Authors' perspective: What is the optimum intake of vitamin C in humans?

Balz Frei, Ines Birlouez-Aragon, and Jens Lykkesfeldt*

Linus Pauling Institute, Oregon State University, Corvallis, OR, USA (BF)

Spectralys Innovation, Paris, France (IBA)

Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark (JL)

*To whom correspondence should be addressed: Balz Frei, Ph.D., Linus Pauling Institute, Oregon State University, 307 Linus Pauling Science Center, Corvallis, OR 97331, USA. Phone: +1 541 737-5075. FAX: +1 541 737-5077. Email: balz.frei@oregonstate.edu.

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KEY WORDS: Coronary heart disease, stroke, cancer, recommended dietary allowance.

Abbreviations:

CHD, Coronary Heart Disease; CRP, C-Reactive Protein; CVD, Cardiovascular Diseases; EPIC, European Prospective Investigation into Cancer and Nutrition; *H. pylori*, *Helicobacter pylori*; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; NHANES, National Health and Nutrition Examination Survey; RCT, Randomized Placebo-controlled Trial; RDA, Recommended Dietary Allowance; SU.VI.MAX, Supplementation en Vitamines et Mineraux Antioxydants; SVCT, Sodium-dependent Vitamin C Transporter.

Definitions:

Phase II Randomized Placebo-controlled Trial: Short-term (weeks or months) intervention study performed on groups of 20-400 subjects designed to assess the safety and efficacy of a drug in lowering risk factors or intermediary markers of disease, *e.g.*, hypertension, vascular dysfunction, chronic inflammation, or *Helicobacter pylori* infection.

Phase III Randomized Placebo-controlled Trial: Long-term (years) intervention study performed on groups of more than 400 subjects, usually in the thousands at multiple study centers, designed to assess the safety and efficacy of a drug in lowering (chronic) disease incidence or mortality.

ABSTRACT: The recommended dietary allowance (RDA) of vitamin C has traditionally been based on the prevention of the vitamin C deficiency disease, scurvy. While higher intakes of vitamin C may exert additional health benefits, the limited Phase III randomized placebo-controlled trials (RCTs) of vitamin C supplementation have not found consistent benefit with respect to chronic disease prevention. To date, this has precluded upward adjustments of the current RDA. Here we argue that Phase III RCTs—designed principally to test the safety and efficacy of pharmaceutical drugs—are ill suited to assess health benefits of essential nutrients; and the currently available scientific evidence is sufficient to determine the optimum intake of vitamin C in humans. This evidence establishes biological plausibility and mechanisms of action for vitamin C in the primary prevention of coronary heart disease, stroke, and cancer; and is buttressed by consistent data from prospective cohort studies based on blood analysis or dietary intake and well-designed Phase II RCTs. These RCTs show that vitamin C supplementation lowers hypertension, endothelial dysfunction, chronic inflammation, and *Helicobacter pylori* infection, which are independent risk factors of cardiovascular diseases and certain cancers. Furthermore, vitamin C acts as a biological antioxidant that can lower elevated levels of oxidative stress, which also may contribute to chronic disease prevention. Based on the combined evidence from human metabolic, pharmacokinetic, and observational studies and Phase II RCTs, we conclude that 200 mg per day is the optimum dietary intake of vitamin C for the majority of the adult population to maximize the vitamin's potential health benefits with the least risk of inadequacy or adverse health effects.

I. INTRODUCTION

Vitamin C is an essential nutrient for humans because we cannot synthesize ascorbic acid in our bodies. The dietary intake recommendations for vitamin C set by health agencies around the world have traditionally been intended to prevent the vitamin C deficiency disease, scurvy. However, the recommended dietary allowance (RDA) for vitamin C set by different health agencies differs widely (1-5), ranging from 40 mg/day for adults in the UK (6) to 75 and 90 mg, respectively, for adult women and men in the US, to 110 mg/day in France and Belgium (7, 8). In addition, some countries have set higher RDAs for certain subpopulations, such as smokers and pregnant or lactating women. Some health agencies, *e.g.*, in France (7) and the US (1, 9), have also started to consider additional health benefits of vitamin C beyond the prevention of scurvy and, therefore, have recently increased their RDA for vitamin C.

The currently available evidence from metabolic, pharmacokinetic, and epidemiologic studies strongly suggests that vitamin C intakes above current RDAs can contribute to the prevention of chronic diseases, in particular cardiovascular diseases (CVD)—principally coronary heart disease (CHD) and stroke—and certain cancers (reviewed in [10, 11]). However, large Phase III randomized placebo-controlled trials (RCTs) (see Definitions), currently considered the "gold standard" for establishing efficacy of dietary supplements in chronic disease prevention, have been disappointing, showing little or no beneficial effect of vitamin C supplementation on CVD or cancer incidence (12-16). However, we (17-19) and others (20, 21) have argued that current Phase III RCTs—designed principally to test safety and efficacy of *pharmaceutical drugs* in disease *treatment*—are ill suited to demonstrate efficacy in disease *prevention* of substances endogenously present in the human body and required for normal metabolism, such as *vitamins and other essential nutrients*. Even if study designs were improved, additional Phase III RCTs of vitamin C as primary intervention of CVD, cancer, and other chronic diseases will likely be cost–prohibitive and not be funded by

federal agencies or private industry, and, hence, will not be conducted in the foreseeable future.

We believe that the lack of apparent proof of benefit from Phase III RCTs should not prevent further adjustments of the RDA for vitamin C, and that the current scientific evidence from human studies is sufficient to justify an increase in recommended intake levels. This paper will review this evidence and conclude with a recommendation for the optimum intake of vitamin C in humans.

