Oxidative damage and defense

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ABSTRACT Increased production of reactive oxygen species is a feature of most, if not all, human disease, including cardiovascular disease and cancer. Dietary antioxidants may be especially important in protecting against human diseases associated with free radical damage to cellular DNA, lipids, and proteins. Ascorbic acid is an effective water-soluble antioxidant, and epidemiologic studies suggest that increased ascorbate nutrition is associated with reduced risk of some degenerative diseases, especially cancer and eye cataracts. Population studies have also shown that high vitamin E intakes are associated with decreased risk of coronary heart disease, possibly as a result of inhibition of atherogenic forms of oxidized low-density lipoprotein. Recent data suggest that β-carotene provides protection against lipid peroxidation in humans, as well as provitamin A activity. Yet, present data are not sufficient to quantitate micronutrient requirements needed to protect against oxidative damage. The antioxidant roles of many food constituents, such as polyphenols, have not been clarified. Most antioxidants can act as prooxidants under certain conditions, and more research is needed to determine the occurrence and importance of this in vivo. The few controlled intervention trials carried out so far have shown mixed results as to the potential of antioxidant supplements for reducing the incidence of chronic diseases. Definitive recommendations on antioxidant intake for disease prevention must await evidence from controlled studies and intervention trials, some currently in progress. Overall, the present data suggest that protection against oxidative damage and related disease is best served by the variety of antioxidant substances found in fruit and vegetables. Am J Clin Nutr 1996;63:985S–90S.

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REACTIVE OXYGEN SPECIES: ORIGINS AND CONSEQUENCES

(This section reflects oral presentation by B Halliwell, 1994.) Free radicals (such as superoxide, O₂⁻; nitric oxide, NO', and hydroxyl, OH') as well as other oxygen-derived species (such as hydrogen peroxide, H₂O₂, and hypochlorous acid, HOCl) are formed constantly in the human body. In addition, the body is exposed to oxidizing air pollutants such as ozone, oxides of nitrogen, tobacco smoke, and motor vehicle exhaust. A variety of antioxidant defenses, including enzymes, have evolved to protect against these reactive oxygen species, but these defenses are not completely efficient. Hence, there is ongoing oxidative damage to DNA, lipids, and proteins in the human body. Indeed, the availability of methods to measure this damage provides a tool to investigate the effects of dietary antioxidant nutrients in vivo. Such antioxidant nutrients, which may include carotenoids, vitamins C and E, flavonoids, and other plant phenolics may be especially important in protecting against human disease. Antioxidants are also used in the food industry, eg, to protect food lipids against oxidative damage. Increased production of reactive oxygen species is a feature of most, if not all, human disease, including cardiovascular disease and cancer. Cells can respond to mild oxidative stress by up-regulating antioxidant defenses and other protective systems, but severe stress can damage DNA, proteins, and lipids and lead to cell transformation or cell death by apoptotic or necrotic mechanisms (1–3).

ANTIOXIDANT PROTECTION AND VITAMIN C REQUIREMENTS

(This section reflects oral presentation by RA Jacob, 1994.) Ascorbic acid provides in vivo antioxidant protection primarily as an aqueous phase peroxyl and oxygen radical scavenger; it is concentrated in tissues and fluids with a high potential for radical generation, such as the eye, brain, liver, lung, heart, semen, and leukocytes. The oxidized or “spent” form of the vitamin (dehydroascorbic acid) is readily converted back to the reduced form by reduced glutathione, NADPH, or both. Some evidence suggests that ascorbate protects against lipid peroxidation by regenerating the reduced form of α-tocopherol, the primary lipid-phase antioxidant (4, 5); however, not all data support this role for ascorbate (6) and it has not yet been shown to be significant in humans. In vitro studies have shown a substantial contribution of ascorbate to the total antioxidant protection in plasma. In one study, ascorbate made up 0–24% of the total peroxyl radical trapping capacity of plasma (the largest contributions were from urate and protein thiols) (7). In another study, the order of antioxidant protection against low-density lipoprotein (LDL) lipid peroxidation was as follows: ascorbate = protein thiols > bilirubin > urate > α-tocopherol (8).

