caloric restriction and longevity regulation. Mechanisms of Ageing and Development 126, 987–1002.

Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA circles–a cause of aging in yeast. Cell 91, 1033–1042.

Vaziri, H., Dessain, S.K., Ng Eaton, E., et al., 2001. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 107, 149–159.

Zhang, J., Lee, S.M., Shannon, S., et al., 2009. The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. The Journal of Clinical Investigation 119, 3048–3058.

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Inhibitory Effect of Food Compounds on Autoimmune Disease

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Recently, research on autoimmune disease has rapidly advanced. Such disease involves an immune response against the body's own antigens, and organ derangement is caused. It is classified into organ–peculiar autoimmune disease and organ nonspecial autoimmune disease based on the type of autoantibody. The former comprises myasthenia gravis, type 1 diabetes mellitus, chronic thyroiditis, etc., and the latter includes systemic lupus erythematosus (SLE), rheumatoid arthritis, etc. The autoimmune response of organ–peculiar autoimmune disease is antigen-specific. Chronic inflammation occurs in the target, lymphocytes and phagocytes permeate, and, finally, the organization is destroyed. Inflammation develops in various organs/organizations, causing nephritis, arthritis, dermatitis, pleurisy, arthritis, etc. For example, an antibody against a component of the nucleus of the cell appears in SLE. Through this phenomenon, the antigen–antibody complex is deposited throughout the body. Multiple environmental factors in addition to genetic factors are associated with these symptom onsets. There are many reports that sex hormones are concerned with these diseases because the pathology deteriorates in pregnancy and autoimmune disease is common for juvenile woman (Kawamoto, 2011).

It is well known that there is a relation between nutrition and the immune function. Many people die due to infectious disease as a result of malnutrition in developing countries. Also, the caloric intake lowering of old people and cancer patients is caused by a reduction of the immune strength. It was shown that malnutrition is closely related to the lowering of immune competence in many studies (Lombardi et al., 2008). Obesity caused by overnutrition also impairs the immune function (Karlsson and Beck, 2010; Samartin and Chandra, 2001). On this basis, nutrition has emphasized to prevent and treat various immune disorders. In recent experiments, food factors such as peptides (amino acid) or oils have been found to prevent SLE crisis (Fujii, 2007; Venkatraman and Meksawan, 2002). In this chapter, the effect of polyunsaturated fatty acids, whose bioactivity has been documented, on autoimmune disease is introduced.

When the effect of linoleic acid and α-linolenic acid on the autoimmune–prone mouse MRL/lpr was investigated, the serum anti-dsDNA antibody value, which is closely related to the crisis of SLE, of the α-linolenic acid group tended to be lower than

Table 34.1 Change in Serum Anti-dsDNA Antibody Titer of Mice with Different Lipid Administrations

Age (weeks)	Soybean oil group (U mL^{-1})	α-Linolenic acid group (U mL^{-1})	Linoleic acid group (U mL^{-1})
8	22.71 ± 3.25[a]	46.24 ± 4.74[b]	62.41 ± 8.16[c]
12	223.82 ± 28.72[a]	234.98 ± 27.18	285.44 ± 20.84[b]
16	740.97 ± 116.95	542.47 ± 73.90	753.99 ± 105.31
20	1967.59 ± 139.67	1849.93 ± 212.83	1954.19 ± 246.62

Source: Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha-linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.
Each mouse was fed with experimental diets starting from 6 weeks old.
Means ± SEM ($n = 5$) within the same line not sharing the same superscript letter are significantly different at $p < 0.05$ by Scheffe's multiple comparison.

in the other groups (Table 34.1). Also, as shown in Figures 34.1 and 34.2, IL–18 and TNF-α, which are related to inflammatory cytokines, showed low value in the α–linolenic acid group. Furthermore, the weight of the axillary lymph node and level of urinary albumin were the lowest in the α–linolenic acid group (Figures 34.3 and 34.4).

