The Interaction of Cigarette Smoking and Antioxidants. Part III: Ascorbic Acid

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Abstract

The requirement for antioxidant nutrients depends on a person's exposure to endogenous and exogenous reactive oxygen species. Since cigarette smoking results in an increased cumulative exposure to reactive oxygen species from both sources, it would seem cigarette smokers might have an increased requirement for antioxidant nutrients. This review examines available evidence of ascorbic acid supplementation and combinations of antioxidants as interventions in smokers and their effect on functional biomarkers of nicotine metabolism, oxidative stress, DNA damage, and endothelial function. (*Altern Med Rev* 2003;8(1):43-54)

Introduction

This review is the third part of an article on the interaction of cigarette smoking and antioxidant nutrients. Parts I and II appeared in previous issues of *Alternative Medicine Review* and discussed the core biomarkers used to assess the physiological benefits, or lack thereof, of antioxidant intervention, as well as: (1) dietary substances with antioxidant activities, including fruits, vegetables, garlic, green tea, and turmeric; (2) supplemental beta-carotene; and (3) supplemental alpha-tocopherol. The reader is referred to the preceding articles for these topics.

Part III reviews intervention and biomarker studies of ascorbic acid conducted in populations of smokers. It also reviews interventions that have provided combinations of two or more antioxidant nutrients to smokers and assessed a particular functional biomarker as a measured endpoint.

Ascorbic Acid

Cigarette smoke contains a range of xenobiotics, including oxidants and free radicals that can increase lipid peroxidation. One estimate suggests cigarette smoke contains on the order of 10^{14} free radicals per inhalation.[1] Free radicals are capable of directly and indirectly inducing oxidative stress in the body. Since ascorbic acid is a critical component of the body's antioxidant defense mechanisms, it has been investigated assessing a range of biomarkers of oxidative stress in smokers. Interventions consisting of ascorbic acid supplementation have also been investigated in smokers assessing other functional biomarkers, including DNA damage, endothelial function, monocyte adhesion, and sperm quality.

Unlike the case of beta-carotene or alpha-tocopherol, currently no long-term intervention trials of ascorbic acid in smokers have been conducted that tracked clinical endpoints such as cancer or heart disease. However, similar to these other nutrients, several shorter interventions assessing changes in biomarkers have been conducted and are reviewed and discussed.

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Intake and Requirements

An association between smoking and lower levels of serum ascorbic acid has been observed repeatedly.[2-6] One explanation for lower serum levels of ascorbic acid observed in smokers would be an inadequate dietary intake. Among other plausible explanations, the lower serum ascorbic acid levels observed in smokers might reflect an altered metabolism of ascorbic acid. Decreased absorption, increased metabolic demand, increased elimination, or any combination of these factors might influence the metabolic fate of ascorbic acid, resulting in the consistent observation of lower serum levels in the smoking population.

In order to clarify the association between smoking and vitamin C, Smith and Hodges compared serum vitamin C levels in smokers and non-smokers in relation to dietary and supplemental intake of vitamin C using data from the Second National Health and Nutrition Examination Survey (NHANES II). Their results suggest a slightly lower mean daily intake of vitamin C among smokers. Assessment of serum vitamin C levels also suggest that, given a comparable dietary intake of vitamin C, serum levels of vitamin C among smokers tend to be lower than those of non-smokers.[7]

While their analysis did not allow for a definitive conclusion as to the cause of the lower serum ascorbic acid levels in smokers, their observation of lower serum levels in smokers despite comparable dietary intakes to non-smokers led them to conclude that smokers as a group might be at risk of having a relative deficiency of vitamin C. As a result of this relative deficiency, they proposed the available evidence might warrant a recommendation for a higher daily intake of ascorbic acid in smokers. After analyzing data, Smith and Hodges suggest that an average smoker would need an additional 59 mg daily of dietary vitamin C above the mean daily intake of 65 mg/day to attain serum vitamin C levels comparable to non-smokers.[7]

Schectman also analyzed data from NHANES II and reported the following with respect to ascorbic acid and its interactions with cigarette smoking:

✓ Smokers tend to have lower levels of serum ascorbic acid than non-smokers.

✓ Ascorbic acid intake (dietary and supple mental combined) tends to be lowest in subjects smoking the most cigarettes.

