Annu. Rev. Nutr. 14:371–91

PHARMACOLOGY OF VITAMIN C

Howerde E. Sauberlich
Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama 35294

KEY WORDS: ascorbic acid, health, functions, medical uses

CONTENTS

INTRODUCTION

This review focuses on the potential pharmacological benefits of vitamin C. The majority of the scientific publications considered in this review appeared

0199-9885/94/0715-0371$05.00

between 1987 and 1993. Related reviews have also been published in recent years (10, 24, 44, 58, 68, 76, 112, 139, 150).

VITAMIN C DEFICIENCY

Scurvy is a consequence of a deficiency in vitamin C and was described in ancient writings of the Egyptians, Greeks, and Romans (26, 37). The disease, a scourge of early sea voyagers, was first encountered on the voyages of Vasco da Gama in 1498. Scurvy is characterized by bleeding gums, impaired wound healing, petechiae, perifollicular hemorrhage, anemia, arthralgia and joint effusions, fatigue, depression, and sudden death (26, 37, 137). It appears to result primarily from the decreased synthesis of collagen (11, 44), a major protein in the body dependent on vitamin C for its biosynthesis. However, this role of vitamin C does not explain all of the manifestations associated with scurvy.

Although overt vitamin C deficiency is seldom seen in the US, the Second National Health and Nutrition Evaluation Survey (NHANES II) found low plasma vitamin C levels among 3% of the population studied (174, 181). Men 25 years of age and older had a higher proportion of low levels than women. African-American men 55–74 years of age had the greatest percentage of low vitamin C levels (16%).

The First National Health and Nutrition Examination Survey (NHANES I) gathered nutrition information on 11,348 noninstitutionalized US adults 25–74 years of age. Examination of the vitamin C intake data obtained on this cohort using a standardized mortality ratio revealed a strong inverse relationship with increasing vitamin C intake for males and a weak inverse relationship for females for all causes of death (45). Interestingly, regression analyses indicated that the inverse relationship of total mortality to vitamin C intake was stronger and more consistent in this NHANES population than that of total mortality to serum cholesterol and dietary fat.

BIOCHEMICAL FUNCTIONS OF VITAMIN C

Antioxidant Properties

Ascorbic acid is probably the most effective, least toxic antioxidant identified in mammalian systems (54, 55, 57, 149). It is a water-soluble, chain-breaking antioxidant that reacts directly with superoxide, hydroxyl radicals, and singlet oxygen (10). In laboratory studies, ascorbic acid completely protected lipids in plasma and low-density lipoprotein (LDL) against peroxidative damage (54, 55, 57, 93). Ascorbic acid is superior to other water-soluble plasma antioxidants (uric acid and bilrubin) as well as to the lipoprotein-associated antioxidants (α-tocopherol, β-carotene, and lycopene).

Oxidative processes may damage DNA, enzymes, proteins, and other macromolecules (36, 72, 151, 156). Vitamin C is more effective than vitamin E in preventing oxidation by NO_2, whereas vitamin E appears more protective than vitamin C against O_3 (101). Vitamin C can also interact with the tocopheroxy radical to regenerate tocopherol (108, 135). Consequently, ascorbic acid may be important in protecting against oxidative stress-related diseases and degeneration associated with aging, including coronary heart disease, cataract formation, and cancer (16, 36, 66, 72, 111, 112, 131, 149, 151, 156). An intake of ~ 140 mg/day of ascorbic acid appears necessary to saturate the total body vitamin C pool (81).

Other Functions of Vitamin C

Vitamin C has numerous biologic functions (15, 44, 83). Foremost, it is essential for collagen synthesis and for glycosaminoglycan synthesis of proteoglycans (12, 117). Vitamin C also helps maintain various enzymes (e.g. certain monoxygenases and dioxygenases) in their required reduced forms (53, 61, 111, 112, 131). For example, ascorbic acid serves as a reductant for the iron prosthetic group of prolyl and lysyl hydroxylases (111, 112). The prolyl hydroxylases convert prolyl residues in collagen to hydroxyproline. Hydroxyproline enables collagen to form a triple-helical conformation in the body and to polymerize it into functional collagen fibers (12). Consequently, impaired collagen production associated with a vitamin C deficiency results in impaired wound healing and scurvy.

Additionally, ascorbic acid participates in the biosynthesis of carnitine (119) and neuroendocrine peptides (38, 42). Carnitine biosynthesis requires the participation of ε-N-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase, both of which require ascorbic acid as a cofactor. Ascorbic acid also serves as a cofactor for the dopamine β-hydroxylase and peptidylglycine α-amidating monooxygenase systems, which participate in the biosynthesis of norepinephrine and various α-amidated peptides. The many biochemical functions of vitamin C have been the subject of several recent reviews (11, 16, 25, 44, 77, 111, 112, 131, 149).

VITAMIN C PREPARATIONS

Vitamin C Supplements

Vitamin C is the most commonly available single supplement used in the US. Tablets providing 500 mg of vitamin C are the most widely available single-entity supplement (113, 145, 148, 159). For the first 3 months of 1993, sales of 34 million dollars were reported for vitamin C supplements. Ascorbic acid production is estimated to exceed 40,000 tons yearly (37). Vitamin C is used extensively in the food and feed industry, in pharmaceuticals, and in nonnutritional manufacturng activities. The epimer of L-ascorbic acid, erythorbic acid, is commonly used as a food additive, particularly in meats and beverages (141). However, erythorbic acid has little or no antiscorbutic activity for humans (140).

Derivatives of Vitamin C

L-ascorbate-2-sulfate is stored in fish and some shrimp. It has vitamin C activity for fish such as trout, salmon, and catfish. However, L-ascorbate-2-sulfate is ~ 20× more stable than ascorbic acid. Hence, L-ascorbate-2-sulfate has been used in the formulation of feeds fed these fish (43, 147).

Sodium ascorbate and ascorbyl palmitate reportedly have the same biological activity as ascorbic acid (147). However, L-ascorbate-2-phosphate is considerably more stable to air than L-ascorbic acid. This compound is an active source of vitamin C in the guinea pig and rhesus monkey. For the channel catfish, L-ascorbate-2-phosphate is equivalent, on a molar basis, to the activity of ascorbic acid (43). L-ascorbyl-2-polyphosphate has also been used in animal feeds (50). This form of vitamin C has considerable stability against oxidation.

BIOAVAILABILITY OF VITAMIN C

Information on the bioavailability of vitamin C in foods is limited (136). In general, ascorbic acid in fruits and vegetables is apparently readily available and absorbed. Ascorbic acid ingested as cooked broccoli, orange juice, or fruit or in synthetic form seemed equally bioavailable (98). Conversely, ascorbic acid in raw broccoli was 20% less bioavailable. In addition to cooking and food processing, which result in a loss of vitamin C, several other factors may influence the ascorbic acid content of food as consumed (153). Distribution of vitamin C may vary considerably within a single fruit or vegetable. Changes in content occur with maturation, postharvest storage, and cellular tissue damage. Samples of individual foods show a large range of vitamin C content even for those collected from the same source and the same regions of the country (171). Based on isotopic studies, supplemental vitamin C intakes of 100 mg/day or less are absorbed 80–90%.

POTENTIAL PHARMACOLOGICAL USES OF VITAMIN C

Vitamin C and Cataracts

Cataracts are the leading cause of blindness worldwide (179). The Framingham, Massachusetts study found a 15% prevalence of cataracts in people 55 years of age and older (94). The incidence rose to more than 45% in subjects over 75 years of age. Cataract extraction is the most frequently performed surgery among the elderly, accounting for one million operations annually (91).

Evidence indicates that antioxidants may play an important role in the etiology of senile cataract (24, 59, 87–89, 91, 97, 130, 157, 165, 172, 173). However, most of this evidence has been derived from epidemiologic studies (128, 162). Vitamin C has been of particular interest because of the high levels of ascorbic acid in the human lens (28, 130, 164, 173).

Subjects consuming less than 125 mg/day of ascorbic acid have a fourfold increase in risk of developing cataracts compared with persons consuming more than 500 mg/day (87). In a prospective study involving 50,528 US women, the risk for cataract was 45% lower among women who had taken vitamin C supplements for 10 or more years (73). The supplements most often used contain 250–500 mg of ascorbic acid. The results of this ongoing study appear to support the antioxidant hypothesis of cataract prevention.