Based on previous research, the crisis of autoimmune disease might be affected by oils whose fatty acid composition differs (Mineo et al., 1998; Spurney et al., 1994; Venkatraman and Chu, 1999). But these data have not clarified the effect for this actual disease by the fatty acid, because the content of fatty acid as a target is different.

MRL/lpr, which is a mouse model of SLE, also develops not only glomerulonephritis but also polyarteritis and polyarthritis rheumatica. Further, systemic lymph node enlargement, anti–DNA antibody, and proteinuria appear with the time course. Finally, it dies early due to the progression of nephropathy. IL–18, which induces the production of inflammatory cytokines, is concerned with joint inflammation in lymphocytic
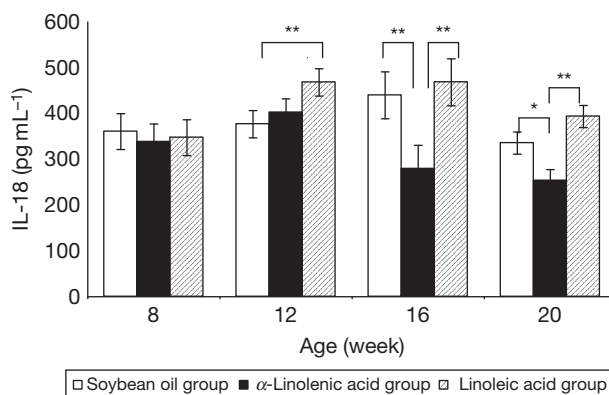
Figure 34.1 Change in serum IL-18 concentration. Serum IL-18 was measured by ELISA at 450 nm. Values are the means ± SEM ($n = 5$), [*]$p < 0.05$, [**]$p < 0.01$. Each diet was fed from 6 weeks of age. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha-linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.
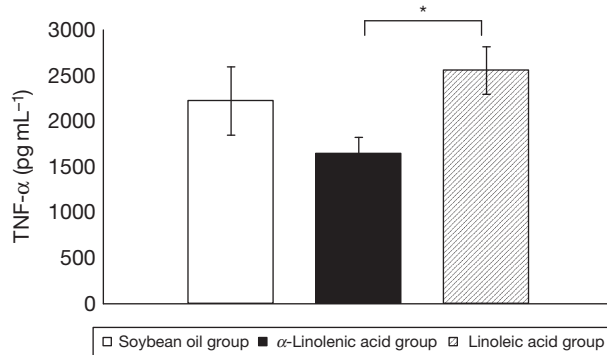
Figure 34.2 Change in serum TNF-α concentration of mice 2 h after the intraperitoneal injection of LPS (10 mg kg^{-1} body weight). Mice were fed for 6 weeks with the experimental diets. Values are the means $\pm$ SEM ($n=5$), $^*p < 0.05$. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha-linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.
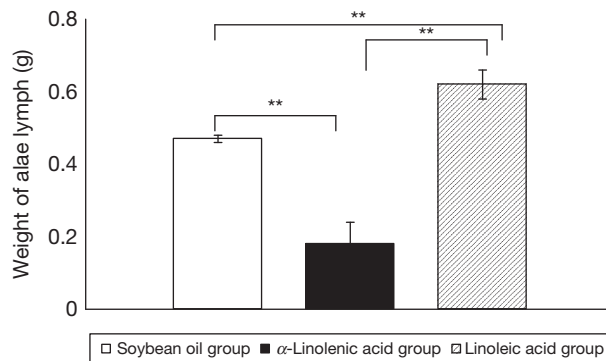
Figure 34.3 Weight of alae lymph nodes of mice. Mice were fed the experimental diets for 10 weeks. Values are the means $\pm$ SEM ($n=5$), $^{**}p < 0.01$. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha-linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.

infiltration, synovial membrane proliferation, and damage of cartilage by osteoclasts. Based on the data, α–linolenic acid delays the crisis of SLE. It was reported that this phenomenon could be confirmed when oil rich in α–linolenic acid was administered.

