Chapter 9

Smoking Depletes Vitamin C: Should Smokers Be Recommended to Take Supplements?

9

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Contents

9.1 Introduction

Smoking has been identified as one of the major risk factors in human diseases such as atherosclerosis and several cancers (Doll et al. 1994; Mosca et al. 1997; Palmer 1985; Stein et al. 1993), yet approximately one third of the Western World's adult population continues to smoke (WHO Health for All Database 2000). Among other factors, oxidative stress has been suggested to play an important role as initiator of the pathological conditions resulting from tobacco smoking (Colditz et al. 1987; Frei et al. 1991; Genkinger et al. 2004; Gey 1986; Hirvonen et al. 2000; Macfarlane et al. 1995; Poulsen et al. 1998; Pryor and Stone 1993). Because cigarette smoke has been shown to result in increased oxidative stress as measured by a variety of biochemical markers, it has been speculated that increased consumption of fruits and vegetables rich in antioxidants or even specific antioxidant supplements could perhaps be particularly beneficial to smokers (Ames 1998; Ames and Gold 1998; Ames and Wakimoto 2002; Ames et al. 1995;). Indeed, numerous reports have shown that cigarette smokers have lower plasma concentrations of almost all low-molecular-weight antioxidants (Eiserich et al. 1995; Kallner et al. 1981; Lykkesfeldt et al. 1997; Munro et al. 1997). This condition results from at least two factors, one of diet and one of smoking (Dietrich et al. 2003; Lykkesfeldt et al. 1996, 2000; Schectman 1993; Schectman et al. 1991). Thus, because of the consumption of a diet containing more fat and less fruits and vegetables, smokers have a lower intake of a variety of phytonutrients, compared with nonsmokers (Dallongeville et al. 1998; Faruque et al. 1995; Jarvinen et al. 1994; Larkin et al. 1990; Ma et al. 2000; Marangon et al. 1998a; Morabia and Wynder, 1990; Preston, 1991; Serdula et al. 1996; Zondervan et al. 1996). However, in addition to dietary differences, it has been shown in studies correcting for dietary intakes of antioxidants, that in particular, vitamin C is further depleted by the smoke itself (Dietrich et al. 2003; Lykkesfeldt et al. 1996, 2000; Schectman et al. 1991). So the question remains: Should supplementation with vitamin C among smokers be a higher priority for health professionals?

A high intake of fruits and vegetables has long been associated with a reduced risk of developing cardiovascular disease and cancer (Knekt et al. 1991b). A critical evaluation of the cancer-related literature by the International Agency for Research on Cancer under the World Health Organization recently confirmed this and concluded that there was evidence, albeit limited, for a beneficial effect of fruits and vegetables on some types of cancers (mouth, pharynx, esophagus, stomach, colorectal, larynx, lung, ovary, bladder and kidney), but insufficient evidence for a beneficial effect on all other cancer types (International Agency for Research on Cancer WHO 2005). It has been suggested that particularly the antioxidant content of the fruits and vegetables could be responsible for the beneficial effect. In agreement with this notion, longitudinal studies have linked low plasma concentrations of antioxidants to increased risk of developing, e.g., lung and prostate cancer and cardiovascular disease (Eichholzer et al. 1996; Knekt et al. 1991a, 1994).

In contrast, large prospective studies using antioxidant supplements have been less promising. Thus, none of the major clinical studies using mortality or morbidity as end points has found significant positive effects of supplementation with such as vitamin C, vitamin E, or β-carotene (Blot et al. 1993; Heart Protection Study Collaborative Group 2002; Miller III et al. 2005). In contrast to the negative results of these larger studies, many smaller or more specific studies suggest a beneficial role of vitamin C supplemen-

tation. For example, the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study found that in male smokers, a combination of vitamins C and E could retard atherosclerotic progression in hypercholesterolemic subjects as measured by carotid intima thickness (Salonen et al. 2000, 2003). Also, high doses of vitamin C given by infusion have been shown to improve the endothelial dysfunction typically observed in smokers (Traber et al. 2000). Moreover, numerous studies using surrogate markers of disease such as, e.g., lipid peroxidation have shown a positive effect of antioxidant supplementation (Carr and Frei 1999b). Finally, most in vitro studies have suggested a positive effect with increasing vitamin C concentrations (Carr and Frei 1999a).

Thus, whereas unequivocal evidence appears to exist supporting the observation that smoking results in lower plasma concentrations of vitamin C, the clinical significance of this depletion—beyond that of scurvy—remains to be clarified. Studies of the possible beneficial effect of antioxidant supplementation to smokers have produced ambiguous data. However, it should not be overlooked that a substantial part of the population in the developed countries apparently suffers from subclinical vitamin C deficiency that may well affect both short- and long-term health, and could easily be cured. This chapter examines the clinical significance of long-term low vitamin C status in smokers and the current pros and cons of supplementing smokers with vitamin C.