II. BIOLOGICAL FUNCTIONS OF VITAMIN C

Vitamin C has several well-known biological functions required for normal metabolism and cell function, including its role as an electron donor for at least nine monooxygenase and dioxygenase enzymes involved in pro-collagen hydroxylation, carnitine and norepinephrine biosynthesis, amidation of peptide hormones, tyrosine metabolism, and hydroxylation of hypoxia inducible factor-1α (22-24). Based on these enzymatic and other biological functions of vitamin C, beneficial cause-and-effect relationships between vitamin C intake and a range of health effects are known to date, including normal energy-yielding metabolism, collagen synthesis, non-heme iron absorption, and normal functioning of the nervous system (2, 24, 25).

The classical symptoms of scurvy are largely related to impaired collagen synthesis, such as impaired wound healing, gingivitis, perifollicular hemorrhages, ecchymoses, and petechiae (1). The principal symptoms of inadequate vitamin C intake without the classical symptoms of scurvy are malaise and fatigue or lethargy, which may be difficult to detect clinically (1, 26, 27). These latter symptoms may result from diminished levels of carnitine, which is needed for fatty acid transport and subsequent β-oxidation in mitochondria for ATP synthesis, and from decreased synthesis of the neurotransmitter, norepinephrine.

In addition, vitamin C is a strong antioxidant, acting as an effective scavenger of free radicals and other reactive oxygen and nitrogen species under physiological conditions (11, 24, 28-31). While the cause-and-effect relationship between vitamin C's antioxidant functions and related health effects remains to be established, such a relationship is suggested by the protection of biological macromolecules from oxidative damage that might otherwise causally contribute to the pathogenesis of numerous chronic and acute diseases (2). Therefore, it is plausible that vitamin C, through its antioxidant actions and other biological mechanisms, plays a role in preventing certain cancers, enhancing immune function, and ameliorating chronic inflammatory conditions, such as atherosclerosis and resulting CVD (10). The possible role of vitamin C in chronic disease prevention will be further discussed below (section IV.).

III. VITAMIN C STATUS OF THE GENERAL POPULATION

While the vitamin C deficiency disease scurvy nowadays is thought to be rare in the developed world (32), several surveys suggest that a substantial proportion of the general population does not meet current estimated average requirements (33-36). Consistently, recent studies in Europe, Canada, and the US (37-41) have shown that blood levels of vitamin C in Western populations are far from optimal, with a high prevalence of both severely deficient (<11 µmol/L) and marginally deficient (11-23 or 28 µmol/L) vitamin C plasma levels 42). Severely deficient vitamin C plasma levels correlate with clinical features of scurvy, while marginally deficient levels may be associated with early signs of scurvy, such as gingival inflammation, fatigue, and—in infants—bone abnormalities (1, 42). In the MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) project, 24% of the study population exhibited marginal vitamin C deficiency (<22.7 µmol/L) and 20% had severe deficiency (<11.4 µmol/L) (38). In a French population survey from 1988, 3-

46% of the population was marginally deficient (11-23 µmol/L) and 3-12% severely deficient (<11 µmol/L) (39). Similarly, a recent study showed that 33% of young Canadians had marginal (11-28 µmol/L) and 14% deficient (<11 µmol/L) vitamin C plasma levels (40). Finally, in the 2003-2004 National Health and Nutrition Examination Survey (NHANES), 7% of the general population in the US was vitamin C deficient (<11.4 µmol/L) (41).

The RDA is defined as "the dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group" (1), and hence represents the nutritional needs of the general population. However, some subgroups may have increased vitamin C needs due to decreased absorption or increased utilization or excretion. Smokers, for example, have lower plasma levels of vitamin C than non-smokers, even after adjustment for dietary vitamin C intake (1, 23, 38, 43); this is thought to be due to increased oxidative stress in smokers (1, 44). In the MONICA study, 36% of male smokers and 23% of female smokers exhibited severe vitamin C deficiency (38). Pregnant women also have an increased need for vitamin C, especially in the last trimester, to meet the needs of the growing fetus (1, 45). Furthermore, during lactation, up to 20 mg/day of vitamin C is secreted in breast milk, requiring an increased vitamin C intake to meet both the mother's and infant's needs (45).

Other subpopulations that appear to have an increased need for vitamin C are older adults, children, and exercisers. Older adults may have lower intestinal absorption of vitamin C than younger subjects (46, 47), although other studies have suggested that older subjects have normal plasma vitamin C levels when dietary vitamin C intake is adequate (39, 48-50). However, there is agreement that older individuals often do not get enough vitamin C from their diet. An adequate intake of vitamin C also is important for children to ensure normal functioning of the immune system, normal growth of bones and other collagen-containing structures, and normal energy metabolism. Related to the latter function of vitamin C in

energy-yielding fat metabolism, vitamin C needs might be higher in people undergoing strenuous physical exercise or suffering from emotional or other types of stress (2).

IV. POTENTIAL HEALTH BENEFITS ASSOCIATED WITH VITAMIN C INTAKES EXCEEDING CURRENT RDAs

A. Epidemiologic Evidence

1. Observational Studies

In addition to the well-known biological and metabolic functions of vitamin C and related health effects described above, an increasing body of evidence suggests that there are additional health benefits associated with above-RDA intakes of vitamin C. Epidemiologic studies have found inverse associations between plasma or serum levels of vitamin C and incidence or risk of chronic diseases, in particular CVD (51-58) and cancer (59-62). Although many epidemiologic studies based on dietary intake of vitamin C exist supporting these results from studies based on blood analysis, this review focuses mainly on the latter, because plasma or serum levels of vitamin C is a much more accurate indicator of body vitamin C status than dietary intake estimated from food frequency questionnaires or food diaries. Dietary intake studies are inherently imprecise due to human recall error, and also because they neither take into account vitamin C loss during food storage and preparation (63, 64) nor polymorphisms affecting vitamin C bioavailability and metabolism, such as sodium-dependent vitamin C transporter 1 (SVCT1), glutathione *S*-transferases, and haptoglobin genotype (40, 63, 65).

a. Cardiovascular Diseases

With respect to observational studies based on blood analysis that support a role of vitamin C in CVD prevention (Table 1), an investigation of participants in NHANES II