Next, the effects of the feeding of a diet rich in oils containing omega–3 or omega–6 unsaturated fatty acids on SLE using MRL/lpr mice were investigated. The oil composition in the experimental diet was linseed oil containing 57% α–linolenic acid (omega–3) or a modified oil with the same proportion of γ–linolenic acid (omega–6).

Spleen swelling was suppressed by the administration of α–linolenic acid–rich or γ–linolenic acid–rich oils. The spleen weight of the α–linolenic acid–rich group, divided
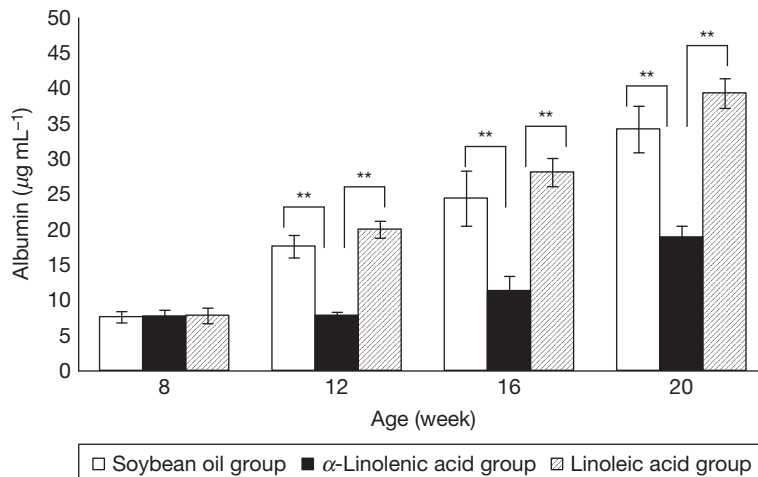
Figure 34.4 Effect of the oils on the albumin concentration of urine. Values are the means $\pm$ SEM ($n=5$), $^{**}p < 0.01$. Each diet was fed from 6 weeks of age. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha-linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.
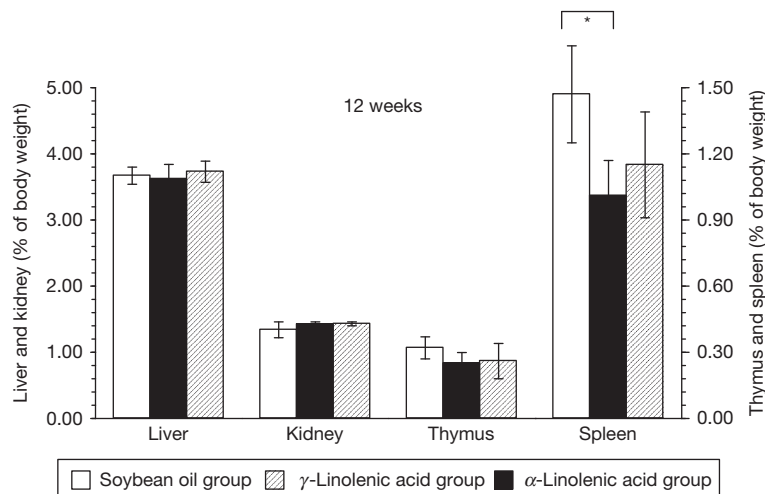
Figure 34.5 Effect of the experimental diets on the weights of the liver, kidney, thymus, and spleen of mice at 4, 8, and 12 weeks after the administration of the diets. Values with bar are the means $\pm$ SE ($n=5$). *The weights of the spleen in soybean oil and γ-linolenic acid groups were significantly different at $p < 0.05$. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006a. Influences of modified borage oil rich in g-linolenic acid and linseed oil rich in a-linolenic acid on MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 125–130.

by the body weight, showed a significantly lower value than the control group (soybean oil group). Also, the number of spleen cells showed no significant difference (Figure 34.5).