9.2 The Antioxidant Activity of Vitamin C

Vitamin C, or ascorbic acid, is a simple, low-molecular-weight carbohydrate, yet its enediol structure provides it with a highly complex chemistry. Of its many chemical properties, the monoanion ascorbate—the predominant form of vitamin C at physiological pH—fulfills the criteria of an effective antioxidant (Halliwell 1996). In fact, the electron donor properties of ascorbate account for all its known functions. It has a complicated redox chemistry that involves comparatively stable radical intermediates and is heavily influenced by the acidic properties of the molecule. It has been known for many years that ascorbate is easily oxidized by molecular oxygen. The two-electron oxidation product in this reaction is dehydroascorbic acid (DHA), which also has antiscorbutic properties and is readily converted back to amino acids in vivo by both chemical and enzymatic means (Poulsen et al. 2004). Further oxidation/hydrolysis renders the vitamin inactive by leading to the irreversible formation of 2,3-diketoglulonate as well as oxalate, threonate, and other products (Poulsen et al. 2004). DHA has a half-life of only a few minutes at physiological pH (Bode et al. 1990). Consequently, highly efficient ways of regenerating ascorbate in vivo have evolved. These processes are of major importance in maintaining ascorbate in its active reduced state—in particular in species that lack the ability to synthesize ascorbate such as humans.

Vitamin C plays a pivotal role in the antioxidant defense. The molecule has been called the most important water-soluble antioxidant in biological fluids (Frei et al. 1989, 1990) and has earned this honor for several reasons. Chemically speaking, both ascorbate and its one-electron oxidation product ascorbate free radical have remarkably low one-electron reduction potentials of +282 and –174 mV, respectively (Buettner 1993), placing ascorbate at the bottom of the antioxidant hierarchy. This means that on top of the ability to reduce virtually all physiologically relevant oxidants, ascorbate is capable of regenerating other antioxidants such as vitamin E from the α-tocopheroxyl radical and

glutathione from the glutathiyl radical back into their active states (Buettner and Schafer 2004). Equally importantly, the relative stability of the ascorbate free radical renders it a harmless intermediate incapable of inducing free radical damage itself. Instead, at physiological pH, the ascorbate free radical primarily disproportionates via dimer formation into one molecule of ascorbate and one of DHA (Buettner and Schafer 2004). DHA is subsequently recycled by means of either glutathione- or NADPH-dependent dehydroascorbic acid reductases (Del Bello et al. 1994; May et al. 1997, 2001; Park and Levine 1996; Wells and Xu, 1994; Wells et al. 1990, 1995).

Whereas the more electrochemical evidence outlined above suggests that ascorbate is indeed a unique antioxidant, in vitro experiments have confirmed that ascorbate plays a key role in the antioxidant defense as a whole. For example, Frei and coworkers have shown under a variety of oxidizing conditions—including that of cigarette smoke—that ascorbate is the only antioxidant capable of completely preventing lipid oxidation in plasma and once ascorbate is depleted; lipid hydroperoxides are formed despite the presence of other plasma antioxidants such as α-tocopherol and β-carotene (Frei et al. 1988, 1991; Gokce and Frei 1996; Lynch et al. 1994; McCall and Frei 1999). These and other findings suggest that the presence of adequate amounts of vitamin C in particular could be of major importance for the maintenance of redox homeostasis in vivo.

9.3 Vitamin C Homeostasis in Smokers

It has been known since the 1950s that smoking results in lower vitamin C in plasma (Pelletier 1968, 1970); however, the precise mechanism of this effect has still not been fully characterized.

Several epidemiological studies have demonstrated that smokers have a lower intake of phytonutrients—including antioxidants—compared with nonsmokers (Dallongeville et al. 1998; Faruque et al. 1995; Jarvinen et al. 1994; Larkin et al. 1990; Marangon et al. 1998b; Morabia and Wynder 1990; Preston 1991; Serdula et al. 1996; Zondervan et al. 1996). This has hampered the ability to distinguish between effects of smoking and diet on the depletion of antioxidants consistently observed among smokers. In a large epidemiological study, the National Health and Nutrition Examination Survey (NHANES), Schectman et al. compared the vitamin C intake and plasma vitamin C concentrations of smokers consuming >1, 1, or <1 pack of cigarettes per day, and nonsmokers, who had stopped smoking <1 year ago, >1 year ago, or had never smoked (Schectman et al. 1990). The results showed that smokers and nonsmokers who had stopped smoking within the last year had a markedly lower intake of vitamin C, compared with that of never smokers. However, as the effect of smoking on the plasma concentration of vitamin C persisted even when corrected for dietary intake, they concluded that depletion of vitamin C as a result of smoking presumably occurs predominantly via mechanisms independent of dietary intake (Schectman et al. 1990). In agreement with this conclusion, clinical studies adjusting for differences in vitamin C intake, and those assessing populations with similar fruit and vegetable intake have found that smokers have lower plasma vitamin C concentrations than have nonsmokers (Dietrich et al. 2001; Lykkesfeldt et al. 2000; Marangon et al. 1998a). Moreover, smoking cessation quickly results in a substantial elevation of plasma vitamin C concentrations, apparently independent of dietary changes (Lykkesfeldt et al. 1996). These results all suggest that smoking per se predisposes to lower vitamin C status.

In their latest report, the Food and Nutrition Board (2000) recommended that the RDA for vitamin C for smokers be increased to 125 and 110 mg/day for men and women, respectively, compared with those for nonsmokers of 90 and 75 mg/day. The basis for this recommendation was primarily the study by Kallner and coworkers (1981) showing increased metabolic turnover of vitamin C in smokers, compared with nonsmokers. They found a vitamin C turnover among smokers of 70 mg/day, compared with 36 mg/day in nonsmokers, suggesting that smokers need an additional 34 mg vitamin C per day.