(n=8,453) concluded that individuals with normal or high serum vitamin C levels (45.4 and 79.5 μmol/L, respectively) had a "marginally" significant 21-25% lower risk of CVD-related deaths and a significant 25-29% lower risk of all-cause mortality, compared to participants with low serum levels of vitamin C (<0.4 mg/dl [<~23 μmol/L]) (55). Furthermore, a prospective nested case-control study found a 33% lower risk of developing CHD in subjects with the highest plasma vitamin C levels (mean, 77.1 μmol/L) compared to those with the lowest levels (mean, 27.6 μmol/L) over an average of six years of follow-up (51). The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study found that plasma vitamin C concentrations in 8,860 men and 10,636 women were inversely related to mortality from all causes and CVD (66). In this study, each 20 μmol/L increase in plasma vitamin C was associated with about a 20% and 30% reduction, respectively, in risk of all-cause and CVD mortality (66). Several other studies also support the association of increased vitamin C plasma or serum levels (mean values ranging from 49.5 to 85.2 μmol/L) and decreased CVD risk (56-58) (Table 1). Consistent with these findings, in young type 1 diabetic patients, a poor vitamin C status was found to be associated with an increase in several early cardiovascular risk markers (67).

Many studies have shown an association between higher vitamin C plasma concentrations with a reduced risk of stroke or stroke-related mortality (Table 1). Gale *et al.* (52) observed a 30% lower risk of death from stroke in old adults with vitamin C levels >27.8 μmol/L at baseline compared to those with vitamin C levels ≤27.8 μmol/L. This association has been confirmed among 20,649 participants in the EPIC-Norfolk study, where the risk of stroke over 10 years of follow-up was 42% lower in subjects with baseline plasma vitamin C levels ≥66 μmol/L compared to those with vitamin C levels <41 μmol/L (53). Similarly, in a Japanese cohort, subjects with vitamin C levels ≥64 μmol/L had a 41% lower risk of stroke than those with vitamin C ≤40 μmol/L over 20 years of follow-up (54). A Finish study

reported a more than two-fold increased risk of stroke in subjects with plasma levels <28.4 µmol/L compared to subjects with vitamin C levels >65.0 µmol/L (68). In addition, Polidori *et al.* (69) observed that plasma vitamin C levels were decreased in patients with intracranial hemorrhage or head trauma (mean, 29.0 and 31.3 µmol/L, respectively) compared to healthy young or older adult subjects (56.9 and 51.6 µmol/L, respectively). Plasma vitamin C levels in hemorrhagic stroke and head trauma patients also were inversely correlated with brain lesion size (69).

It has been suggested that plasma or serum levels of vitamin C are merely a marker of fruit and vegetable intake and, hence, the observed inverse associations with CVD risk may be due to increased fruit and vegetable consumption, not vitamin C *per se* (53, 66). However, the association between fruit and vegetable intake and CHD or stroke risk appears to be considerably weaker than the association with vitamin C plasma levels. Two recent meta-analyses by He and colleagues (70, 71) found that the pooled relative risk for CHD and stroke, respectively, was 17% and 26% lower in subjects consuming more than 5 daily servings of fruits and vegetables compared to those consuming less than 3 servings per day. In part, this weak association may be explained by the fact that many commonly consumed fruits and vegetables contain little vitamin C, such as apples, bananas, tomatoes, potatoes, and carrots. In contrast, data from the EPIC-Norfolk study showed that the relative CHD risk was 68% and 93% lower, respectively, in men and women in the top quintile of mean plasma vitamin C concentration (72.6 µmol/L in men and 85.1 µmol/L in women) compared to those in the lowest quintile (20.8 µmol/L and 30.3 µmol/L, respectively); and the relative risk for stroke was 42% lower in subjects in the top (78.1 µmol/L) compared to the bottom (28.2 µmol/L) quartile of plasma vitamin C (53, 66). In addition, the Nurses' Health Study found an inverse association between CVD risk and vitamin C *supplement* use, which was strongest and statistically significant among women who took >400 mg/day and for more than 10 years

(29% and 30% lower relative risk, respectively) (72). Therefore, it is very likely that vitamin C is one of the main cardioprotective factors in fruits and vegetables and *by itself* exerts health benefits in CHD and stroke.

b. Cancer

With regard to vitamin C and cancer chemoprevention, among participants of NHANES II, men with the lowest serum vitamin C levels (<28.4 μmol/L) had a 62% higher risk of cancer-related deaths and a 57% higher risk of all-cause mortality after 12-16 years of follow-up than men with the highest vitamin C levels (≥73.8 μmol/L) (59). In a case-control study nested within the EPIC study (EPIC-EURGAST), subjects with the highest plasma vitamin C levels (≥51.0 μmol/L) had a 45% lower risk of gastric cancer than those with the lowest plasma vitamin C levels (<29.0 μmol/L) (60). The EPIC-Norfolk study found a 53% lower cancer mortality among men with vitamin C plasma levels in the highest compared to the lowest quintile (mean, 72.6 μmol/L *vs.* 20.8 μmol/L), while women in the highest *vs.* lowest quintile had a 27% decreased risk, although the trend across quintiles was not significant (66).

Several case-control studies also have found significantly lower plasma or serum levels of vitamin C in cancer patients compared to healthy controls, *e.g.*, in patients with multiple myeloma (61). Among 50 patients with advanced cancer, almost one-third were observed to have severe vitamin C deficiency, which was associated with shorter survival and increased levels of inflammatory markers (62). In the latter two studies (61, 62) the lower plasma vitamin C levels in cancer patients may have been a consequence rather than a contributing cause of the disease, a well-recognized limitation of case-control studies. This limitation does not apply, however, to all the prospective cohort studies and nested case-control studies discussed above and listed in Table 1 (73).