The levels of ascorbic acid present in the lens may reflect the dietary intake of the vitamin. In a study of 42 patients scheduled for cataract lens surgery, supplementation with 2 g/day of ascorbic acid for 2–4 weeks resulted in a significant increase in ascorbic acid concentrations in the lens, aqueous humor, and plasma (164). The average lens and aqueous humor ascorbic acid concentrations ranged from 36–63 and 21–35 times the levels found in plasma, respectively (28, 164).

Other studies have also found a reduced risk of senile cataracts with an increased intake of vitamin C (73, 96, 129, 163). However, it is evident from many of the studies that factors other than vitamin C (e.g. vitamin E, carotenoids, and smoking) are also involved in cataract formation (34, 73, 96, 129, 163, 176). In fact, 20% of all cataract cases in the US have been associated with smoking (176).

Vitamin C supplementation may prove beneficial if elevated levels of ascorbic acid reduce the risk of cataract. Intervention trials evaluating this hypothesis are currently underway (164). Recently, the results of two nutrition intervention trials were reported (157). The trials were conducted in Linxian, China, a generally nutritionally deprived population. The objective of these trials was to determine whether selected vitamin/mineral supplements would affect the risk of developing age-related cataracts. Findings from the two trials suggest that vitamin/mineral supplements may decrease the risk of nuclear cataract formation. The design of the study did not warrant assignment of beneficial effects from a specific nutrient. The investigators stated that "Until the results from less nutritionally deprived populations are available, it would be premature to translate these findings into nutritional recommendations for the U.S. population." (157).

Vitamin C and Diabetes Mellitus

In diabetic patients, lower plasma levels of ascorbic acid and an altered turnover of ascorbic acid have been reported (5, 155). Moreover, normal human volunteers subjected to an i.v. glucose tolerance test exhibited significant decreases in the ascorbic acid concentrations in mononuclear and polymorphonuclear leukocytes (32, 114). The biological importance of these changes is unknown.

Vitamin C and Blood Pressure

High intakes of ascorbic acid have been reported to be associated with lower blood pressure (23, 49, 58, 83, 84, 106, 133, 134, 168). In a study conducted in Georgia on 168 healthy normatensive or asymptomatic hypertensive subjects, plasma concentrations of ascorbic acid were significantly inversely related to systolic and diastolic blood pressure (106). The results indicated that for a 100 μmol/liter increase in plasma vitamin C concentration, both the systolic and diastolic blood pressure decreased 8 mm Hg. This small inverse association of plasma vitamin C concentration and blood pressure corroborates the results of numerous other studies (23, 83–85, 110, 168).

Of these studies, the most extensive was the Kuopio Ischaemic Heart Disease Risk Factor Study conducted in Finland on 722 Finnish men 54 years of age (133, 134). Plasma ascorbic acid concentrations had a moderate, independent inverse relationship with resting blood pressure. However, these observations do not prove causality. Carefully conducted long-term intervention trials are necessary to determine whether vitamin C status is a factor in the development or treatment of hypertension. If ascorbic acid does play a role in the etiology of hypertension, its mechanism of action remains speculative at present.

Several small ascorbic acid supplementation trials reported a slight drop in blood pressure (95, 168). For the most part, however, the changes did not reach statistical significance. In another small study conducted in Georgia, 13 men and 8 women received one 500 mg vitamin C tablet twice daily for 4 weeks (49). After 4 weeks on the supplements, both the diastolic blood pressures and the systolic blood pressures were significantly lower than the values observed at the start of the study. Plasma concentrations of ascorbic acid were significantly inversely related to blood pressure.

A larger randomized field trial of vitamin C supplementation on blood pressure was conducted in the Boston area (84). This trial studied 417 nonsmoking subjects 20–60 years of age. Which ascorbic acid supplementation regimen was used is unclear since blood pressure results were related to concentrations of plasma vitamin C observed. Nevertheless, the results indicated that for each 30 μmol/liter increment in plasma ascorbic acid concentration, blood pressure levels were 1.9–5.5% lower, HDL-cholesterol levels 3.7–9.5% higher, and LDL-cholesterol levels 4.1% lower.

Another study conducted in the Boston area investigated the effect of vitamin C intake on blood pressure in the elderly (83–85). For this study, investigators interviewed 761 noninstitutionalized individuals 60–100 years of age and obtained information on their vitamin C intakes from diet records and questionnaires. The results revealed that individuals consuming 240 mg/day or more of vitamin C had an ~ 50% lower prevalence of elevated blood pressure (systolic >160 mm Hg; diastolic >100 mm Hg) compared with those consuming less than 60 mg/day. Although this study and others support an association of vitamin C with blood pressure, the limitations of epidemiologic and observational endeavors do not permit the establishment a causal relationship. Certainly, additional investigations are required in order to assign any clinical significance to these reports.

Vitamin C, Lipoproteins, and Coronary Heart Disease

Results of various studies support the hypothesis that the oxidative modification of LDL is an important process in the genesis of the atherosclerotic lesion (92, 107, 108). Hence, ascorbic acid, α-tocopherol, and β-carotene, micronutrients with antioxidant properties, could inhibit LDL oxidation and thereby prevent the progression of atherosclerosis (54, 60, 66, 92, 93, 149). Jialal & Grundy and Moore et al recently reviewed evidence supporting this concept (92, 105).

In general, the evidence linking vitamin C to cardiovascular disease is circumstantial but suggestive of an association between vitamin C and cardiovascular disease (168), which is certainly plausible. In addition to functioning as a plasma antioxidant, human studies indicate that ascorbic acid is involved in cholesterol metabolism, the synthesis of prostacyclin by the vessel wall endothelium, and the maintenance of vascular integrity (150).

A significant inverse correlation was observed between plasma ascorbic acid levels and coronary artery disease mortality in an epidemiological study (62, 64, 124). The Basel Prospective Study obtained blood samples from 2974 men (65). The samples were analyzed soon after collection for vitamins C and E and carotene. During 12 years of follow-up, 132 men died from ischemic heart disease and 31 died from cerebrovascular disease. Men with low plasma carotene concentrations had a significantly higher risk of death from ischemic heart disease (RR = 1.53). Men with low vitamin C concentrations (22.7 μmol/liter) also appeared to have an increased risk (RR = 1.25). However, men with low levels of both vitamin C and carotene had the highest risk of death from ischemic heart disease (RR = 1.96) (65). No significant association with cardiovascular risk was observed for vitamin E, an outcome that may have been related to the unusually high plasma E concentrations found in this population. Patients with atherosclerotic vascular disease exhibited significantly lower vitamin C levels in aorta compared with controls (41). However, these observations do not prove causality.

Results from a preliminary study involving 17 subjects indicated that acute smoking exerted an oxidative stress on plasma lipoproteins for which higher plasma levels of vitamin C or vitamin E had a protective effect (74). In the Edinburgh Artery Study of 1,592 adult men and women, dietary intake information was obtained via a dietary questionnaire (39). Higher intakes of vitamin C were significantly related to a lower ankle brachial pressure index among those who had smoked but not among nonsmokers.

Other investigations have examined the possible association between dietary or plasma ascorbic acid and plasma lipids (54, 60, 63, 66, 79, 90, 93, 122). The majority, but not all (2, 125), of these studies reported an apparent decreased risk of coronary heart disease with an increased consumption of vitamin C. However, epidemiological studies are often plagued by uncontrolled confounding variables and seldom establish causality.

A number of studies in recent years have reported a reduction in plasma cholesterol concentration following vitamin C supplementation (2). In contrast, other studies have failed to observe any influence of high doses of ascorbic acid on plasma cholesterol concentrations (2). More recently, the influence of dietary supplementation of 200 or 2000 mg/day of ascorbic acid on serum lipids was studied (2). Twenty-seven female long-stay hospital patients in Finland were investigated in a crossover design that permitted the use of each supplement level for a six-week period. The vitamin C supplements had no apparent effect on serum cholesterol, high-density lipoprotein (HDL)-cholesterol, or triglyceride levels.