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Vitamin C intake and human disease

Oxidative damage mediated by free radicals has been associated with a wide variety of human disease conditions. It is difficult to show clear cause-and-effect relations between the diseases and antioxidant status because oxidative damage is subtle and difficult to measure, and the associated diseases develop slowly over many years. Evidence suggests that antioxidant protection from vitamin C may reduce the risk or slow development of certain diseases, including cancer, coronary heart disease, and age-related eye and neurodegenerative diseases. Epidemiologic studies consistently show strong correlations between fruit and vegetable intake and reduced risk of disease. Hence, dietary guidelines, such as those from the US Department of Agriculture, the National Cancer Institute, and the American Heart Association, recommend five or more daily servings of fruit and vegetables to reduce the risk of heart disease and certain kinds of cancer. The problem remains that associations of certain nutrient intakes (and even plasma vitamin concentrations) with reduced disease risk do not prove that the relation is causal. Thus, controlled intervention trials in human subjects are needed to provide convincing evidence that increased intakes of vitamin C, or other micronutrients, would by themselves or in combination reduce the incidence of disease.

Substantial epidemiologic evidence indicates that higher ascorbate intakes are associated with reduced risk of developing cancer, particularly cancer of the stomach, esophagus, or oral cavity (9). Ascorbate inhibits the in vivo formation of carcinogenic nitrosamines, especially in the stomach. Yet, results from the 6-y micronutrient intervention trial in Linxian, China, showed no benefit from a supplement of vitamin C and molybdenum in a population with a high incidence of esophageal and stomach cancers (10). Although in vitro studies suggest ascorbate to be an effective antioxidant against plasma lipid peroxidation, results from the Nurses and Health Professionals Studies showed vitamin E and not vitamin C consumption to be associated with reduced coronary disease (8, 11–13). A review summarized the evidence linking vitamin C to reduced heart disease risk as largely circumstantial but suggestive of an association (14). Similarly, the evidence linking increased ascorbate intakes to reduced occurrence of cataract and age-related macular degeneration is suggestive, but not wholly consistent or conclusive. In recent studies, dietary carotenoids or a combination of antioxidants showed stronger associations with reduced risk for these eye disorders than did vitamin C (15–17).

Recommended intakes of vitamin C

Worldwide recommended intakes for vitamin C are 30–100 mg/d, based on maintenance of adequate body reserves that preclude classical scurvy symptoms. Present data suggesting specific antioxidant-related health benefits from higher consumption of vitamin C alone are not sufficiently convincing to suggest an increase in recommended intakes. Among many dietary and physiologic antioxidant systems, there is no compelling reason to consider vitamin C to be especially important. The glutathione system seems to be of much greater importance for antioxidant protection because its regeneration derives ultimately from glucose; it is under enzymatic regulation via glutathione reductase and glutathione peroxidase, and it regenerates other antioxidants in vivo, including ascorbate (18). The dietary intake of other antioxidants besides ascorbate, such as carotenoids and polyphenol-type compounds, exceeds that of ascorbate, yet their relative contributions to overall antioxidant protection are largely unknown. Although higher vitamin C intakes may be recommended for certain groups such as smokers and those under a variety of stresses, the present data suggest that antioxidant protection of the population at large is best served by the variety of antioxidant substances found primarily in fruit and vegetables. Hence, a public health strategy for antioxidant protection seems to be better served by dietary guidelines than by changes in recommended intakes of specific nutrients.

HOMOCYSTEINE, A PUTATIVE PROXIDANT, AND FOLATE REQUIREMENTS

(This section reflects oral presentation by ME Swendsen, 1994.) Data from human studies suggest that plasma homocysteine is a sensitive, functional measure of folate deficiency and that the current recommended dietary allowance (RDA) for folic acid of 200 µg/d (19) may be insufficient to preclude moderate elevations of plasma homocysteine in the US population. Several recent studies have shown that hyperhomocysteinemia is an independent risk factor for occlusive heart disease (20–22). The mechanism by which hyperhomocysteinemia influences the atherosclerotic process has not yet been clarified but one hypothesis based on cell-culture studies is that homocysteine acts as a prooxidant (23). A copper-catalyzed oxidation of homocysteine with the generation of hydrogen peroxide has been observed in endothelial cells. This process has also been shown to promote oxidation of LDL, additional evidence that homocysteine functions as a prooxidant. Under normal conditions, homocysteine, a product of the demethylation of S-adenosylmethionine, is remethylated intracellularly to methionine by reactions requiring either betaine or S-methyltetrahydrofolate (with the coenzyme vitamin B-12) as methyl donors (24). Alternatively, homocysteine is condensed with serine to form cystathionine in a vitamin B-6-catalyzed, irreversible reaction and further metabolized, eventually to sulfate. Among the dietary factors associated with the development of hyperhomocysteinemia, folate deficiency has been studied most extensively. Plasma homocysteine concentration is markedly increased in cases of folate deficiency and also in subjects with low-normal concentrations of plasma folate (25, 26). Plasma homocysteine is reduced after folate administration. Several investigators have suggested that plasma homocysteine concentrations may be useful as an indicator of folate status.