✓ Even after adjusting for intake, serum ascorbic acid levels remain low, suggest ing an independent effect of smoking on ascorbic acid metabolism that might re sult in a functional hypovitaminosis state.

After comparing smokers to non-smokers, Schectman concluded a daily intake of 200 mg vitamin C by smokers was needed to approximate the same risk of hypovitaminosis for vitamin C found among non-smokers with a daily intake of 60 mg.[3]

The conclusions of these independent researchers are remarkably similar. The lower serum ascorbic acid observed among smokers, as a population, is a result of both a marginally inadequate daily intake and an apparent increased metabolic demand. To meet this increased metabolic demand Smith and Hodges, as well as Schectman, conclude that a higher daily vitamin C intake is warranted in smokers.[3,7]

Unfortunately, many smokers, left to their own devices, do not appear to consistently meet the existing suggested intakes for vitamin C-rich foods, and far fewer meet the higher proposed intakes. In the NHANES II data, only 50 percent of smokers consumed 60 mg of vitamin C daily and only 10 percent had intakes (including diet and supplement contributions) of 200 mg or greater daily. Daily intake also tends to be lowest in the population with possibly the greatest need – the heaviest smokers.[3]

One way to increase daily intake of vitamin C is to increase fruit and vegetable consumption. Schectman suggested the intake of fruits and vegetables needed to provide adequate vitamin C to offset the adverse effects of smoking appears to be in the range of 20-25 servings per week.[3] However, as discussed in Part I of this article, epidemiological evidence has consistently indicated

Table 1. *Mean Serum Ascorbic Acid Levels in Smokers Subsequent to Different Doses of Ascorbic Acid Supplementation*

Dosage Group	Baseline	Week 1	Week 4
0	45.4 micromol/L	45.4 micromol/L	39.7 micromol/L
200 mg	34.1 micromol/L	79.4 micromol/L	90.8 micromol/L
1000 mg	39.7 micromol/L	73.8 micromol/L	96.5 micromol/L

Adapted From: Dawson EB, Evans DR, Harris WA, McGanity WJ. The effect of ascorbic acid supplementation on the nicotine metabolism of smokers. *Preventive Medicine* 1999;29:451-454.

that chronic cigarette smokers, as a group, consume fewer fruits and vegetables than non-smokers. This trend has been demonstrated in smokers in the United Kingdom,[8] Canada,[9] the United States,[10] and the Netherlands.[11] The available evidence also indicates the heaviest smokers invariably consume even fewer fruits and vegetables than those who smoke fewer cigarettes daily.[11,12]

Currently, intervention studies aimed at tracking adherence to long-term increased dietary consumption of fruits and vegetables in smokers are lacking. Until evidence is available indicating an ability to adhere long-term to the quantity of fruit and vegetable intake suggested, it seems unreasonable to assume all smokers, especially heavy smokers, are going to sustain the needed dietary modifications. Assuming the suggested increased intakes (124-200 mg vitamin C daily[3,7]) are more appropriate for smokers because of an increased metabolic need, if a smoker is unwilling or unable to begin or sustain food consumption habits needed to reach this level of intake, supplemental vitamin C is likely warranted.

Pharmacokinetics of Ascorbic Acid in Smokers

Oral supplementation with ascorbic acid has been consistently shown to raise serum levels of ascorbic acid in smokers.[2-6] Preliminary evidence does not support a conclusion that this increase is dose-dependent. It appears a maximum increase in serum levels of ascorbic acid is achieved following a dose of 200 mg and no significant additional increase is obtained with a substantially higher oral dose.

Dawson et al evaluated the changes in serum ascorbic acid levels subsequent to two different doses of ascorbic acid in smokers. The subjects were adult men between the ages of 20 and 35 years with a smoking habit of a minimum of one pack of cigarettes daily. Subjects were randomized into three groups and provided with placebo or 200 mg or 1,000 mg ascorbic acid daily for four weeks. After one week, the serum ascorbic acid levels had increased an average of 100 percent in the group receiving 200 mg daily of ascorbic acid. By the end of the four-week supplementation period the average increase in serum ascorbic acid in these subjects was 166 percent above baseline. The subjects receiving 1,000 mg of ascorbic acid daily had average increases of 72 percent and 143

percent, respectively, at the same two time points. The increases in serum ascorbic acid were statistically significant at both doses of ascorbic acid at all time points when compared with the subjects receiving placebo.[13]