The relationship between plasma vitamin C concentrations and the risk of angina pectoris was studied in a population of adult men from the Edinburgh, Scotland area. Plasma concentrations of vitamins C, E, and A and of carotene were determined for 110 cases of angina pectoris and 394 controls (123, 124). Plasma concentrations of vitamins C and E and of carotene were significantly inversely related to the risk of angina. The risk was greater with low vitamin E plasma levels than with low vitamin C plasma levels. These observations are interesting, but confirmatory studies are needed. Perhaps some populations with a high incidence of coronary heart disease should modify their diets to ensure an ample intake of vitamin C and vitamin E.

The Health Professionals Follow-Up Study involved 39,910 US males between the ages of 40 and 75 (125). In this prospective investigation, dietary information was obtained about all individuals through a food-frequency dietary questionnaire. No blood analyses were performed. During four years of follow-up, 667 cases of coronary heart disease were documented. Evaluation of the dietary data revealed a lower risk of coronary heart disease among men with higher intakes of vitamin E. In contrast, higher intakes of vitamin C were not associated with a lower risk of coronary heart disease. Unfortunately, plasma levels of vitamin C and vitamin E were not determined. Such information would have helped validate the estimated dietary intakes of vitamins C and E.

Vitamin C and Cancer

Epidemiologic evidence consistently and strongly indicates that vitamin C has a protective effect against a variety of human tumors (13, 16, 25, 40). Many different mechanisms have been proposed to explain the potential beneficial and protective effect of vitamin C (25, 40, 77). An important factor in cancer initiation is the oxidative and free-radical damage to DNA and cell membranes. As a water-soluble antioxidant, ascorbic acid traps free radicals and reactive oxygen molecules and reduces nitrite. For example, vitamin C blocks the formation of N-nitrosamines and nitrosamides, chemicals that induce cancer in experimental animals and probably in humans as well (25, 40, 44, 52, 77, 101, 102, 112, 144, 149, 152, 154, 160, 161).

Studies conducted since 1987 on the relationship of vitamin C and cancer were recently reviewed in depth (138, 139). We refer the reader to those articles for information on this interesting aspect of vitamin C metabolism. Other recent reviews also discuss the association between vitamin C and cancer, including earlier studies (13–15, 25, 40, 52, 77, 152). Nevertheless, a few comments on this subject seem appropriate.

Perhaps the most consistent epidemiological finding has been an association between high intakes of vitamin C or foods rich in vitamin C and a reduced risk of stomach cancer. As noted elsewhere, this relationship may have been mediated through the action of vitamin C in blocking the formation of nitrosamines and other carcinogens in the stomach.

Several studies have reported that low intakes of vitamin C are associated with an increased risk of cervical cancer. This relationship was especially apparent in smokers. The results of some studies suggest that, in addition to vitamin C, folate and carotenes may convey protective effects. Higher intakes of fruits and juices rich in vitamin C also reduced the risk of esophageal cancer. For example, one report documented a significant inverse correlation between the intake serum levels of vitamin C and the incidence of esophageal cancer.

Although results have not been consistent, some studies noted an inverse association between the risk of lung cancer and the intake of vitamin C. However, other studies observed little or no protective effect of vitamin C on the risk of lung cancer. Many of the studies had design problems that precluded a definitive link between lung cancer and vitamin C. Whether vitamin C is effective in reducing the incidence of breast cancer remains uncertain. Although a number of studies indicated that diets with high calculated vitamin

C intakes exert a protective effect, several other investigators found no such effect.

The protective effect of vitamin C against pancreatic cancer was equivocal. A few studies reported some protective effect, but dietary associations with this form of cancer have been difficult to investigate. Similarly, vitamin C was not associated with reproducible significant changes in the risk for colon, prostate, bladder, or ovarian cancers.

Epidemiologic data provide the main source of evidence for a protective effect of vitamin C against some cancers. However, the studies frequently indicate that in addition to vitamin C, other nutrients in the diet, such as vitamin E, carotenoids, and folacin, may participate in the protective effect. The importance of each as a protective factor is difficult to evaluate from epidemiologic information.

Vitamin C and Iron Metabolism

We have known for some years that ascorbic acid enhances the absorption of nonheme iron (104). Nonheme iron usually represents more than 90% of the iron in the diet (69, 70). Consequently, ascorbic acid is an important promotor of the absorption of dietary iron (180). The mechanism of action of vitamin C is apparently related to its ability to prevent the formation of insoluble and unabsorbable iron compounds or to reduce ferric to ferrous ions (70, 71). The maximum benefit seems to be attained with an intake of 50–100 mg of vitamin C (70, 71). The effects of ascorbic acid supplementation on iron absorption were studied in a group of iron-depleted young women (78). Iron absorption improved 11% with vitamin C supplementation. However, one must keep in mind that the subjects received 500 mg of ascorbic acid with each of three meals, or 1500 mg/day. Lower levels of ascorbate intake were not studied.

The inhibitory effects of phytates and tannic acid on nonheme iron absorption were studied in a group of 199 housewives in Johannesberg, South Africa (158). Phytate-containing maize bran or tannic acid was incorporated into bread at graded levels. Both phytate and tannic acid produced a marked dose-dependent inhibition of iron absorption. The inhibitory effects were overcome by supplementing each meal with 30–50 mg of ascorbic acid.

Clark et al described an unusual case involving a seven-year-old white male child with iron-deficiency anemia, folate deficiency, and scurvy (35). The anemia was refractory to iron supplementation until folate and ascorbic acid were included in the treatment regimen. This case exemplifies the multiple interactions among ascorbic acid, iron, and folate.

Vitamin C Supplementation and Physical Performance

Athletes continually search for a "magic" compound that may give them a competitive edge. Vitamin C is one ingredient that has been investigated for this purpose for many years (177, 178). A review of these studies nevertheless revealed no significant effect of vitamin C supplements on physical performance (152, 178).

Vitamin C and Parkinson's Disease

The cause of Parkinson's disease is unknown. One theory suggests that oxidant stress may contribute to neuron degeneration in patients (46). Accordingly, decreasing the formation of free radicals or increasing the scavenging of free radicals in the brain would slow progression of the disease.

An open-labeled pilot trial of high-dose ascorbic acid and α-tocopherol was conducted on 66 patients with early Parkinson's disease prior to levodopa therapy (46–48). The patients received 3.0 g/day of vitamin C and 3200 IU of vitamin E. The point at which levodopa became necessary for the patients treated with vitamins C and E was compared with that for another group of patients followed elsewhere who did not receive the antioxidant vitamins. Levodopa therapy was required two and a half years later in the group receiving the antioxidants. The results of this pilot study suggest that the antioxidants slowed the progression of Parkinson's disease. However, confirmatory controlled clinical trials using double blind randomization techniques are needed (48). Moreover, whether both vitamin C and vitamin E are required must be determined in order to obtain the indicated benefits. Of note, all of the patients in the trial eventually required levodopa or a dopamine agonist.

Vitamin C and Respiratory Symptoms

The possible role of vitamin C in the prevention and treatment of respiratory symptoms has been of interest for some time (103). Early studies failed to demonstrate a beneficial effect of large doses of ascorbic acid on asthma or airway constriction. More recent studies indicate that an increased intake of vitamin C may have a protective effect against the adverse effects of air pollution and may reduce the occurrence of respiratory symptoms such as bronchitis, wheezing, and respiratory tract infection (19–21, 101, 116, 146a, 175). Evaluation of data obtained on 2526 adults during the First National Health and Nutrition Examination Survey (NHANES 1) revealed that dietary vitamin C intake was positively and significantly associated with the level of pulmonary function ($P = 0.01$) (146b). However, these views are largely hypotheses that need to be investigated further and quantified under controlled conditions.

Chronic respiratory symptoms and reduced levels of pulmonary function have been associated with cigarette smoking (146a, 175). Although smoking increases the requirement for vitamin C, no systematic study has been conducted to elucidate the mechanism for the adverse effects of smoking on this vitamin (6, 9, 51b, 101, 109, 142). Evidence suggests that the decrease in vitamin C status in cigarette smokers may be due to the vitamin's role in inactivating the numerous oxidants in cigarette smoke (1, 22, 56, 101, 118).