To determine the dietary folate intake that prevents elevated plasma homocysteine concentrations, folate in amounts equivalent to 13%, 84%, and 220% of the current RDA of 200 µg/d was fed over a 108-d period to 10 healthy males residing in a metabolic unit (27). Thirteen percent of the RDA was provided by a low-folate diet that included soybean protein, oil, and crystalline amino acids. This diet was supplemented with pteroylglutamic acid to attain 84% of the RDA, and the diet providing 220% of the RDA for folate consisted of foods typical of the US diet. Whereas all 10 subjects had normal
homocysteine concentrations initially (< 14 μmol/L; range: 6–12 μmol/L), 4 subjects developed high homocysteine concentrations (15–21 μmol/L) after 4 wk of the low-folate diet. These high concentrations persisted even after 2 wk of intake of 84% of the RDA, but decreased to normal (< 14 μmol/L) within 9 d of consumption of the high-folate diet. Mean red blood cell folate concentrations were not reduced over the course of the study. These results indicate that hyperhomocysteinemia may provide a sensitive indicator of folate status. They also suggest that the current folate RDA for adult males may not provide the expected margin of protection against hyperhomocysteinemia.

**ANTIOXIDANT NUTRIENTS AND CANCER: RESULTS OF RECENT INTERVENTION TRIALS**

(This section reflects oral presentation by D Alberts, 1994.) Cancer causes more than half a million deaths per year in the United States, and if trends continue will be a major cause of death by the year 2000. Most cancers are believed to arise during a long-term process that begins with the somatic mutation of a normal cell and proceeds by the replication and clonal expansion of this genetically altered cell to form a benign tumor. Further initiation or mutation can result in progression to malignancy. Small studies (on < 500 patients) suggested that several nutrients are effective in delaying cancer initiation or progression. Successful interventions include the use of high doses of retinyl palmitate on non-small-cell lung cancer and of 13-cis retinoic acid (Accutane; Roche Laboratories, Nutley, NJ) on oral leukoplasia and head and neck cancer (28). However, these analogues have toxic side effects, including dry skin, calcification of spinal ligaments, and teratogenicity. Vitamin E appears to prevent recurrence of oral leukoplasia without the toxic side effects (29), but β-carotene has been less successful (30). The success of these and other chemoprevention studies has led to several phase III chemoprevention trials involving antioxidant nutrients. Phase III trials are large, randomized, placebo-controlled, long-term studies of the efficacy of pharmacologic agents in preventing cancer initiation or replication.

The results of two phase III trials in Linxian, China, and Finland with the antioxidants β-carotene and vitamin E were reported recently. In the Linxian Study (10), cancer risk in a poor Chinese population with low intakes of several nutrients was investigated. Nearly 30 000 people from the general population were supplemented with retinol and zinc, riboflavin and niacin, vitamin C and molybdenum, or β-carotene, vitamin E, and selenium in a 2 × 4 factorial design (including placebo) for 5.5 y. Vitamin supplement doses were one to two times the US RDA (26). Stomach cancer [relative risk (RR) = 0.79] and mortality (RR = 0.91) were significantly reduced in the group receiving β-carotene, vitamin E, and selenium. In the study in Finland, risk was investigated in elderly male long-term smokers through use of high doses of vitamin supplements (50 mg vitamin E and 20 mg β-carotene/d) for 5 y (31). Vitamin E supplementation appeared to have no effect on cancer risk or mortality. β-Carotene appeared to increase the risk of lung cancer.

There are several lessons to be learned from these results. First, the pharmacology of the nutrient being studied must be completely understood. Antioxidants may be double-edged swords. For example, β-carotene is an antioxidant under normal physiologic conditions (low oxygen pressure and low concentration), but it has been reported to be a prooxidant at high concentrations and oxygen pressures (32, 33). Therefore, the high β-carotene concentrations used in the Finnish study may have enhanced oxidative damage in the lung, where oxygen concentrations are high. Vitamin E may also be a double-edged sword regarding skin cancer because esterified forms (but not α-tocopherol) may be tumor promoters when used topically (34). Second, it may not be appropriate to formulate relatively short-term interventions for long-term (30–40 y), heavy smokers. Finally, a multiple-nutrient intervention is more likely to protect against cancer than intervention with a single nutrient. Five to eight servings of fruit and vegetables a day is probably the best approach.

