Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans[1–3]

Anitra C Carr and Balz Frei

ABSTRACT The current recommended dietary allowance (RDA) for vitamin C for adult nonsmoking men and women is 60 mg/d, which is based on a mean requirement of 46 mg/d to prevent the deficiency disease scurvy. However, recent scientific evidence indicates that an increased intake of vitamin C is associated with a reduced risk of chronic diseases such as cancer, cardiovascular disease, and cataract, probably through antioxidant mechanisms. It is likely that the amount of vitamin C required to prevent scurvy is not sufficient to optimally protect against these diseases. Because the RDA is defined as "the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all healthy individuals in a group," it is appropriate to reevaluate the RDA for vitamin C. Therefore, we reviewed the biochemical, clinical, and epidemiologic evidence to date for a role of vitamin C in chronic disease prevention. The totality of the reviewed data suggests that an intake of 90–100 mg vitamin C/d is required for optimum reduction of chronic disease risk in nonsmoking men and women. This amount is about twice the amount on which the current RDA for vitamin C is based, suggesting a new RDA of 120 mg vitamin C/d. *Am J Clin Nutr* 1999;69:1086–107.

KEY WORDS Antioxidant, cancer, cardiovascular disease, cataract, DNA, lipid, protein, recommended dietary allowance, vitamin C, adults

INTRODUCTION

Vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body (1). Humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via the glucuronic acid pathway (2). Thus, vitamin C must be obtained through the diet. The vitamin is especially plentiful in fresh fruit, in particular citrus fruit, and vegetables (3). A lack of vitamin C in the diet causes the deficiency disease scurvy (4). This potentially fatal disease can be prevented with as little as 10 mg vitamin C/d (5), an amount easily obtained through consumption of fresh fruit and vegetables.

The current recommended dietary allowance (RDA) for vitamin C is 60 mg/d for healthy, nonsmoking adults (6). The RDA is determined by the rate of turnover and rate of depletion of an initial body pool of 1500 mg vitamin C and an assumed absorption of ≈85% of the vitamin at usual intakes (7). This amount provides an adequate margin of safety: 60 mg/d would prevent the development of scurvy for ≈1 mo with a diet lacking vitamin C (7). The RDAs are determined primarily on the basis of prevention of deficiency; because scurvy is not a major health problem in the United States, this goal is clearly accomplished by the current RDA for vitamin C. Nevertheless, ≈25% of men and women in the United States consume <60 mg vitamin C/d and ≈10% of adults consume <30 mg/d (3).

The molecular mechanisms of the antiscorbutic effect of vitamin C are largely, although not completely, understood. Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters (8, 9). Procollagen-proline dioxygenase (proline hydroxylase) and procollagen-lysine 5-dioxygenase (lysine hydroxylase), 2 enzymes involved in procollagen biosynthesis, require vitamin C for maximal activity (10). Posttranslational hydroxylation of proline and lysine residues by these enzymes is essential for the formation and secretion of stable collagen helixes. A deficiency of vitamin C results in a weakening of collagenous structures, causing tooth loss, joint pains, bone and connective tissue disorders, and poor wound healing, all of which are characteristics of scurvy (8). Two dioxygenases involved in the biosynthesis of carnitine also require vitamin C as a cofactor for maximal activity (8). Carnitine is essential for the transport of activated long-chain fatty acids into the mitochondria; as a result, vitamin C deficiency results in fatigue and lethargy, early symptoms of scurvy. In addition, vitamin C is used as a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to norepinephrine catalyzed by dopamine β-monooxygenase (8). Depression, hypochondria, and mood changes frequently occur during scurvy and could be related to deficient dopamine hydroxylation.

The activities of several other enzymes are known to be dependent on vitamin C, although their connection to scurvy has not yet been clearly established. These enzymes include the mono- and

dioxygenases involved in peptide amidation and tyrosine metabolism (8, 9). Vitamin C has also been implicated in the metabolism of cholesterol to bile acids via the enzyme cholesterol 7α-monooxygenase and in steroid metabolism in the adrenals (8, 9). Hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P450 is also enhanced by reducing agents such as vitamin C (9).

The role of vitamin C in the above metabolic pathways is to reduce the active center metal ion of the various mono- and dioxygenases (8, 9). Unlike other water-soluble vitamins, vitamin C acts as a cosubstrate in these reactions, not as a coenzyme. The ability to maintain metal ions in a reduced state is related to the redox potential of vitamin C (11). Reduction of iron by vitamin C has also been implicated in enhanced gastrointestinal absorption of dietary nonheme iron (8, 12). Other proposed activities of vitamin C include maintenance of enzyme thiols in a reduced state and sparing of glutathione, an important intracellular antioxidant and enzyme cofactor (13), and tetrahydrofolate, a cofactor required for catecholamine biosynthesis (9).

Many biochemical, clinical, and epidemiologic studies have indicated that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer, and cataract (5, 14, 15). The amount of vitamin C required to prevent scurvy may be less than the amount necessary to maintain optimal health and reduce the incidence of chronic disease morbidity and mortality. The Food and Nutrition Board of the US National Academy of Sciences has changed the criteria for establishing RDAs from prevention of deficiency disease to prevention of chronic diseases. Therefore, in this review we address whether the current RDA of 60 mg vitamin C/d is sufficient for optimally reducing the risk of chronic diseases such as cardiovascular disease and cancer, as well as cataract. We also address whether the activity of vitamin C in these conditions is due to its antioxidant properties or to other proposed mechanisms.

VITAMIN C AS AN ANTIOXIDANT

Vitamin C is an important water-soluble antioxidant in biological fluids (16, 17). An antioxidant has been defined as "any substance that, when present at low concentrations compared to those of an oxidizable substrate (e.g., proteins, lipids, carbohydrates and nucleic acids), significantly delays or prevents oxidation of that substrate" (11). The definition proposed by the Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board is that "a dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans" (18). Vitamin C readily scavenges reactive oxygen and nitrogen species, such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxide radicals, and hypochlorous acid (11), thereby effectively protecting other substrates from oxidative damage. Although vitamin C also reacts rapidly with hydroxyl radicals (rate constant $>10^9$ L·mol^{-1}·s^{-1}), it is nevertheless unable to preferentially scavenge this radical over other substrates (19). The reason for this is that hydroxyl radicals are extremely reactive and will combine indiscriminately with any substrate in their immediate environment at a diffusion-limited rate. Vitamin C can also act as a coantioxidant by regenerating α-tocopherol (vitamin E) from the α-tocopheroxyl radical, produced via scavenging of lipid-soluble

radicals (20, 21). This is a potentially important function because in vitro experiments have shown that α-tocopherol can act as a prooxidant in the absence of coantioxidants such as vitamin C (21, 22). However, the in vivo relevance of the interaction between vitamin C and vitamin E is unclear. Vitamin C has also been shown to regenerate urate, glutathione, and β-carotene in vitro from their respective one-electron oxidation products, ie, urate radicals, glutathiyl radicals, and β-carotene radical cations (11, 23).

Two major properties of vitamin C make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate (282 mV) and its one-electron oxidation product, the ascorbyl radical (−174 mV), which is derived from the ene-diol functional group in the molecule (11). These low reduction potentials enable ascorbate and the ascorbyl radical to react with and reduce basically all physiologically relevant radicals and oxidants. For this reason, vitamin C has been said to be "at the bottom of the pecking order" and "to act as the terminal water-soluble small molecule antioxidant" in biological systems (24). The second major property that makes vitamin C such an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species (Eq *1*). The ascorbyl radical readily dismutates to form ascorbate and dehydroascorbic acid (Eqs *1* and *2*), or is reduced back to ascorbate by an NADH-dependent semidehydroascorbate reductase (9, 20, 25). The 2-electron oxidation product of ascorbate, dehydroascorbic acid, can itself be reduced back to ascorbate by glutathione, the glutathione-dependent enzyme glutathione:dehydroascorbate oxidoreductase [glutathione dehydrogenase (ascorbate), or glutaredoxin], or the NADPH-dependent selenoenzyme thioredoxin reductase (9, 20, 25). Alternatively, dehydroascorbic acid is rapidly and irreversibly hydrolyzed to 2,3-diketogulonic acid (DKG) (Eq *3*) (11).
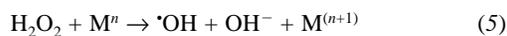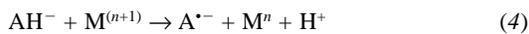
$$AH^- \leftrightarrow A^{\bullet-} \leftrightarrow A \qquad (1)$$

$$A^{\bullet-} + A^{\bullet-} \rightarrow AH^- + A \qquad (2)$$

$$A \rightarrow DKG \rightarrow \text{oxalate, threonate, etc} \qquad (3)$$

where equation *1* shows the reversible 1- and 2-electron oxidation of ascorbate (AH$^-$) to the ascorbyl radical (A$^{\bullet-}$) and dehydroascorbic acid (A), respectively; equation *2* shows the dismutation of the ascorbyl radical to form ascorbate and dehydroascorbic acid; and equation *3* shows the hydrolysis of dehydroascorbic acid to DKG, which then decomposes to oxalate, threonate, and many other products.

Vitamin C has been recognized and accepted by the US Food and Drug Administration (FDA) as one of 4 dietary antioxidants, the other 3 being vitamin E, the vitamin A precursor β-carotene, and selenium, an essential component of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase. The Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board (26) has, in principle, concurred with this definition, and in addition will consider other carotenoids. New regulations were recently published in which the FDA stated that vitamin C serves as an effective free radical scavenger to protect cells from damage by reactive oxygen molecules (27). Statements of the antioxidant properties of vitamin C are also appearing on food labels and in nutrient content claims throughout the United States.

Although substantial scientific evidence exists regarding the antioxidant and health effects of vitamin C in humans, further investigations of the role of vitamin C both in vitro and in vivo

are warranted, particularly because vitamin C, being a redox-active compound, can act not only as an antioxidant, but also as a prooxidant in the presence of redox-active transition metal ions (11). Reduction of metal ions such as iron or copper by vitamin C in vitro (Eq *4*) can result in the formation of highly reactive hydroxyl radicals via reaction of the reduced metal ions with hydrogen peroxide, a process known as Fenton chemistry (Eq *5*). Lipid hydroperoxides may also be broken down by the reduced metal ions, resulting in the formation of lipid alkoxyl radicals (Eq *6*) that can initiate and propagate the chain reactions of lipid peroxidation (28). The mechanism shown in equation *5*, however, requires the availability of free, redox-active metal ions and a low ratio of vitamin C to metal ion, conditions unlikely to occur in vivo under normal circumstances (11, 15, 28). Furthermore, it was shown recently that in biological fluids such as plasma, vitamin C acts as an antioxidant toward lipids even in the presence of free, redox-active iron (29).

$$AH^- + M^{(n+1)} \rightarrow A^{\bullet -} + M^n + H^+ \qquad (4)$$

$$H_2O_2 + M^n \rightarrow {}^{\bullet}OH + OH^- + M^{(n+1)} \qquad (5)$$

$$LOOH + M^n \rightarrow LO^{\bullet} + OH^- + M^{(n+1)} \qquad (6)$$

where equation *4* shows the reduction of redox-active metal ions [$M^{(n+1)}$] by ascorbate to form the ascorbyl radical and the reduced metal (M^n), equation *5* shows the production of highly reactive hydroxyl radicals (${}^{\bullet}OH$) from the reaction of hydrogen peroxide (H_2O_2) with the reduced metal ions, and equation *6* shows the reaction of lipid hydroperoxides (LOOH) with reduced metal ions to form alkoxyl radicals ($LO^{\bullet}$).