Vitamin C and Immunity

The status of several vitamins reportedly influences immunity (29, 51a, 68). Vitamin A has proved particularly beneficial in protecting children in developing countries against diarrhea, respiratory diseases, and various infections (3, 17). In contrast, a review of the many trials led to the conclusion that supplements of vitamin C (1–2 g/day) appeared ineffective in preventing the common cold (7, 76, 169). However, evidence indicates that supplemental vitamin C may decrease the severity of symptoms and the duration of cold episodes (76, 169), a benefit that may be related to the antioxidant properties of ascorbic acid.

Because immunity declines with age, the benefits of nutritional supplementation have been studied in the elderly. However, epidemiological surveys have failed to establish a meaningful relationship between vitamin status and impairment of immunity in healthy subjects (30, 57, 67). In a double blind randomized study to assess the efficacy of a multivitamin supplement in preventing common infections, 218 noninstitutionalized, independent subjects over 60 years of age were recruited from the Nevers region of France (31). The treatment group received daily for four months a commercially available multivitamin tablet that contained 90 mg of ascorbic acid. Although the supplement improved the vitamin status of the supplemented groups over that of the control (placebo) group, no significant difference in the incidence of infections was noted between the two groups. These findings support the results of an earlier study (67). Similarly, partial depletion of vitamin C in healthy men under controlled conditions of a metabolic unit failed to produce immunological changes (80).

In contrast, the effect of combined dietary supplementation with vitamins A, E, and C on cell-mediated immune function was studied in a group of elderly long-stay patients (115). This controlled trial investigated 30 patients who had been hospitalized for more than 3 months. The patients were randomly assigned to receive for 28 days either a placebo or a supplement that provided daily 100 mg of vitamin C, 8,000 I.U. of vitamin A, and 50 mg of vitamin E. The vitamin supplement produced a significant improvement in cell-mediated immune function as reflected by an increase in the absolute number of T cells, T4 subsets, and T4 to T8 ratio. However, because a combined supplement was used, investigators could not determine which vitamin(s) was responsible for the improvement.

Adoptive immunotherapy used in cancer treatment involves the infusion of interleukin-2 (a lymphokine and T-cell activator) and activated lymphocytes (99, 100, 132). Lymphocytes are isolated from the patient and cultured in the presence of interleukin to generate lymphokine-actived killer cells. Because of the stressful nature of this treatment, vitamin C status was followed in a group of 15 treated patients (99, 100). Rapid and severe hypovitaminosis C occurred in the patients during the course of treatment. Plasma ascorbic acid became undetectable in 12 of the 15 patients, although 13 patients had normal concentrations of vitamin C at the outset of therapy. Plasma vitamin E concentrations remained normal throughout the therapy. The mechanism of action and the clinical significance of these changes remain speculative.

Idiopathic Thrombocytopenic Purpura

Idiopathic thrombocytopenic purpura is a relatively common illness of uncertain origin. Evidence indicates that it may be caused by the immune destruction of platelets. The most common treatment has been adrenocorticosteroid therapy or splenectomy. In a clinical trial, a group of refractory patients with idiopathic thrombocytopenic purpura was treated with ascorbic acid (18). The patients were given 2 g of ascorbic acid each morning for an indefinite period of time. Nine of eleven patients responded favorably while receiving the ascorbic acid supplements. Seven had a complete response. Patients have remained on the ascorbate supplementation for as long as two and a half years without any apparent adverse effects. Although the mechanism of action is uncertain, the degree of success should warrant additional studies to confirm these findings.

Sickle Cell Anemia

In patients with sickle cell anemia, sickle cell membranes are more oxidatively stressed than the membranes of normal cells (105), i.e. sickle cells spontaneously produce more reactive oxygen species, including hydrogen peroxide, superoxide anion, and hydroxy radical (105). In a study of 18 sickle cell patients, 50% had low leukocyte ascorbic acid levels consistent with a vitamin C deficiency (33). The investigators concluded that utilization of vitamin C had increased in these patients, since their dietary intakes of vitamin C appeared adequate. The clinical significance of these observations remains unknown, but confirmation studies are necessary.

VITAMIN C TOXICITY

Patients with glucose-6-phosphate dehydrogenase deficiency who undergo high-dose ascorbic acid therapy are at risk of developing hemolysis (121). Thus, although a 32-year old patient of Nigerian ancestry tolerated the i.v. administration 40 g of ascorbic acid three times weekly for a month along with a daily oral intake of vitamin C, acute hemolysis developed shortly after the i.v. dose was increased to 80 g (121).

Other examples of vitamin C toxicity are few (8, 75, 126, 127). This low number attests to the safety of ascorbic acid in view of its widespread use in supplements and as food additions. The average adult in the US ingests more than three times the recommended dietary allowance for vitamin C (i.e. >180 mg/day). At an intake of 180 mg of ascorbic acid, more than 75% is absorbed. Pharmacokinetic studies have previously indicated that the absorption capacity of the intestine appears to be attained with oral intakes of ~ 3 g/day of ascorbic acid. The kidney effectively eliminates excess absorbed ascorbic acid, thereby preventing any appreciable increase in tissue levels of vitamin C.

The average subject excretes ~ 15–30 mg/day of oxalate, ~ 50% of which is derived from vitamin C metabolism. With a daily intake of 5 g of ascorbic acid, urinary oxalate excretion increased only an average of 14.8 mg (143). Consequently, one may safely conclude that high intakes of vitamin C do not pose a risk for calcium oxalate stone formation in most healthy subjects, excluding those prone to kidney stones (167, 170). Investigators have postulated that these individuals have abnormal ascorbic acid metabolism or oxalate absorption. Hence, subjects with recurrent stone formation and patients with renal impairment or on chronic hemodialysis should avoid high doses of vitamin C (4, 27, 167, 170).

Patients receiving continuous ambulatory peritoneal dialysis or hemodialysis frequently have low plasma or whole-blood concentrations of vitamin C. Ascorbic acid supplements may be prescribed to improve their vitamin C status. Supplements of 50 mg/day of ascorbic acid restored normal whole-blood vitamin C levels without increasing plasma oxalate concentrations. However, administration of 500 mg/day of vitamin C for three weeks resulted in a significant rise in mean plasma oxalate concentrations (30.3 ± 3.5 to 48.4 ± 20.3 μmol/liter) (166). Other effects of vitamin C toxicity, such as systemic conditioning, vitamin B-12 deficiency, or hyperuricosuria, appear rare, unconfirmed, or unfounded (8, 75, 126, 127).

Several animal studies have reported an apparent antagonism of ascorbic acid toward copper metabolism and function. In a closely controlled study with 11 young male volunteers, intakes of 605 mg/day of ascorbic acid for three weeks resulted in a 21% reduction in the oxidase activity of serum (82). However, with the use of stable isotope ^{65}Cu, the ascorbic acid supplement neither depressed intestinal copper absorption nor altered overall body copper status. The clinical importance of this effect remains unknown.

SUMMARY

A better understanding of the functions of ascorbic acid would help clarify the magnitude of the influence of this vitamin on health-related conditions. Many of the purported benefits require confirmation as well as a knowledge of the mechanism of action. The majority of investigations of the association of vitamin C with various types of cancer, with cardiovascular risk, and with cataract formation were epidemiologic studies. Often it was not possible to discern whether the apparent protective effect was due to vitamin C, vitamin

E, or carotene, or to a combined effect of these nutrients or of additional factors. Human intervention trials may provide definitive and quantitative assessments of the role of vitamin C in health maintenance. We need to gain a more thorough understanding of the interactions of vitamin C with other nutrients, such as vitamin E and carotenoids, in order to appreciate the role of vitamin C in disease prevention. Investigators are increasingly recognizing the diverse functions of vitamin C in the body in addition to its role in collagen synthesis.

However, the functional consequences of these many important roles of vitamin C remain essentially unknown. Excluding scurvy, the health consequences of inadequate vitamin C status are not well characterized. Nonetheless, epidemiologic evidence suggests a role for vitamin C in cancer and heart disease as well as in a number of other diseases.

ACKNOWLEDGMENTS

This work was supported in part by the USPHS NIH grant 5P01-CA28103. We deeply appreciate the assistance of Ms. Janine Smith in the preparation of the manuscript.

