

Free Radicals, Antioxidants, and Nutrition

Yun-Zhong Fang, Sheng Yang, and Guoyao Wu, PhD

From the Department of Biochemistry and Molecular Biology, Beijing Institute of Radiation Medicine, Beijing, China; the Division of Animal Nutrition, Department of Animal Science, China Agricultural University, Beijing, China; and the Department of Animal Science and Faculty of Nutrition, Texas A&M University, College Station, Texas, USA

Radiation hazards in outer space present an enormous challenge for the biological safety of astronauts. A deleterious effect of radiation is the production of reactive oxygen species, which result in damage to biomolecules (e.g., lipid, protein, amino acids, and DNA). Understanding free radical biology is necessary for designing an optimal nutritional countermeasure against space radiation-induced cytotoxicity. Free radicals (e.g., superoxide, nitric oxide, and hydroxyl radicals) and other reactive species (e.g., hydrogen peroxide, peroxynitrite, and hypochlorous acid) are produced in the body, primarily as a result of aerobic metabolism. Antioxidants (e.g., glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A, and tea polyphenols) and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidases) exert synergistic actions in scavenging free radicals. There has been growing evidence over the past three decades showing that malnutrition (e.g., dietary deficiencies of protein, selenium, and zinc) or excess of certain nutrients (e.g., iron and vitamin C) gives rise to the oxidation of biomolecules and cell injury. A large body of the literature supports the notion that dietary antioxidants are useful radioprotectors and play an important role in preventing many human diseases (e.g., cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes). The knowledge of enzymatic and non-enzymatic oxidative defense mechanisms will serve as a guiding principle for establishing the most effective nutrition support to ensure the biological safety of manned space missions. *Nutrition* 2002;18:872–879. ©Elsevier Science Inc. 2002

KEY WORDS: oxidative stress, diets, nutrition support, space flight

INTRODUCTION

Radiation hazards in outer space, such as the Earth's radiation belt (primarily protons and electrons captured by the geomagnetic field), solar space radiation (primarily protons and α -particles), and galactic space radiation (primarily protons, α -particles, and heavier nuclei), present an enormous challenge for the biological safety of manned space missions.¹ A deleterious effect of radiation is the production of reactive oxygen species (ROS), which include superoxide anion (O_2^- , a free radical), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2).² These reactive species may contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein oxidation, and muscle injury) and to metabolic and morphologic changes (e.g., increased muscle proteolysis and changes in the central nervous system) in animals and humans during space flight.^{3–5} Although space radiation hazard can be reduced primarily by radiation shielding,¹ dietary antioxidants may be useful radioprotectors to protect astronauts against radiation-induced tissue lethality and other deleterious effects.^{6–8}

For designing an optimal nutritional countermeasure against space radiation, it is necessary to fully understand the mechanisms responsible for the production and removal of free radicals and other reactive species. Since the discoveries of the catalytic function of superoxide dismutase (SOD) in 1969⁹ and the arginine-dependent synthesis of nitric oxide ($\cdot NO$), a nitrogen free radical, in 1988,¹⁰ there has been rapid progress in the field of free radical biology.^{11–13} Over the past three decades, the free radical theory has greatly stimulated interest in the role of dietary antioxidants in

preventing many human diseases including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes.^{12–16}

The knowledge of enzymatic and non-enzymatic oxidative defense mechanisms will serve as a guiding principle for establishing the most effective nutrition support to ensure the biological safety of manned space missions. With this proposition, the major objective of this article is to review recent advances in free radical biology and antioxidant nutrients, with emphasis on oxidative defense systems against radiation-induced radical damage.

BASIC CONCEPTS OF FREE RADICAL BIOLOGY AS APPLIED TO NUTRITION

What Are Free Radicals?

Free radicals are defined as molecules having an unpaired electron in the outer orbit.¹² They are generally unstable and very reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl ($RO_2^{\cdot}$), alkoxyl ($RO^{\cdot}$), and hydroperoxyl ($HO_2^{\cdot}$) radicals. Nitric oxide and nitrogen dioxide ($\cdot NO_2$) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide, hypochlorous acid ($HOCl$), hypobromous acid ($HOBr$), and peroxynitrite ($ONOO^-$). ROS, reactive nitrogen species (RNS), and reactive chlorine species are produced in animals and humans under physiologic and pathologic conditions.¹⁷ Thus, ROS and RNS include radical and non-radical species.

Biological Roles of Free Radicals

Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on the

Correspondence to: Guoyao Wu, PhD, Department of Animal Science, Texas A&M University, 2471 TAMU, College Station, TX 77843-2471, USA. E-mail: g-wu@tamu.edu