Although there is no convincing evidence for a prooxidant effect of vitamin C in humans, there is substantial evidence for vitamin C's antioxidant activity. Interestingly, the antioxidant activity of vitamin C is not directly related to its antiscorbutic effect. Therefore, if the antioxidant activity of vitamin C is accepted as occurring in vivo and considered to be relevant to human health by the Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board, then scurvy should not be used as the sole criterion for nutritional adequacy or to determine the required or optimal amount of vitamin C. In this section of the review, therefore, we address the following questions: *1*) Is oxidative damage to biological macromolecules relevant to human chronic diseases? *2*) What is the evidence that vitamin C acts as an antioxidant in humans? *3*) Are there other mechanisms, besides those directly related to the antiscorbutic and antioxidant activities of vitamin C, by which vitamin C could affect chronic disease incidence, mortality, or both? and *4*) Does vitamin C lower chronic disease incidence, mortality, or both?

Is oxidative damage to biological macromolecules relevant to human chronic diseases?

Oxidative damage to biomolecules, such as lipids, DNA, and proteins, has been implicated in many chronic diseases, in particular, cardiovascular disease, cancer, and cataract, respectively (30).

LDL oxidation and cardiovascular disease

Oxidative processes have been strongly implicated in atherosclerosis, myocardial infarction, and stroke (31). The oxidative modification hypothesis of atherosclerosis is currently the most widely accepted model of atherogenesis. LDL, the major carrier of cholesterol and lipids in the blood (32), infiltrates the intima of lesion-prone arterial sites, where it is oxidized over time by

oxidants generated by local vascular cells or enzymes (33) to a form that exhibits atherogenic properties. Minimally oxidized LDL and cytokines can activate endothelial cells to express surface adhesion molecules, primarily vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 as well as monocyte chemotactic protein 1, which cause circulating monocytes to adhere to the endothelium and migrate into the artery wall (31). The monocytes subsequently differentiate into macrophages in response to macrophage colony stimulating factor, the expression of which by vascular cells also is enhanced by modified LDL. The oxidized LDL further inhibits the egress of macrophages from the artery wall, where the cells recognize and readily take up the oxidized LDL through a process mediated by scavenger receptors (31). Unlike the normal apolipoprotein B/E LDL receptor recognizing native LDL, the scavenger receptors on macrophages that recognize modified LDL are not tightly regulated; as a result, the macrophages are converted into foam cells, a component of fatty streaks and the hallmark of atherosclerosis.

It is still uncertain which factors are responsible for the oxidation of LDL in vivo. LDL can be oxidized into a potentially atherogenic form in vitro through metal-ion-dependent oxidation of its lipid component with subsequent modification of apolipoprotein B-100 by reactive aldehyde products of lipid peroxidation, particularly malondialdehyde and 4-hydroxynonenal (34). Whether catalytic metal ions are available in the early lesion in vivo remains a matter of debate (28). Several metal-ion-independent mechanisms that are primarily enzymatic in nature have been proposed; these include mechanisms involving 15-lipoxygenase and myeloperoxidase (33, 35). The problems of comparing LDL oxidation in vitro with LDL oxidation in vivo were discussed in 2 recent reviews (36, 37).

Several lines of direct evidence point to the formation and existence of oxidized LDL in vivo. Antibodies to aldehyde-modified LDL recognize epitopes in human plaques (38) and LDL extracted from these lesions reacts with antibodies to oxidized LDL and has characteristics identical to those of LDL oxidized in vitro. Aldehyde-modified LDL has also been detected in plasma, as have autoantibodies to oxidized LDL (31). Antibodies to hypochlorous acid–modified protein have detected epitopes in lesions, suggesting an alternative or additional mechanism of LDL oxidation (39). F_2-isoprostanes, stable markers of lipid oxidation, have been detected in lesions (40, 41). Other oxidized lipids also increase with age and severity of atherosclerosis (42). Indirect evidence for in vivo oxidation has come from antioxidant supplementation studies in animals that show reduced lesion formation and reduced LDL oxidation (43, 44). In addition, numerous epidemiologic studies have indicated that dietary antioxidants reduce the incidence of cardiovascular disease in humans, as discussed below.

DNA oxidation and mutagenesis and carcinogenesis

Carcinogenesis is a multistage process. Free radicals and oxidative processes have been implicated in both the initiation and the promotion of carcinogenesis (5, 45). The oxidative hypothesis of carcinogenesis asserts that many carcinogens can generate free radicals that damage cells, predisposing these cells to malignant changes (46). Antioxidants, by neutralizing free radicals and oxidants, can prevent cell damage and subsequent development of cancer. DNA contains reactive groups in its bases that are highly susceptible to free radical attack (47) and oxidative DNA damage

can lead to deleterious mutations (2). It has been proposed that oxidative damage to DNA occurs continuously in vivo at a rate of $\approx 10^4$ oxidative hits per cell per day (2). Most oxidative lesions are efficiently repaired by specific DNA glycosylases, but repair is not 100% efficient and the number of lesions accumulates with age. When the cells divide, the lesions become fixated and mutations and cancer may result (2). The existence of numerous DNA glycosylases specific for oxidized bases (47) indicates that the latter are deleterious to human survival and therefore must be repaired.

There are >20 different oxidative DNA lesions, of which 8-oxoguanine is one of the most abundant and best studied (2). 8-Oxoguanine is formed by hydroxyl radical, peroxynitrite, or singlet oxygen attack of guanine in the C-8 position and is highly mutagenic (2). Normally guanine forms a base pair with cytosine, but 8-oxoguanine tends to pair with adenine. After replication, a transverse mutation occurs whereby G-C base pairs are replaced by A-T base pairs. This may contribute to the formation of cancer if the lesion occurs at a critical position in an important gene, such as a tumor suppressor or growth factor gene (2). Smokers, who have a higher risk of lung cancer than nonsmokers, have elevated concentrations of 8-oxo-2′-deoxyguanosine (8-oxodG) (48–50). 8-Oxoguanine has also been detected in women with breast cancer (51, 52). Although direct evidence for the link between DNA oxidation and cancer is still lacking (53), many epidemiologic studies have suggested that dietary intake of antioxidant vitamins, mainly from fruit and vegetables, protects against different types of cancer.

Protein oxidation and cataract

Cataract is a dysfunction of the lens resulting from opacification, which impedes the transmission of light (54). About 98% of the solid mass of the lens is protein, predominantly crystallins. These proteins are long lived and undergo minimal turnover; as a result, cataract formation is primarily age related. Oxidation of the lens proteins as a result of chronic exposure to ultraviolet light and oxygen has been implicated in this process (54, 55). Smoking, which is known to produce oxidative stress, is also associated with enhanced cataract risk (56, 57). Evidence of lens protein oxidation includes loss of sulfhydryl and tryptophan residues with age as well as formation of disulfides and other covalent cross-linkages. Deamination and acidification also occur, as well as formation of advanced glycation end products, particularly in persons with diabetes (54, 58). The oxidized proteins accumulate, aggregate, and eventually precipitate, producing the sequelae of cataract.

The lens contains multiple antioxidant defenses, such as high concentrations of vitamin C and glutathione, and antioxidant enzymes such as superoxide dismutase, catalase, and the glutathione peroxidase-reductase system (54, 55). Secondary defenses include proteolytic enzymes that selectively degrade damaged proteins. With aging, however, antioxidant concentrations in the lens, including concentrations of vitamin C, may be reduced (54) and the antioxidant enzymes are prone to inactivation, resulting in increased protein oxidation in older lenses. Proteolytic activity is also reduced in older lenses, resulting in accumulation of damaged proteins (54). Therefore, supplementation with antioxidants such as vitamin C may reduce the risk of cataract.

What is the evidence that vitamin C acts as an antioxidant in humans?

The most conclusive evidence that vitamin C acts as an antioxidant in humans has come from supplementation studies using specific biomarkers of oxidative damage to lipids, DNA, and proteins.

Because these specific oxidative biomarkers have only recently been developed and continue to be evaluated, only relatively few studies have investigated the effects on these biomarkers of supplementation with antioxidant micronutrients, including vitamin C.

Lipid oxidation

The most commonly used assay for lipid oxidation, although perhaps the least specific, measures the aldehyde peroxidation product malondialdehyde and other aldehydes by reaction with thiobarbituric acid (the so-called thiobarbituric acid–reactive substances, or TBARS, assay) (59, 60). Other products of lipid peroxidation, such as conjugated dienes and lipid hydroperoxides, are often measured (61, 62). TBARS and conjugated dienes are also commonly used to measure the oxidizability, or susceptibility to oxidation, of LDL (63). The oxidizability of LDL is determined by measuring the lag time and propagation rate of lipid peroxidation in LDL exposed in vitro to copper ions or other oxidants and is dependent on the antioxidant content and lipid composition of LDL (32, 63). Specific biomarkers of lipid peroxidation are the F_2-isoprostanes [in particular 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF$_{2\alpha}$)], which are formed from nonenzymatic, radical-mediated oxidation of arachidonyl-containing lipids (59). Increased concentrations of F_2-isoprostanes have been detected in persons with diabetes, in smokers, in persons with hypercholesterolemia (64, 65), and in LDL exposed in vitro to various types of oxidative stress (66).

Numerous studies in humans have investigated the effects on the oxidizability of LDL of vitamin C supplementation in combination with vitamin E or β-carotene or both (67). Studies have been carried out in smokers (68–70), nonsmokers (71, 72), and persons with hypercholesterolemia or cardiovascular disease (73, 74). In all cases, a significant reduction in LDL oxidizability was observed. It is, however, difficult to determine the relative contribution of vitamin C in these studies because of the presence of the cosupplements, of which vitamin E appears to be the major contributor to protection of LDL. This is because vitamin E is the most abundant lipid-soluble antioxidant associated with LDL (32).

Several studies of LDL oxidizability have also been carried out with vitamin C as the only supplement (**Table 1**). Although 2 studies found no effects (80, 81), 2 other studies (76, 84) found a significant reduction in the oxidizability of LDL obtained from persons supplemented with vitamin C. It is difficult to rationalize these findings, however, because vitamin C, being water soluble, is removed from the LDL during isolation from plasma. One possible explanation is the postulated role of vitamin C in either sparing or recycling vitamin E (15, 84). As mentioned above, this activity is readily observed in vitro, but evidence for sparing or recycling of vitamin E by vitamin C in vivo is inconclusive (15, 83, 87).