Literature Cited

1. Anderson R. 1991. Assessment of the roles of vitamin C, vitamin E, and β-carotene in the modulation of oxidant stress mediated by cigarette smoke-activated phagocytes. *Am. J. Clin. Nutr.* 53:358S–361S
2. Aro A, Kyllastinen M, Kostiainen E, Gref CG, Elfving S, Uusitalo U. 1988. No effect on serum lipids by moderate and high doses of vitamin C in elderly subjects with low plasma ascorbic acid levels. *Ann. Nutr. Metab.* 32: 133–37
3. Arthur P, Kirkwood B, Ross D, Morris S, Gyapong J, et al. 1992. Impact of vitamin A supplementation on childhood morbidity in northern Ghana. *Lancet* 339:361–62
4. Balcke P, Schmidt P, Zazgornik J, Kopsa H, Haubenstock A. 1984. Ascorbic acid aggravates secondary hyperoxalemia on chronic hemodialysis. *Ann. Intern. Med.* 101:344–45
5. Banerjee A. 1982. Blood dehydroascorbic acid and diabetes mellitus in human beings. *Ann. Clin. Biochem.* 19:65–70
6. Barrett B, Gunter E, Wang M. 1991. Ascorbic acid status in women who smoke during pregnancy. *J. Am. Diet. Assoc.* 90:A15
7. Beisel WR. 1982. Single nutrients and immunity. *Am. J. Clin. Chem.* 35:417–68
8. Bendich A. 1992. Safety issues regarding the use of vitamin supplements. *Ann. N.Y. Acad. Sci.* 669:300–12
9. Bendich A. 1993. Lifestyle and environmental factors that can adversely affect maternal nutritional status and pregnancy outcomes. *Ann. N.Y. Acad. Sci.* 678:255–65
10. Bendich A, Machlin LJ, Scandurra O, Burton GW, Wayner DM. 1986. The antioxidant role of vitamin C. *Adv. Free-Radical Biol. Med.* 2:419–44
11. Berg RA, Kerr JS. 1992. Nutritional aspects of collagen metabolism. *Annu. Rev. Nutr.* 12:369–90
12. Bird TA, Schwartz NB, Peterkofsky B. 1986. Mechanism for the decreased biosynthesis of cartilage proteoglycan in the scorbutic guinea pig. *J. Biol. Chem.* 261:11166–72
13. Block G. 1991. Epidemiologic evidence

regarding vitamin C and cancer. *Am. J. Clin. Nutr.* 54:1310S–1314S

14. Block G. 1991. Vitamin C and cancer prevention: the epidemiologic evidence. *Am. J. Clin. Nutr.* 53:270S–282S
15. Block G. 1992. Vitamin C status and cancer. Epidemiologic evidence of reduced risk. *Ann. N.Y. Acad. Sci.* 669: 280–92
16. Block G. 1992. The data support a role for antioxidants in reducing cancer risk. *Nutr. Rev.* 50:207–13
17. Bloem MW, Wedel M, Egger RJ, Speek AJ, Schrijver J, et al. 1990. Mild vitamin A deficiency and risk of respiratory tract diseases and diarrhea in preschool and school children in northeastern Thailand. *Am. J. Epidemol.* 131:332–39
18. Brox AG, Howson-Jan K, Fauser AA. 1988. Treatment of idiopathic thrombocytopenic purpura with ascorbate. *Br. J. Haematol.* 70:341–44
19. Bucca C, Rolla G, Arossa W, Caria E, Elia C, et al. 1989. Effect of ascorbic acid on increased bronchial responsiveness during upper airway infection. *Respiration* 55:214–19
20. Bucca C, Rolla G, Farina JC. 1992. Effect of vitamin C on transient increase of bronchial responsiveness in conditions affecting the airways. *Ann. N.Y. Acad. Sci.* 669:175–87
21. Bucca C, Rolla G, Oliva A, Farina J-C. 1990. Effect of vitamin C on histamine bronchial responsiveness of patients with allergic rhinitis. *Ann. Allergy* 65: 311–14
22. Bui MH, Sauty A, Collet F, Leuenberger P. 1992. Dietary vitamin C intake and concentrations in the body fluids and cells of male smokers and nonsmokers. *J. Nutr.* 122:312–16
23. Bulpitt CJ. 1990. Vitamin C and blood pressure. *J. Hypertens.* 8:1071–75
24. Bunce GE. 1990. Nutritional factors in cataract. *Annu. Rev. Nutr.* 10:233–54
25. Byers T, Perry G. 1992. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annu. Rev. Nutr.* 12:139–59
26. Carpenter KJ. 1986. *The History of Scurvy and Vitamin C.* New York: Cambridge Univ. Press. 288 pp.
27. Chalmers AU, Cowley DM, Brown JM. 1986. A possible etiological role for ascorbate in calculi formation. *Clin. Chem.* 32:333–36
28. Chandra DB, Varma R, Ahmad S, Varma SD. 1986. Vitamin C in the human aqueous humor and cataracts. *Int. J. Vitam. Res.* 56:165–68
29. Chandra RK. 1981. Immunodeficiency in undernutrition and overnutrition. *Nutr. Rev.* 39:225–31
30. Chavance M, Herbeth B, Fournier C, Janot C, Vernhes G. 1989. Vitamin status, immunity and infections in an elderly population. *Eur. J. Clin. Nutr.* 43:827–35
31. Chavance M, Herbeth B, Lemoine A, Zhu B-P. 1993. Does multivitamin supplementation prevent infections in healthy elderly subjects? A controlled trial. *Int. J. Vitam. Nutr. Res.* 63:11–16
32. Chen MS, Hutchinson L, Pecordaro RE, Lee WY, Labbe RF. 1983. Hyperglycemia induced intracellular depletion of ascorbic acid in human mononuclear leukocytes. *Diabetes* 32:1078–81
33. Chiu D, Vichinsky E, Ho S-L, Liu T, Subin BH. 1990. Vitamin C deficiency in patients with sickle cell anemia. *Am. J. Pediatr. Hematol./Oncol.* 12:262–67
34. Christen WG, Manson JE, Seddon JM, Glynn RJ, Buring JE, et al. 1992. A prospective study of cigarette smoking and risk of cataract in men. *J. Am. Med. Assoc.* 268:989–93
35. Clark NG, Sheard NF, Kelleher JF. 1992. Treatment of iron-deficiency anemia complicated by scurvy and folic acid deficiency. *Nutr. Rev.* 50:134–37
36. Cross CE, Halliwell B, Borish ET, Psyor WA, Ames BN, et al. 1987. Oxygen radicals and human disease. *Ann. Intern. Med.* 107:526–45
37. Davies MB, Austin J, Partridge DA. 1991. *Vitamin C: Its Chemistry and Biochemistry.* Herts, U. K.: Royal Soc. Chem. Distrib. Centre. 154 pp.
38. Diliberto EJ Jr, Daniels AJ, Viveros OH. 1991. Multicompartmental secretion of ascorbate and its dual role in dopamine β-hydroxylation. *Am. J. Clin. Nutr.* 54: 1163S–1172S
39. Donnan PT, Thomson M, Fowkes FGR, Prescott RJ, Housley E. 1993. Diet as a risk factor for peripheral arterial disease in the general population: the Edinburgh Artery Study. *Am. J. Clin. Nutr.* 57:917–21
40. Dorgan JF, Schatzkin A. 1991. Antioxidant micronutrients in cancer prevention. *Hematol. Oncol. Clin. North Am.* 5(1):43–68
41. Dubick MA, Hunter GC, Gasey SM, Keen CL. 1987. Aortic ascorbic acid, trace elements, and superoxide dismutase activity in human aneurysmal and occlusive disease. *Proc. Soc. Exp. Biol. Med.* 184:138–43
42. Eipper BA, Mains RE. 1991. The role of ascorbate in the biosynthesis of neuroendocrine peptides. *Am. J. Clin. Nutr.* 54:1153S–1156S
43. El Naggar GO, Lovell RT. 1991. L-ascorbyl-2-monophosphate has equal anti-

scorbutate activity as L-ascorbic acid but L-ascorbyl-2-sulfate is inferior to L-ascorbic acid for channel catfish. *J. Nutr.* 121:1622–26