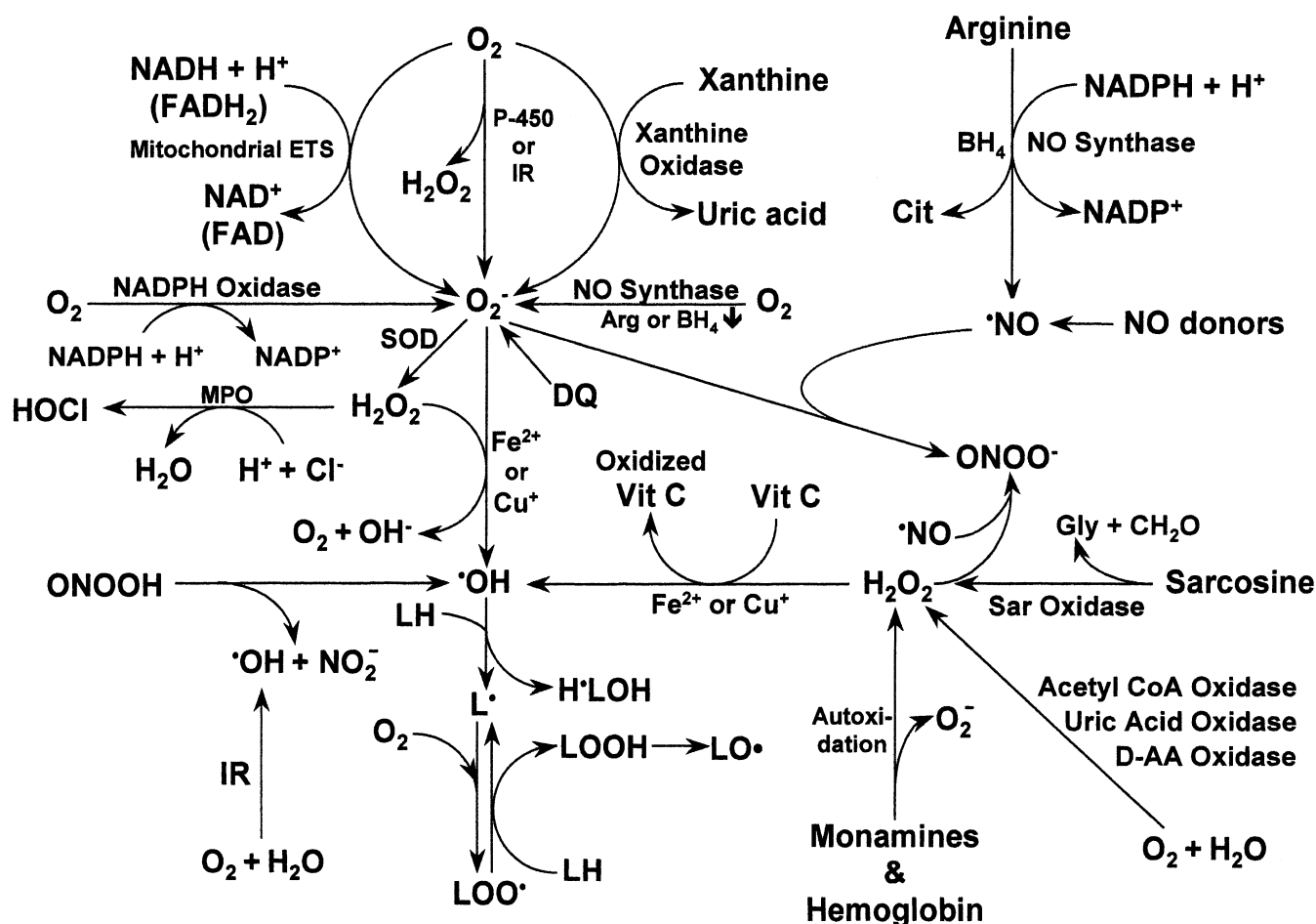


FIG. 1. Production of oxygen and nitrogen free radicals and other reactive species in mammalian cells. AA, amino acid; Arg, L-arginine; BH₄, (6R)-5,6,7,8-tetrahydro-L-biopterin; CH₂O, formaldehyde; Cit, L-citrulline; DQ, diquat; ETS, electron transport system; FAD, flavin adenine dinucleotide (oxidized); FADH₂, flavin adenine dinucleotide (reduced); Gly, glycine; H₂O₂, hydrogen peroxide; HOCl, hypochlorous acid; H'LOH, hydroxy lipid radical; IR, ionizing radiation; L[•], lipid radical; LH, lipid (unsaturated fatty acid); LO[•], lipid alkoxyl radical; LOO[•], lipid peroxy radical; LOOH, lipid hydroperoxide; MPO, myeloperoxidase; NAD⁺, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); NADP⁺, nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); NO, nitric oxide; O₂⁻, superoxide anion radical; OH, hydroxyl radical; ONOO⁻, peroxynitrite; P-450, cytochrome P-450; PDG, phosphate-dependent glutaminase; Sar, Sarcosine; SOD, superoxide dismutase; Vit C, vitamin C; Vit E, vitamin E (α-tocopherol).

organisms.¹⁸ For example, oxygen radicals exert critical actions such as signal transduction, gene transcription, and regulation of soluble guanylate cyclase activity in cells.^{19,20} Also, NO is one of the most widespread signaling molecules and participates in virtually every cellular and organ function in the body.²¹ Physiologic levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone, and hemodynamics.²¹ In addition, NO produced by neurons serves as a neurotransmitter, and NO generated by activated macrophages is an important mediator of the immune response.²² However, as oxidants and inhibitors of enzymes containing an iron-sulfur center, free radicals and other reactive species cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA), which leads to cell injury and death.^{18,22} For example, radiation-induced ROS markedly alter the physical, chemical, and immunologic properties of SOD,² which further exacerbates oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system.¹⁸ Thus, there are "two faces" of free radicals in biology in that they serve as signaling and regulatory

molecules at physiologic levels but as highly deleterious and cytotoxic oxidants at pathologic levels.²²

Production of Free Radicals

Oxygen is required for the generation of all ROS, RNS, and reactive chlorine species.²² The major reactions for the production of oxygen and nitrogen free radicals in the body are illustrated in Fig. 1. NO is formed from L-arginine by one of the three NO synthase (NOS) isoforms: nNOS (originally identified as constitutive in neuronal tissue; also known as NOS-I or NOS-1), iNOS (originally identified as being inducible by cytokines in activated macrophages and liver; also known as NOS-II or NOS-2), and eNOS (originally identified as constitutive in vascular endothelial cells; also known as NOS-III or NOS-3).²³ All NOS isoforms require oxygen, tetrahydrobiopterin, nicotinamide adenine dinucleotide phosphate (NADPH), calmodulin, flavin adenine dinucleotide (oxidized; FAD), flavin mononucleotide (FMN), and heme for catalytic activity, whereas Ca²⁺ is essential for nNOS and eNOS activity.²³ In contrast, superoxide is generated from O₂ by multiple pathways: 1) NADPH oxidation by NADPH oxidase; 2) oxidation of xanthine or hypoxanthine by xanthine oxidase; 3) oxidation of reducing equivalents (e.g., nicotinamide adenine dinu-

cleotide [reduced; NADH], NADPH, and FADH₂ [FAD reduced]) via the mitochondrial electron transport system; 4) autooxidation of monamines (e.g., dopamine, epinephrine, and norepinephrine), flavins, and hemoglobin in the presence of trace amounts of transition metals; 5) one-electron reduction of O₂ by cytochrome P-450; and 6) one-electron reduction of O₂ by nNOS or eNOS when arginine or tetrahydrobiopterin is deficient.^{12,17,22,23}

Superoxide dismutase converts O₂⁻ to H₂O₂. Hydrogen peroxide is also produced through two-electron reduction of O₂ by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme A oxidase, or uric acid oxidase.^{13,17,22} Further, the oxidation of sarcosine in the pathway of glycine metabolism leads to H₂O₂ formation (Fig. 1). In the presence of water and oxygen, ionizing radiation results in the production of O₂⁻, H₂O₂, and [•]OH, with [•]OH being the major deleterious ROS.² NO can react with O₂⁻ or H₂O₂ to form ONOO⁻, whose oxidant potential is greater than that of O₂⁻ or H₂O₂ alone.^{18,22} As a strong oxidant, HOCl is generated from H₂O₂ and Cl⁻ by myeloperoxidase (a heme enzyme) particularly in immunologically activated phagocytes.

When free radicals and other reactive species (e.g., [•]OH, HOO[•], ONOO⁻) extract a hydrogen atom from an unsaturated fatty acyl chain (e.g., ω -6 polyunsaturated fatty acid [PUFA]), a carbon-centered lipid radical (L[•]) is produced. This is followed by the addition of oxygen to L[•] to yield a lipid peroxyl radical (LOO[•]). LOO[•] further propagates the peroxidation chain reaction by abstracting a hydrogen atom from a nearby unsaturated fatty acid. The resulting lipid hydroperoxide (LOOH) can easily decompose to form a lipid alkoxyl radical (LO[•]). This series of ROS-initiated lipid peroxidation reactions with the production of lipid peroxyl and alkoxyl radicals, collectively called *chain propagation*, occurs in mammalian cells, such that oxygen free radicals may cause damage far in excess of their initial reaction products.