Several studies investigated the effects of vitamin C supplementation on lipid oxidation markers in smokers (Table 1). Smokers have higher concentrations of lipid oxidation products (65, 77) and lower plasma concentrations of vitamin C than do nonsmokers (88, 89). It has therefore been proposed that supplementation with antioxidant vitamins may inhibit smoking-related lipid oxidation. Three of 6 studies in smokers reported a reduction in the markers of lipid oxidation with vitamin C supplementation (75–77). The study by Reilly et al (77) is particularly noteworthy because it measured lipid oxidation with the specific biomarker 8-epi-PGF$_{2\alpha}$. Reilly et al found that supplementation of heavy smokers with 2000 mg vitamin C/d for only 5 d significantly reduced the urinary excretion of 8-epi-PGF$_{2\alpha}$.

TABLE 1
Vitamin C supplementation and biomarkers of lipid oxidation in humans[1]

Reference	Subjects	Vitamin C dose	Duration	Plasma change	Findings
Harats et al, 1990 (75)	17 Smokers	1000 mg/d	2 wk	2.0-fold	↓ Plasma and LDL TBARS
		1500 mg/d	4 wk	2.3-fold	↓ Plasma and LDL TBARS
Fuller et al, 1996 (76)	19 Smokers (9 Placebo)	(<30 mg/d)	(2 wk)	(Baseline)	—
		1000 mg/d	4 wk	3.9-fold	↓ LDL oxidizability[2] (TBARS, CD)
Reilly et al, 1996 (77)	5 Heavy smokers	2000 mg/d	5 d	ND	↓ Urine 8-epi-PFG$_{2\alpha}$
Mulholland et al, 1996 (78)	16 Female smokers (8 placebo)	1000 mg/d	14 d	3.0-fold	Serum TBARS: no change
Cadenas et al, 1996 (79)	21 Healthy males	1000 mg/d	30 d	ND	Urine TBARS: no change
Nyyssönen et al, 1997 (80)	59 Male smokers (19 placebo)	500 mg/d	2 mo	1.3-fold	LDL oxidizability[2] (CD), plasma TBARS: no change
		500 mg/d (SR)	2 mo	1.5-fold	LDL oxidizability[2] (CD): no change; ↑ plasma TBARS
Samman et al, 1997 (81)	8 Male smokers	(40 mg/d)	(2 wk)	(Baseline)	—
		1000 mg/d	2 wk	2.0-fold	LDL oxidizability[2] (CD): no change
Anderson et al, 1997 (82)	48 Nonsmokers	60 mg/d	14 d	1.2-fold	↓ Plasma MDA-HNE (NS)
		6000 mg/d	14 d	1.8-fold	↓ Plasma MDA-HNE (NS)
Wen et al, 1997 (83)	20 Nonsmokers (9 placebo)	1000 mg/d	4 wk	2.2-fold	LDL oxidizability (TBARS): no change; ↓ plasma MDA
Harats et al, 1998 (84)	36 Healthy males	(50 mg/d)	(1 mo)	(Baseline)	—
		500 mg/d	2 mo	3.8-fold	↓ LDL oxidizability[2] (CD)
Naidoo and Lux 1998 (85)	15 Volunteers	250–1000 mg/d	8 wk	1.5–2.0-fold	↓ Plasma MDA
Gokce et al, 1999 (86)	46 CAD patients (25 placebo)	500 mg/d	1 mo	2.3-fold	Plasma 8-epi-PGF$_{2\alpha}$: no change

[1] TBARS, thiobarbituric acid–reactive substrates; CD, conjugated dienes; 8-epi-PFG$_{2\alpha}$, 8-epi-prostaglandin F$_{2\alpha}$; ND, not determined; SR, slow release; MDA, malondialdehyde; HNE, hydroxynonenal; CAD, coronary artery disease.
[2] Measured by the lag time and propagation rate of in vitro lipid peroxidation.

An equal number of studies with vitamin C supplementation have been carried out in healthy individuals or nonsmokers. A reduction in lipid oxidation was observed with some markers (83–85), whereas a nonsignificant decrease (82) or no change was observed with others (79, 83). Finally, a recent study in coronary artery disease patients supplemented with 500 mg vitamin C/d for 1 mo found no change in plasma 8-*epi*-PGF$_{2\alpha}$ concentrations (86). With one exception (80), none of the studies listed in Table 1 found an increase in lipid oxidation markers with vitamin C supplementation.

An antioxidant role of vitamin C in vivo is also suggested by animal studies in guinea pigs or genetically scorbutic (Osteogenic Disorder Shionogi, or ODS) rats, which, like humans, are unable to synthesize vitamin C (90–93). Guinea pigs fed a diet marginally deficient in vitamin C have significantly higher concentrations of endogenous malondialdehyde than do animals fed diets supplying 20–40 times the amount of vitamin C required to avoid scurvy (90). Vitamin C–deficient guinea pigs exhale significantly higher amounts of breath ethane when exposed to an oxidative stress than do vitamin C–sufficient animals (92). Note that human smokers supplemented with 600–1000 mg vitamin C/d in combination with vitamin E and β-carotene for 3–8 wk exhaled lower amounts of breath pentane and ethane (68, 94). Genetically scorbutic ODS rats

fed a vitamin C–free diet have higher concentrations of plasma and liver TBARS and lipid peroxides than do rats fed vitamin C–supplemented diets (91). In a more recent study (93), however, supplementation of ODS rats with vitamin C, in contrast with vitamin E, did not protect against the endogenous formation of TBARS.

Finally, in vitro experiments with human plasma have shown that the formation of F$_2$-isoprostanes and lipid hydroperoxides by aqueous peroxyl radicals, stimulated neutrophils, gas-phase cigarette smoke, or redox-active iron does not occur until most or all of the endogenous vitamin C has been depleted (29, 66, 95). Vitamin C has also been shown to protect isolated LDL in vitro from oxidation by various radicals and oxidants (31). It is not surprising that vitamin C effectively prevents oxidation of isolated LDL and lipoproteins in plasma because, as explained above, vitamin C effectively scavenges most aqueous reactive oxygen and nitrogen species before they can interact with and oxidize other substrates, including lipids.

DNA oxidation

Of the >20 known oxidative DNA lesions, the most commonly measured markers of in vivo DNA oxidation are 8-oxoguanine and its respective nucleoside 8-oxodG (96). These modified bases have been detected in cells such as lymphocytes and are also

excreted in urine (96). The tissue pool represents a steady state between oxidation and cellular repair mechanisms, whereas the excreted products represent the total net damage and repair. The 2 methods most commonly used to detect 8-oxoguanine and 8-oxodG are gas chromatography–mass spectroscopy (GC-MS) and HPLC with electrochemical detection (HPLC-EC) (97). These methods, however, can generate artificially high amounts of oxidation products because of lengthy extraction, hydrolysis, and derivatization procedures, especially with GC-MS (97). Indirect methods for measuring DNA damage by using specific repair endonucleases in combination with single-cell gel electrophoresis (the comet assay) have shown baseline concentrations of DNA oxidation products up to 1000-fold lower than those measured by GC-MS (96, 97). Antibodies against 8-oxopurines are another potentially useful method for detecting DNA oxidation (98).

DNA oxidation, as determined by 8-oxodG in cells, is increased in cases of oxidative stress such as smoking and is correlated with reduced plasma concentrations of the antioxidant vitamins C and E (99). It is therefore conceivable that supplementation with vitamin C may ameliorate oxidative damage to DNA. Six of 7 studies shown in **Table 2** (82, 100–102, 104, 105) represent cell measurements, ie, steady state damage. Five of these studies showed a significant reduction in ≥1 marker of oxidative DNA damage in vitamin C–supplemented subjects (100–102, 104, 105).

In one of the more recent studies, a significant decrease in lymphocyte 8-oxoguanine concentrations was observed after vitamin C supplementation with 500 mg/d; in contrast, an increase in the less established marker 8-oxoadenine was observed (104). These authors thus suggested that vitamin C supplementation may have a prooxidant effect. However, lymphocyte 8-oxoguanine concentrations in this study were 30 lesions/10^5 guanine bases, which is ≥30-fold higher than currently accepted values (97), and 8-oxoadenine concentrations appeared to be extremely high as well. Therefore, the data seem to largely reflect ex vivo oxidation of the DNA before or during GC-MS analysis. In addition, other major problems with this study have been identified (106, 107).

Another recent study using GC-MS showed a significant reduction in both 8-oxoguanine and 8-oxoadenine in healthy persons supplemented for 12 wk with either 60 or 260 mg vitamin C/d in combination with 14 mg Fe/d, although other modified bases were increased, including 5-hydroxycytosine and thymine glycol (105). Once again, however, baseline concentrations of 8-oxoguanine and 8-oxoadenine were high and comparable with those measured by Podmore et al (104). Furthermore, there were no control groups included given iron alone or placebo, and vitamin C supplementation in the study did not always result in significant changes in plasma vitamin C concentrations (105). Therefore, the interpretation of the data from these 2 studies (104, 105) is uncertain.

TABLE 2
Vitamin C supplementation and biomarkers of oxidative DNA damage in humans[1]

Reference	Subjects	Vitamin C dose	Duration	Plasma change	Findings
Fraga et al, 1991 (100)	10 Men	(250 mg/d)	(7–14 d)	(Baseline)[2]	—
		5 mg/d	32 d	0.5-fold[2]	↑ Sperm 8-oxodG (HPLC-EC)
		10–20 mg/d	28 d	0.5-fold[2]	↑ Sperm 8-oxodG (HPLC-EC)
		60–250 mg/d	28 d	Baseline[2]	↓ Sperm 8-oxodG (HPLC-EC)
Green et al, 1994 (101)	6 Subjects	35 mg/kg	Single dose	ND	↓ Ex vivo[3] lymphocyte DNA damage (comet assay)
Panayiotidis and Collins, 1997 (102)	6 Smokers, 6 nonsmokers	1000 mg	Single dose	ND	↓ Ex vivo[4] lymphocyte DNA damage (comet assay)
Anderson et al, 1997 (82)	48 Nonsmokers	60 mg/d	14 d	1.2-fold	Lymphocyte DNA damage, in vivo
		6000 mg/d	14 d	1.8-fold	and ex vivo[4]: no change (comet assay)
Prieme et al, 1997 (103)	18 Male smokers	500 mg/d	2 mo	ND	Urine 8-oxodG: no change (HPLC-EC)
	20 Male smokers	500 mg/d (SR)	2 mo	ND	Urine 8-oxodG: no change (HPLC-EC)
Podmore et al, 1998 (104)	30 Subjects	500 mg/d	6 wk	1.6-fold	↓ Lymphocyte 8-oxogua (GC-MS), ↑ lymphocyte 8-oxoade (GC-MS)
Rehman et al, 1998 (105)	10 Healthy subjects	60 mg/d + 14 mg Fe/d	12 wk	1.1-fold	↓ Leukocyte 8-oxogua, 8-oxoade, 5-methylhydantoin, 5-hydroxyuracil, and 5-chlorouracil; ↑ leukocyte thymine glycol, 5-hydroxycytosine, and 5-methyluracil (GC-MS)
Rehman et al, 1998 (105)	10 Healthy subjects	260 mg/d + 14 mg Fe/d	12 wk	1.1-fold	↓ Leukocyte 8-oxogua, 5-hydroxyuracil, and 5-chlorouracil; ↑ leukocyte thymine glycol and 5-hydroxycytosine (GC-MS)

[1] 8-oxodG, 8-oxo-2′-deoxyguanosine; EC, electrochemical detection; ND, not determined, SR, slow release; 8-oxogua, 8-oxoguanine; GC-MS, gas chromatography–mass spectroscopy; 8-oxoade, 8-oxoadenine.
[2] Seminal plasma.
[3] Ex vivo challenge by ionizing radiation.
[4] Ex vivo challenge by hydrogen peroxide.