44. Englard S, Seifter S. 1986. The biochemical functions of ascorbic acid. *Annu. Rev. Nutr.* 6:365–406
45. Enstrom JE, Kanim LE, Klein MA. 1992. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 3:194–202
46. Fahn S. 1989. The endogenous toxic hypothesis of the etiology of Parkinson's disease and a pilot trial of high dosage antioxidants in an attempt to slow the progression of the illness. *Ann. N.Y. Acad. Sci.* 570:186–96
47. Fahn S. 1991. An open trial of high-dosage antioxidants in early Parkinson's disease. *Am. J. Clin. Nutr.* 53:380S–382S
48. Fahn S. 1992. A pilot trial of high-dose alpha-tocopherol and ascorbate in early Parkinson's disease. *Ann. Neurol.* 32: S128–S132
49. Feldman EB, Gold S, Greene J, Moran J, Xu G, et al. 1992. Ascorbic acid supplements and blood pressure: a four-week pilot study. *Ann. N.Y. Acad. Sci.* 669:342–48
50. Felton SP, Halver JE. 1989. Separation of three commercial forms of viatmin C (ascorbic acid, ascorbic-2-sulfate and ascorbate-2-polyphosphate) by HPLC. *Proc. Soc. Exp. Biol. Med.* 190:217–18

51a. Fidanza F, Sarchielli P, Jordan P, Luden E, Schalch W, Weimann BJ. 1991. Vitamin nutritional status and immunocompetence of elderly in Perugia (Italy): an epidemiological approach. *Int. J. Vitam. Nutr. Res.* 61:224–31

51b. Food and Nutrition Board, National Research Council. 1989. *Recommended Dietary Allowances*, pp. 115–24. Washington, D.C.: Natl. Acad. Press. 10th ed.

52. Forman D. 1991. The etiology of gastric cancer. *Int. Agency Res. Cancer Sci. Publ.* 105:22–32
53. Franceschi RT. 1992. The role of ascorbic acid in mesenchymal differentiation. *Nutr. Rev.* 50:65–70
54. Frei B. 1991. Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *Am. J. Clin. Nutr.* 54:1113S–1118S
55. Frei B, England L, Ames BN. 1989. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. USA* 86:6377–81
56. Frei B, Forte TM, Ames BN, Cross CE. 1991. Gas phase oxidants of cigarette smoke induced lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. *Biochem. J.* 277:133–38
57. Frei B, Stocker R, Ames BN. 1988. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. USA* 85:9748–52
58. Gaby SK, Singh VN. 1991. Vitamin C. In *Vitamin Intake and Health: A Scientific Review*, eds. SK Gaby, A Bendich, VN Singh, LJ Machlin, pp. 103–61. New York: Dekker
59. Garland DL. 1991. Ascorbic acid and the eye. *Am. J. Clin. Nutr.* 54:1198S–1202S
60. Gaziano JM, Manson JE, Buring JE, Hennekens CH. 1992. Dietary antioxidants and cardiovascular disease. *Ann. N.Y. Acad. Sci.* 669:249–59
61. Geesin JC, Hendricks LJ, Falkenstein PA, Gordin JS, Berg RA. 1991. Regulation of collagen synthesis by ascorbic acid: characterization of the role of ascorbate-stimulated lipid peroxidation. *Arch. Biochem. Biophysics.* 290:127–32
62. Gey KF, Brubacher GB, Stahelin HB. 1987. Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. *Am. J. Clin. Nutr.* 45:1368–77
63. Gey KF, Moser UK, Jordan P, Stahelin HB, Eichholzer M, Ludin E. 1993. Increased risk of cardiovascular disease at suboptinal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *Am. J. Clin. Nutr.* 57: 787S–797S
64. Gey KJ, Puska P, Jordan P, Moser U. 1991. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* 53: 326S–334S
65. Gey KF, Stahelin HB, Eichholzer M. 1993. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study. *Clin. Invest.* 71:3–6
66. Gey KF, Stahelin HB, Puska P, Evans A. 1987. Relationship of plasma level of vitamin C to mortality from ischemic heart disease. *Ann. N.Y. Acad. Sci.* 498: 110–23
67. Goodwin JS, Gary PJ. 1983. Relationship between megadose vitamin supplementation and immunological function in a healthy elderly population. *Clin. Exp. Immunol.* 51:647–53
68. Gross RL, Newberne PM. 1980. Role of nutrition in immunologic function. *Physiol. Rev.* 60:188–302
69. Hallberg L. 1981. Biovailability of di-

etary iron in man. *Ann. Rev. Nutr.* 1:123–47

70. Hallberg L, Brune M, Rossander-Hulthen L. 1987. Is there a physiological role of vitamin C in iron absorption? *Ann. N.Y. Acad. Sci.* 498:324–32
71. Hallberg L, Brune M, Rossander L. 1989. The role of vitamin C in iron absorption. In *Elevated Dosages of Vitamins: Benefits and Hazards*, eds. P Walter, G Brubacher, H Stahelin, pp. 103–8. Toronto: Hans Huber
72. Halliwell B. 1987. Oxidants and human disease. Some new concepts. *FASEB J.* 1:358–64
73. Hankinson SE, Stampfer MJ, Seddon JM, Colditz GA, Rosner B, et al. 1992. Nutrient intake and cataract extraction in women: a prospective study. *Br. Med. J.* 305:335–39
74. Harats D, Ben-Naim M, Dabach Y, Hollander G, Havivi E, et al. 1990. Effect of vitamin C and E supplementation on susceptibility of plasma lipoprotein to peroxidation induced by acute smoking. *Atherosclerosis* 85:47–54
75. Hathcock JN. 1991. Safety of vitamin and mineral supplements. In *Micronutrients in Health and in Disease Prevention*, eds. A Bendich, CE Butterworth Jr, pp. 439–50. New York: Dekker
76. Hemila H. 1992. Vitamin C and the common cold. *Br. J. Nutr.* 67:3–16
77. Henson DE, Block G, Levine M. 1991. Ascorbic acid: biologic functions and relation to cancer. *J. Natl. Cancer Inst.* 83:547–50
78. Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH. 1990. Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. *Am. J. Clin. Nutr.* 51:649–55
79. Itoh R, Yamada K, Oka J, Echizen H, Suyama Y, Murakami K. 1990. Serum ascorbic acid and HDL cholesterol in a healthy elderly Japanese population. *Int. J. Vitm. Nutr. Res.* 60:360–65
80. Jacob RA, Kelley DS, Pianolto FS, Swendseid ME, Henning SM, et al. 1991. Immunocompetence and oxidant defense during ascorbate depletion of healthy men. *Am. J. Clin. Nutr.* 54: 130S–139S
81. Jacob RA, Skala JH, Omaye ST. 1987. Biochemical indices of human vitamin C status. *Am. J. Clin. Nutr.* 46:818–26
82. Jacob RA, Skala JH, Omaye ST, Turnlund JR. 1987. Effect of varying ascorbic acid intakes on copper absorption and ceruloplasmin levels of young men. *J. Nutr.* 117:2109–15
83. Jacques PF. 1992. Relationship of vitamin C status to cholesterol and blood pressure. *Ann. N.Y. Acad. Sci.* 669:205–14
84. Jacques PF. 1992. Effects of vitamin C on high-density lipoprotein cholesterol and blood pressure. *J. Am. Coll. Nutr.* 11:139–44
85. Jacques PF. 1992. A cross-sectional study of vitamin C intake and blood pressure in the elderly. *Int. J. Vitm. Nutr. Res.* 62:252–55
86. Deleted in proof
87. Jacques PF, Chylack LT Jr. 1991. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. *Am. J. Clin. Nutr.* 53:352S–355S
88. Jacques PF, Chylack LT, McGandy RB, Hartz SC. 1988. Antioxidant status in persons with and without senile cataract. *Arch. Opthalmol.* 106:337–40
89. Jacques PF, Hartz SC, Chylack LT, McGandy RB, Sadowski JA. 1988. Nutritional status in persons with and without senile cataract: blood vitamin and mineral levels. *Am. J. Clin. Nutr.* 48: 152–58
90. Jacques PF, Hartz SC, McGandy RB, Jacob RA, Russell RM. 1987. Ascorbic acid, HDL, and total plasma cholesterol in the elderly. *J. Am. Coll. Nutr.* 6:169–74
91. Jacques PF, Taylor A. 1991. Micronutrients and age-related cataracts. See Ref. 75, pp. 359–79
92. Jialal I, Grundy SM. 1992. Influence of antioxidant vitamins on LDL oxidation. *Ann. N.Y. Acad. Sci.* 669:237–48
93. Jialal I, Vega GL, Grundy SM. 1990. Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoproteins. *Atherosclerosis* 82: 185–91
94. Kahn HA, Leiborvitz HM, Ganley JP, Kini MM, Calton T, et al. 1977. The Framingham Eye Study. I. Outline and major prevalence findings. *Am. J. Clin. Epidemiol.* 196:17–32
95. Koh ET. 1984. Effect of vitamin C on blood parameters of hypertensive subjects. *J. Okla. State Med. Assoc.* 77:177–82
96. Leske MC, Chylack LT Jr, Wu S-Y. 1991. The lens opacities case-control study risk factors for cataract. *Arch. Ophthalmol.* 109:244–51
97. Lohman W. 1987. Ascorbic acid and cataract. 1987. *Ann. N.Y. Acad. Sci.* 498: 307–11
98. Mangels AR, Block G, Frey CM, Patterson BH, Taylor PR, et al. 1993. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of