The mitochondrial electron transport system is a source of superoxide.²² Because NADH, NADPH, and FADH₂ are produced almost exclusively via the aerobic metabolism of protein, fat, and glucose, an increase in dietary energy intake enhances mitochondrial free radical production, which results in oxidative stress. Thus, calorie restriction reduces the generation of free radical species and retards aging in animals.¹¹ Under physiologic conditions, approximately 1% to 3% of the O₂ consumed by the body is converted into superoxide and other ROS.¹¹ Throughout the life cycle, any person may be at a risk of oxidative stress induced by high rates of oxygen use (e.g., strenuous work and competitive sports), the autoimmune activation of immune system cells (e.g., respiratory burst of polymorphonuclear and mononuclear cells), and environmental factors (e.g., pollutants containing NO, nitrogen dioxide, and hydroxyl radicals). Prolonged exposure to free radicals, even at a low concentration, may result in the damage of biologically important molecules and potentially lead to DNA mutation, tissue injury, and disease.^{18,22} Thus, although molecular oxygen is absolutely essential for aerobic life, it can be toxic under certain conditions. This phenomenon has been termed the *oxygen paradox*.¹²

Scavenging of Free Radicals

The removal of free radicals is achieved through enzymatic and non-enzymatic reactions (Fig. 2). NO is rapidly oxidized by oxy-hemoglobin to form NO₃ (nitrate), the major end stable oxidation product of NO in the body.²⁴ NO also reacts with glutathione (reduced; GSH) to form nitrosothiol or with heme to yield heme-NO. Physiologically, nitrosothiol can serve as a vehicle to transport NO in plasma, thereby increasing the biological half-life of physiologic concentrations of NO.²⁵ In addition, tyrosine residues of proteins can be nitrosylated by NO or its derivative peroxynitrite. Moreover, GSH can scavenge ONOO⁻ with the formation of oxidized glutathione (GS-SG), which is converted back to GSH by the NADPH-dependent glutathione reductase.²⁶

The principal defense systems against oxygen free radicals are SOD, GSH, GSH peroxidases, glutathione reductase, catalase (a heme enzyme), and antioxidant nutrients. Vitamin E can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA, thereby breaking the radical chain reaction and preventing the peroxidation of PUFA in cellular and subcellular membrane phospholipids. As a reducing agent, vitamin C reacts with a vitamin E radical to yield a vitamin C radical while regenerating vitamin E. Like a vitamin E radical, a vitamin C radical is not a reactive species because its unpaired electron is energetically stable.¹³ A vitamin C radical is converted back to vitamin C by GSH.

Glutathione, the most abundant thiol-containing substance of low molecular weight in cells, is synthesized from glutamate, cysteine, and glycine. *N*-acetylcysteine is a stable, effective precursor of cysteine for intracellular GSH synthesis.²⁶ Interestingly, almost all dietary glutamate is catabolized by the small intestinal mucosa in the first pass.²⁷ Thus, the hydrolysis of glutamine to glutamate by glutaminase and the production of glutamate from α -ketoglutarate and branched-chain amino acids via transamination are two major sources of plasma and cellular glutamate for GSH synthesis. As a major component of the cellular antioxidant system, GSH has the following characteristics: 1) GSH in the diet can be partly absorbed from the small intestine and can be synthesized *de novo*, so that GSH is an exogenous and endogenous antioxidant; 2) although glutathione radical (GS[•]) formed from the oxidation of GSH is a pro-oxidant radical, GS[•] can react with another GS[•] to yield GS-SG, which is then reduced to GSH by the NADPH-dependent glutathione reductase; 3) GSH can react with a variety of xenobiotic electrophilic compounds in the catalytic reaction of glutathione-S-transferase; 4) GSH effectively scavenges ROS (e.g., lipid peroxyl radical, peroxynitrite, and H₂O₂) directly and indirectly through enzymatic reactions; 5) GSH can conjugate with NO, resulting in the formation of a S-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO; and 6) GSH interacts with glutaredoxin and thioredoxin (thiol-proteins), which play important roles in the regulation of cellular redox homeostasis.

Cytochrome C and SOD catalyze the formation of O₂ from O₂⁻ (Fig. 2). A coproduct of SOD is H₂O₂, which is converted to H₂O by catalase and the selenium-dependent GSH peroxidase. Lipid hydroperoxides are detoxified to alcohols by GSH peroxidase. Another type of GSH peroxidase (phospholipid peroxide GSH peroxidase) acts on phospholipid peroxides in membrane structures.²⁶ Other ROS and RNS scavengers include uric acid (a metabolite of purines), salicylate, mannitol, carotenoid, ubiquinone, bilirubin (a product of hemoglobin catabolism), α -lipoate, arginine, citrulline, glycine, taurine, histidine, creatine (a metabolite of arginine, glycine and methionine), carnosine (β -alanyl-L-histidine, which is abundant in skeletal muscle), tetrahydrobiopterin (a metabolite of guanosine triphosphate and thus glutamine), phytate, and tea polyphenols.^{22,28-33}

Glucose metabolism via the pentose cycle plays a crucial role in providing NADPH and, hence, maintaining the normal ratio of [GSH]² to [GSSG] and a normal redox state in cells. When the intracellular concentration of GSH decreases and that of GS-SG increases, the cellular demand for NADPH increases markedly.²⁶ This necessitates an increase in glucose metabolism via the pentose cycle. A deficiency of intracellular NADPH may exacerbate an imbalance between the production and scavenging of free radicals. When rates of free radical production are greater than the scavenging rates, oxidative damage likely occurs in cells and tissues.

Assessments of Free Radical Activity

There are multiple, complex methods for assessing free radical activity depending on experimental conditions, the availability of analytical facilities, and the investigator's interest.¹⁶ In view of the lack of any "gold standard" assays of free radical activity, three