While the percent increase in ascorbic acid would appear to indicate the lower dose was more effective at increasing serum ascorbic acid in smokers, the higher percent increase is a direct result of the lower average baseline ascorbic acid that existed in the 200 mg per day group as opposed to the 1,000 mg group prior to supplementation (34.1 µMol/L versus 39.7 µMol/L, respectively). The post supplementation serum ascorbic acid levels in both the 200-mg and 1,000-mg ascorbate groups were actually comparable, when expressed in quantitative terms of µMol/L at week 1 (79.4 µMol/L versus 73.8 µMol/L) and week 4 (90.8 µMol/L versus 96.5 µMol/L) post-supplementation.[13] Statistical analysis was not conducted comparing these quantitative values. See Table 1 for a summary of mean serum ascorbic acid levels at the different time points at the varying doses of ascorbic acid.

Biomarker Studies: Ascorbic Acid

Short-term interventions with ascorbic acid lasting from weeks to months, assessing changes in biomarkers of nicotine metabolism, oxidative stress, DNA damage, endothelial function, monocyte adhesion, and sperm quality have been conducted among smokers. Taken as a whole, the data suggest supplementation of a smoker with ascorbic acid can positively modify some biomarkers and not others. For a detailed description of the biomarkers discussed, see Part I of this review in the *Alternative Medicine Review,* Volume 7(5).

Nicotine Biomarkers

Nicotine is extensively metabolized, primarily in the liver, by cytochrome P450 enzymes. Research indicates approximately 70 percent of nicotine is metabolized to cotinine metabolites and excreted in the urine; however, a great deal of individual variation in the metabolism of nicotine does exist.[14,15] The amounts of cotinine metabolites excreted in the urine are such a sensitive measure of nicotine intake that these metabolites have been proposed to be a biomarker of cigarette intake.[15, 16]

Dawson et al investigated the impact of different doses of ascorbic acid on the metabolism of nicotine in smokers by measuring urinary excretion of cotinine metabolites as expressed in cotinine equivalents (CE). Subjects were adult men ages 20-35 years, with a smoking habit of a minimum of one pack of cigarettes daily. Subjects were randomized to three groups and provided with placebo or 200 mg or 1,000 mg ascorbic acid daily for four weeks. A dose-dependent effect of ascorbic acid supplementation on urinary excretion of CE over time was observed. While the lower dose of ascorbic acid resulted in a non-significant 10-percent decrease from baseline in urinary CE by the end of week 4, the 1,000-mg daily dose of ascorbic acid resulted in a statistically significant decrease of 47 percent in CE by the end of week 4. Unlike the decrease observed in the two supplemented groups, the placebo group had an increase in urinary CE over the four-week study period. The changes in CE that resulted were independent of nicotine intake, since nicotine intake in the three study groups remained unchanged throughout the observation period.[13]

Biomarkers of Oxidative Stress

Several researchers have examined the effects of supplemental ascorbic acid on biomarkers of oxidative stress. Available evidence has not demonstrated a beneficial effect of supplementation on *in vitro* markers of lipid peroxidation, even when supplementation with ascorbic acid followed a period of dietary depletion of vitamin C in smokers. *In vivo* biomarkers of lipid peroxidation have not improved in smokers subsequent to ascorbic acid supplementation; and in the study of the longest duration (two months) actually worsened subsequent to supplementation.

Fuller et al investigated the effects of ascorbic acid supplementation on biomarkers of oxidative stress on 19 smokers subsequent to a period of vitamin C depletion. Participants were

placed on a low ascorbate diet, defined as less than 30 mg/day of dietary vitamin C, for two weeks, after which subjects were randomly assigned to receive a placebo or 1,000 mg ascorbic acid daily for four weeks. Subjects receiving ascorbate demonstrated a reduction in LDL oxidative susceptibility, as measured by *in vitro* lag time and rate kinetics post-supplementation; however, the differences between the placebo and supplemented groups at 0 and 4 weeks did not reach statistical significance.[17]