synthetic ascorbic acid. *J. Nutr.* 23: 1054–61

99. Marcus SL, Dutcher JP, Paietta E, Ciobanu N, Strauman J, et al. 1987. Severe hypovitaminosis C occurring as the result of adoptive immunotherapy with high-dose interleukin 2 and lymphokine-activated killer cells. *Cancer Res.* 47:4208–12
100. Marcus SL, Petrylak DP, Dutcher JP, Paictta E, Ciobanu N, et al. 1991. Hypovitaminosis C in patients treated with high-dose interleukin 2 and lymphokine-activated killer cells. *Am. J. Clin. Nutr.* 54:1292S–1297S
101. Menzel DB. 1992. Antioxidant vitamins and prevention of lung disease. *Ann. N.Y. Acad. Sci.* 669:141–55
102. Mirvish SS. 1986. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. *Cancer* 58:1842–50
103. Mohsenin V, DuBois AB. 1987. Vitamin C and airways. *Ann. N.Y. Acad. Sci.* 498:259–79
104. Moore CV, Dubach R. 1951. Observation on the absorption of iron from foods tagged with radioiron. *Trans. Assoc. Am. Physicians* 64:245–56
105. Moore RB, Hulgan TM, Green JW, Jenkens LD. 1992. Increased susceptibility of sickle cell membrane Ca^{2+} + Mg^{2+} – ATP'ase to t-butylhydroperoxide: protective effects of ascorbate and desferal. *Am. Soc. Hematol.* 79:1334–41
106. Moran JP, Cohen L, Greene JM, Xu G, Feldman EB, et al. 1993. Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. *Am. J. Clin. Nutr.* 57:213–17
107. Niki E. 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr.* 54: 1119S–1124S
108. Niki E, Yamamoto Y, Komuro E, Sato K. 1991. Membrane damage due to lipid oxidation. *Am. J. Clin. Nutr.* 53:201S–205S
109. Norkus EP, Hsu H, Cehelsky MR. 1987. Effect of cigarette smoking on the vitamin C status of pregnant women and their offspring. *Ann. N.Y. Acad. Sci.* 498:500–1
110. Osilesi O, Trout DL, Ogunwole JO, Glover EE. 1991. Blood pressure and plasma lipids during ascorbic acid supplementation in borderline hypertensive and normotensive adults. *Nutr. Res.* 11: 405–12
111. Padh H. 1990. Cellular functions of ascorbic acid. *Biochem. Cell Biol.* 68: 1166–73
112. Padh H. 1991. Vitamin C: newer insights into its biochemical functions. *Nutr. Rev.* 49:65–70
113. Park YK, Kim I, Yetley EA. 1991. Characteristics of vitamin and mineral supplement products in the United States. *Am. J. Clin. Nutr.* 54:750–59
114. Pecoraro RE, Chen MS. 1987. Ascorbic acid metabolism in diabetes mellitus. *Ann. N.Y. Acad. Sci.* 498:248–58
115. Penn ND, Purkins L, Kelleher J, Heatley RV, Mascie-Taylor BH, Belfield PW. 1991. The effect of dietary supplementation with vitamins A, C, and E on cell-mediated immune function in elderly long-stay patients: a randomized controlled trial. *Age Ageing* 20:169–74
116. Peters EM, Gaetzshe JM, Grobbelaar B, Noakes TD. 1993. Vitamin C supplementation reduces the incidence of post-race symptoms of upper-respiratory-tract infection in ultramarathon runners. *Am. J. Clin. Nutr.* 57:170–74
117. Peterkofsky B. 1991. Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. *Am. J. Clin. Nutr.* 54:1135S–1140S
118. Preston AM. 1991. Cigarette smoking—nutritional implications. *Prog. Food Nutr. Sci.* 15:183–217
119. Rebouche CJ. 1991. Ascorbic acid and carnitine biosynthesis. *Am. J. Clin. Nutr.* 54:1147S–1152S
120. Deleted in proof
121. Rees DC, Kelsey H, Richards JDM. 1993. Acute haemolysis induced by high dose ascorbic acid in glucose-6-phosphate dehydrogenase deficiency. *Br. Med. J.* 306:841–42
122. Riemersma RA, Oliver M, Elton RA, Alfthan G, Vartiainen E, et al. 1990. Plasma antioxidants and coronary heart disease: vitamins C and E, and selenium. *Eur. J. Clin. Nutr.* 44:143–50
123. Riemersma RA, Wood DA, Macintyre CCA, Elton DA, Gey KF, Oliver MF. 1989. Low plasma vitamins E and C. Increased risk of angina in Scottish men. *Ann. N.Y. Acad. Sci.* 570:291–95
124. Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF. 1991. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* 337:1–5
125. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. 1993. Vitamin E consumption and the risk of coronary heart disease in men. *New Engl. J. Med.* 328:1450–56
126. Rivers JM. 1987. Safety of high-level vitamin C ingestion. *Ann. N.Y. Acad. Sci.* 498:445–54