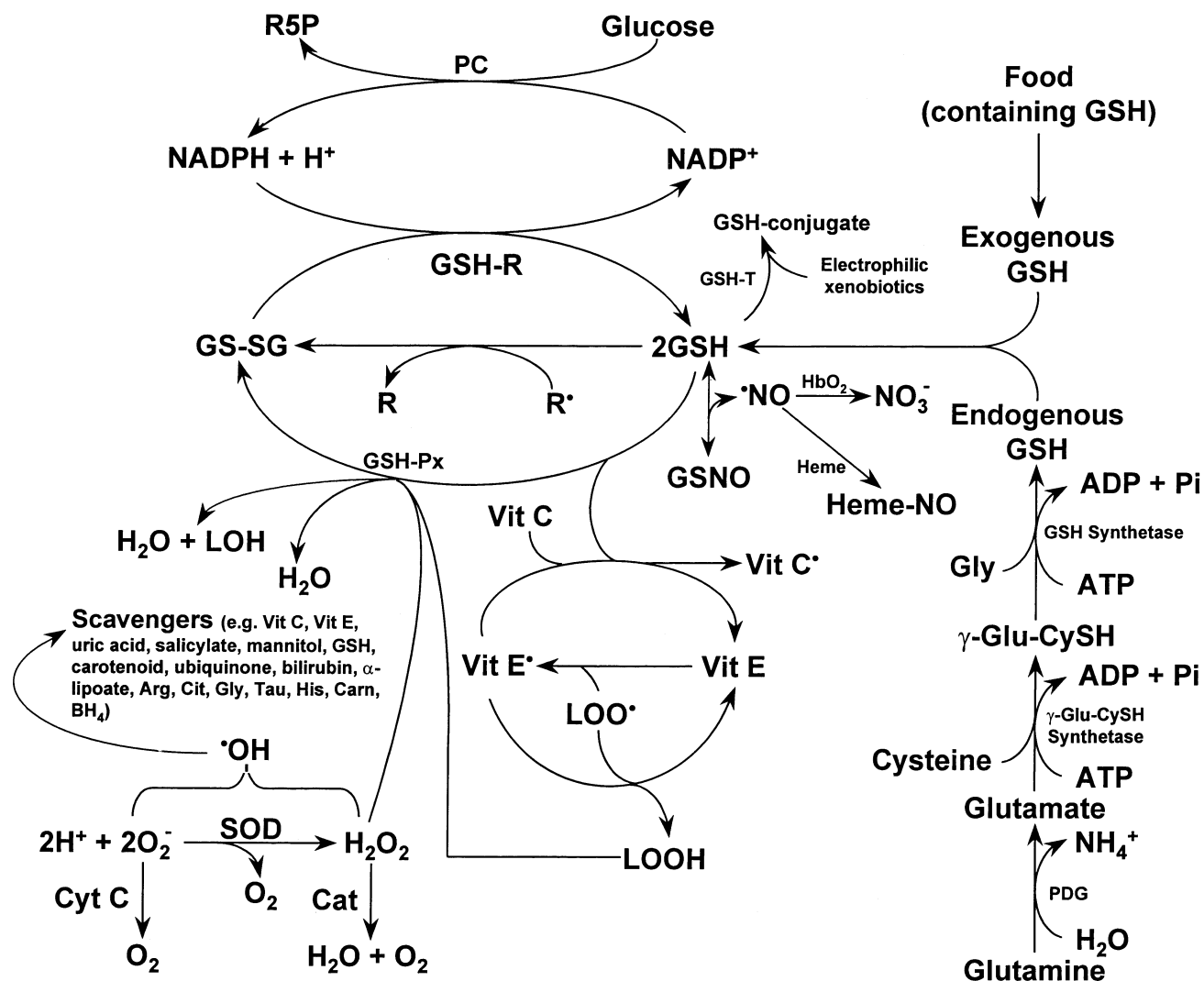


FIG. 2. Removal of oxygen and nitrogen free radicals and other reactive species in mammalian cells. ADP, adenosine diphosphate; Arg, arginine; BH₄, (6R)-5,6,7,8-tetrahydro-L-biopterin; Carn, carnosine; Cat, catalase; Cit, citrulline; Cyt C, cytochrome C; ETS, electron transport system; Glu, L-glutamate; Gly, glycine; γ-Glu-CySH, γ-glutamyl-cysteine; GS-SG, oxidized glutathione (glutathione disulfide); GSH, glutathione (reduced form); GSH-Px, glutathione peroxidase; GSH-R, glutathione reductase; GSH-T, glutathione S-transferase; GSNO, nitrosylated glutathione; HbO₂, oxyhemoglobin; Heme-NO, heme-nitric oxide; His, histidine; LOH, lipid alcohol; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; •NO, nitric oxide; NO₃⁻, nitrate; O₂⁻, superoxide anion radical; ONOO•, peroxynitrite; PC, pentose cycle; R•, radicals; R, non-radicals; R5P, ribulose 5-phosphate; SOD, superoxide dismutase; Tau, taurine; Vit C, vitamin C (ascorbic acid); Vit C•, vitamin C radical; Vit E, vitamin E (α-tocopherol); Vit E•, vitamin E radical.

major approaches have been used: 1) determination of endogenous antioxidant levels; 2) measurement of the products of oxidized macromolecules; and 3) direct detection of free radicals. For assessing endogenous antioxidant capacity, most studies have examined the concentrations of antioxidants (e.g., vitamin E, vitamin C, carotenoids, folate, GSH, and zinc) in plasma and cells and the cellular activities of antioxidant enzymes (e.g., glutathione reductase, SOD, catalase, and glutathione peroxidase). Because GSH is rapidly oxidized to GS-SG by radicals and other reactive species and GS-SG is exported from cells, intracellular [GS-SG]:[GSH]² ratio can provide a valid index of oxidative stress. Assessments of lipid peroxidation have included the analysis of lipid peroxides, isoprostanes, diene conjugates, and breakdown products of lipids (e.g., malonaldehyde, ethane, pentane, and 4-hydroxynonenal). Among these products, malonaldehyde is often used as a reliable marker of lipid peroxidation. For assessing ROS-induced protein oxidation, most investigators have determined the production of protein carbonyls, the loss of free thiol groups in proteins, and

nitration of protein-bound tyrosine residues.¹³ Indeed, protein nitrotyrosine has been widely used as a convenient stable marker for the production of reactive nitrogen-centered oxidants (e.g., NO and peroxynitrite).¹² Specific products of DNA base oxidation such as 8-hydroxydeoxyguanosine, 5-OH cytosine, 8-OH adenine, 8-OH guanine, and thymine glycol have been measured often to assess DNA base oxidation.¹⁶ Importantly, urinary excretion of 8-hydroxydeoxyguanosine may provide a useful, non-invasive means to assess whole-body DNA base oxidation in humans and animals.¹⁶ Direct detection of free radicals has been performed using electron spin resonance and spin trapping techniques.² Although the electron spin resonance technique is suitable for detecting free radicals in solution chemistry, it has limited application to biological tissues owing to their usually high content of water. However, this problem can be overcome by the use of the spin trapping technique, which involves the conversion of highly reactive free radicals to relatively inert radicals, followed by electron spin resonance analysis.¹⁶

EFFECTS OF NUTRIENTS ON FREE RADICAL PRODUCTION AND REMOVAL

Protein

The net loss of body protein, in particular skeletal muscle protein, is likely a major factor responsible for protein malnutrition^{7,8} and possibly deficiencies of some amino acids (e.g., glutamine, arginine, and cysteine) during space flight. Interestingly, radiation directly contributes to the increased muscle proteolysis and muscle atrophy under space flight conditions.³ Amino acids are building blocks for the synthesis of proteins, including antioxidant enzymes. Some amino acids (e.g., arginine, citrulline, glycine, taurine, and histidine), small peptides (e.g., GSH and carnosine), and nitrogenous metabolites (e.g., creatine and uric acid) directly scavenge oxygen free radicals. In addition, available evidence has shown that taurine and taurine chloramine inhibit iNOS expression and inducible NO synthesis in various cell types, including hepatocytes, macrophages, and glial cells.¹⁴ Thus, a dietary deficiency of protein not only impairs the synthesis of antioxidant enzymes but also reduces tissue concentrations of antioxidants, thereby resulting in a compromised antioxidant status.^{26,29} Interestingly, when arginine or tetrahydrobiopterin is deficient, eNOS produces superoxide anion,²² which contributes to oxidative stress in the vasculature and atherosclerosis.²¹

Food intake by astronauts is reduced by approximately 20% during space flight,⁸ which may be suboptimal for long-term manned space missions. Thus, there is a concern about amino acid availability and balance in astronauts. Research with animal models (e.g., rats) has shown that insufficient protein intake results in a deficiency of zinc (a cofactor of Cu,Zn-SOD),^{13,29} indirectly affecting superoxide removal. It is likely that protein malnutrition decreases plasma concentrations of albumin (a transporter of zinc in circulation) and intracellular metallothionein (a major carrier of zinc) due to reduced protein synthesis in the liver and extrahepatic tissues. Remarkably, plasma concentrations of free iron are elevated in most children with kwashiorkor but are not altered in children without the disease.³⁴ These findings suggest that iron-induced free radical injury plays a role in the pathogenesis of cardiovascular abnormalities in patients with kwashiorkor. There is also a significant excess of chelatable iron in bone marrow and the urine of children with kwashiorkor compared with the controls.³⁵ Thus, some investigators have suggested that the abnormal ultrastructure of voluntary muscle in protein-deficient children results from ROS-induced cell damage.³⁵ The increased tissue concentrations of free iron in protein-deficient subjects likely result from low concentrations of iron-binding proteins such as transferrin (extracellular fluid), lactoferrin (extracellular fluid and cytosolic), and ferritin (cytosolic).