The IgM–rheumatoid factor (IgM-RF) antibody levels in the serum of MRL/lpr mice in each group were measured. After the feeding of experimental diets, the production of 3 autoantibodies tended to increase. However, in comparison with the control (soybean oil) group, α-linolenic acid– and γ-linolenic acid–rich groups showed a suppressed tendency in general (Figure 34.6).
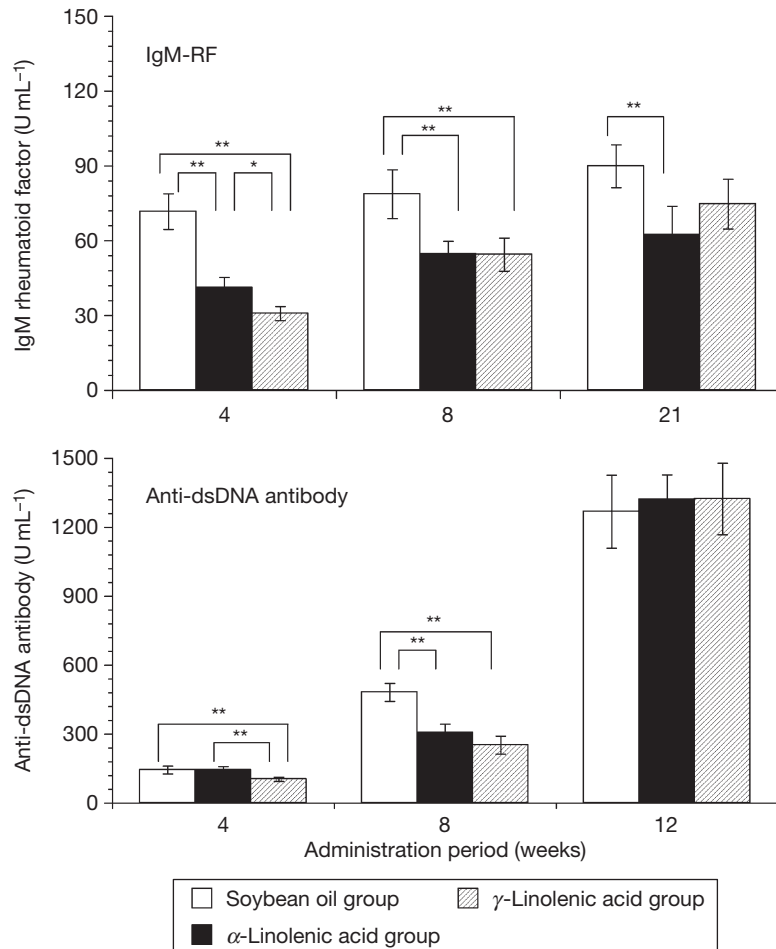
Figure 34.6 Autoantibody titer in serum of MRL/lpr mice. Mice were fed the experimental diets for 4, 8, and 12 weeks, starting from 12 weeks old. Values with bar are the means $\pm$ SE ($n=5$), $^{*}p < 0.05$, $^{**}p < 0.01$. Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006a. Influences of modified borage oil rich in g-linolenic acid and linseed oil rich in a-linolenic acid on MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 125–130.

Table 34.2 Serum Concentration of Inflammatory Cytokines in Mice

	Soybean oil group	α-Linolenic acid group	γ-Linolenic acid group
TNF-α (pg mL^{-1})	1534 ± 197[a]	1027 ± 88[b]	1581 ± 108[a]
IL-1β (pg mL^{-1})	2776 ± 145[a]	833 ± 60[b]	1088 ± 183[b]
IL-6 (pg mL^{-1})	34 040 ± 1651	31 092 ± 1827	32 396 ± 2176