The study by Anderson et al (82) indicated no significant change in DNA damage as assessed by the comet assay after 2 wk of supplementation with 60 or 6000 mg vitamin C/d, either before or after an ex vivo challenge by hydrogen peroxide (Table 2). In contrast, 2 other studies in which the comet assay was used showed reduced susceptibility of lymphocytes to ex vivo oxidation of DNA after supplementation with vitamin C (101, 102). Similar findings were reported in another study in which vitamin E and β-carotene were given as cosupplements (108). Using HPLC-EC, Fraga et al (100) also showed a significant decrease in sperm 8-oxodG after replenishment with vitamin C. A recent animal study of vitamin C (and vitamin E) supplementation of guinea pigs, however, showed no effect on 8-oxodG concentrations in the liver as determined by HPLC-EC (109), despite a 60-fold difference in liver vitamin C concentrations.

The remaining study in Table 2 (103), which represents urinary measurements (ie, the net rate of damage and repair), showed no significant effect of vitamin C supplementation on 8-oxodG concentrations as determined by HPLC-EC. Two other studies in which vitamin C was given in the presence of cosupplements also reported similar findings (49, 110). However, several investigators have questioned the appropriateness of using urinary concentrations of 8-oxoguanosine or 8-oxodG as markers of nucleic acid damage because these products can be derived from nonspecific degradation of RNA or DNA, respectively, from dead cells (47), and the major 8-oxoguanine DNA glycosylase repair product is 8-oxoguanine, not 8-oxodG.

Protein oxidation

Protein oxidation is most commonly measured by determining carbonyl groups, oxidized amino acids, and advanced glycation end products (111, 112). Protein carbonyls can be formed by direct oxidative cleavage of the peptide chain or by oxidation of specific amino acid residues, such as lysine, arginine, proline, and threonine (111). Carbonyls can also be formed indirectly through modification of lysine, histidine, and cysteine residues by α,β-unsaturated aldehydes such as 4-hydroxynonenal in a process called Michael addition, or via Schiff base formation between lysine residues and dialdehydes such as malondialdehyde (111, 113). A variety of oxidized amino acids have been identified in proteins exposed to oxidizing conditions, including methionine sulfoxide, *o*- and *m*-tyrosine, *o,o′*-dityrosine, 3-chlorotyrosine, and 3-nitrotyrosine (112). Advanced glycation end products are generated by reaction of reducing sugars with lysine residues under oxidizing conditions in the Maillard reaction to form carboxymethyl- and carboxyethyl-lysine (111). Advanced glycation end products have been implicated in diseases such as diabetes and in aging (58, 111). The oxidative products of vitamin C may undergo similar reactions, although whether these occur in vivo is uncertain (114).

Few studies have been carried out to investigate the effects of vitamin C supplementation on the in vivo products of protein oxidation. Two animal studies (90, 115) indicated reduced protein carbonyl formation in guinea pigs supplemented with vitamin C, either with (115) or without (90) an endotoxin challenge. Vitamin C supplementation (2000 mg/d for 4–12 mo) of patients with *Helicobacter pylori* gastritis led to a significant reduction in nitrotyrosine concentrations (116). However, another recent human study found no change in urine *o,o′*-dityrosine or *o*-tyrosine concentrations in coronary artery disease patients supplemented with 500 mg vitamin C/d for 1 mo (86). Further studies investigating the effect of vitamin C supplementation on the markers of protein oxidation are clearly required.

Taken together, the evidence reviewed above suggests that vitamin C acts as an antioxidant in humans. Biomarker studies showed reduced lipid oxidation after vitamin C supplementation in both smokers and nonsmokers (Table 1). Several studies also showed decreased steady state DNA oxidation after vitamin C supplementation, although other studies showed either no change or mixed results (Table 2). The latter appears to be primarily due to the technical difficulties of accurately measuring DNA oxidation products by GC-MS and HPLC-EC and eliminating ex vivo artifacts. In addition, the effects of vitamin C supplementation on oxidative markers also critically depend on baseline concentrations of the vitamin. In a study by Levine et al (117) of the pharmacokinetics of vitamin C, it was found that in healthy adult men tissue saturation occurred at vitamin C intakes of ≈100 mg/d, as assessed by vitamin C concentrations in lymphocytes, monocytes, and neutrophils. Kallner et al (118) also reported that the body pool of vitamin C was saturated by an intake of ≈100 mg/d in healthy, nonsmoking men. If tissues are already saturated before vitamin C supplementation because of an intake ≥100 mg/d, then supplementation with any vitamin C dose cannot further reduce oxidative damage, a fact that likely explains some of the discrepant results of the biomarker studies discussed. More attention to this critical issue and more detailed biomarker studies on vitamin C intakes near tissue-saturating concentrations are warranted.

Are there other mechanisms by which vitamin C could affect chronic disease incidence, mortality, or both?

As discussed above, vitamin C has many functions in the body, such as acting as a cosubstrate for several biosynthetic enzymes (8). Therefore, vitamin C may affect chronic disease incidence, mortality, or both by mechanisms that may not be directly related to its role as an antioxidant.

Cardiovascular disease

Hypercholesterolemia is a significant risk factor for cardiovascular disease (43, 119). The relation between vitamin C supplementation, or plasma vitamin C concentrations, and total serum cholesterol has been investigated in several studies (82, 119–123). In one supplementation study, consumption of 1000 mg vitamin C/d for 4 wk resulted in a reduction in total serum cholesterol (120), whereas in another study, supplementation with 60 or 6000 mg/d for 2 wk had no effect (82). Two observational studies found an inverse correlation between vitamin C status and total serum cholesterol concentrations (122, 123). The mechanism for the possible modulating effect of vitamin C on serum cholesterol concentrations is not entirely certain. One putative pathway is through vitamin C's role as a cofactor for cholesterol 7α-monooxygenase, an enzyme involved in the in vivo hydroxylation of cholesterol to form bile acids (8). Vitamin C may also modulate the activity of hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol (43).

The plasma lipoprotein profile is also an important consideration for cardiovascular disease, with decreased concentrations of HDL and increased concentrations of LDL being significant risk factors (43, 119). Numerous observational studies have found a significant association between elevated plasma vitamin C concentrations and increased concentrations of HDL cholesterol and reduced concentrations of LDL cholesterol (119, 121–125). Findings indicated that with every 30-μmol/L increase in plasma vitamin C, HDL was elevated by 4–10% and LDL was reduced by 4%

(125). Similar modulatory effects were reported after supplementation with 1000 mg vitamin C/d for 4 wk (120). Ness et al (121) also found an inverse correlation between vitamin C status and triacylglycerol concentrations. Vitamin C may modulate the activity of lipoprotein lipase (43), although the mechanism is unknown.

The thrombotic risk of cardiovascular disease is associated with increased concentrations of the coagulation factor fibrinogen (126). Two studies found an inverse association between serum vitamin C concentrations and coagulation factors as well as a positive association between low serum vitamin C and elevated fibrinogen and coagulation activation markers (126, 127). Two early studies indicated that supplementation of heart disease patients with 2000–3000 mg vitamin C/d for 1–6 wk increased fibrinolytic activity and reduced platelet adhesiveness (128, 129). In a more recent study (130), healthy volunteers were supplemented with 250 mg vitamin C/d for 8 wk and a nonsignificant decrease in platelet aggregation and an increased sensitivity to PGE_1 were reported. In vitro studies showed that physiologic concentrations of vitamin C may increase PGE_1 and PGI_1 (prostacyclin) production, resulting in a reduction in platelet aggregation and thrombus formation (31), although whether this mechanism is relevant in vivo has yet to be established. Low concentrations of vitamin C are also associated with increased concentrations of plasminogen activator inhibitor 1, a protein that inhibits fibrinolysis (131).

Adhesion of leukocytes to the endothelium is an important initiating step in atherogenesis (31). Smokers have lower plasma vitamin C concentrations than do nonsmokers (88), and monocytes isolated from smokers exhibit increased adhesion to endothelial cells (132, 133). Supplementation of smokers with 2000 mg vitamin C/d for 10 d elevated plasma vitamin C concentrations from 48 to 83 μmol/L and significantly reduced monocyte adhesion to endothelial cells (132). In another study, however, supplementation of smokers with 2000 mg vitamin C 2 h before serum was collected had no significant effect on ex vivo monocyte or endothelial cell adhesion, despite an increase in vitamin C concentrations from 34 to 115 μmol/L (133). Interest-

TABLE 3
Vitamin C supplementation and endothelium-dependent vasodilation in humans[1]

Reference	Subjects	Vitamin C dose	Findings
Ting et al, 1996 (140)	10 Patients with type 2 diabetes, 10 control subjects	24 mg/min (infusion)	Forearm blood flow ↑ by 40% (measured after methacholine infusion)
Levine et al, 1996 (141)	46 CAD patients (20 placebo)	2000 mg (oral), 2.5-fold plasma increase	Brachial artery dilation ↑ by 220% (measured after 2 h)
Heitzer et al, 1996 (142)	10 Chronic smokers, 10 control subjects	18 mg/min (infusion)	Forearm blood flow ↑ by 60% (measured after acetylcholine infusion)
Motoyama et al, 1997 (143)	20 Smokers, 20 nonsmokers	10 mg/min (infusion)	Brachial artery vasodilation ↑ by 70% (measured after 20 min)
Ting et al, 1977 (144)	11 Hypercholesterolemic patients, 12 healthy control subjects	24 mg/min (infusion)	Forearm blood flow ↑ by 30% (measured after methacholine infusion)
Solzbach et al, 1997 (145)	22 Hypertensive patients (5 placebo)	3000 mg (infusion)	Coronary artery vasoconstriction ↓ 160% (measured after acetylcholine infusion)
Hornig et al, 1998 (146)	15 Chronic heart failure patients, 8 healthy control subjects	25 mg/min (infusion)	Radial artery dilation ↑ by 60% (measured after 10 min)
		2000 mg (oral)	Radial artery dilation ↑ by 45% (after 4 wk supplementation)
Timimi et al, 1998 (147)	10 Patients with type 1 diabetes, 10 control subjects	24 mg/min (infusion)	Forearm blood flow ↑ by 40% (measured after methacholine infusion)
Kugiyama et al, 1998 (148)	32 Coronary spastic angina patients, 34 control subjects	10 mg/min (infusion)	Epicardial artery vasoconstriction ↓ 100% (measured after acetylcholine infusion)
Taddei et al, 1998 (149)	14 Essential hypertensive patients, 14 healthy control subjects	24 mg · L forearm tissue^{-1} · min^{-1}	Forearm blood flow ↑ by 26% (measured after acetylcholine infusion)
Ito et al, 1998 (150)	12 Chronic heart failure patients	1000 mg (infusion)	Brachial artery dilation ↑ by 27% (NS)
	10 CAD patients, 10 control subjects	1000 mg (infusion), 10–13-fold plasma increase	Brachial artery dilation ↑ by 128% (measured after 30 min)
Gokce et al, 1999 (86)	46 CAD patients (25 placebo)	2000 mg (oral), 2.8-fold plasma increase	Brachial artery dilation ↑ by 50% (measured after 2 h)
		500 mg/d (oral), 2.3-fold plasma increase	Brachial artery dilation ↑ by 40% (after 4 wk supplementation)

[1] CAD, coronary artery disease.

ingly, supplementation with 7000 mg L-arginine, the physiologic substrate for nitric oxide (NO) synthase, significantly reduced monocyte and endothelial cell adhesion, suggesting an important role for NO. Several in vivo animal studies also suggested an important role for vitamin C in modulating leukocyte and endothelial cell interactions in hamsters exposed to cigarette smoke (134, 135) or injected with oxidized LDL (136).