As mentioned above, the actual mechanisms by which the vitamin C status is compromised remains less well characterized. However, many data point toward increased oxidative stress as the mediator of this effect. One puff of cigarette smoke has been estimated to contain as many as 10^{15} of gas-phase radicals and 10^{14} of tar-phase radicals (Pryor and Stone 1993). Evidently, it has been suggested that oxidative stress arising directly from the toxicity of the smoke itself can increase vitamin C turnover (Pryor 1997). Also, smoke-induced inflammatory responses may indirectly increase oxidative stress, thereby contributing to the turnover of vitamin C (Anderson 1991; Elneihoum et al. 1997; Langlois et al. 2001a; Lehr et al. 1997). Whether directly or indirectly, cigarette smoke results in an increase vitamin C oxidation ratio (%DHA of total vitamin C) among smokers (Lykkesfeldt et al. 1997). This suggests that vitamin C acts as a free radical scavenger in smokers but could also be because of an impairment of the enzymatic recycling of vitamin C. However, in a recent report, it was shown that recycling of vitamin C is, in fact, increased in smokers, and that this activity is not related to differences in vitamin C transport, glutathione concentration, or cellular energy status (Lykkesfeldt et al. 2003b). From these results, it may be concluded that the rate of vitamin C oxidation exceeds that of the DHA reductases in smokers (Lykkesfeldt et al. 2003b). Other studies have suggested that differences in vitamin C status between smokers and nonsmokers be unrelated to altered pharmacokinetics, although possible differences in sodium-dependent vitamin C transport has not been accounted for (Lykkesfeldt et al. 2003a; Viscovich et al. 2004). Taken together with the observed increased oxidative damage among smokers as measured by biochemical markers, the evidence is building up for an oxidative stress mediated depletion of vitamin C.

9.3.1 The RDA for Vitamin C in Smokers

In the debate against or in favor of antioxidant supplementation to smokers, one of the major points of discussion has been how to determine the proper RDA for smokers. Although smokers have been shown to have lower plasma concentrations of several antioxidants, only the RDA for vitamin C has so far been increased. Based on the data outlined above, this seems appropriate because the depletion of antioxidants other than vitamin C appears to result primarily from altered dietary habits. However, the question remains: How is the correct RDA for vitamin C in smokers determined?

As mentioned previously, the Food and Nutrition Board (2000) currently recommends an RDA for vitamin C for smokers of 125 and 110 mg/day for men and women, respectively. However, Schectman and coworkers proposed a higher RDA for smokers, based on their comprehensive material from the second NHANES (II) (Schectman 1993; Schectman et al. 1989, 1990, 1991). By simply plotting the serum level of vitamin C on the daily intake of vitamin C for smokers and nonsmokers separately, they were able to show that smokers would require a daily intake of 200 mg vitamin C to achieve

the serum concentration of nonsmokers consuming 60 mg vitamin C per day (the previous RDA for vitamin C), i.e., resulting in serum levels around 70 μ*M* (Schectman et al. 1990).

In another approach to this problem, Levine and coworkers (1995, 1996b, 1997) studied vitamin C pharmacokinetics in detail. They found that in nonsmokers, consumption of 60 mg of vitamin C per day resulted in a plasma concentration around 25 μ*M*, i.e., considerably less than the concentration indicated by Schectman's data, whereas ingestion of 200 mg vitamin C per day resulted in a plasma concentration of about 66 μ*M* (Levine et al. 1996b). Higher doses did not increase plasma concentrations considerably, and doses above 500 mg were largely excreted unabsorbed. Based on their studies, Levine et al. concluded that the RDA for vitamin C should be increased to 200 mg per day.

Using DHA as biomarker of smoking-induced oxidative stress added a new way of estimating an increased vitamin C requirement in smokers (Lykkesfeldt et al. 1997). Lykkesfeldt et al. (1997) found no DHA in the plasma of nonsmokers regardless of their vitamin C status, whereas a significant inverse correlation between plasma DHA and plasma vitamin C was observed in smokers. Complete reduction of DHA was maintained and not different from that of nonsmokers for plasma vitamin C concentrations higher than 70 μ*M*. Consequently, the data could be interpreted as supporting an RDA for smokers that result in a plasma concentration of about 70 μ*M*. Based on the data from Schectman et al. (1990) and in agreement with their conclusions, this would require an intake of 200 mg vitamin C per day.

An equally important aspect of an increased RDA for vitamin C for smokers is the possibility of getting the recommended amount of vitamin C through the regular diet. Dietary guidelines currently recommend five to nine servings per day of fruits and vegetables (National Research Council 1990). However, several studies suggest that only a relative small proportion of the US population routinely has an intake in the recommended range (Krebs-Smith et al. 1995; Munoz et al. 1997; Rogers et al. 1995). Moreover, as mentioned previously, considerable evidence has been presented to demonstrate that smokers constitute a relative large part of those having a low intake of fruits and vegetables (Dallongeville et al. 1998; Faruque et al. 1995; Jarvinen et al. 1994; Larkin et al. 1990; Marangon et al. 1998b; Morabia and Wynder 1990; Preston, 1991; Serdula et al. 1996; Zondervan et al. 1996). In contrast to this reality, a daily intake of 200 mg vitamin C has been estimated by the Food and Nutrition Board to require the consumption of a minimum of five servings per day of fruits and vegetables. Thus, conforming to the guidelines for fruit and vegetable consumption will result in nonsmokers getting more than 2-fold their current RDA for vitamin C, whereas smokers would get about 1.5 times their RDA and indeed, the 200 mg per day suggested above. However, a significant dietary modification would be required for most smokers to overcome their increased risk of hypovitaminosis C (Schectman 1993). As this may be practically impossible for many, supplementation with vitamin C may be necessary to reduce the prevalence of low plasma ascorbate concentrations of smokers to rates acceptable for nonsmokers. These considerations have warranted studies to investigate the effects of vitamin C supplementation in smokers in particular.