2. Phase III Randomized Placebo-controlled Trials

The consistent associations between high plasma or serum vitamin C status and decreased risk of CHD, stroke, and cancer in the observational epidemiologic literature (Table 1) have prompted a number of intervention studies in which the potential health benefits of vitamin C were explored alone or, much more often, in combination with other "antioxidant vitamins," *i.e.*, vitamin E and β-carotene, or as part of a multivitamin-mineral (Table 2). In fact, of the 16 Phase III RCTs listed in Table 2, only five used vitamin C as a single intervention (15, 16, 77, 82, 83).

In contrast to the consistent findings from observational studies based on blood analysis or dietary intake, most Phase III RCTs investigating CVD, cancer, or other disease morbidity or mortality as endpoints have been unable to show a positive effect of vitamin C supplementation (12-16, 74-84). While the studies did not find harmful effects of vitamin C either, the lack of evidence from Phase III RCTs confirming the observational findings has been detrimental to any further considerations to increase the RDA for vitamin C.

In a recent comprehensive review, we have pointed out several important design issues that have been neglected in the majority of the relevant clinical trials of vitamin C supplementation ([17] and references therein). One predominant issue is that none of the Phase III RCTs has used high plasma vitamin C levels at baseline as an exclusion criterion. Pharmacokinetic studies in humans have shown that plasma and cellular levels of vitamin C are saturable (Figs. 1 and 2) (26, 27). Hence, vitamin C supplementation of subjects who already have high or saturating plasma and body vitamin C status cannot be expected to provide additional health benefits (17). This is a particular problem for Phase III RCTs, as they tend to recruit health-conscious, self-motivated subjects who already eat a healthful diet–

–likely high in vitamin C—and have lower disease rates than the general population; a phenomenon known as the "healthy enrollee effect" (19, 85).

Other important limitations of applying a study design traditionally used for testing safety and efficacy of pharmaceutical drugs to testing health benefits of essential nutrients have been identified and discussed in detail (10, 17-21). For example, in Phase III RCTs of vitamins, even subjects who receive placebo have a life-long exposure to the vitamin and continue to ingest it from their diet throughout the study; hence, there is no true placebo control group in these RCTs, just a "lower-dose" group. This severely limits the statistical power of the study and should be taken into consideration in study design and data interpretation.

In addition, Phase III RCTs of drugs are usually conducted in diseased individuals (secondary prevention) or individuals with elevated risk factors, whereas primary disease prevention studies using supplemental micronutrients are generally conducted in healthy subjects; this requires supplementation for a very long time to accumulate enough disease endpoints. As mentioned above, the Nurses' Health Study found an approximately 30% CVD risk reduction that was statistically significant only in those women who took vitamin C supplements greater than 400 mg/day for 10 years or more (72); however, only two of the 16 Phase III RCTs listed in Table 2 met both of those criteria (15, 16).

Finally, pharmaceutical drugs are xenobiotics that are metabolized very differently from vitamins, for which specific transport mechanisms have evolved; drugs induce phase I-III metabolism and are excreted rapidly in bile and urine, while vitamin C is required for normal metabolism and, hence, is efficiently absorbed from the intestinal tract, reabsorbed from the proximal tubules in the kidneys, and retained in cells and tissues at very high, millimolar concentrations.

These serious issues make it evident that the Phase III RCTs of vitamin C supplementation conducted to date (Table 2) are neither useful for detecting benefits with respect to primary prevention of chronic disease nor informing dietary intake recommendations aimed at maximizing the potential health benefits of vitamin C; instead, these recommendations should be based on human metabolic, pharmacokinetic, and observational studies and Phase II RCTs; with mechanistic underpinnings and biological plausibility also derived from basic and pre-clinical animal studies.

B. Plausible Underlying Mechanisms for the Health Effects of Vitamin C in Chronic Disease Prevention

Numerous mechanisms have been identified that may underlie vitamin C's anti-cancer and cardiovascular health effects suggested by the observational epidemiologic data (Table 1). As mentioned above, vitamin C is a highly effective antioxidant that can protect biological macromolecules from oxidative damage. Several oxidative DNA lesions are known to be mutagenic, and redox-sensitive cell signalling pathways can activate transcription factors that affect cell growth or apoptosis and, hence, carcinogenesis (86). Similarly, oxidative modifications of low-density lipoprotein (LDL) and redox-imbalances in vascular cells have been implicated in the pathogenesis of atherosclerosis (87). Vitamin C has been shown to protect LDL from oxidation by pathophysiologically relevant types of oxidative stress, such as activated leukocytes, leukocyte-derived reactive oxygen species, particularly hypochlorous acid, and cigarette smoke (18, 28, 29). Human studies have found vitamin C supplementation to lower elevated levels of F_2-isoprostanes, an established *in vivo* marker of lipid peroxidation, in both active and passive smokers (88, 89) and non-smokers (90, 91). Similarly, vitamin C supplementation has been shown to lower elevated levels of 8-hydroxy-2'-deoxyguanosine, an established marker of oxidative DNA damage, in hemodialysis patients

(92); and baseline serum levels of ascorbic acid were inversely associated with urinary 8-hydroxy-2'-deoxyguanosine in nonsmoking adults (93).

While the role of oxidative stress in chronic disease causation remains controversial, primarily because of the failure of Phase III RCTs of "antioxidant vitamins" (vitamins C and E and ß-carotene) in chronic disease prevention or treatment (ref. 1; see above for limitations of these trials), vitamin C supplementation has been shown to positively affect risk factors or intermediary markers of CVD and cancer. In particular, several dozen Phase II RCTs (see Definitions) have demonstrated that vitamin C supplementation improves endothelial function and vasodilation in CHD patients or subjects with CVD risk factors, such as hypercholesterolemia, hypertension, smoking, or diabetes (11, 94-97). Endothelial dysfunction and impaired vasodilation are being increasingly recognized as an independent CVD risk factor (98-100). The beneficial effects of vitamin C on endothelial function are likely explained by increased activity of the enzyme, endothelial nitric oxide synthase (eNOS), due to vitamin C-dependent recycling of its essential cofactor, tetrahydrobiopterin (101).