Samman et al also conducted a study examining the impact of ascorbic acid supplementation in smokers following a period of dietary depletion. Eight male smokers (average age 25; mean intake of 19.1 cigarettes daily) were placed on a low vitamin-C diet containing approximately 40 mg daily. After two weeks, diets were supplemented with either placebo or 1 g ascorbic acid daily for two weeks, followed by a 10-day washout period, and a crossover for an additional two-week supplementation period. Despite a doubling of plasma ascorbic acid concentrations, *in vitro* lag time and oxidation rate did not change significantly.[18]

In addition to and consistent with these negative studies measuring the effects of ascorbic acid supplementation on *in vitro* assessments of lipid peroxidation following a period of dietary depletion in smokers, supplementation with ascorbic acid in smokers who were not previously placed on a diet designed to deplete vitamin C stores failed to demonstrate a beneficial effect. Susceptibility of LDL to *in vitro* oxidation was assessed in young smokers (average age 20 years). Inclusion criteria was a smoking history of less than or equal to five years with daily cigarette use of at least 10 cigarettes. Supplementation consisted of 1 g per day ascorbic acid for eight weeks. No statistically significant changes were observed in either *in vitro* lag time or lag rate.[19]

The quantity of thiobarbituric reactive substances (TBARS) formed *in vivo* is an indirect indication of the quantity of malondialdehyde (MDA), and therefore reflects the quantity of metabolic by-products of oxidation of polyunsaturated fatty acids containing three or more double bonds. As such it provides an indication of ongoing lipid peroxidation.

An acute episode of smoking (5-7 cigarettes) results in increased lipid peroxidation determined by *in vivo* measurement of plasma TBARS assessed 90 minutes after the smoking episode.[20] Supplementation for four weeks with 1.5 g/day ascorbic acid reduced the amount of TBARS formed after smoking, suggesting the supplemental ascorbic acid improved both antioxidant reserves and protection from acute oxidative stress exerted by smoking 5-7 cigarettes.[20]

Despite this potentially advantageous effect subsequent to four weeks of supplementation, an increase of plasma TBARS compared to baseline values before the smoking was seen.[20] This study did not provide before-and-after supplementation statistical analysis of these unprovoked changes in plasma TBARS. Nevertheless, these changes are suggestive of increased baseline lipid peroxidation *in vivo* occurring subsequent to ascorbic acid supplementation and are consistent with findings observed by one other research group with respect to this particular biomarker of oxidative stress.

Nyyssonen et al performed a two-month, randomized, single-blind, placebo-controlled clinical trial with the intent of establishing the *in vivo* effect of ascorbic acid supplementation on LDL oxidation in smokers. They assessed *in vivo* MDA production as indicated by TBARS formation, and observed an increase subsequent to supplementation. Study participants were 59 male smokers ages 36-65 years and a daily cigarette use of 11-40 cigarettes. Subjects were randomized to receive either 250 mg twice daily ascorbic acid or slow-release ascorbic acid, or placebo for two months. Both forms of ascorbic acid resulted in substantial increases in plasma levels of reduced ascorbic acid. They observed no significant differences in *in vitro* lipoprotein oxidation assessed by lag time subsequent to intervention with either form of ascorbic acid; however, mean plasma MDA, as assessed by TBARS, increased significantly in the ascorbic acid (not slow release) group compared with the placebo group, indicating a rise in plasma lipid

peroxidation products. A non-significant increase in MDA as assessed by TBARS formation was found in the slow-release group when compared with placebo.[21]

In two shorter trials (four weeks and two weeks, respectively) measuring a similar biomarker of lipid peroxidation, supplementation with ascorbic acid at 500 or 1,000 mg daily did not result in an increase in lipid peroxidation; however, it also failed to show any significant benefit.