In the 2000 revision of the RDA for vitamin C from the Food and Nutrition Board (2000), the overwhelming evidence supporting an important role of vitamin C in the defense against oxidative stress was taken into account for the first time. Indeed, the current RDA for vitamin C is primarily based on the intake necessary to achieve about 80% saturation of neutrophils (Levine et al. 2004). In contrast, previous recommenda-

tions were based primarily on clinical evidence, i.e., the prevention of scurvy and hypovitaminosis C. This acceptance of biochemical evidence in support of a higher RDA for vitamin C may well result in the RDA for smokers being further increased in the future.

9.3.2 Repletion of Vitamin C in Smokers

Ever since it became clear half a century ago that smoking apparently predisposes to lower vitamin C status, there have been speculations regarding the possible beneficial effect of antioxidant supplementation. Although it would seem very unlikely that antioxidant supplementation alone should be able to prevent the multiple deleterious effects of smoking, there has been an obvious interest in trying to counter the depletion of antioxidants in smokers: (1) it fits the basic theory of smoking-induced oxidative stress and damage, (2) oxidative stress has been suggested to play an important role as initiator of the pathological conditions resulting from smoking, and (3) plasma concentrations of antioxidants are easily elevated by daily administration of a multivitamin pill. However, one adverse effect may be that these studies can unintentionally detract the focus from the only known effective remedy against smoking-related diseases: to stop smoking. In other words, the smokers may be waiting for a miracle.

As pointed out above, some controversy still exists on the proper RDA for vitamin C for smokers. However, the fact that an increased RDA for smokers exists may be interpreted as acceptance of the basic idea that vitamin C supplementation can be a treatment of oxidative stress caused by smoking. Numerous studies of vitamin C intervention in smokers have been published over the past four decades (Carr and Frei 1999b; Traber et al. 2000). Most studies have used doses between 500 mg and 2 g of vitamin C per day, and found plasma levels saturating around 70 μmol/l. Sodium-dependent vitamin-C transporter 1 (SVCT1) activity, responsible for both primary absorption in the intestine and secondary reabsorption in the kidney, is dependent on the concentration of vitamin C, and the kidney threshold for active tubular reabsorption is around 70μmol/l (MacDonald et al. 2002; Oreopoulos et al. 1993). Based on the detailed pharmacokinetics provided by Levine et al. (1996b) as well as what could realistically be obtained from a diet rich in fruits and vegetables, it has been shown that only 250 mg of vitamin C per day can restore plasma vitamin C levels in poorly nourished smokers from an average of only 23 μmol/l to that of near saturation around 70 μmol/l (Lykkesfeldt et al. 2000). These data also provide indirect evidence that intestinal and renal SVCT1 activity is not impaired by smoking, and that decreased SVCT1 capacity cannot explain the depletion of vitamin C among smokers.

9.4 Prevalence and Clinical Significance of Low Vitamin C Status

Although the definition of optimal vitamin C status remains a matter of some controversy, the opinions appear to converge around the level of apparent saturation, i.e., a plasma concentration of approximately 70 μmol/l (Carr and Frei 1999b; Levine et al. 1995, 1996b, 1997, 2004). Defining vitamin C deficiency is equally complex, because considerable individual variation exists regarding the relationship between the plasma

concentration of vitamin C and the classical hallmark of severe vitamin C deficiency: scurvy (Newton et al. 1985; Schorah et al. 1979). Moreover, the clinical significance of vitamin C deficiency—beyond that of scurvy—has not been clearly defined. Guidelines developed by the National Survey of Canada suggested categories of "severe vitamin C deficiency" (serum level <11 μmol/l) and "marginal vitamin C deficiency" (serum levels between 11 and 23 μmol/l) (Smith and Hodges 1987). As the RDA for vitamin C has been increased since these categories were put forward in 1987, and considering the believed optimal vitamin C level in plasma of 70 μmol/l, a new category (e.g., for serum levels between 23 and, e.g., 50 μmol/l) should be added and could be termed "suboptimal vitamin C status."

9.4.1 Severe Vitamin C Deficiency

Scurvy is normally the clinical manifestation of prolonged and severe vitamin C deficiency. In nonsmokers, scurvy is prevented by a daily intake of as little as 10 mg of vitamin C (Weber et al. 1996b). Clinical symptoms include follicular hyperkeratosis, petechiae, ecchymoses, coiled hairs, inflamed and bleeding gums, perifollicular hemorrhages, joint effusions, arthralgia, and impaired wound heeling (Chazan and Mistilis 1963). Other symptoms include dyspnea, weakness, fatigue, and depression. Scurvy usually occurs in individuals with plasma concentrations lower than 11 μmol/l, i.e., those diagnosed as having severe vitamin C deficiency. However, far from all individuals with plasma levels <11 μmol/l actually develop clinical scurvy (Newton et al. 1985; Schorah et al. 1979). Thus, the relationship between plasma vitamin C status and scurvy is not entirely clear.

