The Interaction of Cigarette Smoking and Antioxidants. Part II: Alpha-Tocopherol

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Abstract
It is logical that 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, it would seem cigarette smokers would have an increased requirement for antioxidant nutrients. This review examines available evidence of alpha-tocopherol supplementation by smokers and its effect clinically and on *in vitro* biomarkers of oxidative stress.
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Introduction

This review is the second part of an article on the interaction of cigarette smoking and antioxidant nutrients. Part I (*Altern Med Rev* 2002;7(5):370-388) introduced the reader to the hypothesis that a cigarette smoker might benefit by increased antioxidant nutrition and to the core biomarkers used to assess the physiological benefits, or lack thereof, of antioxidant intervention. Dietary substances with antioxidant activities including fruits, vegetables, garlic, green tea, and turmeric were reviewed. Intervention and biomarker studies on supplemental beta-carotene were also reviewed. The reader is referred to the preceding article for these topics.

Part II reviews intervention and biomarker studies of alpha-tocopherol conducted in populations of smokers. Essential fatty acids (EFAs) will also be discussed since some evidence suggests a possibility of interaction between alpha-tocopherol supplementation and EFAs. In some studies cited, mg, rather than IU, dosages are noted. See Table 1 for mg/IU conversions.

Alpha-Tocopherol

Cigarette smoke contains a range of xenobiotics, including oxidants and free radicals that can increase lipid peroxidation. One estimate suggested 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 lipid structures within the body are particularly susceptible to oxidative stress, smokers have been reported to have higher *in vivo* lipid peroxidation. In some cases, specific biomarkers of lipid peroxidation have been reported to correlate with the number of cigarettes smoked daily, with more cigarettes smoked resulting in higher lipid peroxidation.[2]

Alpha-tocopherol is a critical component of the body's antioxidant defense mechanisms, serving as a primary antioxidant in lipid compartments. Among its accepted physiological effects is an ability to protect against lipid peroxidation. The increased free radical exposure and *in vivo* lipid peroxidation associated with smoking would seem to indicate a need for increased alpha-tocopherol intake as an antioxidant counter-measure.

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Unlike beta-carotene, which is routinely low in the serum of smokers, evidence suggests alpha-tocopherol concentrations are equivalent to[3,4] or higher[5,6] in serum and tissue of smokers than non-smokers. Despite similar or elevated levels of this antioxidant compound, smokers have an increased tendency to lipid peroxidation.[2,4] It is possible the amount of alpha-tocopherol in serum and tissues that would be adequate to minimize lipid peroxidation in a non-smoker is incapable of doing a sufficient job to completely offset the increased lipid peroxidation caused by cigarette smoke exposure. This suggests additional alpha-tocopherol, either in the diet or as a supplement, might counter this increased oxidative damage to lipids.

In the Alpha-Tocopherol, Beta-Carotene Study (ATBC Study), baseline data on serum alpha-tocopherol levels was available for 29,102 men. When this cohort was stratified according to serum alpha-tocopherol levels at entry, a 19-percent reduction in lung cancer incidence was observed during the 7.7-year follow-up in the group with the highest versus lowest quintile of serum alpha-tocopherol upon entry.[7] While this suggests the possibility of a clinical benefit from higher serum levels of alpha-tocopherol, it is not possible to determine the specific nature of this interaction or whether increasing alpha-tocopherol intake would be beneficial. The association could be a result of: (1) a direct protective effect of alpha-tocopherol; (2) increased dietary intake of other phytonutrients found in vitamin E-rich foods; (3) a response to other non-dietary factors and/or physiological processes; (4) an indication of better antioxidative processes or lower oxidative stress in the quintile with the highest levels of serum alpha-tocopherol; or (5) additional confounding factors. Because of the inherent ambiguity encountered in observational studies, intervention trials are required to more appropriately identify cause-effect relationships and benefits.

Table 1. Unit Conversions for alpha-Tocopherol

Form of alpha-Tocopherol	International Units (IU)	Milligrams (mg)
dl-alpha-Tocopherol	1.1 IU	1 mg
d-alpha-Tocopherol	1.49 IU	1 mg

Currently, only one large, long-term intervention trial of alpha-tocopherol in smokers has been conducted that tracked clinical endpoints – the ATBC Study conducted in a population of male Finnish smokers. The results of this intervention did not indicate an across-the-board role for alpha-tocopherol in positively modifying clinical endpoints of cancer or heart disease in smokers; however, it suggested the possibility that some clinical endpoints can be positively, while others are negatively, modified by alpha-tocopherol supplementation. The specific findings will be discussed below. Several shorter interventions assessing changes in biomarkers have been conducted and will be reviewed and discussed.