**NEWLY APPRECIATED ANTIOXIDANTS IN FOODS**

(This section reflects oral presentation by B German, 1994.) The reaction of biological macromolecules with oxygen is a thermodynamically favored process—body cells and tissues owe their stability to a kinetic impediment of this reaction. Oxidative processes are also essential biological mechanisms. In fact, our bodies have developed many uses for the oxidative process, accomplishing specific purposes such as programmed cell death (apoptosis), phagocytosis, chemotaxis of immune cells, and signaling the basis for clotting in blood coagulation.

A relevant model for biological oxidation is LDL. These molecules are highly unstable because of their size, large surface area, and high concentration of easily oxidized molecules like polyunsaturated fatty acids. The instability of LDL is related to its important role in diseases such as heart disease. Therefore, antioxidants are essential to life. Oxidation is not a single event, it is an elaborate process with many branches and perturbations where antioxidants can act. Antioxidants can act during initiation or at propagation during the process. When and where one intervenes in this process has significant effects on the rate and products of oxidation. The topology of the molecules involved is also very important.

Dealcoholized wine is a good antioxidant, comparable with α-tocopherol, that inhibits oxidation in the physiologic range of 10 μmol/L. Many molecules found in wines (quercitins, polyphenolics, and flavonoids) have hydroxyl-system structures that are effective in inhibiting oxidation. Flavonoids are potent inhibitors of free radicals. Flavonoids and polyphenols are found in many fruit and vegetables, as well as in wine; both their concentrations and relative compositions in fruit, vegetables, and wines are variable. The structure and specificities of these flavonoids and polyphenolics are great, with differences in orders of magnitude.

The risk of many diseases, including coronary disease, is related to fat in the diet. Fat is energy dense but a low-density source of nutrients, especially nutrients found in plant materials and phytochemicals. A high-fat diet may be unhealthy partly because of this lack of plant material in the diet, instead of because of fat itself. This might explain the so-called French paradox. Worldwide, as fat consumption increases, so does the risk of coronary disease, but France is an outlier. The French consume a diet that is both high in fat and high in plant...
phenolics (from wine). The antioxidant components of wine might attenuate the oxidative stress provided from their highfat diet. Thus, plant phenolics might be important parts of the antioxidant cascade (35).

ARE CAROTENES ESSENTIAL NUTRIENTS?

(This section reflects oral presentation by BJ Burri, 1994.) Carotenes are colored pigments found in yellow and green vegetables such as carrots, spinach, and sweet potatoes. Some carotenes, in particular β-carotene, are precursors of vitamin A. Currently, there is no RDA for carotenes, except as vitamin A precursors. However, many carotenes, including β-carotene and lycopene, are chain-breaking antioxidants and singlet oxygen quenchers in vitro. Thus, carotenes may function as part of the human antioxidant defense system.

Numerous well-designed epidemiologic studies have shown that people who report eating diets rich in carotene-containing foods have lower rates of cancer and heart disease than does the general population (36, 37). However, these studies are not able to conclusively separate the effects of carotenes from the effects of other substances (such as fiber, vitamin C, folate, and other phytochemicals) that may also be associated with low rates of cancer. Thus, epidemiologic studies show many promising correlations between high carotene consumption and improved health status, but they are fundamentally not capable of proving that carotene consumption is beneficial.

Two studies reported the influence of carotene-depleted diets on oxidative defense status. Both a metabolic unit study of adult women and a free-living study of adult men showed that carotene depletion was associated with up to a five-fold increase of plasma thiobarbituric acid–reactive substances, an index of oxidative damage (38, 39). The metabolic unit study also showed that carotene depletion was associated with deleterious changes in other indexes of antioxidant status and oxidative damage in platelets, red blood cells, and LDLS. For example, the production of hexanal, a compound associated with oxidative damage in LDL, showed a significant 19% decrease after carotene repletion (38). The results of the few studies in which high concentrations of β-carotene were fed to subjects are mixed. A supplement of 60 mg β-carotene/d for 2 mo had little or no beneficial effect on oxidative damage in healthy individuals (40). However, other studies suggested that β-carotene supplementation appears to increase immunologic activities (41).

In summary, current evidence suggests that low concentrations of dietary carotenes may be needed to inhibit oxidative damage and decrease oxidation susceptibility, but that β-carotene supplementation of healthy nonsmoking individuals does not seem to improve oxidative status significantly. This suggests that the amounts of carotenes needed for normal oxidative status are easily met by a variety of Western diets in healthy nonsmokers. An alternative but less likely possibility is that carotenes other than β-carotene are involved in the antioxidant defense system. Larger concentrations of β-carotene may be required for normal immunologic functions.