Source: Reproduced from Mei, L., Ohara, A., Matsuhisa, T., 2006a. Influences of modified borage oil rich in g-linolenic acid and linseed oil rich in a-linolenic acid on MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 125–130.
Each mouse was fed experimental diets for 4 weeks starting from 8 weeks old.
Means ± SE ($n = 5$) within the same line not sharing the same superscript letter are significantly different at $p < 0.01$ by Scheffe's multiple comparison.
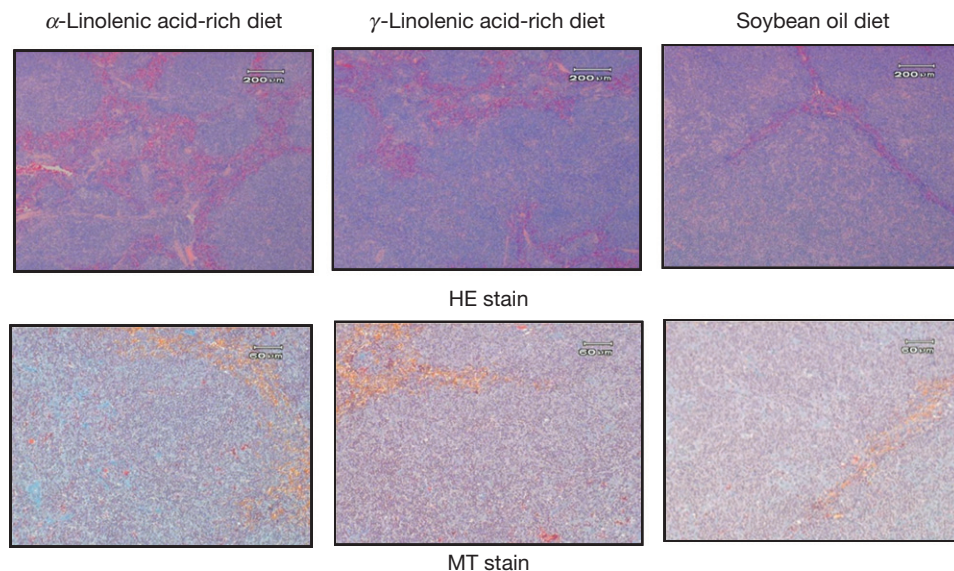
Figure 34.7 Histopathological appearance of the spleen of MRL/lpr mice.

Also, suppression of the production of autoantibody and a decrease in urine albumin were induced. The administration of α-linolenic acid-rich oil also reduced TNF-α, IL-1β, and IL-6 (Table 34.2).

The histopathology of the spleen and kidney on consuming the experimental diet for 8 weeks was observed (Figure 34.7). In the control (soybean oil) group, accompanied by expansion of the splenic white pulp region, narrowing of the splenic red pulp region was observed. On the other hand, in the α-linolenic acid-rich and γ-linolenic acid-rich oil groups, both splenic red and white pulp areas were distinctly observed with no apparent abnormalities (HE stain). However, no dietary group showed the hyperplasia of collagen fibers (blue) (Masson's trichrome stain).

The histopathological appearance of the mouse kidney is shown in Figure 34.8. In the control (soybean oil) and γ-linolenic acid-rich oil groups, moderate perivascular
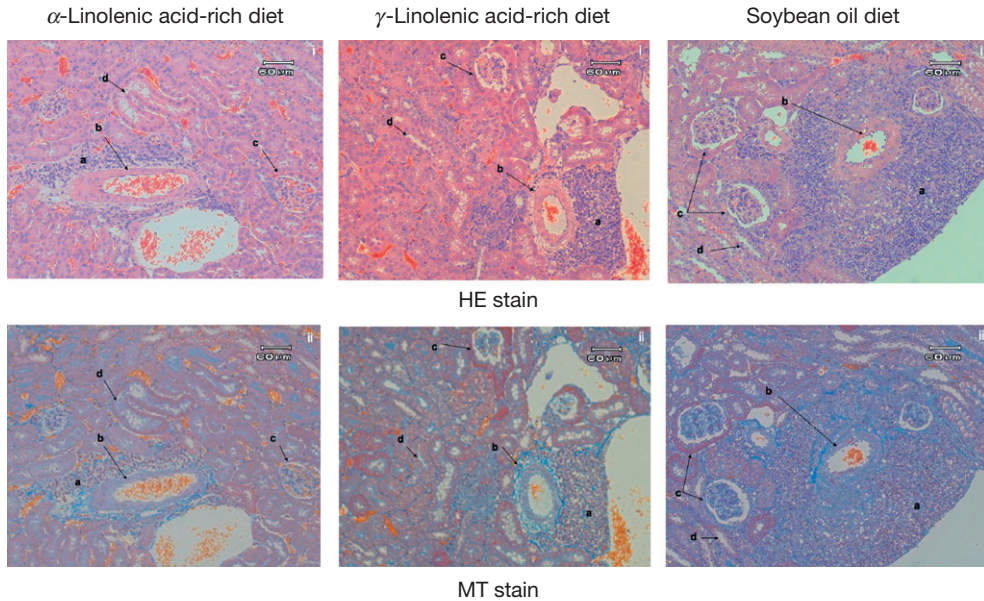
Figure 34.8 Histopathological appearance of the kidney of MRL/lpr mice.