Aghdassi et al conducted a randomized, double-blind, controlled trial on 56 smokers. Intervention consisted of four weeks of supplementation with 500 mg ascorbic acid daily or placebo. In addition to assessment of *in vivo* MDA formation, breath pentane output (BPO) was utilized as a functional biomarker of *in vivo* lipid peroxidation. Pentanes are formed in the body as a result of peroxidation of polyunsaturated fatty acids. A portion of pentanes is volatile and eliminated in respiration; therefore, BPO provides a functional surrogate measure of oxidative stress to lipids. A decrease in BPO suggests an *in vivo* decrease in the formation of lipid peroxidation products. In this study, supplementation with ascorbic acid failed to positively modify either of these two biomarkers of lipid peroxidation.[22]

Mulholland et al randomized 16 female smokers to receive 1 g ascorbic acid or placebo for 14 days. No improvements were observed in serum antioxidant potential or lipoprotein oxidation, as assessed by measuring serum MDA-like material.[23]

F(2)-isoprostanes are prostaglandin-F isomers produced by cyclooxygenase-independent, free-radical peroxidation of arachidonic acid. Their quantity in plasma and urine is a functional indicator of lipid peroxidation. Under circumstances of increased oxidative stress, F(2)-isoprostanes would be expected to increase. A dose-response relationship between number of cigarettes smoked daily and levels of F(2)-isoprostanes in the urine has been reported.[24] Some researchers have suggested this is a better indicator of functional *in vivo* free-radical stress than are *in vitro* assessments using lag time or oxidation rate.[25]

Reilly et al examined the production of 8-epi-prostaglandin (PG) F2 alpha (8-epi-PGF2 alpha), a stable product of lipid peroxidation *in vivo*, and its modulation by 2 g daily ascorbic acid in smokers over a five-day time period. Supplementation significantly decreased urinary levels of 8-epi-PGF2 alpha, suggesting an ability of ascorbic acid to positively modify prostaglandin metabolites formed in the arachidonic acid pathway and to functionally offset the increased free-radical stress of smoking.[26]

Urinary excretion rate of 8-hydroxydeoxyguanosine (8-OHdG), a repair product of DNA that increases subsequent to oxidative damage to DNA, is used as a biomarker to evaluate free radical damage and risk of carcinogenesis. A decrease in 8-OHdG would suggest a decrease in oxidative damage to DNA. Prieme et al investigated the effect of ascorbic acid supplementation on this biomarker in smokers. Two groups of male smokers, ages 35-65 years with a daily smoking habit of greater than 10 cigarettes, were given either 250 mg ascorbic acid or 250 mg slow-release ascorbic acid twice daily for two months. Supplementation with both forms of ascorbic acid resulted in increased plasma ascorbate concentrations; however, neither form of ascorbic acid produced a significant change in the urinary excretion rate of 8-OHdG.[27]

Endothelial Dysfunction

Long-term cigarette smoking results in chronic endothelial dysfunction characterized by impairment in vasodilation. A transient impairment of endothelial function also occurs acutely subsequent to smoking. This smoking-induced disruption in endothelial function is due at least in part to oxidative stress and has been proposed to be a key early event in the promotion of atherosclerosis.[28] An ability to maintain appropriate endothelial function in smokers is considered to be of possible practical relevance in cardiovascular disease prevention.[2]

Several studies have noted improvements in endothelial function subsequent to ascorbic acid supplementation in adults with cardiovascular disease.[29-30] In an attempt to determine the impact of ascorbic acid supplementation on endothelial

function in smokers, Raitakari et al assessed vascular reactivity before and after ascorbic acid supplementation in 20 smokers (average age 36 years). Study participants were randomized to receive 1 g daily ascorbic acid or matching placebo in a double-blind, crossover design. While supplementation resulted in a sustained increase in plasma ascorbate levels, there was no effect of chronic supplementation with this dose of ascorbic acid for eight weeks on any parameter of endothelial function measured.[2]

Adhesion of immune system cells to endothelium has been proposed to be a part of the early pathogenesis of atherosclerosis.[31] Weber et al assessed monocyte adhesion in smokers and nonsmokers and then reassessed the adhesiveness after supplementation with ascorbic acid. Monocyte adhesion was observed to be, on average, higher among smokers. Supplementation with 2 g daily ascorbic acid for 10 days decreased monocyte adhesion to values found in nonsmokers. No effect of ascorbic acid supplementation on monocyte adhesion was observed among nonsmokers.[4]