Furthermore, vitamin C supplementation has been shown to lower plasma levels of C-reactive protein (CRP), an established marker of chronic inflammation and independent risk factor for CVD (102). Consistent with this finding from a Phase II RCT (102), Langlois *et al*. found that serum vitamin C concentrations were low and associated with both increased CRP levels and severity of atherosclerosis in patients with peripheral arterial disease (58). In a recent study, plasma levels of CRP and myeloperoxidase, the enzyme that generates hypochlorous acid in leukocytes, were inversely related to endothelial function and plasma vitamin C levels in normal-weight and obese men (103).Importantly, vitamin C has been demonstrated to affect hypertension, one of the most significant and prevalent risk factors of CHD and stroke. Numerous observational studies reported that plasma levels of vitamin C are

inversely associated with systolic and diastolic blood pressure (10, 104). We found that daily supplementation with 500 mg of vitamin C for 30 days significantly lowered systolic blood pressure in moderately hypertensive patients (105). A recent pooled meta-analysis of 29 Phase II RCTs using a median dose of 500 mg/day of vitamin C, including the above study (105), found that vitamin C supplementation significantly reduces systolic blood pressure in both hypertensive and non-hypertensive subjects by 4.85 and 3.11 mm Hg, respectively (106). Furthermore, vitamin C supplementation significantly reduced diastolic blood pressure in all subjects by 1.48 mm Hg. The authors noted that such reductions in blood pressure would be expected to significantly lower CVD risk in vitamin C supplemented subjects, but that Phase III RCTs have been unable to confirm this (see also Table 2), most likely due to "limitations" in study design. They concluded that "Long-term trials with clinical endpoints are difficult and costly but are still needed to determine whether vitamin C supplementation reduces risk of cardiovascular events" (106).

Vitamin C may also be important in ensuring adequate collagen content of blood vessels and atherosclerotic plaques, thereby supporting vascular integrity and decreasing the risk of plaque rupture (107). Finally, vitamin C has been shown to effectively inhibit cigarette smoke or oxidized LDL-induced leukocyte adhesion to the vascular endothelium *in vivo*, a critical step in the initiation and progression of atherosclerosis (108, 109).

With respect to underlying mechanisms for cancer chemoprevention, in addition to protecting DNA from oxidative damage and lowering chronic inflammation (*vide supra*), vitamin C has long been known to inhibit formation of carcinogenic *N*-nitroso compounds from dietary nitrites and nitrates, which are strongly implicated in the etiology of gastric cancer (110). In addition, a Phase II RCT found that high-dose vitamin C treatment eradicated *Helicobacter pylori* (*H. pylori*) infection in 30% of patients with chronic gastritis (111). Vitamin C has also been shown to enhance the immune response to *H. pylori* infection (112).

These findings have important implications for the prevention and treatment of gastric cancer, as *H. pylori* infection is now recognized as a major risk factor. Finally, it has become clear that vitamin C plays an important role in the hydroxylation, and hence proteosomal degradation and inactivation, of hypoxia inducible factor-1α, which prevents this transcription factor from upregulating genes involved in angiogenesis and, hence, tumor growth and metastasis (113).

V. RECOMMENDATION FOR AN OPTIMUM DAILY INTAKE OF VITAMIN C

The above discussed scientific literature indicates that the highest plasma levels of vitamin C are associated with the greatest health benefits for CHD, stroke, and cancer, as well as all-cause mortality (Table 1). In 10 of the 14 studies listed in Table 1, the mean plasma vitamin C levels in the highest quantile or category of participating subjects were between 64.0-85.2 µmol/L. These data are remarkably consistent with pharmacokinetic data in humans, showing that steady-state levels of plasma vitamin C reach a maximum of 70-80 µmol/L (26, 27). Specifically, in healthy, young adult males and females previously depleted of vitamin C, there is a steep, linear increase in plasma steady-state concentrations of vitamin C from about 10 µmol/L at an intake of 30 mg/day to about 60 µmol/L at 100 mg/day; vitamin C plasma concentrations are near saturation at intakes of 200-400 mg/day and reach a plateau of about 80 µmol/L at intakes of 1,000 and 2,500 mg/day (Figure 1) (9, 26, 27, 114).

Hence, 200 mg/day is the first dose of vitamin C beyond the steep, linear part of the sigmoid dose-response curve and is associated with a plasma concentration of approximately 70 µmol/L (Figure 1). This concentration also falls within the range of 60-100 µmol/L where the human sodium-dependent vitamin C (tissue) transporter 2 (SVCT2) is at its V_{max} (115). Indeed, neutrophils and other circulating cells are saturated with vitamin C at a daily intake of 200 mg (Figure 2), suggesting saturation of all tissues at this dose (9, 17, 26, 27).

Correspondingly, when plasma levels exceed the renal threshold of about 70 µmol/L, there is a sharp increase in the amount of vitamin C excreted in urine (114).

Therefore, a vitamin C intake of at least 200 mg/day can be considered "optimal," because it is the amount of vitamin C that achieves near-saturation of plasma and full saturation of cells and—presumably—tissues. Tissue saturation of vitamin C is desirable because it maximizes the potential health benefits of vitamin C with no risk of inadequacy or adverse health effects (1). Additionally, as indicated above, a daily amount of 200 mg is the first dose beyond the linear part of the sigmoid plasma concentration curve; below this dose, small changes in intake can result in large variations of plasma levels (27, 116). Therefore, recommended intake levels of vitamin C for the general population should not be below 200 mg/day, yet currently all RDAs of vitamin C in the US and various other countries are well below that threshold value.