Although the basic symptoms and cure of the disease have been known for centuries, a significant part of the population in developed countries—and the smokers in particular—suffer from severe vitamin C deficiency and thus have increased risk of experiencing scurvy-like symptoms. Data from the NHANES II (11,592 subjects, collected from 1976–1980) show that of all smokers (including those taking vitamin C supplements), 7.4% suffered from severe vitamin C deficiency, compared with 1.9% of the nonsmokers, giving rise to a risk odds ratio relative to smoking of 3.0 after adjusting for vitamin C intake (Schectman et al. 1989). A Swiss study of more than 4,000 employees came to the same conclusion (Ritzel and Bruppacher 1977). More recent data suggest that the incidence of severe vitamin C deficiency in developed countries is not declining, but may actually be increasing, although improved analytical methodology may also account for a more accurate categorization of the population as older methods may have overestimated vitamin C (Lykkesfeldt, 2002; Washko et al. 1992). Regardless, data from NHANES III (15,769 subjects, collected from 1988 to 1994) showed that in the United States, 14% of males and 10% of females suffered from severe vitamin C deficiency (Hampl et al. 2004). Of smokers, the numbers were 31 and 25%, respectively. The third Glasgow Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) population survey (1,267 subjects) found 26% of males and 14% of females suffered severe vitamin C deficiency (Wrieden et al. 2000). Among the smokers alone, the numbers were 36 and 23% of men and women, respectively (Wrieden et al. 2000). A French population study (1,039 subjects) found severe vitamin C deficiency in 7 to 12% of men and 3 to 5% of women, depending on age group (Hercberg et al. 1994).

The clinical significance of severe vitamin C deficiency extends beyond that of scurvy. In clinical studies in which subjects were made vitamin C deficient, common complaints as gingival inflammation and fatigue were among the most sensitive markers of deficiency (Leggott et al. 1986, Levine et al. 1996b). In a prospective population study, Nyyssönen et al. (1997) found a higher risk of myocardial infarction (relative risk, 3.5) among men with severe vitamin C deficiency constituting about 6% of their Finnish cohort (1,605 subjects). Moreover, Langlois et al. (2001b) recently showed that 14% of patients with peripheral arterial disease suffered from severe vitamin C deficiency as compared with none of the healthy controls and suggested a relationship between vitamin C status and severity of atherosclerosis. In a study with advanced cancer patients, 30% had severe vitamin C deficiency and these patients had shorter survival (Mayland et al. 2005).

9.4.2 Marginal Vitamin C Deficiency

As defined above, plasma concentrations between 11 and 23 μmol/l constitutes a situation of marginal vitamin C deficiency. Hypovitaminosis C has been characterized as having a plasma concentration of vitamin C <23 μmol/l (Schectman 1993), i.e., encompassing both severe and marginal vitamin C deficiency. As with severe vitamin C deficiency, smokers also have increased risk of marginal vitamin C deficiency. Thus in the NHANES II, 19.7% of the smokers showed marginal vitamin C deficiency, compared with 8.2% of the nonsmokers (Schectman et al. 1989). The Scottish MONICA Study found marginal vitamin C deficiency among 30% of smoking men, compared with 22% of nonsmoking men and 30% of smoking women, compared with 16% of nonsmoking women (Wrieden et al. 2000). In the cohort as a whole, the numbers were 26 and 22% for men and women, respectively (Wrieden et al. 2000). In the NHANES III, marginal vitamin C deficiency was found in 20% of males and 17% of females, the upper limit of the group being set at 28 μmol/l (Hampl et al. 2004). In a Parisian cohort, 10–46% of males and 3–15% of females had plasma vitamin C concentrations between 11 and 19 μmol/l, depending on the age group (Hercberg et al. 1994). These data clearly demonstrate that a substantial part of the populations in the developed countries can be diagnosed with vitamin C deficiency.

The clinical significance of marginal vitamin C deficiency—as isolated from severe vitamin C deficiency—has not been thoroughly investigated. In most studies, upper and lower tertiles, quartiles, or quintiles are compared, making it difficult to compare groups between studies. Consequently, the category of marginal vitamin C deficiency can rarely be singled out from all vitamin C deficiency/hypovitaminosis C. With respect to scurvy, clinical cases among people with marginal vitamin C deficiency are rare, but do occur (Hodges et al. 1971; Reuler et al. 1985). Probably more importantly, considerable epidemiological evidence suggests that there may other clinical consequences of marginal vitamin C deficiency. Thus, in a recent reexamination of the NHANES II data combined with a follow-up on vital status 12–16 years later, Loria et al. (2000) found that men in the lowest (<28.4 μmol/l), compared with the highest (>73.8 μmol/l) serum ascorbate quartile had a 57% higher risk of death from any cause and a 62% higher risk of dying from cancer. A similar conclusion was reached by Simon et al. (2001), who also found that severe or marginal vitamin C deficiency was significantly associated with all-cause mortality while being weakly associated with death from cardiovascular disease.

In a 20-year follow study in Britain (730 subjects), significantly higher risk of mortality from stroke was observed in elderly men and women with severe and marginal vitamin C deficiency separately, compared with those with plasma concentrations of vitamin C >28 μmol/l (Gale et al. 1995). The authors concluded that vitamin C status was as strong a predictor of death from stroke as diastolic blood pressure (Gale et al. 1995). An inverse correlation between vitamin C status and stroke was also reported from a study (2,121 subjects) in a rural Japanese population, aged 40 years or more (Yokoyama et al. 2000). In the 12-year follow-up on the Basel Prospective Study, significantly increased risk of ischemic heart disease and stroke was found in individuals with plasma ascorbate <22.7 μmol/l, corresponding to severe or marginal vitamin C deficiency (Gey et al. 1987, 1993a, b).