127. Rivers JM. 1989. Safety of high-level vitamin C ingestion. See Ref. 71, pp. 95–102
128. Robertson J MVD. 1990. Cataract prevention: time for a clinical trial? *Br. J. Clin. Pract.* 44:475–76
129. Robertson J MCD, Donner AP, Trevithick JR. 1989. Vitamin E intake and risk of cataracts in humans. *Ann. N.Y. Acad. Sci.* 570:372–82
130. Robertson J McD, Donner AP, Trevithick JR. 1991. A possible role for vitamin C and E in cataract prevention. *Am. J. Clin. Nutr.* 53:346S–351S
131. Rose RC, Bode AM. 1993. Biology of free radical scavengers: an evaluation of ascorbate. *FASEB J.* 7:1135–42
132. Rosenberg SA. 1986. Adoptive immunotherapy of cancer using lymphokine-activated killer cells and recombinant interleukin-2. In *Important Advances in Oncology*, eds. V Devita, S Hellman, SA Rosenberg, pp. 55–92. Philadelphia: Lippincott
133. Salonen JT. 1991. Dietary fats, antioxidants and blood pressure. *Ann. Med.* 23:295–98
134. Salonen JT, Salonen R, Ihanainen M, Parvianinen M, Seppanen R, et al. 1988. Blood pressure, dietary fats, and antioxidants. *Am. J. Clin. Nutr.* 48: 1226–32
135. Sato K, Niki E, Shimasaki H. 1990. Free radical mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. *Arch. Biochem. Biophys.* 279: 402–5
136. Sauberlich HE. 1985. Bioavailability of vitamins. *Prog. Food Nutr. Sci.* 9: 1–33
137. Sauberlich HE. 1990. Ascorbic acid. In *Present Knowledge in Nutrition*, ed. ML Brown, pp. 132–41. Washington, D.C.: Int. Life Sci. Inst.
138. Sauberlich HE. 1991. *Evaluation of publicly available scientific evidence regarding certain nutrient-disease relationships: 8B. Vitamin C and cancer.* Bethesda, Md: Life Sci. Res. Off., Fed. Am. Soc. Exp. Biol. 53 pp.
139. Sauberlich HE. 1994. Vitamin C and cancer. In *Nutrition and Disease Update: Cancer*, eds. D Kritchevsky, K Carroll, Champaign, Ill.: Am. Oil Chem. Soc. In press
140. Sauberlich HE, Kretsch MJ, Taylor PC, Johnson HL, Skala JH. 1989. Ascorbic acid and erythorbic acid metabolism in nonpregnant women. *Am. J. Clin. Nutr.* 50:1039–49
141. Sauberlich HE, Wood SM, Tamura T, Freeberg LE. 1991. Influence of dietary intakes of erythorbic acid on plasma vitamin C analyses. *Am. J. Clin. Nutr.* 54:1319S–1322S
142. Schectman G, Byrd JC, Hoffmann R. 1991. Ascorbic acid requirements for smokers: analysis of a population survey. *Am. J. Clin. Nutr.* 53:1466–70
143. Schmidt K-H, Hagmaier V, Horning DH, Vuilleumier J-P, Rutishauser G. 1981. Urinary oxalate excretion after large intakes of ascorbic acid in man. *Am. J. Clin. Nutr.* 34:305–11
144. Schorah CJ, Sobala GM, Sanderson M, Collis N, Primrose JN. 1991. Gastric juice ascorbic acid: effects of disease and implications for gastric carcinogenesis. *Am. J. Clin. Nutr.* 53:287S–293S
145. Schutz HG, Read M, Bendel R, Bhalla VS, Harrill I, et al. 1982. Food supplement usage in seven western states. *Am. J. Clin. Nutr.* 36:897–901
146a. Schwartz J, Weiss ST. 1990. Dietary factors and their relation to respiratory symptoms. *Am. J. Epidemiol.* 132:67–76
146b. Schwartz J, Weiss ST. 1994. Relationship between dietary vitamin C intake and pulmonary function in the First National Health and Nutrition Examination Survey (NHANES 1). *Am. J. Clin. Nutr.* 59:110–14
147. Seib PA. 1985. Oxidation, monosubstitution and industrial synthesis of ascorbic acid. *Int. J. Vitam. Nutr. Res.* 55(Suppl. 27):259–306
148. Shapiro LR, Samuels S, Brewlow L, Camacho T. 1983. Patterns of vitamin C intake from food and supplements: survey of an adult population in Alameda County, California. *Am. J. Public Health* 73:773–78
149. Sies H, Stahl W, Sundquist AR. 1992. Antioxidant functions of vitamins: vitamins E and C, beta-carotene, and other carotenoids. *Ann. N.Y. Acad. Sci.* 669:7–20
150. Simon JA. 1992. Vitamin C and cardiovascular disease: a review. *J. Am. Coll. Nutr.* 11:107–25
151. Singh VN. 1992. A current perspective on nutrition and exercise. *J. Nutr.* 122: 760–65
152. Singh VN, Gaby SK. 1991. Premalignant lesions: role of antioxidant vitamins and beta-carotene in risk reduction and prevtion of malignant transformations. *Am. J. Clin. Nutr.* 53:386S–390S
153. Sinha R, Block G, Taylor PR. 1993. Problems with estimating vitamin C intakes. *Am. J. Clin. Nutr.* 57:547–50
154. Sobala GM, Pignatelli B, Schorah CJ, Bartsch H, Sanderson M, et al. 1991. Levels of nitrite, nitrate, N-nitroso com-

pounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of stomach. *Carcinogenesis* 12:193–98

155. Som S, Basu D, Mukherjee S, et al. 1981. Ascorbic acid metabolism in diabetes mellitus. *Metabolism* 30:572–77
156. Southorn PA, Powis G. 1988. Free radicals in medicine. II. Involvement in human disease. *Mayo Clin. Proc.* 63: 390–408
157. Sperduto RD, Hu T-S, Milton RC, Zhao JL, Evertt DF, et al. 1993. The Linxian cataract studies. Two nutrition intervention trials. *Arch. Opthalmol.* 111:1246–53
158. Sregenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, et al. 1991. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am. J. Clin. Nutr.* 53: 537–41
159. Subar AF, Block G. 1990. Use of vitamin and mineral supplements: demographics and amounts of nutrients consumed. *Am. J. Epidemiol.* 132:1091–101
160. Tannenbaum SR, Wishnok JS. 1987. Inhibition of nitrosamine formation by ascorbic acid. *Ann. N.Y. Acad. Sci.* 498: 354–63
161. Tannenbaum SR, Wishnok JS, Leaf CD. 1991. Inhibition of nitrosamine formation by ascorbic acid. *Am. J. Clin. Nutr.* 53:247S–250S
162. Taylor A. 1992. Role of nutrients in delaying cataracts. *Ann. N.Y. Acad. Sci.* 669:111–24
163. Taylor A. 1993. Cataract: relationships between nutrition and oxidation. *J. Am. Coll. Nutr.* 12:138–46
164. Taylor A, Jacques PF, Nadler D, Morrow F, Sulsky SI, Shepard D. 1991. Relationship in humans between ascorbic acid consumption and levels of total and reduced ascorbic acid in lens, aqueous humor, and plasma. *Curr. Eye Res.* 10:751–59
165. Taylor A, Jahngen-Hodge J, Huang LL, Jacques P. 1991. Aging in the eye lens: roles for proteolysis and nutrition in formation of caratact. *Age* 14:65–71
166. Tomson CRV, Channon SM, Parkinson IS, McArdle P, Qureshi M, et al. 1989. Correction of subclinical ascorbate deficiency in patients receiving dialysis: effects on plasma oxalate, serum cholesterol, and capillary fragility. *Clin. Chim. Acta* 180:255–64
167. Trinchieri A, Mandressi A, Luongo P, Longo G, Pisani E. 1991. The influence of diet on urinary risk factors for stones in healthy subjects and idiopathic renal calcium stone formers. *Br. J. Urol.* 67: 230–36
168. Trout DL. 1991. Vitamin C and cardiovascular risk factors. *Am. J. Clin. Nutr.* 53:322S–325S
169. Truswell AS. 1986. Ascorbic acid. *New Engl. J. Med.* 315:709
170. Urivetzky M, Kessaris D, Smith AD. 1992. Ascorbic acid overdosing: a risk factor for calcium oxalate nephrolithiasis. *J. Urol.* 147:1215–18
171. Vanderslice JT, Higgs DJ. 1991. Vitamin C content of foods: sample variability. *Am. J. Clin. Nutr.* 54:1323S–1327S
172. Varma SD. 1987. Ascorbic acid and the eye with special reference to the lens. *Ann. N.Y. Acad. Sci.* 498:280–306
173. Varma SD. 1991. Scientific basis for medical therapy of cataracts by antioxidants. *Am. J. Clin. Nutr.* 53:335S–345S
174. US Dept. Health Hum. Serv., US Dept. Agric. 1986. *Nutrition Monitoring in the United States.* A progress report from the Joint Nutr. Monit. Eval. Comm. DHHS Publ. No. (PHS)86-1255. 356 pp. US Public Health Serv., Washington, D.C.: US Gov. Print. Off.
175. US Public Health Serv. 1984. *The Health Consequences of Smoking: Chronic Obstructive Lung Disease. A report of the Surg. Gen. DHHS publication no. (PHS) 84-50205,* pp. 1–508. Washington, D.C.: US Gov. Print. Off.
176. West S. 1992. Does smoke get in your eyes? (Editorial) *J. Am. Med. Assoc.* 268:989–93
177. Williams MH. 1985. *Nutritional Aspects of Human Physical and Athletic Performance.* Springfield, IL: Thomas. 565 pp.
178. Williams MH. 1989. Vitamin supplementation and athletic performance. See Ref. 127, pp. 163–91
179. World Health Organization. 1982. Prevention of blindness. *Wkly. Epidemiol. Rec.* 57:145–46
180. Worthington-Robertsm B. Monsen ER. 1990. Iron. In *Meat and Health,* eds. AM Pearson, TR Dutson, 6:185–235. New York: Elsevier Appl. Sci.
181. Woteki C, Johnson CL, Murphy R. 1986. Nutritional status of the U.S. population: iron, vitamin C, and zinc. In *What Is America Eating?* pp. 21–39. Washington, D.C.: Food Nutr. Board, Natl. Res. Counc., Natl. Acad. Press