ANTIOXIDANT VITAMINS AND ATHEROSCLEROSIS: A STATUS REPORT

(This section reflects oral presentation by D Steinberg, 1994.) Whether dietary recommendations for nutrients should be made based on their antioxidant activity is a difficult and important problem. No one single proof is sufficient. A scientific basis, experimental and epidemiologic data, and clinical trials are all needed to justify a recommendation. A solid scientific basis exists for explaining how antioxidants might protect against atherosclerosis. The first lesion associated with atherosclerosis is the fatty streak, the result of an accumulation of LDL in macrophages (“foam cells”). The fatty streak is benign but leads to atherosclerotic plaque. Oxidized LDL is much more atherogenic than native LDL in many ways. It is taken up more avidly by macrophages, attracts monocytes, and stimulates release of macrophage colony-stimulating factor (42). Antioxidants can slow the progression of atherosclerosis in several species. Nine studies investigated the effects of antioxidants on atherosclerosis. The tests were conducted in rabbits and monkeys, and used probucol, beta-hydroxy toluene, and vitamin E as the antioxidants. Seven studies yielded positive results, most of which used the antioxidant drug probucol (42).

Epidemiologic studies showed that large vitamin E intakes are associated with decreased risks of coronary heart disease (12, 13). Vitamin C intake was also inversely related to cardiovascular disease and to total mortality in men (43). However, epidemiologic studies can never establish causal relations. Moreover, in these studies, increased vitamin supplement consumption was also associated with indexes of greater health consciousness such as decreased rates of smoking and increased amounts of exercise.

A recently reported clinical intervention trial in Finland, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (31), was designed to test for protection against lung cancer but data on heart disease were also recorded. No evidence of benefit was found with either vitamin E or β-carotene supplementation. However, the subjects were heavy long-term smokers who probably had extremely advanced coronary artery lesions and the intervention was probably too late. The only experimental evidence for the benefit of antioxidants is that they prevent or slow the progression of early fatty streaks and >5 y may be needed to see the effect of antioxidant interventions on clinical expressions of atherosclerosis. The vitamin E dose (50 mg/d) used in the study was probably much too low. Furthermore, there were no data on morbidity or the fate of new lesions. This study underscores the need for caution in making any recommendations for antioxidant supplements.

What is the current status? The benefits that antioxidants may confer must be known before they are recommended, and the benefits are not yet known. Standards of proof should not be abandoned because antioxidants may be of value. Antioxidants such as vitamin E will probably cause no harm, but there have been few long-term studies. Consideration must be given to the consequences of recommending antioxidants such as vitamin E before the proof is there. People may use vitamin E instead of making the more difficult changes such as decreasing dietary fat or quitting smoking. Judgment must be reserved until the data are in.
INFLUENCE OF EXERCISE ON OXIDATIVE BALANCE AND ANTIOXIDANT NUTRIENT REQUIREMENTS

(This section reflects oral presentation by W Evans, 1994.) Catabolic and anabolic processes that occur in skeletal muscle during and after exercise are under the influence of various mediators in which oxygen free radicals are major contributors. During exercise-induced oxidative stress, oxygen free radicals are released in muscles through mitochondrial oxidative phosphorylation or from inflammatory cells, which may trigger and stimulate a host of metabolic events that involve the antioxidant defense system. Activated oxygen species appear to play a key role in exercise-induced injury to muscle membrane components and in the associated alteration of lysosomal and mitochondrial enzyme activity. The magnitude of oxidative damage occurring after exercise is dependent on the rate of oxygen consumption and the dynamic balance of antioxidant and prooxidant cellular mechanisms. Exercise training, in addition to increasing muscle mass and oxidative capacity, improves immune function and mediators that are involved in muscle adaptation. Furthermore, exercise training increases enzymatic antioxidant defense against oxygen free radicals. However, although exercise training induces the production of the principal antioxidant enzymes, vitamin E is consumed by muscle and other tissues during increased physical activity. Vitamin E supplementation reduces the oxidative stress and lipid peroxidation induced by exercise and there is evidence that vitamin E requirements increase with exercise.

With increasing age, the balance between the antioxidant defense system and the deleterious action of oxygen radicals becomes more sensitive to physical stress. There is evidence that the age-related increases in antioxidant enzyme activity are not sufficient to compensate for increases in the rate of lipid peroxidation. This fragile balance implies that the elderly are more susceptible to oxidative muscle damage after the stress of muscle adaptation. Because the overall rate of lipid peroxidation in muscles of old age is higher than enzymatic antioxidant protection, there is increasing evidence that the elderly may benefit from dietary antioxidants.

REFERENCES