Impaired vascular function and relaxation are highly relevant to the clinical expression of atherosclerosis, ie, angina pectoris, myocardial infarction, and stroke. Hypertension is a recognized risk factor for cardiovascular disease (43) and low concentrations of plasma vitamin C have been associated with hypertension (122, 125, 137–139). Several studies reported positive effects of high doses of vitamin C, administered either orally or by intraarterial infusion, on vasodilation (**Table 3**). Four studies (86, 141, 146, 150) investigated vasodilation in patients with cardiovascular disease and found increases of 45–220% in vasodilation after administration of vitamin C (1000–2000 mg oral or 25 mg/min infusion). One of these studies found a 128% increase in brachial artery dilation in coronary artery disease patients, but a nonsignificant 27% increase in chronic heart failure patients (150). Kugiyama et al (148) observed a 100% reversal of epicardial artery vasoconstriction in coronary spastic angina patients infused with 10 mg vitamin C/min. Other studies investigated patients with hypercholesterolemia (144) or hypertension (145, 149), both of which are important risk factors for cardiovascular disease. Infusion of 3000 mg (145) or 24 mg/min (144, 149) vitamin C in these patients resulted in increased blood flow and decreased vasoconstriction. Heitzer et al (142) and Motoyama et al (143) observed increased vasodilation in smokers given infusions of 10–18 mg vitamin C/min. Motoyama et al showed a significant positive correlation ($r = 0.597$, $P = 0.0001$) between serum concentrations of vitamin C and the percentage increase in the brachial arterial diameter of smokers and nonsmokers. Similarly, patients with type 2 and type 1 diabetes had increased blood flow after infusion of 24 mg vitamin C/min (140, 147). Finally, healthy individuals given an oral dose of 1000 mg vitamin C in combination with 800 IU vitamin E had increased vasodilation several hours after a single high-fat meal (151).

Several mechanisms are possible for these positive effects of vitamin C on vasodilation and are most likely related to vitamin C's antioxidant activity. Endothelium-derived relaxing factor, or NO, plays an important role in vasodilation and also inhibits platelet aggregation and leukocyte adhesion (31). NO is rapidly inactivated by reaction with superoxide radicals and release of NO from endothelial cells can be inhibited by oxidized LDL (141). Therefore, vitamin C may spare NO by scavenging superoxide radicals or preventing the formation of oxidized LDL. The latter mechanism is unlikely, however, because of the short time spans involved in the studies listed in Table 3. Furthermore, high concentrations of vitamin C are required to scavenge superoxide radicals in competition with NO because of the large difference in rate constants ($\approx 10^5$ L·mol^{-1}·s^{-1} at pH 7.4 for superoxide radicals with ascorbate compared with $\approx 10^9$ L·mol^{-1}·s^{-1} for superoxide radicals with NO). Nevertheless, millimolar concentrations of vitamin C can be achieved with infusion (117) and superoxide scavenging may, at least in part, explain the beneficial effects of vitamin C on vasodilation in those studies using infusion (140, 142–150). Vitamin C can also maintain intracellular concentrations of glutathione by a sparing effect or regeneration of thiols from thiyl radicals, which may enhance the synthesis of NO or increase the stabilization of NO through formation

of S-nitrosothiol species (141). Like vitamin C, administration of a cysteine delivery agent known to increase intracellular glutathione concentrations enhances vasodilation in patients with coronary artery disease (152).

Cancer

Vitamin C may protect against cancer through several mechanisms in addition to inhibition of DNA oxidation. One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines (153, 154). N-Nitroso compounds are formed by reaction of nitrite or nitrate (common in cured food and cigarette smoke) with amines and amides (153). Nitrosating compounds can also be formed from NO generated by inflammatory cells expressing inducible NO synthase (116, 153, 155, 156). N-Nitroso compounds undergo activation by cytochrome P450–dependent enzymes and have been implicated in gastric and lung cancer (153). Epidemiologic studies have shown an inverse association between vitamin C intake, mainly from fruit and vegetables, and cancers at these sites (157, 158); additionally, vitamin C reduces in vivo nitrosation by scavenging nitrite and hence preventing its reaction with amines to form nitrosamines (153, 154). Concentrations of fecapentaenes, fecal mutagens that have been implicated in colon cancer (155), are also reduced by vitamin C (159).

In addition, vitamin C may reduce carcinogenesis through stimulation of the immune system. Two of the major functions of the immune system are to fight off infections and to prevent cancer (3). It is hypothesized that the immune system recognizes tumor-forming cells as nonself. Cytotoxic T lymphocytes, macrophages, and natural killer cells can lyse tumor cells (3). Free radicals and oxidative products secreted by immune cells can also lyse tumor cells. Vitamin C can protect host cells against harmful oxidants released into the extracellular medium. Therefore, an optimal immune response requires a balance between free radical generation and antioxidant protection.

Vitamin C is taken up by phagocytes and lymphocytes to concentrations up to 100-fold greater than in plasma, and intracellular concentrations of vitamin C are reduced when phagocytes are activated (3). Many studies have investigated the effects of vitamin C on leukocyte function; however, the data are inconsistent and conflicting (160). Vitamin C may modulate the functions of phagocytes, such as chemotaxis (161–164), as well as the activity of natural killer cells and the functions and proliferation of lymphocytes (160, 165, 166). Vitamin C may also affect the production of immune proteins such as cytokines and antibodies as well as complement components (160, 167, 168). An important measure of overall immune function is the delayed-type hypersensitivity response, which may be modulated by antioxidant micronutrients such as vitamin C (3, 169). Jacob et al (159) showed that vitamin C deficiency in men ingesting 5–20 mg vitamin C/d for 32 d significantly reduced delayed-type hypersensitivity responses, but resulted in no significant change in lymphocyte proliferation. Delayed-type hypersensitivity responses did not return to baseline, even after supplementation with 250 mg vitamin C/d for 4 wk.

Does vitamin C lower chronic disease incidence, mortality, or both?

Many epidemiologic studies and a limited number of clinical trials have indicated that dietary intake of, or supplementation with, antioxidant vitamins is associated with a reduction in the incidence of chronic disease morbidity and mortality (5, 14, 15). Because vitamin C acts as an antioxidant and can ameliorate

oxidative damage to lipids, DNA, and proteins, the association of vitamin C with cardiovascular disease, cancer, and cataract, respectively, is of substantial interest. Much of the evidence discussed in the subsequent sections is derived from epidemiologic studies. As such, observed associations between vitamin C intake or plasma concentrations and disease risk are not proof of cause-effect relations; the observed differences may in part reflect differences in dietary behavior or lifestyle patterns. There also may be confounding of interpretations by unmeasured risk factors or imperfect statistical corrections. Furthermore, most of the epidemiologic data are based on dietary vitamin C intake, mainly from fruit and vegetables, and it is difficult to discern whether an observed inverse association with disease incidence is due to vitamin C itself, vitamin C together with other substances in fruit and vegetables, or these other substances themselves, with vitamin C as a surrogate marker. Finally, estimation of vitamin C intake from food-frequency questionnaires has limited accuracy and measurement of vitamin C plasma concentrations, although more accurate than estimation of dietary intake, also has pitfalls and is dependent on proper handling and storage of the samples. These limitations of epidemiologic studies based on dietary intake and plasma concentrations of vitamin C have been discussed previously (158, 170).

Cardiovascular disease

Coronary artery disease and stroke are the leading causes of morbidity and mortality in the United States and other westernized populations. Cardiovascular disease is responsible for nearly one million deaths every year in the United States alone, at the cost of >$15 billion in health care and lost productivity (34). Major risk factors associated with cardiovascular disease are age, male sex, smoking, hypercholesterolemia, hypertension, family history, obesity, and physical inactivity (43, 119). Many epidemiologic studies have shown inverse associations between antioxidant intake, particularly vitamin E, and cardiovascular disease (171). Over the past 15 y, several prospective cohort studies have been published on the association between vitamin C intake and the risk of cardiovascular disease (**Table 4**). Some of these were reviewed previously by Enstrom (14) and Gey (15), as well as by others (5, 43, 171). Because the purpose of this review is to propose an RDA for vitamin C based on chronic disease incidence, only studies that stated actual amounts of vitamin C intake are considered further here.

TABLE 4
Vitamin C intake associated with reduced cardiovascular disease risk (prospective cohort studies)[1]

Reference	Population (duration)	Endpoint (events)	Risk and associated dietary intake of vitamin C
Enstrom et al, 1986 (172)	3119 Men and women (10 y)	CVD (127 deaths)	>250 compared with <250 mg/d: no ↓ risk
Enstrom et al, 1992 (173) and Enstrom, 1993 (174)	4479 Men (10 y) 6809 Women (10 y)	CVD (558 deaths) CVD (371 deaths)	>50 mg/d + regular supplement: ↓ risk by 42% >50 mg/d + regular supplement: ↓ risk by 25%
Manson et al, 1992 (175) and Manson et al, 1993 (176)	87 245 Female nurses (8 y)	CAD (552 cases) Stroke (183 cases)	>359 compared with <93 mg/d: ↓ risk by 20% (NS) >359 compared with <93 mg/d: ↓ risk by 24%
Rimm et al, 1993 (177)	39 910 Male health professionals (4 y)	CAD (667 cases)	392 compared with 92 mg/d median: no ↓ risk
Fehily et al, 1993 (178)	2512 Men (5 y)	CVD (148 cases)	>67 compared with <35 mg/d: ↓ risk 37% (NS)
Knekt et al, 1994 (179)	2748 Finnish men (14 y) 2385 Finnish women (14 y)	CAD (186 deaths) CAD (58 deaths)	>85 compared with <60 mg/d: no ↓ risk >91 compared with <61 mg/d: ↓ risk by 51%
Gale et al, 1995 (180)	730 UK elderly men and women (20 y)	Stroke (125 deaths) CAD (182 deaths)	>45 compared with <28 mg/d: ↓ risk by 50% >45 compared with <28 mg/d: ↓ risk by 20% (NS)
Kritchevsky et al, 1995 (181)	4989 Men (3 y) 6318 Women (3 y)	Carotid atherosclerosis Carotid atherosclerosis	>982 compared with <56 mg/d: ↓ intima thickness >728 compared with <64 mg/d: ↓ intima thickness
Pandey et al, 1995 (182)	1556 Men (24 y)	CAD (231 deaths)	>113 compared with <82 mg/d: ↓ risk by 25%
Kushi et al, 1996 (183)	34 486 Women (7 y)	CAD (242 deaths)	>391 compared with <112 mg/d (total)[2]: no ↓ risk; >196 compared with <87 mg/d (dietary): no ↓ risk; regular supplement compared with no supplement: no ↓ risk
Losconczy et al, 1996 (184)	11 178 Elderly men and women (6 y)	CAD (1101 deaths)	Regular supplement compared with no supplement: no ↓ risk
Sahyoun et al, 1996 (185)	725 Eldery men and women (10 y)	CVD (101 deaths)	>388 compared with <90 mg/d: ↓ risk by 62% (NS)
Mark et al, 1998 (186)[3]	29 584 Chinese men (5 y)	Stroke	180 mg/d supplement: no ↓ risk (+ 30 μg Mo/d cosupplement)

[1] CVD, cardiovascular disease; CAD, coronary artery disease.
[2] Intake from diet plus supplements.
[3] Trial (not prospective cohort study).