High-protein diets, provided mainly from animal sources, have been popular as a "new" strategy for weight loss in obese patients since the 1960s. Although there are no long-term scientific studies of their overall efficacy and safety,³⁶ such diets may lead to oxidative stress in humans on the basis of the following considerations. First, homocysteine, an independent risk factor for cardiovascular disease, increases endothelial superoxide production and induces oxidative stress in the vasculature.¹⁴ Second, increasing protein intake has recently been shown to stimulate the generation of ROS and lipid peroxidation in human polymorphonuclear leukocytes and mononuclear cells.³⁷ Third, increasing dietary protein intake increases whole-body NO production by constitutive and inducible NOS in rats.²⁴

Lipids

PUFAs are prone to be oxidized by free radicals and other ROS.³⁸ Thus, high intake of PUFA may render the organism more susceptible to lipid peroxidation, which may be alleviated by dietary

supplementation of antioxidants such as vitamin C, vitamin E, and carotenoids.^{13,17} Increasing extracellular concentrations of fatty acids and low-density lipoproteins induces iNOS expression in many cell types including pancreatic β -cells, vascular smooth muscle cells, and macrophages.¹⁴ Similarly, feeding a high saturated-fat diet to rats increases iNOS activity in liver and colon³⁹ and stimulates free radical production and oxidative DNA damage in skeletal muscle mitochondria and the whole body.^{40,41}

Epidemiologic studies have shown that consumption of fish oil, rich in ω -3 PUFA, reduces the risk of cardiovascular disease in humans.⁴² This effect of fish oil results in part from an inhibition of lipogenesis and stimulation of fatty acid oxidation in the liver. Interestingly, like other PUFAs, fish oil can be easily peroxidized to form hydroperoxides and would increase oxidative stress. To understand this apparent paradox of fish oil, recent work has shown that, in contrast to ω -6 PUFAs, ω -3 PUFAs are inhibitors of free radical generation.^{14,43} For example, Takahashi et al.⁴³ reported that long-term (6 mo) feeding of a diet rich in fish oil increases the expression of antioxidant genes (glutathione-S-transferases, uncoupling protein-2, and Mn-SOD) in mouse liver and upregulates the expression of lipid-catabolism genes. In addition, ω -3 PUFAs (docosahexaenoic acid, eicosapentaenoic acid, and α -linolenic acid) inhibit iNOS expression and inducible NO synthesis by cytokine-activated macrophages.^{44,45} Thus, ω -3 PUFA exerts its beneficial effect on cardiovascular function through two mechanisms: 1) decreasing plasma triacylglycerol concentration and 2) inhibiting free radical production.

Vitamins

Many vitamins inhibit NO production by iNOS, in support of their known antiatherogenic and antineuroinflammatory roles.¹⁴ For example, vitamin A inhibits iNOS gene transcription in vascular smooth muscle cells,⁴⁶ endothelial cells,⁴⁷ cardiac myocytes,⁴⁷ and mesangial cells.⁴⁸ 1,25-Dihydroxyvitamin D₃, vitamin K₂, and niacin inhibit iNOS expression in inflammatory cells of the brain (macrophages, microglia, and astrocytes),⁴⁹ the bleomycin-mouse model of lung fibrosis,⁵⁰ and vascular smooth muscle cells,⁵¹ respectively. In addition, many carotenoids suppress iNOS expression and inducible NO synthesis in activated macrophages and promyelocytic HL-60 cells.⁵² By reducing NO generation by iNOS, these vitamins play an important role in preventing radical-induced cytotoxicity.

Vitamins also directly scavenge ROS and upregulate the activities of antioxidant enzymes. Among them, vitamin E has been recognized as one of the most important antioxidants. Vitamin E inhibits ROS-induced generation of lipid peroxyl radicals, thereby protecting cells from peroxidation of PUFA in membrane phospholipids, from oxidative damage of plasma very low-density lipoprotein, cellular proteins, DNA, and from membrane degeneration.⁵³ Consequently, a dietary deficiency of vitamin E reduces the activities of hepatic catalase, GSH peroxidases, and glutathione reductase,⁵⁴ induces liver lipid peroxidation, and causes neurologic and cardiovascular disorders,^{55,56} all of which can be reversed by dietary vitamin E supplementation. In support of the critical antioxidant role of vitamin E, Yokota et al.⁵⁷ recently demonstrated increases in brain lipid peroxidation and neurodegeneration in mice with a deficiency of α -tocopherol transfer protein.

The antioxidant role of physiologic concentrations of vitamin C has been well established in the literature.⁵⁸ For example, on the basis of the formation of 8-hydroxydeoxyguanosine in DNA exposed to irradiation or $\cdot$ OH, Fischer-Nielsen et al.⁵⁹ found that vitamin C exhibits a protective effect against free radical-induced oxidative damage. Other water-soluble vitamins are crucial for oxidative defense. For example, a series of studies by Fang and coworkers demonstrated that irradiation increases the oxidation of proteins, amino acids, lipids, vitamin C, and folate in rats.^{60–64} As

such, the availability of vitamin C and folate is markedly reduced in irradiated rats.^{60,62} A deficiency of folate in these rats is consistent with an increase in the urinary excretion of formiminoglutamate,⁶¹ an histidine metabolite whose further catabolism requires tetrahydrofolate. Importantly, dietary supplementation of vitamin B12 and folic acid reduces radical-induced radiation damage, improves blood leukocyte cell counts, and decreases the mortality of irradiated rats.^{60–64} Remarkably, a deficiency of dietary choline increases hepatic concentrations of 8-hydroxydeoxyguanosine (an indicator of DNA damage) and promotes the growth of hepatocellular carcinomas in rats, which can be prevented by dietary supplementation of choline or vitamin C.⁶⁵ Note that vitamin B12, folic acid, and choline participate in one-carbon unit metabolism and, hence, are essential for the methylation of DNA and proteins. Further, folate, vitamin B6, and vitamin B12 are required for homocysteine metabolism by serving as cofactors for methionine synthase (B12), cystathionine synthase (B6), and cystathionase (B6) and as a substrate (5-methyltetrahydrofolate) for methionine synthase. Because homocysteine contributes to oxidative stress in endothelial cells, these vitamins help reduce the risk of cardiovascular disease in humans and animals.¹⁴

By serving as components of NADP⁺/NADPH, NAD⁺/NADH, and FAD/FADH₂, nicotinamide and riboflavin play important roles in protecting organisms from oxidative stress.^{17,58} For example, as a cofactor for transketolase of the pentose cycle, thiamine is essential for NADPH generation. NADPH and FAD are cofactors for glutathione reductase, the major enzyme responsible for the regeneration of GSH from GS-SG. In addition, NADPH is tightly bound to catalase and is thus necessary to maintain the enzyme function. Further, NADPH is required for the production of endothelial NO,⁶⁶ and physiologic concentrations of NO inhibit superoxide production by vascular endothelial cells.³⁰

Minerals

The role of minerals in enzyme functions has been studied extensively in nutrition and biochemistry. For example, magnesium is a cofactor for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, two pentose-cycle enzymes catalyzing the production of NADPH from NADP⁺. Thus, a deficiency of dietary magnesium reduces glutathione reductase activity and results in radical-induced protein oxidation (indicated by the generation of protein carbonyls) and marked lesions in tissues (e.g., skeletal muscle, brain, and kidney).^{67,68}

Iron is the most abundant trace element in the body, and almost all iron occurs bound to proteins. Free iron concentrations are particularly low for two reasons: 1) Fe³⁺ is not water soluble, and 2) Fe²⁺ participates in the generation of free radicals (Fig. 1). Thus, an increase in extracellular or intracellular iron concentrations, which can result from dietary protein deficiency, dietary iron loading, low concentrations of iron-binding proteins, or cell injury, promotes ROS production, lipid peroxidation, and oxidative stress.⁶⁹ Increasing the extracellular concentration of non-heme iron also enhances iNOS protein expression and inducible NO synthesis in many cell types, including cultured proximal tubule cells and macrophages,¹⁴ further exacerbating oxidative damage via peroxynitrite generation.