In addition to the pharmacokinetic studies of Levine *et al*. comparing vitamin C dose to plasma levels (26, 27), the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) study measured plasma levels and assessed dietary intake of vitamin C in 5,625 French subjects (14, 117). This Phase III RCT found that plasma vitamin C levels did not further increase significantly above 57 and 65 µmol/L in men and women, respectively, at dietary intakes of about 175 mg/day or higher. These data are comparable to the near-saturation level of about 70 µmol/L observed by Levine *et al.* at a daily dose of 200 mg (26, 27). These similarities are remarkable given the large inter-individual differences in vitamin C bioavailability and the different study designs, *i.e*., depletion-repletion studies giving defined doses of vitamin C to experimental subjects under highly controlled conditions (26, 27) *versus* RCTs such as the SU.VI.MAX, which estimated vitamin C intakes of free-living subjects using food questionnaires (117, 118).

The SU.VI.MAX data were used to determine the most recent RDA of vitamin C in France. Based on the premise that plasma saturation is "optimal for prevention of degenerative pathologies," and using the standard method for calculating RDAs, the French RDA for vitamin C was set at 110 mg/day (117). This was based on the mean dietary intake of vitamin C associated with plasma saturation in a sub-population of 700 men and women in the SU.VI.MAX study, which was estimated to be 80 mg/day (117). To this value, 30% was added—corresponding to two theoretical standard deviations—to cover the needs of 97.5% of the total population, which resulted in an RDA of 110 mg/day. However, considering the real standard deviation of about 60% observed in this sub-population, an estimated daily intake of 180 to 250 mg of vitamin C would be required to truly cover the needs of 97.5% of the population (7).

Another argument supporting our proposed optimum daily intake of 200 mg is that bioavailability of vitamin C is 100% of a 200-mg dose but declines significantly at higher doses, with only approximately 75 and 50% bioavailable, respectively, of a 500 and 1,250-mg dose (27). These data indicate that intestinal vitamin C transport mechanisms in humans, primarily SVCT1 (24), have evolved to fully absorb up to about 200 mg of vitamin C. In addition, vitamin C is reabsorbed in the proximal renal tubules by SVCT1, which helps maintain plasma levels at a maximum concentration of 70-80 μmol/L; this concentration is reached in humans by an intake of about 200 mg/day of vitamin C (see above).

Finally, a daily intake of 200 mg of vitamin C may be achieved without the need for supplementation through the consumption of the recommended five to nine servings of fruit and vegetables. For example, a survey in France of 4,000 subjects representative of the French socio-demographic distribution found that consuming 430 g of fruit and vegetables provides about 100 mg of vitamin C (119). Adding a daily 200-mL glass (6.8 fl. oz.) of orange juice, either processed or freshly squeezed, increases the vitamin C intake by about 60

or 100 mg, respectively. Moreover, steam cooking vegetables preserves their vitamin C content, while frying or pan-cooking results in substantial loss (120). Therefore, a diet including five to nine servings of fruit and raw or steam-cooked vegetables and 200 mL of fresh orange juice could provide the 200-mg vitamin C dose proposed.

When proposing an increased intake of vitamin C, not only potential health benefits but also potential risks need to be considered. At an intake of 200 mg/day, no safety issues have been observed, and safety and toxicity assessments report no evidence of harm at vitamin C intakes up to 3 g/day (1, 121, 122). Even in individuals at increased risk of toxicity, *e.g.*, hemochromatosis heterozygotes and subjects with thalassemia or prior kidney stones, adverse health effects tend to occur only at doses above 1 g/day (123). Therefore, most health agencies agree on a tolerable upper intake level of 1-2 g/day, 5 to 10-fold higher than the 200 mg/day proposed here as optimum intake (1, 8, 45).

VI. CONCLUSIONS

Recent literature has attempted to determine the intake of vitamin C in humans that would be needed to not only prevent clinical deficiency but also help protect against chronic disease. While randomized, placebo-controlled Phase III trials have found limited or no health benefits of vitamin C supplementation for chronic disease treatment or prevention, these trials suffer from serious limitations that make positive outcomes nearly impossible. Since it is unlikely that further and better-designed Phase III RCTs of vitamin C supplementation will be forthcoming in the foreseeable future, we contend that dietary intake recommendations for vitamin C should be based on currently available data from human metabolic, pharmacokinetic, and observational studies and Phase II RCTs.

As discussed in this perspective, a role of vitamin C in the primary prevention of coronary heart disease, stroke, and certain cancers, in particular gastric cancer, is biologically

plausible and strongly supported by observational epidemiologic data based on blood analysis (51-62, 66-69) and numerous well-designed Phase II RCTs (11, 88-92, 94-97, 102, 105, 106, 111) showing benefits of vitamin C in reducing hypertension, endothelial dysfunction, chronic inflammation, oxidative stress, and *H. pylori* infection. This evidence establishes a highly favorable benefit-to-risk ratio for vitamin C in human health promotion and chronic disease prevention. Based on these considerations and human pharmacokinetic data, we propose that 200 mg is the optimum daily intake of vitamin C for the majority of the adult population. This proposed amount of vitamin C is consistent with the concept that the recommended dietary allowance should maximize potential health benefits with the least risk of inadequacy or adverse health effects (1).

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Table 1. Observational studies reporting positive health effects associated with elevated plasma or serum levels of vitamin C

Ref.	Study population	Mean vitamin C level associated with health effect	Disease outcome	Main results
(55)	8,453 adults	45.4 µmol/L (normal) and 79.5 µmol/L	CVD, all-cause mortality	Subjects with normal or saturating serum ascorbic acid levels (45.4 µmol/L and 79.5 µmol/L, respectively) had a "marginally" significant 21-25% decreased risk of fatal CVD and a significant 25-29% decreased risk of all-cause mortality compared to subjects with low serum ascorbic acid levels (17.0 µmol/L).
(51)	979 cases and 1794 controls	77.1 µmol/L	CHD	Subjects with the highest vitamin C plasma levels (highest quartile, mean, 77.1 µmol/L) had a 33% lower risk of CHD compared to those in the lowest quartile (mean, 27.6 µmol/L).
(66)	19,496 men and women	72.6 µmol/L in men and 85.1 µmol/L in women	CVD, cancer, all-cause mortality	Subjects in the highest quintile of plasma ascorbic acid (72.6 µmol/L in men and 85.1 µmol/L in women) had about half the risk of total mortality and a greater than 60% lower risk of CVD mortality compared to those in the lowest quintile (20.8 µmol/L and 30.3 µmol/L, respectively), using an age and sex-adjusted Cox regression model. Mean plasma ascorbic acid by quintiles was inversely associated with mortality from all-causes and CVD in men and women, and from cancer in men. A 20 µmol/L increase in plasma ascorbic acid was associated with about a 20% reduction in risk of all-cause mortality.