Effect of Ascorbic Acid on Sperm Quality

In an effort to determine the effects of supplemental ascorbic acid on sperm quality, 75 men, ages 20-35 years with a smoking habit of a minimum of one pack per day, were randomly divided into one of three supplementation groups: placebo, 200 mg ascorbic acid daily, or 1,000 mg ascorbic acid daily for four weeks. Table 2 summarizes the results of this study. Microscopic observation of sperm quality indicated that, while placebo had no impact, supplementation with ascorbic acid produced an improvement in sperm quality. Statistically significant decreases in the percent of total sperm agglutination and abnormal morphology were observed in subjects receiving the highest dose of ascorbic acid. Insignificant trends toward increased sperm count and improved sperm quality were observed in other measured parameters for both doses of ascorbic acid. Taken as a whole, the parameters assessed appear to indicate ascorbic acid had a positive, dose-dependent effect on sperm quality in smokers.[32]

Antioxidant Combinations

Several studies have examined the impact of various combinations of antioxidants upon biomarkers in smokers. Because of the interactions between, and recycling of, antioxidants, it appears a combination of antioxidants is more advantageous than an intervention with a single antioxidant nutrient in smokers; however, available evidence is currently not sufficient to warrant any definitive conclusion with respect to this theory.

Biomarker Studies: Antioxidant Combinations

Susceptibility of LDL to *in vitro* oxidation was assessed in young smokers, average age 20 years. Inclusion criteria were a smoking history of five years or less and daily cigarette use of at least 10 cigarettes. Daily supplementation consisted of either 1 g ascorbic acid, 400 IU d-alpha-tocopheryl acetate, a combination of both ascorbic acid and d-alpha-tocopheryl acetate, or placebo for eight weeks. Subjects who received vitamin E alone had a significant increase in the lag phase of *in vitro* LDL oxidation but no change in oxidation rate. The vitamin C and placebo groups had no changes in LDL oxidation kinetics. The group receiving vitamins E and C had no change in lag time but a significant reduction in LDL oxidation rate. While supplementation with d-alpha-tocopheryl acetate increased plasma and LDL levels of alpha-tocopherol, it also resulted in a statistically significant decrease in plasma and LDL beta-carotene and lycopene. This decrease in levels of carotenes was not found in subjects who received the same amount of d-alpha-tocopheryl acetate in combination with ascorbic acid.[19]

The diet of smokers was supplemented with a tomato-based juice with added ascorbic acid (600 mg), alpha-tocopherol (400 IU), and beta-carotene (30 mg). BPO, *in vitro* susceptibility of LDL to copper-mediated oxidation, and plasma total peroxyl radical trapping potential were assessed. Juice with no added vitamins was used as the placebo. A significant decrease in BPO and a significant improvement in the resistance of LDL to *in vitro* oxidation were observed, as assessed

Table 2. *Sperm Motility, Viability, Agglutination, 24-hour Viability and Abnormal Morphology in Smokers before and after Four Weeks of Supplementation with Ascorbic Acid or Placebo*

	Placebo		200 mg AA		1000 mg AA	
	Wk 0	Wk 4	Wk 0	Wk 4	Wk 0	Wk 4
Count ($X10^6$/mL)	64	37	45	56	50	67
Motility (% of sperm showing forward motility)	38%	29%	38%	45%	40%	42%
Viability (% of total sperm count)	52%	36%	49%	61%	47%	63%
Agglutination (% of sperm count clumped together)	12%	11%	8%	7%	12%	4%*
24-hr viability (% of total sperm count)	16%	13%	15%	17%	14%	23%
Abnormal morphology	50%	56%	46%	48%	54%	36%*

* P less than or equal to 0.1
Adapted from: Dawson EB, Harris WA, Teter MC, Powell LC. Effect of ascorbic acid supplementation on the sperm quality of smokers. *Fertil Steril* 1992;58:1034-1039.

by lag time and lag rate in subjects receiving the juice fortified with antioxidant vitamins. Plasma total peroxyl radical trapping potential values did not change in response to antioxidant fortification.[33]

Reilly et al examined the production of 8-epi-PGF2 alpha and its short-term modulation by either vitamin E (400 IU twice daily), ascorbic acid (1 g twice daily), or a combination of both vitamins at the same doses for five days in chronic cigarette smokers. The smokers ranged in age from 20-47 years and participants smoked a daily mean of 25 cigarettes. While ascorbic acid significantly decreased urinary levels of 8-epi-PGF2 alpha in smokers, suggesting an ability for vitamin C to positively modify prostaglandin metabolism and counter oxidative stress in smokers, the dose of vitamin E used had no significant effect. When both supplements were given in combination for five days, results were statistically significant and comparable to those observed with ascorbic acid supplementation alone.[26]