Copper and zinc, and manganese are indispensable metals for the activities of Cu,Zn-SOD and Mn-SOD, respectively.⁵⁸ Therefore, dietary deficiencies of these minerals markedly decrease tissue Cu,Zn-SOD and Mn-SOD activities and result in peroxidative damage and mitochondrial dysfunction.⁵⁸ A deficiency of copper or zinc in rats also enhances cytochrome P-450 activity in microsomes of liver and lung, stimulates ROS generation, and increases intestinal iNOS expression.^{70,71} Such effects render the animal more susceptible to lipid peroxidation⁷⁰ and gastrointestinal infection.⁷¹

There is a rich and exciting history of selenium nutrition and biochemistry. Since the discovery of glutathione peroxidase as a

selenium-dependent enzyme in 1973, selenium has been identified as an essential cofactor for selenoprotein P and other selenoproteins.⁵⁸ Strikingly, a dietary deficiency of selenium markedly decreases tissue glutathione peroxidase activity by 90% and results in peroxidative damage and mitochondrial dysfunction.⁷² Likewise, a dietary deficiency of selenium impairs the function of other selenoproteins, which include iodothyronine deiodinases and thioredoxin reductase.¹⁷ The essential role of selenium in the removal of free radicals and the maintenance of normal human health is epitomized by the etiology of Keshen disease in China.⁷³ This disease (named after the village where human cases were initially reported) was discovered by Chinese scientists to result from a severe deficiency of dietary selenium owing to a deficiency of soil selenium.⁷³ Remarkably, dietary supplementation of selenium successfully prevents the deficiency of selenium in humans and cures Keshen disease,⁷³ thus exemplifying the significance of basic antioxidant research in nutritional and medical practices.

Phytochemicals

Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals. Among them, phenolic and polyphenolic compounds, such as flavonoids and catechin in edible plants, exhibit potent antioxidant activities.^{13,74} A large body of the literature has documented the beneficial effects of tea polyphenolic compounds on scavenging free radicals and on their role in the prevention and therapy of disease. First, tea polyphenols enhance red blood cell resistance to oxidative stress in vitro and in vivo.⁷⁵ Second, tea polyphenols effectively scavenge superoxide and hydroxyl radicals and inhibit oxidative modification of low-density lipoprotein.¹⁵ Third, dietary supplementation of tea polyphenols decrease serum concentrations of total cholesterol and malondialdehyde (an indicator of lipid peroxidation) and increase serum concentrations of high density lipoprotein in humans.⁷⁶ As such, tea polyphenols are beneficial for the treatment of coronary heart disease, hypertension, and type 2 diabetes.⁷⁶ Fourth, tea polyphenols inhibit the growth and induce apoptosis of several human cancer cell lines in vitro.^{77,78} In vivo studies have demonstrated that dietary supplementation of tea polyphenols dose-dependently decreases S180 sarcoma weight in mice and increases the survival time of the mice with Ehrlich's adenocarcinoma.¹³ Fifth, tea polyphenols enhance Cu,Zn-SOD activity, decrease malondialdehyde and lipofuscin concentrations in the brain of houseflies,⁷⁹ and increase the average life span of male and female houseflies.⁸⁰

β -Carotene and other carotenoids, such as α -carotene, γ -carotene, and β -cryptoxanthin, are potent antioxidants of plant origins.⁵⁸ For example, β -carotene reacts with a peroxy radical to form a resonance-stabilized carbon-centered radical within its conjugated alkyl structure, thereby inhibiting the chain propagation effect of ROS. Although lycopene, lutein, canthaxanthin, and zeaxanthin do not possess provitamin A activity, their antioxidant actions are similar to, or even greater than, those of β -carotene.^{13,58} Recent work has shown that tocotrienol exerts a hypocholesterolemic effect, thereby reducing plasma levels of atherogenic apolipoprotein B and low-density lipoproteins.⁸¹ Further, the phytoestrogen biochanin A dose-dependently inhibits iNOS expression, inducible NO synthesis and the growth of the MCF-7 human breast cancer cell line.¹⁴ Also, glucosamine, which has been used to treat arthritis in humans and dogs, inhibits inducible NO synthesis by suppressing iNOS protein expression in activated macrophages and many tissues (e.g., liver and lung).⁸² Interestingly, a diet rich in brussels sprouts (300 g/d) markedly decreases the urinary excretion of 8-hydroxydeoxyguanosine in humans, indicating a reduction of DNA oxidation.⁸³ Similarly, dietary supplementation of cabbage and broccoli extracts to rats decreases free radical-induced tissue damage brought about by irradiation.⁸⁴ Moreover, phytic acid has a high chelation potential

and can be supplemented to diets for suppressing iron-catalyzed oxidative reactions and potentially for reducing the incidence of colonic cancer and inflammatory bowel disease.⁸⁵ Collectively, these studies suggest that phytochemicals may be used as effective antioxidants for improving human health and preventing carcinogenesis and cardiovascular disease.

PERSPECTIVES AND FUTURE DIRECTIONS

With the development of long-term manned space missions and permanent space habitats, the protection of astronauts from radiation injury has emerged as a crucial issue of biological safety in space travel.^{4,8,86} The adverse effects of long-term exposure to the space environment such as the very intense random solar energetic particles and the low-density heavy-ion flux of the galactic cosmic rays, which increase the risk of tissue injury, DNA damage, and cancer induction,^{3,5} are a major obstacle to human space exploration.⁸⁶ Because of the existing weight limitations of spacecraft and the expensive costs of shielding requirements,¹ developing an effective nutritional countermeasure against radiation may be beneficial for maintaining the health of astronauts during space flight and after their return to the land. An important concept that has emerged from this review is that dietary antioxidants and other nutrients play an important role in preventing cells from radical-induced cytotoxicity. However, it is prudent to recognize that excess levels of some well-known antioxidants may have potential hazardous effects. For example, an excess of dietary selenium results in toxicity in humans (loss of hair and nails, lesions of the skin and nervous system, and even death).⁷³ Also, dietary supplementation of high doses of vitamin C (260 mg/d) plus iron (14 mg ferrous sulfate/d) for 12 wk causes DNA oxidation in human leukocytes.⁸⁷ Thus, knowledge of free radical biology will serve as a guiding principle for establishing the most effective nutrition support to ensure the safety of manned space missions.

There are many basic and applied research problems in the areas of nutrition and antioxidants that are highly relevant to space flight. Examples of such studies would include 1) studies of the effects of microgravity and radiation on the metabolism of nutrients (e.g., amino acids, glucose, fatty acids, minerals, and vitamins) at molecular, cellular, and whole-body levels; 2) effects of microgravity and radiation on nutrient requirements by humans and animal models; and 3) the use of a mixture of antioxidant vitamins (e.g., vitamins E, C, and A) and other nutrients (e.g., arginine, citrulline, taurine, creatine, *N*-acetylcysteine, tea polyphenols, and ω -3 PUFA) in preventing oxidative stress in humans and animal models under space flight conditions.