(56)	6,624 adults	85.2 µmol/L	Stroke, CHD	Subjects in the highest category of serum vitamin C (saturation, 85.2 µmol/L) had a 26% reduction in stroke and 27% reduction in CHD prevalence compared to the lowest category (low to marginal, 17.0 µmol/L).
(57)	1605 men	64.8 µmol/L	Myocardial infarction	Subjects with the lowest vitamin C plasma levels (deficiency, <11.4 µmol/L) had a 4-fold higher risk of myocardial infarction compared to subjects with the highest levels (>64.8 µmol/L), after adjustment for age, season, and year of examination.
(58)	85 patients with peripheral arterial disease (PAD), 106 hypertensives without PAD, and 113 healthy subjects	51.7 µmol/L (healthy) and 49.6 µmol/L (hypertensives without PAD)	Peripheral arterial disease	Serum ascorbic acid concentrations were low among PAD patients (median, 27.8 µmol/L) despite comparable smoking status and dietary intake with the other groups (median, 51.7 µmol/L in healthy subjects and 49.6 µmol/L in hypertensive patients without PAD).
(52)	730 men and women	>27.8 µmol/L	Stroke	Subjects with the highest vitamin C plasma levels (>27.8 µmol/L) had a 30% lower risk of death from stroke compared to subjects with lower vitamin C levels.
(53)	20,649 men and women	78.1 µmol/L	Stroke	Subjects in the top quartile of baseline plasma vitamin C (78.1 µmol/L) had a 42% lower risk of stroke than those in the bottom quartile (28.2 µmol/L), independent of age, sex, BMI, systolic blood pressure, smoking, alcohol consumption, cholesterol, social class, physical activity, diabetes, myocardial infarction, or supplement use.

(54)	2,121 men and women	64.0 µmol/L	Stroke	Subjects with the highest vitamin C serum levels (≥64 µmol/L, top quartile) had a 41% lower risk of all stroke than those with the lowest levels (≤40 µmol/L, bottom quartile). The corresponding risk reductions for cerebral infarction and hemorrhagic stroke were 49% and 55%,
(68)	2419 middle aged men	>65.0 µmol/L	Stroke	Men with the lowest plasma levels of vitamin C (<28.4 µmol/L, bottom quartile) had a 2.4-fold higher risk of any stroke compared with men with highest plasma levels of vitamin C (>65.0 µmol/L, top quartile), after adjustment for age and examination month.
(69)	13 patients with intracranial hemorrhage (ICH), 15 patients with head trauma (HT), and 40 healthy controls	51.6 µmol/L in young adult control subjects and 56.9 µmol/L in older control subjects	Intracranial hemorrhage, head trauma	ICH and HT patients had significantly lower plasma levels of vitamin C compared with healthy subjects (29.0-31.3 µmol/L in patients compared to 51.6-56.9 µmol/L in healthy controls). Brain lesion size was inversely associated with plasma ascorbic acid concentration.
(59)	7,071 men and women	≥73.8 µmol/L in men, ≥85.2 µmol/L in women	Cancer, all-cause mortality	Men in the lowest quartile (<28.4 µmol/L) had a 57% higher risk of dying from any cause and 62% higher risk of dying from cancer than men in the highest quartile (≥73.8 µmol/L).
(60)	215 cases and 416 controls	>82.0 µmol/L in cases, >75.0 µmol/L in controls	Gastric cancer	Plasma vitamin C levels were inversely associated with gastric cancer risk, which was significant in the highest versus the lowest quartile in both groups (5^{th}–95^{th} percentile: 11.0–82.0 µmol/L in cases and 12.0–75.0 µmol/L in controls).

(62)	50 men and women with advanced cancer	>11 µmol/L	Cancer survival, inflammatory markers	Low dietary intake, low albumin, high platelet count, high CRP level, and shorter survival were all significantly associated with low plasma vitamin C concentrations (<11 µmol/L).

Table 2. Phase III randomized placebo-controlled trials using vitamin C as part of the intervention

Ref.	Study population	Design	Supple-mentation period (yrs)	Vitamin C dose (mg/day)	Interventions (1.), major disease outcomes (2.), and main results (3.)
12	29,584 adults aged 40 yrs or older	½ (2 x 2 x 2 x 2)	5.25	120	1. Specific vitamin/mineral combinations of retinol+Zn; riboflavin+niacin; vitamin C+Mo; β-carotene+Se+vitamin E 2. Cancer incidence and disease-specific mortality 3. No effect of vitamin C+Mo supplementation
13	20,536 adults aged 40 yrs or older with CHD or other occlusive arterial disease or diabetes	2 x 2	5	250	1. Vitamins C+E+β-carotene (antioxidant vitamins); cholesterol-lowering therapy 2. Major coronary events (for overall analyses) and fatal or non-fatal vascular events (for subcategory analyses) 3. No effect of antioxidant vitamin supplementation
14	13,017 adults aged 35 yrs or older	Parallel	7.54	120	1. Vitamins C+E+β-carotene+Zn+Se (antioxidant vitamins and minerals) *vs.* placebo 2. Incidence of cancer, ischemic cardiovascular disease, and all-cause mortality 3. No effect of supplementation with antioxidant vitamins and minerals
15	8,171 women aged 40 yrs or older and with prior CVD or high CVD risk	2 x 2 x 2	9.4	500	1. Vitamin C; vitamin E; β-carotene 2. Myocardial infarction, stroke, coronary revascularization, or CVD death 3. No effect of vitamin C supplementation
16, 124	14,641 men aged 50 yrs or older	2 x 2	10	500	1. Vitamin C; vitamin E 2. Cardiovascular events, myocardial infarction, stroke, or CVD death; incidence of prostate and total cancer 3. No effect of vitamin C supplementation
74	4,757 adults aged 55 yrs or older	2 x 2	6.3	500	1. Vitamins C+E+β-carotene (antioxidant vitamins); zinc 2. Age-related cataract, lens opacity, or vision loss 3. No effect of antioxidant vitamin supplementation
75	3,411 adults aged 35-69 yrs	2 x 2 x 2	3.25	500	1. Multivitamins; garlic; anti-*Helicobacter pylori* treatment 2. Gastric cancer mortality, cancer, CVD death 3. No effect of multivitamin supplementation
76	3,318 adults aged 40 yrs or older with esophageal dysplasia	Parallel	6	180	1. Multivitamins-minerals *vs.* placebo 2. Esophageal or gastric cardia death, cancer, cerebrovascular disease 3. No effect of multivitamin-mineral supplementation