An antioxidant mixture containing vitamin C (515 mg average daily intake), alpha-lipoic acid (95 mg average daily intake), and vitamin E (mixture containing approximately 371 mg alpha-tocopherol, 171 mg gamma-tocopherol, 50 mg alpha-tocotrienol, 184 mg gamma-tocotrienol, and 18 mg delta-tocotrienol,) was given daily for two months in a randomized double-blind, placebo-controlled trial. Subjects were both genders (average age 46 years; average of 23 cigarettes daily with no less than 15 daily); 39 subjects received the antioxidant mixture, 42 received vitamin C only, and 45 received placebo. Participants had an average smoking history of 27

years. Increases in plasma ascorbic acid (69.9%) were found subsequent to supplementation in the vitamin C group. Increases in plasma ascorbic acid (89.7%) and alpha-tocopherol (33.8%), and a decrease in gamma-tocopherol (29.6%) were observed subsequent to supplementation in the mixed group. Plasma F(2)-isoprostanes were measured as a biomarker of oxidative stress and were found decreased by a statistically significant 11.1 percent in subjects receiving only vitamin C. No significant decrease was observed in subjects receiving the antioxidant mixture.[34]

Phagocytosis of bacteria and other foreign material is associated with the so-called "respiratory burst reaction (RBR)," in which superoxide radicals are formed. It has been hypothesized that smokers, as a result of high exposure to exogenous foreign particles in cigarette smoke, might have a higher RBR in alveolar macrophages and neutrophils of the peripheral circulation, and that this increased tendency toward RBR might have potentially long-term pathological consequences secondary to accelerated oxidative damage to DNA.[35]

In order to evaluate this hypothesis, Clausen compared the peripheral blood RBR of smokers to non-smokers and then assessed the effects of antioxidant supplementation on RBR. Study subjects consisted of 10 smokers and 10 nonsmoking controls, ages 20-45 years. At baseline, compared with the nonsmoking controls, smokers had a significantly higher RBR. Smokers were then given a placebo for 10 days followed by an additional 10-day period of antioxidant supplementation. The antioxidant intervention consisted of 200 mcg selenium (as selenomethionine) and 1,000 mg alpha-tocopherol daily. When compared to placebo, the combination of selenium and alpha-tocopherol resulted in a statistically significant reduction in RBR, reducing values to levels approximating those found in the nonsmoking controls.[35]

Welch et al conducted a trial that assessed biomarkers of DNA strand damage and oxidative damage to DNA in smokers. A combination of ascorbic acid (350 mg) and RRR-alpha-tocopherol (250 mg) was unable to positively modify DNA damage in mononuclear leukocytes, as assessed by comet assay. DNA damage, as assessed by 8-OHdG in mononuclear leukocytes, was also not statistically influenced by the combination of these two antioxidants.[36]

Conclusion

Available evidence suggests smokers as a group have a slightly lower mean daily intake of vitamin C. Evidence also suggests smokers have an increased metabolic demand for vitamin C and require a higher daily intake than nonsmokers to have similar blood levels. These observations have led to suggestions that smokers require a daily intake of vitamin C in the range of 124-200 mg daily. Since the majority of smokers fail to consume sufficient vitamin C-rich foods to meet these levels, it would seem supplementation with ascorbic acid is warranted. Unfortunately, available interventions with ascorbic acid in smokers have not produced uniformly positive results.

Results suggest supplementation with ascorbic acid might depress cytochrome P450 metabolism of nicotine dose-dependently over time. Since the cotinine metabolites of nicotine formed by cytochrome P450 are potentially more toxic than nicotine, a possible protective role of supplementation with ascorbic acid is suggested; however, until reliable data is available that evaluates the impact of reducing cotinine equivalents on disease endpoints such as cancer or heart disease, the absolute clinical relevance of these preliminary observations cannot be determined.