Much of the data on free radical biology have been generated from in vitro studies and need to be verified in vivo.^{12-14,23,88} The recent advances in biochemistry and molecular biology techniques provide new, powerful tools for studying the expression of tissue antioxidant enzymes and for elucidating the mechanisms of the actions of antioxidants. For example, a number of knockout mice models, such as SOD, glutathione peroxidase, and eNOS null mice, have been developed to determine the specific roles of the defense systems under physiologic conditions.^{21,88} This is an exciting time for antioxidant research in the space station era. The future holds great promise for discoveries of new knowledge about free radical biology and for turning basic knowledge into practical use for ensuring space flight safety.

ACKNOWLEDGMENTS

The authors thank Tony Haynes for assistance in manuscript preparation and Frances Mutscher for office support. The excellent comments of Drs. Xin-Gen Lei, Joanne R. Lupton, Wilson G. Pond, and Nancy D. Turner on this manuscript are gratefully appreciated. Work in G. Wu's laboratory is supported, in part, by

grants from United States Department of Agriculture, American Heart Association, and Juvenile Diabetes Research Foundation. G. Wu is an Established Investigator of the American Heart Association.

REFERENCES

- Kovalev EE. Radiation protection during space flight. *Aviat Space Environ Med* 1983;54:S16
- Fang YZ. Effect of ionizing radiation on superoxide dismutase in vitro and in vivo. In: Fang YZ, (English edition). Beijing: Atomic Energy Press, 1991. *Advances in free radical biology and medicine. Vol 1:1*
- Ilyina-Kakueva EI, Portugalov VV. Combined effect of space flight and radiation on skeletal muscles of rats. *Aviat Space Environ Med* 1977;48:115
- Newberg AB. Changes in the central nervous system and their clinical correlates during long-term spaceflight. *Aviat Space Environ Med* 1994;65:562
- George K, Durante M, Wu H, et al. Chromosome aberrations in the blood lymphocytes of astronauts after space flight. *Radiat Res* 2001;156:731
- Weiss JF. Pharmacologic approaches to protection against radiation-induced lethality and other damage. *Environ Health Perspect* 1997;105(suppl 6):1473
- Lane HW, Schulz LO. Nutritional questions relevant to space flight. *Annu Rev Nutr* 1992;12:257
- Stein TP. Nutrition in the space station era. *Nutr Res Rev* 2001;14:87
- McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969;244:6049
- Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988;333:664
- Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:1397
- Gilbert DL. Fifty years of radical ideas. *Ann NY Acad Sci* 2000;899:1
- Fang YZ. Free radicals and nutrition. In: Fang YZ, Zheng RL, eds. *Theory and application of free radical biology*. Beijing: Scientific Press, 2002;647
- Wu G, Meininger CJ. Regulation of nitric oxide synthesis by dietary factors. *Annu Rev Nutr* 2002;22:61
- Fang YZ, Sun CP, Tian XH. Effect of Lu-Duo-Wei on scavenging superoxide and hydroxyl radicals in vitro. *Am J Chin Med* 1998;26:153
- Jackson MJ. An overview of methods for assessment of free radical activity in biology. *Proc Nutr Soc* 1999;58:1001
- Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. *Br J Nutr* 2001;85(suppl 2):S67
- McCord JM. The evolution of free radicals and oxidative stress. *Am J Med* 2000;108:652
- Zheng M, Storz G. Redox sensing by prokaryotic transcription factors. *Biochem Pharmacol* 2000;59:1
- Lander HM. An essential role for free radicals and derived species in signal transduction. *FASEB J* 1997;11:118
- Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* 1999;34:879
- Fridovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann NY Acad Sci* 1999;893:13
- Wu G, Morris SM, Jr. Arginine metabolism: nitric oxide and beyond. *Biochem J* 1998;336:1
- Wu G, Flynn NE, Flynn SP, Jolly CA, Davis PK. Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. *J Nutr* 1999;129:1347
- Rassaf T, Preik M, Kleinbongard P, et al. Evidence for in vivo transport of bioactive nitric oxide in human plasma. *J Clin Invest* 2002;109:1241
- Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 1999;27:916
- Wu G. Intestinal mucosal amino acid catabolism. *J Nutr* 1998;128:1249
- Lawler JM, Barnes WS, Wu G, Song W, Demaree S. Direct antioxidant properties of creatine. *Biochem Biophys Res Commun* 2002;290:47
- Machilin LJ, Bandito A. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J* 1987;1:441
- Wu G, Meininger CJ. Arginine nutrition and cardiovascular function. *J Nutr* 2000;130:2626
- Lass A, Suessenbacher A, Wolkart G, Mayer B, Brunner F. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. *Mol Pharmacol* 2002;61:1081
- Akashi K, Miyake C, Yokota A. Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. *FEBS Lett* 2001;508:438
- Redmond HP, Wang JH, Bouchier-Hayes D. Taurine attenuates nitric oxide- and reactive oxygen intermediate-dependent hepatocyte injury. *Arch Surg* 1996;131:1280