Seven of the 12 prospective cohort studies listed in Table 4 showed a significant inverse association between vitamin C intake and cardiovascular or cerebrovascular disease risk (173–176, 179–182, 185). Several studies observed a reduced risk with moderate intakes of vitamin C between 45 and 113 mg/d (178–180, 182). Knekt et al (179) reported a 51% lower risk of coronary artery disease in women consuming >91 mg vitamin C/d than in those consuming <61 mg/d, although no association was observed in men consuming similar amounts. Daily intakes >91 mg had no additional protective effect in this study. In a population of elderly men and women, Gale et al (180) found that daily intakes of >45 mg vitamin C were associated with a 50% lower risk of stroke than were intakes <28 mg/d, although there was only a nonsignificant 20% reduction in coronary artery disease in this study. Pandey et al (182) observed a moderate but significant 25% lower risk of coronary artery disease in men consuming >113 mg vitamin C/d than in those consuming <82 mg/d. Finally, Fehily et al (178) observed a nonsignificant 37% lower risk of cardiovascular disease in men or women consuming moderate amounts of >67 mg vitamin C/d than in those consuming <35 mg/d.

Numerous studies reported a reduced risk of cardiovascular disease with vitamin C intakes considerably higher than those in the above-mentioned studies (173–176, 181, 185, 187). Enstrom et al (173) showed a risk reduction in cardiovascular disease of 42% in men and 25% in women consuming >50 mg vitamin C/d from the diet plus regular supplements, corresponding to ≈300 mg total vitamin C/d (174). An earlier study by Enstrom et al (172) indicated that intakes of vitamin C >250 mg/d were not associated with an additional risk reduction for cardiovascular disease, although subsequent reanalysis of the data indicated that intakes >750 mg/d were associated with a reduction in overall mortality (173). Sahyoun et al (185) reported a significant 62% lower risk of cardiovascular disease in a population of elderly men and women consuming >388 mg vitamin C/d than in those consuming <90 mg/d. Similar intakes were reported by Manson et al (175, 176, 187) in a cohort of female nurses, although only a moderate risk reduction of 24% was observed for stroke, whereas the risk reduction for coronary artery disease was nonsignificant. Finally, Kritchevsky et al (181) measured carotid artery wall thickness as a measure of atherosclerosis and found significantly decreased intima thickness in men and women aged >55 y consuming, respectively, >982 or >728 mg vitamin C/d than in those consuming <56 or <64 mg/d.

Interestingly, several epidemiologic studies indicated no association between vitamin C intake or supplementation and risk of cardiovascular disease (177, 183, 184, 186). Losconczy et al (184) and Kushi et al (183) observed no effect on coronary artery disease risk with regular vitamin C supplementation. Kushi et al (183) and Rimm et al (177), in 2 large epidemiologic studies, also reported no additional reduction in risk of coronary artery disease with vitamin C intakes of ≈200 and 400 mg/d, respectively, compared with intakes of ≈90 mg/d. One intervention trial found no reduction in risk of stroke or hypertension in a population of Chinese men and women supplemented with 180 mg vitamin C/d and 30 μg Mo/d for 5 years (186).

The study by Levine et al (117), which found that tissue saturation in healthy men occurred at vitamin C intakes of ≈100 mg/d, may explain why several of the above-mentioned studies showed no protective effect of dietary vitamin C intakes >90 mg/d (177, 179, 183) or vitamin C supplementation (183, 184, 186). Because an intake of 90 mg vitamin C/d results in near tissue saturation, increasing vitamin C intake over this amount may have only a small or no additional effect on tissue concentrations and hence disease risk. Thus, the totality of evidence from prospective cohort studies to date suggests that there is only a minimal intake requirement for vitamin C to optimally reduce the risk of cardiovascular disease, and that there is little or no additional benefit from vitamin C intakes >90–100 mg/d, likely because of tissue saturation at this level (117).

Several investigators studying cardiovascular disease have measured plasma concentrations of vitamin C (**Table 5**), which is a considerably more accurate and reliable measure of body vitamin C status than dietary intake estimated from questionnaires. One prospective cohort study indicated that plasma vitamin C concentrations >23 μmol/L are associated with moderate, statistically nonsignificant reductions of 20% and 22%, respectively, in the risk of coronary artery disease and stroke (189, 190). Similarly, Gale et al (180) observed a statistically significant 30% lower risk of death from stroke in subjects with plasma vitamin C concentrations >28 μmol/L than in those with concentrations <12 μmol/L; however, no association was observed with coronary artery disease risk. Larger risk reductions of 39–60% were observed for coronary artery disease, myocardial infarction, and angina pectoris with vitamin C concentrations >11–57 μmol/L (188, 191, 193). Furthermore, patients with these conditions were found to have significantly lower plasma vitamin C concentrations than control subjects or survivors (188, 191, 192, 194). Interestingly, in the studies reporting an inverse association between plasma vitamin C concentrations and angina pectoris (188) and coronary artery disease (191), the association was substantially reduced after adjustment for smoking. This finding is to be expected given the known effect of smoking on plasma vitamin C concentrations (196) and suggests that smoking may increase cardiovascular disease risk in part by lowering vitamin C concentrations.

Another study observed a risk reduction of 47% for cardiovascular disease mortality with plasma concentrations >89 μmol/L compared with <52 μmol/L (185). A large study by Simon et al (195), comprising 6624 men and women enrolled in the second National Health and Nutrition Examination Survey, showed 26% and 27% risk reductions for stroke and coronary artery disease, respectively, with saturating serum vitamin C concentrations of 63–153 μmol/L compared with low to marginal concentrations of 6–23 μmol/L. In a comprehensive recent review article, Gey (15) proposed that plasma vitamin C concentrations ≥50 μmol/L provide optimal benefit with regard to cardiovascular disease, and this number seems to be in good agreement with most of the studies listed in Table 5. Most interestingly, a plasma vitamin C concentration of 50 μmol/L is achieved by a dietary intake of ≈100 mg vitamin C/d (117), in good agreement with the suggested protective intake of 90–100 mg/d derived from diet-based prospective cohort studies (Table 4) and the amount required for tissue saturation (117).

Cancer

More than half a million deaths occur annually from cancer in the United States (197). Lung cancer resulting from smoking causes 30% of all US cancer deaths; colon-rectum, breast, and prostate cancers account for another 25% of deaths (197). The major risk factors for cancer are smoking, chronic inflammation, and an unbalanced diet. A multitude of epidemiologic studies have shown that increased consumption of fresh fruit and vegetables is associated with a reduced risk of most types of cancer (157, 158). Fruit and vegetables contain many constituents that may contribute to protection against cancer, including antioxidant vitamins. Over the years, numerous

TABLE 5
Plasma concentration of vitamin C associated with reduced cardiovascular disease risk[1]

Reference and type of study	Population (duration)	Endpoint (events)	Risk and associated plasma concentration of vitamin C	Estimated intake[2]
Riemersma et al, 1991 (188): case-control	110 Men, 394 control subjects	Angina pectoris	>57.4 compared with <13.1 μmol/L: ↓ risk by 39%	100 compared with 35 mg/d
			35.3 μmol/L in controls compared with 28.1 μmol/L in cases	75 compared with 65 mg/d
Eichholzer et al, 1992 (189) and Gey et al, 1993 (190): prospective cohort	2974 Swiss men (12 y)	CAD (132 deaths)	>22.7 μmol/L: ↓ risk by 20% (NS)	55 mg/d
		Stroke (31 deaths)	>22.7 μmol/L: ↓ risk by 22% (NS)	55 mg/d
Gale et al, 1995 (180): prospective cohort	730 UK elderly men and women (20 y)	Stroke (117 deaths)	>27.8 compared with <11.9 μmol/L: ↓ risk by 30%	65 compared with 35 mg/d
		CAD (170 deaths)	>27.8 compared with <11.9 μmol/L: no ↓ risk	65 compared with 35 mg/d
Singh et al, 1995 (191): cross-sectional	595 Indian men and women	CAD (72 cases)	>42.6 compared with <15.2 μmol/L: ↓ risk by 55%	80 compared with 40 mg/d
			37.8 μmol/L in controls compared with <20.3 μmol/L in cases	75 compared with 50 mg/d
Sahyoun et al, 1996 (185): prospective cohort	725 Elderly men and women (10 y)	CVD (75 deaths)	>88.6 compared with <51.7 μmol/L: ↓ risk by 47%	>400 compared with 95 mg/d
Halevy et al, 1997 (192): case-control	137 Cases, 70 controls	CAD	35.9 μmol/L in controls compared with 31.3 μmol/L in cases	75 compared with 70 mg/d
Nyyssönen et al, 1997 (193): prospective cohort	1605 Finnish men (8 y)	MI (70 cases)	>11.4 μmol: ↓ risk by 60%	35 mg/d
Vita et al, 1998 (194): case-control	149 CAD patients	Unstable angina, MI	42.5 μmol/L in controls compared with 33.6 μmol/L in cases	80 compared with 70 mg/d
Simon et al, 1998 (195): prospective cohort	6624 Men and women	CAD	63–153 compared with 5.7–23 μmol/L: ↓ risk 27%	125 to >400 compared with <30 to 55 mg/d
		Stroke	63–153 compared with 5.7–23 μmol/L: ↓ risk 26%	125 to >400 compared with <30 to 55 mg/d

[1] CVD, cardiovascular disease; CAD, coronary artery disease; MI, myocardial infarction.
[2] According to data from Levine et al (117).

case-control studies have been carried out to investigate the role of vitamin C in cancer prevention; these have been reviewed by Block (157) and Fontham (158). Most case-control studies showed a consistent inverse association between vitamin C intake and cancers of the oral cavity, larynx-pharynx, and esophagus, as well as of the lung, stomach, and colon-rectum. Of the hormone-dependent cancers, only breast cancer was inversely associated with vitamin C intake, in contrast with ovary and prostate cancers. Although interesting and consistent in their results, these case-control studies may be intrinsically biased because of their retrospective design (158). Therefore, only prospective cohort studies, some of which were reviewed by Enstrom (14) and Gey (15), that also stated actual dietary intakes are considered further in this section (**Table 6**).