The impact of ascorbic acid on various biomarkers of oxidative stress assessed to date seems to suggest this vitamin as a single intervention is incapable of mitigating all of the unwanted physiological effects caused by cigarette smoking. Supplementation appears to be unable to positively modify *in vitro* LDL oxidation kinetics or *in vivo* lipid peroxidation as assessed by either TBARS formation or BPO. Despite the inability to positively modify these *in vitro* and *in vivo* biomarkers of oxidative stress, ascorbic acid supplementation appears to exert a positive impact on F2-isopentanes, suggesting a beneficial impact on prostaglandin formation and a functional ability to act as an *in vivo* antioxidant in stabilizing

membrane arachidonic acid. DNA damage as assessed by 8-OHdG was not changed by supplementation with ascorbic acid.

Ascorbic acid appears unable to positively modify endothelial function; however, at least over a short period of supplementation (10 days), it has a beneficial impact on monocyte adhesion to endothelial cells. The discrepancy between these observed results with respect to endothelial function in a population of smokers and those previously observed in persons with hypertension or existing coronary artery disease might be a result of the different study population or stage of endothelial dysfunction, since the smokers in this population did not have hypertension or clinically evident atherosclerosis. While ascorbic acid might have a role in more advanced cardiovascular disease in smokers and warrants research in that specific population, it appears to be unable to reverse the endothelial dysfunction caused by smoking and, as such, appears unable to provide complete vascular protection for smokers.

While the ability of supplementation with ascorbic acid to normalize monocyte adhesion in smokers over a short period of time (10 days) suggests a potential benefit in atherosclerosis prevention, whether ascorbic acid would be capable of sustaining this effect over a long-period of supplementation is currently unknown. Since monocyte adhesion is not related to the severity of atherosclerosis in persons with existing atherosclerosis, but to the presence of risk factors such as smoking,[37] the long-term clinical relevance of reducing monocyte adhesion, especially with respect to prevention of heart disease in smokers, is also currently unknown.

There is no clear consensus on the most appropriate dose of ascorbic acid for smokers. In all likelihood, the optimum dose varies among individuals and would depend on number of cigarettes smoked, diet, lifestyle, genetics, and other factors. Supplementation of ascorbic acid to smokers consistently increases serum levels of ascorbic acid; however, this increase does not appear to be dose-dependent above a 200-mg dose. Despite the plateau of serum ascorbic acid at this dose, the studies indicating a positive effect on a measured biomarker used higher doses. Improvements in F2-isopentanes were reported at doses of 515 mg daily ascorbic acid for two months and 2 g daily for five days. The improvement in monocyte adhesion occurred at a studied dose of 2 g daily for 10 days. The study that assessed sperm quality utilized two doses of ascorbic acid (200 mg and 1 g daily). The only statistically significant improvements, a decrease in the percent of total sperm agglutination and abnormal morphology, were observed in subjects receiving the highest dose of ascorbic acid. These findings suggest a dose range of 500-2,000 mg daily of ascorbic acid, at least over short periods of time, is capable of positively modifying some aspects of function in smokers.

Although ascorbic acid is an antioxidant and generally considered to be non-toxic, it appears to be capable of functioning as a pro-oxidant with respect to TBARS formation in smokers. Since, in the longest study that assessed TBARS formation, supplementation with 500 mg ascorbic acid daily for two months as a sole antioxidant intervention resulted in a statistically significant increase in the formation of byproducts of lipid peroxidation, it seems prudent to monitor this biomarker for possible unwanted effects, if ascorbic acid is supplemented long term at daily doses of 500 mg or more in smokers.

Available evidence on antioxidant combinations is not sufficiently comprehensive to arrive at definitive conclusions. While ascorbic acid, given in conjunction with alpha-tocopherol, appears to prevent the decrease in carotenes that resulted from giving alpha-tocopherol exclusively to smokers and positively modify aspects of *in vitro* LDL-lipid peroxidation kinetics, the combination was not effective in preventing DNA damage. Furthermore, a combination of mixed tocopherols, lipoic acid, and ascorbic acid was less effective in decreasing F2-isopentanes than was ascorbic acid alone. The combination of ascorbic acid, alpha-tocopherol, and beta-carotene, added to a tomato-juice base, had a positive impact on BPO, suggesting the possibility this combination of antioxidant vitamins added to a juice base reduces lipid peroxidation *in vivo*. The single study

that assessed a combination of alpha-tocopherol and selenium noted a normalization of RBR. More comprehensive and long-term biomarker and clinical endpoint assessments are required before an optimal dose and combination of antioxidants can be prescribed for smokers.

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