34. Dempster WS, Sive AA, Rosseau S, Malan H, Heese HV. Misplaced iron in Kwashiorkor. *Eur J Clin Nutr* 1995;49:208
35. Brooks SE. Ultrastructure of voluntary muscle in childhood malnutrition. *West Indian Med J* 1995;44:133
36. Jeor STS, Howard BV, Prewitt TE, et al. Dietary protein and weight reduction. A statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Circulation* 2001;104:1869
37. Mohanty P, Ghanim H, Hamouda W, et al. Both lipid and protein intakes stimulate generation of reactive oxygen species by polymorphonuclear cells leukocytes and mononuclear cells. *Am J Clin Nutr* 2002;75:767
38. Henning B, Toborek M, McClain CJ. High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. *J Am Coll Nutr* 2001;20:97
39. Wan GH, Ohnmi S, Kato N. Increased hepatic activity of inducible nitric oxide synthase in rats fed on a high-fat diet. *Biosci Biotechnol Biochem* 2000;64:555
40. Haegele AD, Briggs SP, Thompson HJ. Antioxidant status and dietary lipid unsaturation modulate oxidative DNA damage. *Free Radic Biol Med* 1994;16:111
41. Sreekumar R, Unnikrishnan J, Fu A, et al. Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. *Am J Physiol Endocrinol Metab* 2002;282:E1055
42. Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 2001;73:673
43. Takahashi M, Tsuboyama-Kasao N, Nakatani T, et al. Fish oil alters liver gene expression to defend against PPAR- α activation and ROS production. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G338
44. Khair-Eldin T, Sicher SC, Vazquez MA, et al. Transcription of the murine iNOS gene is inhibited by docosahexaenoic acid, a major constituent of fetal and neonatal sera as well as fish oil. *J Exp Med* 1996;183:1241
45. Ohata T, Fukuda K, Takahashi M, et al. Suppression of nitric oxide production in lipopolysaccharide-stimulated macrophage cells by ω -3 polyunsaturated fatty acids. *Jpn J Cancer Res* 1997;88:234
46. Hirokawa K, Oshaughnessy KM, Ramrankha P, Wilkins MR. Inhibition of nitric oxide synthesis in vascular smooth muscle by retinoids. *Br J Pharmacol* 1994;113:1448
47. Grosjean S, Devaux Y, Seguin C, et al. Retinoic acid attenuates inducible nitric oxide synthase (NOS2) activation in cultured rat cardiac myocytes and microvascular endothelial cells. *J Mol Cell Cardiol* 2001;33:933
48. Datta PK, Lianos EA. Retinoic acids inhibit inducible nitric oxide synthase expression in mesangial cells. *Kidney Int* 1999;56:486
49. Garcion E, Nataf S, Berod A, Darcy F, Brachet P. 1,25-Dihydroxyvitamin D-3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. *Mol Brain Res* 1997;45:255
50. Gurujeyalakshmi G, Wang YJ, Giri SN. Suppression of bleomycin-induced nitric oxide production in mice by taurine and niacin. *Nitric Oxide Biol Chem* 2000;4:399
51. Sano M, Fujita H, Morita I, Uematsu H, Murota S. Vitamin K-2 (menatetrenone) induces iNOS in bovine vascular smooth muscle cells: no relationship between nitric oxide production and γ -carboxylation. *J Nutr Sci Vitam* 1999;45:711
52. Murakami A, Nakashima M, Koshiba T, et al. Modifying effects of carotenoids on superoxide and nitric oxide generation from stimulated leukocytes. *Cancer Lett* 2000;149:115
53. Topinka J, Bincova B, Sram RJ, Erin AN. The influence of α -tocopherol and pyritinol on oxidative DNA damage and lipid peroxidation in human lymphocytes. *Mutat Res* 1989;225:131
54. Chow CK, Reddy K, Tappel AI. Effect of dietary vitamin E on the activity of glutathione peroxidase in vitro and in vivo studies. *J Clin Invest* 1969;48:1957
55. Muller DP. Antioxidant therapy in neurological disorders. *Adv Exp Med Biol* 1990;264:475
56. Carr AC, Zhu BZ, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α -tocopherol (vitamin E). *Circ Res* 2000;87:349
57. Yokota T, Igarashi K, Uchiyama T, et al. Delayed-onset ataxia in mice lacking α -tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. *Proc Natl Acad Sci USA* 2001;98:15185
58. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. *J Am Chem Soc* 1998;75:199
59. Fischer-Nielsen A, Poulsen HE, Loft S. 8-Hydroxydeoxyguanosine in vitro: effects of glutathione, ascorbate, and 5-aminosalicylic acid. *Free Radic Biol Med* 1992;13:121
60. Fang YZ, Wang PF, Lai YF, et al. Effect of γ -irradiation on the metabolism of ascorbic acid. *Acta Nutr Sinica* 1982;9:31
61. Lai YF, Fang YZ, Wang R, et al. Studies on the effect of vitamin B₁₂ and folic acid on radiation damage. 1. Urinary excretion of formiminoglutamic acid. *Acta Nutr Sinica* 1982;4:145
62. Hu B, Huang SF, Fang YZ, et al. The effects of vitamin B₁₂ and folic acid on radiation damage. 2. The level of folate in rat blood. *Acta Nutr Sinica* 1983;5:161
63. Fang YZ, Lai YF, Cao WQ. The effect of vitamin B₁₂ and folic acid on radiation damage. 3. Nitrogen metabolism. *Acta Nutr Sinica* 1983;5:347
64. Fang YZ, Lai YF, Bin Hu B, et al. Studies on the effect of vitamin B₁₂ and folic acid on radiation damage. 4. Body weight, leucocyte counts and mortality of rats. *Acta Nutr Sinica* 1984;6:59
65. Hinrichsen LI, Floyd RA, Sudilovsky O. Is 8-hydroxydeoxyguanosine a mediator of carcinogenesis by a choline-devoid diet in the rat liver? *Carcinogenesis* 1990;11:1879
66. Wu G, Haynes TE, Li H, Yan W, Meininger CJ. Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. *Biochem J* 2001;353:245
67. Rock E, Astier C, Lab C, et al. Dietary magnesium deficiency in rats enhance free radical production in skeletal muscle. *J Nutr* 1995;128:1205
68. Mickel HS, Oliver CN, Starke-Reed PE. Protein oxidation in magnesium deficiency rat brains and kidneys. *Biochem Biophys Res Commun* 1993;196:596
69. Dabbagh AJ, Mannion T, Lynch SM, et al. The effect of iron overload on rat plasma and liver oxidant status in vitro. *Biochem J* 1994;300:799
70. Hammermueller JD, Bray TM, Bertger WJ. Effect of zinc and copper deficiency on microsomal NADPH-dependent active oxygen generation in rat lung and liver. *J Nutr* 1984;117:894
71. Wepnir RA. Zinc deficiency, malnutrition and the gastrointestinal tract. *J Nutr* 2000;130:1388S
72. Xia Y, Hill KE, Burk RF. Effect of selenium deficiency on hydroperoxide-induced glutathione release from the isolated perfused rat heart. *J Nutr* 1985;115:733
73. Yang GQ, Wang SZ, Zhou RH, Sun SZ. Endemic selenium intoxication of humans in China. *Am J Clin Nutr* 1983;37:872
74. Decker EA. The role of phenolics, conjugated linoleic acid, carnosine and pyrrol-quinolinequinone as nonessential dietary antioxidants. *Nutr Rev* 1995;53:49
75. Voudin KA, Shukitt-Hale B, Mackonnons, Kalt W, Joseph JA. Polyphenols enhance red blood cell resistance to oxidative stress in vitro and in vivo. *Biochim Biophys Acta* 2000;1523:117
76. Fang YZ, You YG, Chen GM, et al. Clinical observation on health care effects of tea polyphenol compound (Lu-Duo-Wei): prevention of coronary heart disease, hypertension and type II diabetes. Paper presented at the Symposium on Tea and Anticarcinogenesis, Shanghai, 1998;40
77. Fang YZ, Chen GM, Chen AM, et al. Effect of tea polyphenol compound (Lu-Duo-WEI) on inhibition of tumor growth. Paper presented at the Symposium on Tea and Anticarcinogenesis, Shanghai, 1998;38
78. Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis* 1998;19:611
79. Cui X, Dai XG, Li WB, Zhang BL, Fang YZ. Effects of Lu-Duo-Wei capsules on superoxide dismutase activity and contents of malondialdehyde and lipofuscin in the brain of the housefly. *Am J Chin Med* 2000;28:259
80. Cui X, Dai XG, Li WB, Zhang BL, Fang YZ. Effects of Lu-Duo-Wei capsules on prolonging life span of housefly and *Drosophila melanogaster*. *Am J Chin Med* 1999;27:407
81. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K. Tocotrienol. A review of its therapeutic potential. *Clin Biochem* 1999;32:309
82. Meininger CJ, Kelly KA, Li H, Haynes TE, Wu G. Glucosamine inhibits inducible nitric oxide synthesis. *Biochem Biophys Res Commun* 2000;279:234
83. Verhagen H, Poulsen HE, Loft S, et al. Reduction of oxidative DNA-damage in human by brussels spouts. *Carcinogenesis* 1995;16:969
84. Fang YZ, Lai YF, Hu B, et al. Protective effect of liver, cabbage and yeast on radiation damage. *Acta Nutr Sinica* 1987;9:150
85. Graf E, Eaton JW. Antioxidant functions of phytic acid. *Free Radic Biol Med* 1990;8:61
86. Simonsen LC, Wilson JW, Kim MH, Cucinotta FA. Radiation exposure for human Mars exploration. *Health Phys* 2000;79:515
87. Rehman A, Collis CS, Yang M, et al. The effects of iron and vitamin C co-supplementation on oxidative damage of DNA in healthy volunteers. *Biochem Biophys Res Commun* 1998;246:293
88. Lei XG. In vivo antioxidant role of glutathione peroxidase: evidence from knockout mice. *Methods Enzymol* 2002;347:213