Pharmacokinetics of alpha-Tocopherol in Smokers

Pharmacokinetic data suggests alpha-tocopherol metabolism might differ between smokers and non-smokers. Compared with non-smokers, an oral dose of alpha-tocopherol resulted in a lower quantity of vitamin E in the circulation at 6-, 12-, and 27-hours post-supplementation. The authors commented that, "Whether this decrease was a result of an impairment in absorption, modified handling of vitamin E by the liver, increased clearance rate and deposition into tissues, or some combination of these and other factors was not able to be determined based upon the constraints of this study."[8] An independent study suggested at least part of this altered metabolism is a result of increased deposition of vitamin E into tissues. After investigating the pharmacokinetics of alpha-tocopherol in smokers and observing an increased

rate of disappearance from the systemic circulation, Traber et al speculated that, "The increased disappearance might have been secondary to increased functional need of vitamin E as an antioxidant to ameliorate the oxidative stress associated with smoking."[9] This finding and hypothesis appear to correspond to the findings of Hilbert et al who reported bronchoalveolar lavage levels of alpha-tocopherol 20-fold higher among the smokers when compared with the non-smokers they examined.[6] Taken together the various observations suggest the pharmacokinetics of vitamin E might be altered in an adaptive manner in smokers in order to concentrate antioxidant defenses in areas that come in direct or higher contact with smoke-derived free radical stress.

Regardless of the reason for the altered pharmacokinetics, supplementation of alpha-tocopherol to smokers appears to further increase serum levels by two- to threefold (depending on the dose), LDL concentrations by two- to threefold, and lung tissue concentrations by 20 percent.[6,10,11]

Evidence suggests supplementation with pharmacological doses of alpha-tocopherol alters metabolism of several other antioxidant compounds in smokers. Fuller et al reported an eight-week intervention with 400 IU alpha-tocopherol daily resulted in decreased concentrations of beta-carotene and lycopene in LDL, suggesting the possibility of an unwanted impact on the metabolism of these carotenoids by exclusive alpha-tocopherol supplementation in smokers. This decrease was not observed when smokers were given 1 g ascorbic acid daily in combination with alpha-tocopherol.[12]

Brown et al reported a 20-week intervention with doses of alpha-tocopherol ranging from 70-1,050 mg d-alpha-tocopheryl acetate daily initially increased plasma concentrations of ascorbic acid at week 10; however, by week 20 plasma ascorbic acid levels were below baseline levels in all supplemented groups. The largest decreases were observed in persons taking the higher doses of alpha-tocopherol (140, 560, and 1,050 mg daily).[13]

Hoshino et al reported that two weeks of daily supplementation with 800 IU dl-alpha-tocopherol reduced plasma gamma- and beta-tocopherols significantly during the supplementation period but rebounded to presupplementation levels after four weeks of discontinuing alpha-tocopherol supplementation.[3] This decrease in gamma-tocopherol secondary to supplementation with alpha-tocopherol in smokers is consistent with results reported in non-smokers receiving exclusively alpha-tocopherol as a form of supplemental vitamin E.[14,15]

While the clinical relevance of these alterations in beta-carotene, ascorbic acid, and gamma- and beta-tocopherol in smokers secondary to exclusive supplementation of alpha-tocopherol is currently unknown, these observations point to a need for improved pharmacokinetic data on the interactions between alpha-tocopherol supplementation and other antioxidant nutrients in smokers.

Clinical Outcome Studies: alpha-Tocopherol Intervention Trials
Alpha-Tocopherol Beta-Carotene Study (ATBC Study)

The only long-term intervention study to date that examined the clinical outcomes in a population of cigarette smokers subsequent to supplementation with alpha-tocopherol is the ATBC Study.