Several studies investigated the association of moderate intakes of vitamin C with cancer risk. Kromhout et al (199) reported a significant 64% risk reduction of lung cancer with vitamin C intakes >83 mg/d. Similarly, 3 studies found that individuals who developed lung cancer had lower dietary intakes of vitamin C than healthy individuals, who in all 3 studies consumed >82 mg vitamin C/d (198, 200, 207). However, the differences in vitamin C intake between cases and controls was statistically

significant in only 1 of the 3 studies (207). Pandey et al (182) observed a significant 39% lower risk of all cancers in men consuming >113 mg vitamin C/d than in those consuming <82 mg/d. A vitamin C intake >50 mg/d from the diet plus regular supplements, totaling ≈300 mg/d (174), was found to be associated with a moderate 21% risk reduction of all cancers in men compared with a dietary intake of <49 mg/d, although no significant effect was observed in women (173). In a large epidemiologic study, Graham et al (202) observed no significant reduction in breast cancer risk in women consuming >79 mg vitamin C/d.

Interestingly, virtually all of the studies in which vitamin C intakes were >87 mg/d in the lowest intake group (quantile) found no or nonsignificant effects on cancer risk reduction with higher intakes of vitamin C (172, 185, 203, 204, 206). Only one study found a significant protective effect: Shibata et al (201) observed a moderate 24% lower risk of all cancers in women consuming >225 mg vitamin C/d than in those consuming <155 mg/d, although no effect was observed in men. In good agreement with these studies, vitamin C supplementation was not associated or nonsignificantly associated with reduced cancer risk in several prospective cohort studies (184, 201, 203, 206), although Bostick

TABLE 6
Vitamin C intake associated with reduced cancer risk (prospective cohort studies)

Reference	Population (duration)	Cancer site (events)	Risk and associated dietary intake of vitamin C
Shekelle et al, 1981 (198)	1954 Men (19 y)	Lung (33 cases)	101 mg/d in noncases compared with 92 mg/d in cases (NS)
Enstrom et al, 1986 (172)	3119 Men and women (10 y)	All cancers (68 deaths)	>250 compared with <250 mg/d: no ↓ risk
Kromhout, 1987 (199)	870 Dutch men (25 y)	Lung (63 deaths)	83–103 compared with <63 mg/d: ↓ risk by 64%
Knekt et al, 1991 (200)	4538 Finnish men (20 y)	Lung (117 cases)	83 mg/d in noncases compared with 81 mg/d in cases (NS)
Enstrom et al, 1992 (173)	4479 Men (10 y)	All cancers (228 deaths)	>50 mg/d + regular supplement: ↓ risk by 21%
	6869 Women (10 y)	All cancers (169 deaths)	>50 mg/d + regular supplement: no ↓ risk
Shibata et al, 1992 (201)	4277 Men (7 y)	All cancers (645 cases)	>210 compared with <145 mg/d: no ↓ risk; 500 mg/d supplement compared with no supplement: no ↓ risk
	7300 Women (7 y)	All cancers (690 cases)	>225 compared with <155 mg/d: ↓ risk by 24%; 500 mg/d supplement compared with no supplement: no ↓ risk
Graham et al, 1992 (202)	18586 Women (7 y)	Breast (344 cases)	>79 compared with <34 mg/d: no ↓ risk
Hunter et al, 1993 (203)	89494 US female nurses (8 y)	Breast (1439 cases)	>359 compared with <93 mg/d (total)[1]: no ↓ risk; regular supplement compared with no supplement: no ↓ risk; supplement ≥10 y compared with no supplement: no ↓ risk
Bostick et al, 1993 (204)	35215 Women (5 y)	Colon (212 cases)	>392 compared with <112 mg/d (total)[1]: ↓ risk (NS); >201 compared with <91 mg/d (diet): no ↓ risk; >60 mg/d supplement compared with no supplement: ↓ risk by 33%
Blot et al, 1993 (205)[2]	29584 Chinese men and women (5 y)	Esophageal-stomach	120 mg/d supplement: no ↓ risk (+ 30 μg Mo/d cosupplement)
Pandey et al, 1995 (182)	1556 Men (24 y)	All cancers (155 deaths)	>113 compared with <82 mg/d: ↓ risk by 39%
Losconczy et al, 1996 (184)	11178 Elderly men and women (6 y)	All cancers (761 deaths)	Regular supplement compared with no supplement: no ↓ risk
Sahyoun et al, 1996 (185)	<725 Elderly men and women (10 y)	All cancers (57 deaths)	>388 compared with <90 mg/d: no ↓ risk
Kushi et al, 1996 (206)	34387 Women (5 y)	Breast (879 cases)	>392 compared with <112 mg/d (total)[1]: no ↓ risk; >198 compared with <87 mg/d (diet): no ↓ risk; regular supplement compared with no supplement: no ↓ risk
Yong et al, 1997 (207)	3968 Men and 6100 women (19 y)	Lung (248)	82 mg/d in noncases compared with 64 mg/d in cases

[1]Intake from diet plus supplements.
[2]Trial (not prospective cohort study).

et al (204) observed a 33% reduction in colon cancer risk in a large population of women consuming >60 mg supplemental vitamin C/d. No association was apparent when supplements were taken for >10 y by patients with breast cancer (203). The Linxian trial found no significant effect of supplementing a population of Chinese men and women with 120 mg vitamin C/d and 30 μg Mo/d for 5 y on the risk of cancers of the esophagus or stomach (205). The results of clinical trials, however, depend on the use of sufficient doses, sufficient durations, and low baseline concentrations of vitamin C.

In summary, in most of the studies in Table 6 that reported no significant reduction in cancer risk, intakes of vitamin C in the lowest quantile were >86 mg/d; those studies that reported significant risk reductions (173, 182, 199, 207) found this effect in

individuals with vitamin C intakes ≥80–110 mg/d. As discussed above, this intake range of 80–110 mg/d is associated with vitamin C tissue saturation in healthy men (117). With one exception (204), studies investigating consumption of supplemental vitamin C, including the Linxian trial (205), did not show a protective effect against cancer, possibly because the dietary intake of vitamin C was already sufficient for tissue saturation. Therefore, the consensus protective intake emerging from the studies in Table 6 appears to be ≈80–110 mg vitamin C/d. More studies investigating cancer risk in persons with low vitamin C intakes are warranted.

Several case-control and prospective cohort studies have investigated the association between plasma concentrations of vitamin C and cancer risk (**Table 7**). Four studies found signifi-

TABLE 7
Plasma concentration of vitamin C associated with reduced cancer risk

Reference and type of study	Population (duration)	Cancer site	Risk and associated concentration of vitamin C	Estimated intake[1]
Stahelin et al, 1984 (208): case-control	129 Cases, 258 controls	All sites	51.5 μmol/L in controls compared with 44.9 μmol/L in cases	95 compared with 85 mg/d
Romney et al, 1985 (209): case-control	46 Cases, 34 controls	Cervix	42.6 μmol/L in controls compared with 20.5 μmol/L in cases	80 compared with 50 mg/d
Eichholzer et al, 1996 (210): prospective cohort	2974 Swiss men (17 y)	All sites (290 deaths)	>22.7 compared with <22.7 μmol/L: ↓ risk by 19% (NS)	55 mg/d
		Colon (22 deaths)	>22.7 compared with <22.7 μmol/L: ↓ risk by 42% (NS)	55 mg/d
		Stomach (28 deaths)	>22.7 compared with <22.7 μmol/L: no ↓ risk	55 mg/d
		Lung (87 deaths)	>22.7 compared with <22.7 μmol/L: ↓ risk by 45% (NS)	55 mg/d
		Prostate (30 deaths)	>22.7 compared with <22.7 μmol/L: no ↓ risk	55 mg/d
Sahyoun et al, 1996 (185): prospective cohort	<725 Elderly men and women (10 y)	All sites (57 deaths)	>88.6 compared with <51.7 μmol/L: ↓ risk by 32%	>400 compared with 95 mg/d
Ramaswamy and Krishnamoorthy, 1996 (211): case-control	100 Cases, 50 controls	Breast	112.5 μmol/L in controls compared with 35.8 μmol/L in cases	>400 compared with 75 mg/d
	100 Cases, 50 controls	Cervix	112.5 μmol/L in controls compared with 27.6 μmol/L in cases	>400 compared with 65 mg/d
Erhola et al, 1997 (212): case-control	57 Cases, 76 controls	Lung	46.5 μmol/L in controls compared with 34.0 μmol/L in cases	85 compared with 75 mg/d
Comstock et al, 1997 (46): case-control	258 Cases, 515 controls	Lung	69.8 μmol/L in controls compared with 59.0 μmol/L in cases (NS)	400 compared with 150 mg/d
Webb et al, 1997 (213): cross-sectional	1400 Men and women	Stomach (29 cases)	47.6 μmol/L in controls compared with 40.6 μmol/L in cases (NS)	90 compared with 80 mg/d

[1] According to data from Levine et al (117).

cantly higher concentrations of vitamin C in control subjects or survivors than in patients with cancer (208, 209, 211, 212), who in all 4 studies had plasma vitamin C concentrations <45 μmol/L. Two recent studies also found lower plasma vitamin C concentrations in cancer patients than in control subjects, but the differences were nonsignificant (46, 213). Because of the retrospective nature of case-control studies, it is not clear whether decreased plasma vitamin C concentrations in cancer patients are a cause or a consequence of the disease. In a prospective cohort study, Sahyoun et al (185) found a 32% lower risk of cancer in elderly persons with concentrations of vitamin C >89 μmol/L than in those with concentrations <52 μmol/L. The Basel study investigated cancers at several sites over 7–17 y of follow-up in a population of 2974 Swiss men (210, 214, 215). Vitamin C concentrations >23 μmol/L were associated with a nonsignificant reduction in risk of cancer at all sites after 17 y (210). Nonsignificant reductions of 42% and 45% in colon cancer and lung cancer risk, respectively, were observed. No associations were observed for risk of stomach cancer or prostate cancer, the latter being a hormone-dependent cancer and hence less likely to be affected by diet, including vitamin C (157). In a comprehensive review article, Gey (15) suggested that plasma vitamin C concentrations >50 μmol/L are associated with protection against

cancer, which is consistent with the findings of the studies listed in Table 7 and an intake of ≈100 mg/d (117).

Cataract

Cataract is the leading cause of blindness in the world (216), accounting for 50% of significant visual impairment among adults in developed countries (54). In the United States alone, more than one million cataract extractions are performed annually at a cost of >$5 billion. Cataract extraction is the most frequently performed surgery in elderly Americans and is the largest single item of Medicare expenditure, accounting for 12% of the budget (54, 216). The population at greatest risk of cataract is persons aged ≥55 y, with 5% of persons aged ≥65 y and 50% of persons aged ≥75 y having visually significant cataract.