inflammatory lymphocyte and macrophage infiltration was observed in the cortical region. There was no aberration in the glomeruli or renal tubules. The α-linolenic acid–rich oil group showed mild perivascular inflammatory lymphocyte and macrophage infiltration in the cortical region. No aberration was noted in the glomeruli or renal tubules (HE stain). However, in all dietary groups, light collagen fiber (blue) was observed in the perivascular inflammatory cell infiltration region of the cortex area. Collagen fibril formation was not observed (Masson's trichrome stain).

The MRL/lpr mouse spontaneously shows glomerulonephritis, polyarteritis, and polyarthritis. This crisis is similar to human SLE and chronic articular rheumatism. The symptoms of lymphoma, serum anti-DNA antibody, rheumatoid factor (RF) antibody, and proteinuria appear. Finally, damage of the kidney advances, and then they die. Autoantibodies detected in MRL/lpr mouse are RF and immune complex, and RF forms anti-DNA antibody and complex as a nephritis primitivity antibody. It also induces inflammation by inflammatory cytokines such as TNF-α, IL-1, and IL-6, and the crisis of SLE nephritis may be promoted (Mukaida and Matsushima, 1992). In previous reports, the effect of α-linolenic acid on the antibody production of lymphocytes was assessed. The administration of α-linolenic acid was evaluated regarding whether it suppressed or progressed production (Huang et al., 1992; Hurst et al., 2010; Jacintho et al., 2009). In our experiment, the production of serum TNF-α and IL-1β significantly reduced on the administration of α-linolenic acid. As the reason, α-linolenic acid is

an essential fatty acid and n–3 series polyunsaturated fatty acid. Alpha-linolenic acid is converted into EPA and DHA. It is well known that this reaction competitively inhibits the metabolism of the n-6 system, and the production of PGE_2 and LTB_4 is suppressed. Further, inflammatory cytokine production such as IL–1β, IL–6, and TNF-α is suppressed (Belury et al., 1989; Garg et al., 1990; Walloschke et al., 2010).

The weak effect of γ–linolenic acid was also shown. It has been confirmed that the production of eicosanoid derived from arachidonic acid is suppressed by the competitive inhibition of the enzyme, which synthesizes arachidonic acid from γ–linolenic acid. Based on this, it appears to suppress inflammatory cytokines through the metabolism of γ–linolenic acid. Also, inflammatory symptoms such as atopic dermatitis and rheumatism are improved by the administration of γ–linolenic acid. The administration of γ–linolenic acid suppresses IL–18 production, and it suppresses the production of inflammatory cytokines and production of IgE (Walloschke et al., 2010). Therefore, the administration of α–linolenic acid or γ–linolenic acid is linked to the improvement of rheumatism and atopic dermatitis. Through the control of urinary albumin quantities, anti–dsDNA antibodies, inflammatory cytokines, α–linolenic acid, and γ–linolenic acid improved the symptoms of glomerulonephritis.

From these results, it was considered that the effect of α–linolenic acid and γ–linolenic acid is favorable for the health status of the mouse. Especially, α–linolenic acid was remarkable (Mei et al., 2006a,b).