9.4.3 Suboptimal Vitamin C Status

Based on the increased RDA for vitamin C as well as the indication that a plasma concentration of vitamin C of about 70 μmol/L is currently considered optimal for health, a new category of suboptimal vitamin C status is reasonable for those individuals with plasma concentrations between 23 and about 50 μmol/l. The obvious rationale for this additional category could be that if 70 μmol/l is optimal, e.g., 35 μmol/l is probably not, and therefore, investigations into the clinical significance of a suboptimal vitamin C status are warranted. However, limited data are available and need to be extracted from the few studies discriminating between the concentrations of suboptimal and optimal vitamin C status.

In the Coronary Artery Risk Development in Young Adults (CARDIA) Study, Simon et al. (2004) divided 2,637 subjects (originally enrolled as young adults, aged 18–30 years) into four groups with respect to plasma vitamin C levels, and their "low normal" group (between 23 and 45 μmol/l) corresponds approximately that of suboptimal vitamin C status. At the 10-year follow-up, 26% of the smokers suffered from severe or marginal vitamin C deficiency, whereas about 40% had suboptimal vitamin C status. Among the never smokers, the numbers were 8 and 33%, respectively (Simon et al. 2004). At a 15-year follow-up, they found that low vitamin C status (as measured at 10 years) was associated with a higher prevalence of coronary artery calcium among men but not women. Statistics was not performed individually on the male low-normal group having an odds ratio of 2.09 to 1, compared with the "saturation" group (plasma vitamin C level >62.5 μmol/l). In a larger population sample of 8,453 subjects, aged 30 years or older from the NHANES II, a similar prevalence for vitamin C deficiency or suboptimal vitamin C status among smokers was observed. Thirty percent of the smokers suffered from severe or marginal vitamin C deficiency, whereas 35% had suboptimal vitamin C status (plasma vitamin C between 23 and 55 μmol/l). Among never smokers, the numbers were 9 and 31%, respectively (Simon et al. 2001).

Several large prospective studies have shown an inverse relationship between plasma vitamin C status and risk of cardiovascular disease and/or all-cause mortality (Eichholzer et al. 1996; Gale et al. 1995; Khaw et al. 2001; Loria et al. 2000; Nyyssonen et al. 1997; Riemersma et al. 1991; Sahyoun et al. 1996; Singh et al. 1995). However, no studies have investigated the specific clinical significance of suboptimal vitamin C status as compared with the optimal. Thus, it remains to be established if the biochemical evidence pointing toward an optimal plasma level around 70 μmol/l can be backed up in larger epidemio-

logical studies or clinical trials. Clearly, the effects of suboptimal, compared with optimal vitamin C status are likely to be at most moderate and presumably relevant only in the long perspective, if at all. Thus, it is debatable if studies aimed at clarifying such a limited risk are feasible bearing the high cost in mind. On the other hand, the problems potentially associated with low vitamin C status affects a large percentage of the population and can be readily and inexpensively cured.

9.5 The Pros of Vitamin C Supplementation to Smokers

As outlined above, it is well established that smoking predisposes to low vitamin C status. However, whereas it is questionable if smokers in general are capable of conforming to such a significant modification of their diet as would be required to comply with the dietary guidelines (Schectman 1993), moderate amounts (250 mg) of vitamin C given as supplement saturates even poorly nourished smokers (Lykkesfeldt et al. 2000). Thus, the inability of most smokers to comply with dietary guidelines indirectly supports the recommendation of supplements. In addition, supplementing smokers with moderate amounts of vitamin C is safe. Moreover, the bioavailability of vitamin C from supplements is not different from that of vitamin C from natural sources (Johnston and Luo 1994; Mangels et al. 1993). The amount needed to supply smokers is far from the tolerable upper intake level (UL) of 2 g/day of the Food and Nutrition Board's most recent reference values (2000). A large body of evidence agrees that ingestion of <500 mg/day poses no significant risk to human health, and even several-fold higher daily doses of vitamin C have indicated low toxicity (Johnston 1999). Unfortunately, those currently eating supplements are the least likely to need them (Kirk et al. 1999; McNaughton et al. 2005; Sinha et al. 1994). One might add that they are also the least likely to benefit from them, as saturation kinetics will result in excretion of surplus amounts. Supplementing smokers with vitamin C will shift this balance toward those most likely to need and benefit from them. In the NHANES II, 63% of those suffering from severe or marginal vitamin C were smokers and in this group; only 1% of both smokers and nonsmokers used vitamin C or multivitamin supplements (Loria et al. 2000). In the NHANES III, only 7% of male and 5% of female users of any supplement suffered from severe vitamin C deficiency (Hampl et al. 2004). Thus, supplementing smokers with vitamin C will substantially lower the risk of smokers suffering from scurvy-like symptoms, including those of weakness, fatigue, and depression.