77	976 adults aged 29-69 yrs with precancerous gastric lesions	2 x 2 x 2	6	2,000	1. Anti-*Helicobacter pylori* therapy; β-carotene; vitamin C 2. Progression/regression of multifocal non-metaplastic atrophy or intestinal metaplasia 3. No effect of vitamin C supplementation plus therapy over therapy alone
78	910 men and women aged 65 yrs or older	Parallel	1	60	1. Multivitamins-minerals *vs.* placebo 2. Contact with primary care physician for infections, self-reported days of infection, and quality of life 3. No effect of multivitamin-mineral supplementation
79	864 adults less than 80 yrs and with prior colorectal adenoma	2 x 2 x 2	4	1,000	1. β-Carotene; β-carotene+vitamin E; β-carotene+vitamins C+E (antioxidant vitamins) 2. Colorectal adenoma incidence 3. No effect of antioxidant vitamin supplementation
80	725 men and women aged 65 yrs or older	2 x 2	2	120	1. Zn+Se; vitamins C+E+β-carotene (antioxidant vitamins) 2. Infectious morbidity and mortality 3. No effect of antioxidant vitamin supplementation, except for lower antibody titers after influenza vaccination
81	652 men and women aged 60 yrs or older	2 x 2	1	60	1. Multivitamins-minerals; vitamin E 2. Incidence and severity of self-reported acute respiratory tract infections 3. No effect of multivitamin-mineral supplementation
82	520 men and women aged 45-69 yrs	2 x 2	6	250	1. Vitamin E; vitamin C 2. Carotid artery intima-media thickness 3. No overall effect of vitamin supplementation; less atherosclerotic lesion progression in supplemented hypercholesterolemic men
83	439 men and women aged 40-69 yrs with chronic gastritis	2 x 2	5	500	1. β-Carotene; vitamin C 2. Serum pepsinogen (PG) level and *Helicobacter pylori* infection 3. Favorable, significant change in PGI/II ratio in vitamin C supplemented subjects
84	423 postmenopausal women with prior coronary stenoses	2 x 2	3	1,000	1. Estrogen; vitamins C+E (antioxidant vitamins) 2. Change in minimum lumen diameter of coronary arteries assessed by angiography 3. No effect of antioxidant vitamin supplementation

Figure Legends

Figure 1. Two hundred milligrams of vitamin C (vertical dotted line) as optimum daily intake based on a) near-saturating plateau plasma vitamin C concentration of ≥70 μM (shaded area) and b) first dose beyond the steep, linear increase in plasma concentration at vitamin C intakes of 30-100 mg/day (Adapted from [26, 27, 104]).

Figure 2. Neutrophils and other circulating cells are saturated with vitamin C at an intake level ≥200 mg/day (dotted line, shaded area), as indicated by intracellular ascorbic acid concentrations (millimolar) (Adapted from [26]).

Figure 1

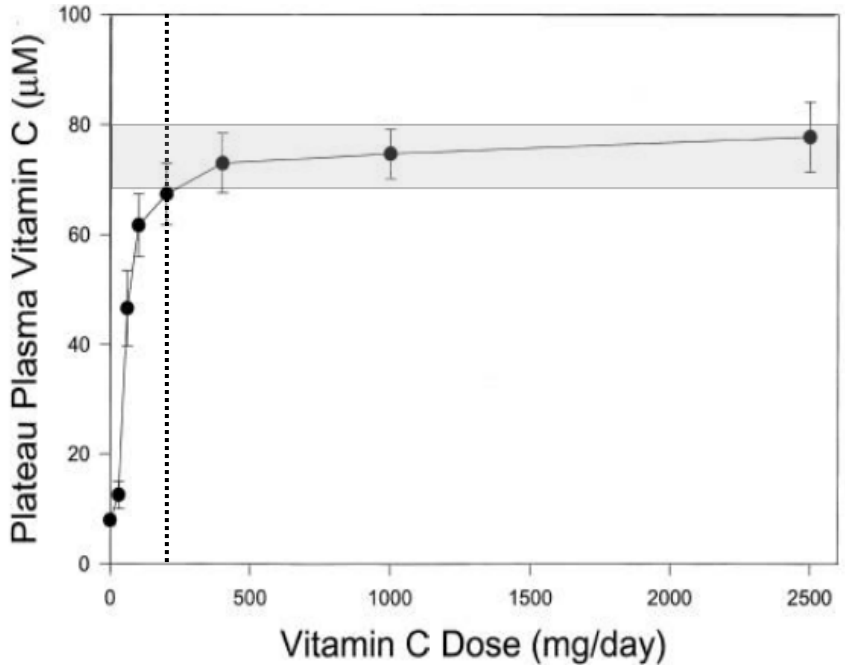

Figure 2