This study was a randomized, double-blind, placebo-controlled, primary-prevention trial designed to determine whether daily supplementation with alpha-tocopherol, beta-carotene, or both, would reduce the incidence of cancer, especially lung cancer, among cigarette smokers. The study included 29,133 male smokers, age 50-69 years at entry (average age 57.2 years). All subjects smoked five or more cigarettes daily with an average daily cigarette intake of 20.4 cigarettes. Participants had an average smoking history of 35.9 years. Subjects were recruited from southwestern Finland. Participants were randomly assigned to one of four intervention groups: (1) basic capsule (placebo consisting of 7 mg ascorbic acid and 2 mg alpha-tocopherol); (2) 50 mg alpha-tocopherol as a 50-percent powder of synthetic dl-alpha-tocopherol

added to the basic capsule; (3) 20 mg beta-carotene as 10-percent water-soluble beadlets of synthetic beta-carotene added to the basic capsule; or (4) 50 mg alpha-tocopherol and 20 mg beta-carotene added to the basic capsule. Follow-up occurred over a 5-8 year time interval.[16,17]

The results of the ATBC Study suggest supplementation of a smoker's diet with alpha-tocopherol might be beneficial, have no effect, or be potentially harmful depending on which clinical endpoint is studied. Supplementation appeared to decrease the risk of prostate and colon cancer, have no effect on lung cancer, and increase the risk of stomach and bladder cancer. With respect to cardiovascular disease, the alpha-tocopherol intervention resulted in an increased incidence and mortality from hemorrhagic strokes but fewer deaths from ischemic heart disease and ischemic stroke. When all-cost mortality was calculated, participants receiving alpha-tocopherol as an active intervention had two-percent (p=0.6) higher mortality during the trial than did participants receiving placebo.[16]

While total strokes were not significantly impacted by the alpha-tocopherol intervention, the type of stroke had a significant association with supplementation. Leppala et al analyzed the data on the 28,519 members of the ATBC Study who had no previous history of stroke in an effort to explore the relationship between alpha-tocopherol supplementation and the incidence and mortality from stroke. During the follow-up time period (median length of six years) the researchers observed a statistically significant 50-percent increased risk of incidence of subarachnoid hemorrhage (p=0.07) and a 181-percent increase in risk for fatal subarachnoid hemorrhage (p=0.01) among subjects receiving alpha-tocopherol. Alpha-tocopherol supplementation also resulted in an increased risk of fatal intracerebral hemorrhage of 64 percent (p=0.09). In contrast to the elevated risk for hemorrhagic strokes, a 14-percent reduction in risk for ischemic stroke (p=0.03) was observed. Despite these trends, the net effects of supplementation on the incidence (fewer incidents of strokes in those receiving alpha-tocopherol) and mortality (higher mortality from strokes in those

receiving alpha-tocopherol) from all types of strokes combined did not reach statistical significance.[18]

Since alpha-tocopherol is known to have an effect on platelet function, assuming these trends are consistent across all populations of smokers, it suggests that smokers at risk for thrombotic conditions might possibly benefit from an intervention including alpha-tocopherol. Conversely, it suggests that alpha-tocopherol should be avoided at these dose levels in high-risk candidates for hemorrhagic strokes.

The influence of alpha-tocopherol supplementation on the incidence of common cold episodes was observed in a subset of 21,796 male smokers over a four-year follow-up period. While, for the cohort as a whole, supplementation had no effect on the incidence of common colds, a slight decrease in incidence (RR = 0.95, 95% CI = 0.90-1.00) was observed for subjects 65 years and older. The reduced incidence was most apparent in a subset of older subjects who lived in cities and smoked fewer than 15 cigarettes daily (RR = 0.72, 95% CI = 0.62-0.83).[19]

Biomarker Studies: alpha-Tocopherol

Short-term interventions with alpha-tocopherol, assessing changes in biomarkers of oxidative stress, DNA damage, and endothelial function, have been conducted among smokers. These interventions utilized different populations of smokers, varied the dose and duration of alpha-tocopherol supplementation, and even utilized varying biomarkers of oxidative stress and DNA damage. Taken as a whole, the data suggests supplementation of a smoker with alpha-tocopherol can positively modify some biomarkers and not others; however, even among the biomarkers it seems to positively modify, vitamin E appears unable to completely offset the increased oxidative stress caused by chronic cigarette smoking. For a detailed description of the biomarkers discussed, see Part I of this review in the previous issue of *Alternative Medicine Review*.