Another part of the rationale for supplementing smokers with vitamin C is based on the so-called oxidation theory of atherosclerosis in which oxidized low-density lipoprotein (LDL) lipid is thought to play a key role in the initiation and progression of the disease (reviewed recently by Witting and Stocker 2004). Other hallmarks of atherosclerosis include endothelial dysfunction and leukocyte adhesion to the endothelium, both of which are increased in smokers (Kalra et al. 1994; Lehr et al. 1993; Morrow et al. 1995; Vita et al. 1990; Weber et al. 1996a; Zeiher et al. 1991).

As mentioned previously, increased oxidative stress and damage have consistently been observed in smokers. This oxidative stress has been suggested to play an important role as initiator of the pathological conditions resulting from smoking (Colditz et al. 1987; Frei et al. 1991; Genkinger et al. 2004; Gey 1986; Hirvonen et al. 2000; Macfarlane et al. 1995; Poulsen et al. 1998; Pryor and Stone 1993). With respect to atherosclerosis, lipid oxidation has been suggested as an early marker (Steinberg 1997). Consistent with

this theory, increasing amounts of F_2-isoprostanes, a marker of lipid oxidation, have been found in smokers (Morrow et al. 1995). Increased amounts of malondialdehyde, another frequently used—albeit more unspecific—marker of lipid peroxidation, has also been found in smokers (Lykkesfeldt et al. 2004). In a recent survey, low vitamin C status was among the strongest predictors of lipid peroxidation (Block et al. 2002). In agreement, vitamin C supplementation has been shown to decrease lipid oxidation in smokers (Dietrich et al. 2002; Helen and Vijayammal, 1997; Motoyama et al. 1997; Nyyssonen et al. 1994; Panda et al. 2000; Valkonen and Kuusi 2000), although not all studies have found an effect (Jacob et al. 2003; Kaikkonen et al. 2001). However, the clinical significance of increased lipid oxidation remains to be further established.

Moving from biomarkers to clinically more relevant studies, vitamin C has been found to improve endothelial dysfunction in smokers, apparently by increasing the bioavailability of nitric oxide (NO) (Antoniades et al. 2003; Carr and Frei 2000; Frei 1999; Heitzer et al. 1996; Kaufmann et al. 2000; Lehr et al. 1997; Levine et al. 1996a; Salonen et al. 1991; Schindler et al. 2000) although conflicting reports also exists (Pellegrini et al. 2004; Raitakari et al. 2000; Scott et al. 2005; Van Hoydonck et al. 2004). The mechanism was initially thought to involve direct scavenging of superoxide radicals (May 2000). However, although intracellular concentrations of ascorbate could potentially be high enough for ascorbate to be able to compete with NO for the reaction with superoxide, the reaction kinetics are unfavorable for ascorbate (Jackson et al. 1998). More recently, it has been shown that vitamin C in physiological amounts can increase the production of NO substantially in human endothelial cell in culture (Heller et al. 1999). The mechanism is believed to involve a vitamin C mediated increase in tetrahydrobiopterin, which increases NO activity via endothelial NO synthase (eNOS) in a dose-dependent manner (Heitzer et al. 2000; Heller et al. 1999; Huang et al. 2000a). High plasma concentrations achieved by infusion have been reported to improve endothelial-dependent vasodilation in, e.g., smokers and patients with type 1 and 2 diabetes and coronary artery disease (Chambers et al. 1999; Gokce et al. 1999; Heitzer et al. 1996; Hornig et al. 1998; Ito et al. 1998; Kugiyama et al. 1998; Levine et al. 1996a; Motoyama et al. 1997; Solzbach et al. 1997; Taddei et al. 1998; Timimi et al. 1998; Ting et al. 1996, 1997). Although unphysiologic levels are achieved, this line of research shows interesting potential—in particular for smokers.

Clinical evidence that vitamin C can actually prevent atherosclerosis is limited, and studies have not been performed in particular with subjects selected for low vitamin C status. In the ASAP Study, the effect of vitamin C, vitamin E, or a combination on carotid intima thickness was studied as a marker of a atherosclerotic progression (Salonen et al. 2000). The authors found that 3 years of supplementation with vitamins C and E in combination—but not vitamin C alone—significantly decreased the intima progression rate in men but not women. Similar results were found at the 6-year follow-up, and the authors concluded that supplementation with vitamins C and E in combination slows down atherosclerotic progression in hypercholesterolemic men (Salonen et al. 2003). Also, another study in women found no effect of vitamins C and E in combination for almost 3 years on the progression of coronary atherosclerosis (Waters et al. 2002).

9.6 The Cons of Vitamin C Supplementation to Smokers

The main problem in recommending vitamin C supplements to smokers can be summarized by looking at two separate observations: (1) smoking predisposes to low vita-

min C status and (2) smoking increases the risk of developing chronic diseases such as atherosclerosis and cancer, and every second smoker will die from the habit (Doll et al. 1994; Mosca et al. 1997; Palmer 1985; Stein et al. 1993). The problem is that limited evidence links the two observations, a situation that mainly boils down to the classic issue in epidemiology: Is smoking-induced vitamin C depletion a cause or a consequence of smoking-related diseases?