REFERENCES

Belury, M.A., Patrick, K.E., Locniskar, M., Fischer, S.M., 1989. Eicosapentaenoic and arachidonic acids: comparison of metabolism and activity in murine epidermal cells. Lipids 24, 423–429.

Fujii, T., 2007. T cell vaccination and peptide therapy in systemic rheumatism disease (in Japanese). In: Okumura, K., Hirano, T., Sato, N. (Eds.), Annual Review Immune 2008. Chugai-igakusha Co., Ltd, Tokyo, pp. 265–272.

Garg, M.L., Thomson, A.B.R., Clandinin, M.T., 1990. Interaction of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. Journal of Lipid Research 31, 271–277.

Huang, S.C., Misfeldt, M.L., Fritsche, K.L., 1992. Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. Journal of Nutrition 122, 1219–1231.

Hurst, S., Zainal, Z., Caterson, B., Hughes, C.E., Harwood, J.L., 2010. Dietary fatty acids and arthritis. Prostaglandins Leukotrienes and Essential Fatty Acids 82 (4–6), 315–318.

Jacintho, T.M., Gotho, H., Gidlund, M., et al., 2009. Anti-inflammatory effect of parenteral fish oil lipid emulsion on human activated mononuclear leukocytes. Nutricion Hospitalaria 24 (3), 288–296.

Karlsson, E.A., Beck, M.A., 2010. The burden of obesity on infectious disease. Experimental Biology and Medicine 235 (12), 1412–1424.

Kawamoto, H., 2011. Allergy and autoimmune diseases. In: Kawamoto, H. (Ed.), Immunology (in Japanese). Yodosha Co., Ltd, Tokyo, pp. 178–194.

Lombardi, V.R.M., Lombardi, C., Cacabelos, R., 2008. Nutrition, immune system activation and anti-cancer strategies in animals and humans. Current Topics in Pharmacology 12 (2), 1–30.

Mei, L., Ohara, A., Matsuhisa, T., 2006a. Influences of modified borage oil rich in γ-linolenic acid and linseed oil rich in α-linolenic acid on MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 125–130.

Mei, L., Ohara, A., Matsuhisa, T., 2006b. Effects of linoleic acid, alpha–linolenic acid and soybean oil on the autoimmune factors of MRL/lpr mice. Japanese Journal of Food Chemistry (in Japanese) 13 (3), 141–145.

Mineo, S., Matsuo, N., Konishi, Y., 1998. Cytokines and n–3 polyunsaturated fatty acids. Foods & Food Ingredients Journal of Japan (in Japanese) 178, 61–68.

Mukaida, N., Matsushima, K., 1992. Special issue: molecular biology of autoimmune diseases. Molecular biology of inflammatory cytokine and auto immune diseases. The Journal of Experimental Medicine 10, 142–148.

Samartin, S., Chandra, R.K., 2001. Obesity, overnutrition and the immune system. Nutrition Research 21, 243–262.

Spurney, R.F., Ruiz, P., Albrightson, C.R., Pisetsky, D.S., Coffman, T.M., 1994. Fish oil feeding modulates leukotriene production in murine lupus nephritis. Prostaglandins 48 (5), 331–348.

Venkatraman, J.T., Chu, W.-C., 1999. Effects of dietary ω3 and ω6 lipids and vitamin E on proliferative response, lymphoid cell subsets, production of cytokines by spleen cells, and splenic protein levels for cytokines and oncogenes in MRL/MpJ-lpr /lpr mice. Journal of Nutritional Biochemistry 10 (10), 582–597.

Venkatraman, J.T., Meksawan, K., 2002. Effects of dietary ω3 and ω6 lipids and vitamin E on chemokine levels in autoimmune-prone MRL/MpJ-lpr /lpr mice. Journal of Nutritional Biochemistry 13 (8), 479–486.

Walloschke, B., Fuhrmann, H., Schumann, J., 2010. Enrichment of RAW264.7 macrophages with essential 18-carbon fatty acids affects both respiratory burst and production of immune modulating cytokines. Journal of Nutritional Biochemistry 21 (6), 556–560.

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