Several epidemiologic studies have investigated the association of vitamin C intake with the incidence of cataract (**Table 8**). Two case-control studies indicated a strong inverse association between high intakes of vitamin C and cataract (217, 218). Robertson et al (217) found that intakes of >300 mg vitamin C/d were associated with a 70% reduced risk of cataract. Similarly, Jacques and Chylack (218) found that daily intakes of >490 mg were associated with a 75% lower risk of cataract than intakes <125 mg/d. These investigators also measured plasma concen-

TABLE 8
Vitamin C intake or plasma concentration with reduced cataract risk

Reference and type of study	Population (duration)	Endpoint	Risk and associated intake or plasma concentration of vitamin C
Dietary intake			
Robertson et al, 1989 (217): case-control	175 Cases, 175 controls	Cataract	>300 mg/d supplement: ↓ risk by 70%
Jacques and Chylack, 1991 (218): case-control	77 Cases, 35 controls	Cataract	>490 compared with <125 mg/d: ↓ risk by 75%
Hankinson et al, 1992 (219): prospective cohort	50828 Women (8 y)	Cataract (493 extractions)	705 compared with 70 mg/d: no ↓ risk; supplements for >10 y: ↓ risk by 45 %
Vitale et al, 1993 (220): longitudinal	660 Men and women (6 y)	Cataract	>261 compared with <115 mg/d: no ↓ risk
Sperduto et al, 1993 (221): trial	3249 Chinese men and women (5 y)	Cataract	120 mg/d supplement: ↓ risk by 22% (NS) (+ 30 µg Mo/d cosupplement)
Jacques et al, 1997 (222): cross-sectional	247 Women (10 y)	Cataract	>359 compared with <93 mg/d: no ↓ risk; supplements for >10 y: ↓ risk by 77–83%
Plasma concentration			
Jacques and Chylack, 1991 (218): case-control	77 Cases, 35 controls	Cataract	>90 compared with <40 µmol/L: ↓ risk by 71% (>400 compared with 80 mg/d)[1]
Vitale et al, 1993 (220): longitudinal	660 Men and women (6 y)	Cataract	>80 compared with <60 µmol/L: no ↓ risk (>400 compared with 150 mg/d)[1]

[1]Corresponding estimated dietary intake according to data from Levine et al (117).

trations of vitamin C: concentrations >90 µmol/L were associated with a 71% lower risk than concentrations <40 µmol/L. In contrast, Vitale et al (220) found no protective effect of 260 mg vitamin C/d over that of 115 mg/d and no association with plasma concentrations >80 µmol/L compared with <60 µmol/L.

Two studies, one of them involving >50000 women (219), indicated that vitamin C has a positive effect on cataract risk when supplements are taken for ≥10 y; risk reductions of 45% (219) and 77–83% (222) were reported. To date, one intervention trial has been carried out to determine the effect of vitamin C intake on cataract formation, among other diseases (221). In this trial, a large population of Chinese adults was supplemented with 120 mg vitamin C/d in conjunction with 30 µg Mo/d for 5 y, resulting in a nonsignificant reduction in cataract risk of 22%. A significant 36% risk reduction was observed in the same trial when a multivitamin and mineral supplement was consumed (221). Several other investigators reported reductions in cataract risk of ≈30–60% when multivitamin supplements or antioxidant combinations were consumed (216, 223–226). In the largest study, which included 17744 men (216), it was noted that although vitamin C supplements alone did not show an association with cataract, the numbers of that particular subpopulation were small.

As mentioned earlier, epidemiologic studies can provide only a semiquantitative estimation of vitamin C intake depending on the accuracy of dietary recall questionnaires; additionally, in many of the above-mentioned studies, intake at only one time point was assessed. Determination of plasma concentrations of vitamin C may more accurately reflect body stores of the vitamin. The protective concentrations of vitamin C in most of the diet-based cataract studies were relatively high (217–219, 222). Why amounts of vitamin C well above those resulting in tissue saturation (117) should reduce cataract is uncertain. Elderly persons may require higher intakes of vitamin C because of reduced bioavailability (54). Lens concentrations of vitamin C are related

to dietary intake and can be significantly increased with supplementation (54). Eye tissues may become saturated with vitamin C at intakes between 150 and 250 mg/d (54).

At this stage it is difficult to propose a protective vitamin C intake with respect to cataract because of the limited number of prospective cohort studies and the wide range of protective concentrations reported. The only intervention trial conducted showed nonsignificant reductions in cataract risk with 120 mg vitamin C/d (221). Providing for additional vitamin C intake in this study from the diet, and considering the intake required for eye tissue saturation (54), it is plausible that ≈150–200 mg vitamin C/d provides optimal protection against cataract. This estimate is consistent with the higher intakes in the epidemiologic studies. Long-term supplementation for ≥10 y may be of benefit in reducing the incidence of age-related cataract (219, 222).

SPECIAL POPULATIONS

Several populations warrant special attention with respect to vitamin C requirements. These include smokers, pregnant and lactating women, and the elderly. Persons with iron-overload conditions, such as homozygous hemochromatosis, and requiring treatment of β-thalassemia may also have different requirements (11, 227). Vitamin C requirements in severely iron-overloaded persons are complicated by safety issues, however, and it is beyond the scope of this review to discuss the tolerable upper intake level of vitamin C.

Smokers

A significant amount of research has indicated that smokers have a higher requirement for vitamin C than do nonsmokers (5, 8). Vitamin C concentrations are lower in smokers than in nonsmokers and are inversely related to cigarette consumption (88,

89, 228, 229). The lower vitamin C status of smokers is most likely due to increased turnover of the vitamin as a result of increased oxidative stress (88, 89, 196, 230). In one study, vitamin C supplementation (2000 mg/d for 5 d) significantly reduced the amount of urinary F_2-isoprostanes, an indicator of oxidative stress that is elevated in smokers, whereas vitamin E had no effect (77). The RDA for smokers is 100 mg vitamin C/d (6), although it has been proposed that smokers require ≥2–3-fold the current RDA of 60 mg/d to maintain plasma vitamin C concentrations comparable with those in nonsmokers (2, 5, 89, 231).

Pregnant and lactating women

Women who are pregnant or lactating also require a higher intake of vitamin C to maintain their plasma vitamin C concentrations near those of other women (8, 232). The higher requirement is probably due to active placental vitamin C transport, whereby vitamin C concentrations are significantly higher in cord blood and in newborn infants than in the mothers, and to additional loss of vitamin C through milk (8, 233). The current RDAs for women during pregnancy and lactation are 80 and 100 mg/d, respectively (6). If a new RDA for healthy, nonsmoking persons is adopted, then recommended intakes for pregnant and lactating women may also need to be adjusted accordingly.

The elderly

The elderly are prone to vitamin C deficiency, probably because of dietary habits (5, 8, 227). The elderly also appear to have a higher requirement for vitamin C (232), although the evidence is inconsistent, suggesting that further study is required. Oxidative processes have been implicated in aging (30) and it has been proposed that antioxidants may have beneficial effects on cognitive functions in the elderly. In one cross-sectional study there was no association between cognitive function and intakes of vitamin C ≥160 mg/d compared with intakes <70 mg/d (234). However, in another cross-sectional and longitudinal study, high plasma vitamin C concentrations were associated with better memory performance (235). A recent cohort study also showed that consumption of vitamin C supplements was associated with a lower prevalence of severe cognitive impairment (236). Finally, 2 other recent studies found that patients with Alzheimer disease have low plasma vitamin C concentrations despite an adequate diet and that supplementation with vitamin C may lower the risk of Alzheimer disease (237, 238).

SUMMARY AND CONCLUSIONS

Vitamin C is required for the optimal activity of several important biosynthetic enzymes and is therefore essential for various metabolic pathways in the body. A deficiency of this vitamin results in the symptoms of scurvy and death. Vitamin C acts as a cosubstrate for several mono- and dioxygenases and oxidases and maintains the active-site metal ions of these enzymes in the reduced state. Vitamin C also acts as an efficient scavenger of aqueous radicals and oxidants, thus protecting other biomolecules from oxidative damage. In addition, vitamin C can spare or recycle glutathione and vitamin E, 2 other important physiologic antioxidants.

Oxidative biomarker studies indicate that vitamin C protects against in vivo oxidation of lipids and DNA in humans, particularly in persons exposed to enhanced oxidative stress, such as smokers (Tables 1 and 2). Numerous epidemiologic studies strongly suggest that vitamin C lowers the incidence of and mortality from 2 of the most prevalent human diseases: cardiovascular disease (Tables 4 and 5) and cancer (Tables 6 and 7). This role of vitamin C in lowering disease incidence is most likely derived from its antioxidant activity, although other mechanisms may also contribute. In addition, vitamin C seems to have a substantial effect on cataract formation (Table 8), again most likely through an antioxidant mechanism. As such, the potential of adequate vitamin C nutriture to benefit public health and reduce the economic and medical costs associated with these chronic diseases is enormous.

If the antioxidant function of vitamin C is accepted as relevant to and important for human health, then morbidity and mortality from cancer, cardiovascular disease, and cataract in addition to scurvy must be used as criteria for determining vitamin C requirements. Therefore, the current RDA of 60 mg/d must be reevaluated and adjusted if justified by the available data. The totality of evidence from the human studies presented in Tables 4–7 strongly suggests that a dietary intake of 90–100 mg vitamin C/d is associated with reduced risk of cardiovascular disease and cancer; there is no indication that 46 mg/d is adequate, ie, the amount on which the current RDA of 60 mg/d is based (7). Therefore, we suggest that the RDA for vitamin C be doubled to 120 mg/d. Even higher intakes of vitamin C, and possibly supplementation, may be required to reduce cataract risk (Table 8), although the evidence is less secure because of the limited number of studies. Furthermore, chronic 500-mg/d doses or acute 1–3-g doses of vitamin C significantly improve vasoreactivity (Table 3), an important consideration for the clinical expression of cardiovascular and cerebrovascular disease (eg, angina pectoris, myocardial infarction, and stroke).

One might argue that the suggested RDA of 120 mg vitamin C/d for optimal risk reduction of cardiovascular disease and cancer is derived solely from epidemiologic studies and not clinical trials, and epidemiologic studies cannot establish causality, but merely show associations. However, these data are the best available for estimating vitamin C adequacy in humans. Clinical trials will not provide this information for several reasons: *1*) it is neither practical nor economically feasible to examine a range of vitamin C supplemental doses, ie, to perform detailed dose-response studies; *2*) the beneficial effects of vitamin C with respect to cardiovascular disease and cancer appear to be derived from intakes well within the dietary range, ie, supplementation has little or no effect; and *3*) without knowledge of exact baseline concentrations or intakes of vitamin C, total intakes cannot be determined. Thus, although clinical trials provide valuable information regarding the usefulness of supplements, they are unlikely to provide the data necessary to determine the RDA for vitamin C, because it appears to be well within the dietary range. Nevertheless, properly designed clinical trials, ie, double-blind, placebo-controlled, randomized trials of vitamin C supplementation in populations with low to very low vitamin C status, would be useful to provide the "proof of concept" that vitamin C can lower morbidity or mortality from cardiovascular disease, cancer, and cataract. Whether such trials are economically and logistically feasible and will be conducted in the foreseeable future is uncertain.

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