The few large prospective intervention studies have consistently found no positive effect of vitamin C supplementation on morbidity and mortality (Blot et al. 1993; Greenberg et al. 1994; Heart Protection Study Collaborative Group 2002). However, none of them used vitamin C as a single substance but included β-carotene or vitamin E as well as other antioxidant vitamins and substances. Regardless, the use of vitamin C and other antioxidants for protection against, e.g., cardiovascular disease in high-risk individuals as those in the British Heart Protection Study is difficult to justify (Heart Protection Study Collaborative Group 2002). Notable for the present discussion is that the subjects included in the Heart Protection Study (20,536 subjects) did generally not suffer from severe or marginal vitamin C deficiency, but rather represented the general population in terms of vitamin C status and thus were less likely to benefit from the intervention. In contrast, the subjects included in the vitamin C part of the Linxian (China) Study (about 18,000 subjects) presumably suffered from vitamin C deficiency in general, although this assumption is based on screening of a sample of only 49 individuals showing an average plasma vitamin C concentration of 8.6 μmol/l (Blot et al. 1993). Following 5 years of intervention with 120 mg vitamin C and 30 mg molybdenum per day, the plasma concentration of vitamin C increased to an average of 46.6 μmol/l, i.e., far from saturation. No effect of the intervention was observed on cancer development or mortality while endpoints such as scurvy symptoms were not included. Only 1% of the deaths among the study participants were attributed to ischemic heath disease, thus limiting the evaluation of intervention effects on this endpoint. As pointed out by the authors, the 5-year duration of the study may have been too short to monitor any effects on cancer incidence, but it might be added that the 120 mg of vitamin C was apparently insufficient to saturate the intervention group with vitamin C, and this may also have impacted the results. Moreover, the main causes of death in Linxian differ from those of the developed countries in that the incidence of cardiovascular disease is considerably lower and that of, e.g., epithelial cancers is extraordinarily high presumably because of dietary and lifestyle differences.

With respect to cancer prevention, clinical intervention trials with vitamin C using surrogate endpoints such as oxidative DNA damage have also been unpromising (reviewed recently by Poulsen et al. 2004). Thus, most studies found no effect of vitamin C supplementation on markers of DNA damage, typically in urine or lymphocytes (Anderson et al. 1997; Brennan et al. 2000; Green et al. 1994; Huang et al. 2000b; Jacobson et al. 2000; Lee et al. 1998; Panayiotidis and Collins, 1997; Porkkala-Sarataho et al. 2000; Prieme et al. 1997; Proteggente et al. 2000; Vojdani et al. 2000; Welch et al. 1999; White et al. 2002; Witt et al. 1992), although positive reports also exist (de la Asuncion et al. 1996; Howard et al. 1998; Moller et al. 2004; Rehman et al. 1998; Schneider et al. 2001).

A different aspect of vitamin C supplementation is the actual timing. For example, should the dose be increase in case of disease or should supplementation be discontinued? High plasma concentrations achieved by infusion have been found to improve, e.g., endothelial-dependent vasodilation. However, vitamin C homeostasis is normally under a tight control, maintaining the concentration within a narrow range at steady state (Levine et al. 2004). This could suggest that higher "unphysiologic" levels are potentially either directly toxic perhaps because of the often-discussed prooxidant effect

of vitamin C, or indirectly because putative regulatory function could be lost when vitamin C concentrations in general approach regulatory levels. Excess levels of oxidants are important for, e.g., activation of internal cellular cascades of apoptosis that again are involved in the protective mechanisms that kill cancer cells and also critical for effective cancer treatment (Blumenthal et al. 2000; Kuipers and Lafleur 1998; Weijl et al. 1997). As pointed out by Zeisel (2004), indiscriminate use of high-dose antioxidant supplements should probably be avoided, at least until the potential risks and benefits have been more clearly characterized.

A completely different reservation against recommending vitamin C supplementation to smokers may be that such action could be interpreted as a potential "cure" for smoking-related diseases and thereby take the wind out of the long-preferred and simple message to the smokers: The only known effective remedy against smoking-related diseases is to quit in time. If vitamin C supplements are eventually recommended particularly to smokers, it is crucial that the potential perspectives—or lack of—are kept in mind and communicated unambiguously. Clearly, vitamin C supplements only have the potential of relieving a relative small part of the problems associated with smoking.

9.7 Conclusion

Whereas considerable literature has identified an inverse correlation between vitamin C status and mortality, the current evidence is primarily of epidemiological nature and thus lacks the ability to establish causality. None of the large prospective intervention studies with vitamin C have been able to establish this causality, which should be kept in mind regardless of the fact that the studies were not designed to look at poorly nourished people with a Western lifestyle, i.e., the group most likely to immediately benefit from such intervention.

However, the fact that a substantial part of the population of the developed countries are suffering from subclinical vitamin C deficiency taken together with the potential health problems and expenses for society associated with this condition as well as its easy and inexpensive cure, demonstrates that action is urgently needed to establish if vitamin C supplements to this part of the population is associated with improved health.

Those presumably in need of vitamin C supplements are the least likely to ingest them. Smokers that currently do not take supplements constitute the largest subpopulation that would potentially benefit from vitamin C supplements because a major proportion remains at increased risk of vitamin C deficiency because of poor dietary habits and the enormous voluntary oxidant exposure. A likely short-term benefit from such a supplementation may be a substantially decreased risk of suffering from scurvy-like symptoms, including weakness, fatigue, and depression. Whether a long-tern benefit exists in terms of lower incidence of cardiovascular disease and cancers can only be established by large controlled clinical trials. Thus, it is important that future studies focus on individuals with a low daily intake of vitamin C. Controlled studies are needed to clarify further the long-term consequences of low vitamin C status in both smokers and the general population as well as the clinical effects of moderate supplementation with vitamin C to these specific individuals. There appears to be a good chance of a value-for-money health benefit for a considerable part of the population.

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