Several researchers have looked at the effects of supplementing alpha-tocopherol on biomarkers of oxidative stress. Duthie et al supplemented the diet of 20 male smokers (cigarette intake of 15-25 cigarettes daily) with 1,000 mg alpha-tocopherol acetate daily for 14 days in an attempt to determine whether this intervention could lower the elevated erythrocyte lipid peroxidation found among these smokers. While supplementation was able to reduce thiobarbituric reactive substances (TBARS), it was only able to slightly lower the quantity of conjugated dienes formed. Although the observed reduction in TBARS suggested some ability to counter lipid peroxidation, the inability to substantially alter conjugated diene formation indicates supplementation with alpha-tocopherol alone was not able to completely offset the increased lipid peroxidation caused by smoking.[4]

A somewhat similar observation on the ability of vitamin E (600 IU dl-alpha-tocopheryl acetate daily for four weeks) to positively modify *in vitro* lag time but have no significant impact on *in vitro* lag rate of conjugated diene formation in smokers was reported by Mol et al.[11]

Porkkala-Sarataho et al conducted a two-month intervention with daily doses of 200 mg RRR-alpha-tocopheryl acetate among a population of male smokers (average age 47; mean of 21 cigarettes daily). They observed an increased *in vitro* lag time suggesting increased ability of LDL to resist oxidative stress. Despite this apparently positive change found *in vitro*, supplementation resulted in no improvement of *in vivo* plasma malondialdehyde (MDA).[10] Since *in vivo* plasma MDA represents a breakdown product of lipid hydroperoxides and largely reflects metabolic by-products of oxidation of polyunsaturated fatty acids (PUFAs) containing three or more double bonds, the positive *in vitro* results on lag time do not appear to be an accurate reflection of what was actually occurring in the body during the two months of supplementation.

Fuller et al conducted an eight-week intervention in 30 smokers (at least 10 cigarettes daily for less than five years) by providing 400 IU d-alpha-tocopheryl acetate daily. Supplementation resulted in a significant increase in LDL lag time but no change in oxidation rate, neutrophil superoxide anion production as assessed by respiratory burst reaction, or conjugated diene formation in LDL. Weighing these seemingly paradoxical findings the authors concluded that since supplementation showed no significant effects on the more physiologically relevant neutrophil respiratory burst function and conjugated diene formation, their findings appeared to cast doubt on the ability of alpha-tocopherol supplementation to reduce oxidative stress in smokers *in vivo*.[12]

In addition to this possible lack of functional antioxidant activity, supplementation of 400 IU alpha-tocopherol for eight weeks resulted in a decreased concentration of LDL beta-carotene and lycopene,[12] suggesting the possibility of an unwanted impact on the metabolism of these carotenoids by exclusive alpha-tocopherol supplementation in smokers.

In a slightly longer intervention (10 weeks), Brown et al reported a decrease in plasma concentration of TBARS subsequent to supplementation with 280 mg dl-alpha-tocopheryl acetate daily to male Scottish smokers. They also observed elevated plasma concentrations of conjugated dienes among the smokers pre-intervention and a significant decrease subsequent to intervention with alpha-tocopherol.[20]

The quantity of TBARS is an indirect indication of the quantity of MDA and therefore reflects the quantity of metabolic by-products of oxidation of PUFAs containing three or more double bonds, and conjugated dienes formed *in vivo* are also reflective of ongoing lipid peroxidation. Therefore, the decreases reported by this study suggest the alpha-tocopherol intervention protected PUFAs *in vivo* from oxidative stress induced by smoking.

In a subsequent study of longer intervention (20 weeks) in 50 Scottish male smokers, Brown et al varied the dose of alpha-tocopherol. Smokers and non-smokers were supplemented with one of the following doses of d-alpha-tocopheryl acetate: 70, 140, 560, or 1,050 mg daily. In the smokers each dose was associated with a significant decrease in susceptibility of

erythrocytes to *in vitro* lipid peroxidation as determined by comparing baseline to post-supplementation hydrogen peroxide-induced MDA concentrations. Contrary to what might be expected, no statistically significant dose response was observed (Table 1).[13] This suggests, at least for this *in vitro* biomarker of resistance to oxidative stress, no additional benefit is accrued by virtue of giving doses above the lowest dose administered (70 mg daily).

Brown et al also reported a substantial negative dose response among the non-smokers. Among non-smokers, supplementation of the highest dose of alpha-tocopherol (1,200 mg daily) for 20 weeks resulted in a statistically significant increase in lipid peroxidation, suggesting pro-oxidant activity. In both the smokers and non-smokers, 20 weeks of supplementation resulted in a decease in plasma concentrations of ascorbic acid. Whether this decrease was because of an unwanted effect of exclusive alpha-tocopherol supplementation on ascorbic acid metabolism; an increased ability to concentrate ascorbic acid in tissues; an increased utilization of ascorbic acid to reduce oxidized alpha-tocopherol; or some other factor, cannot be determined by this study.[13]

Researchers conducted a two-year randomized, double-blind, placebo-controlled study involving 128 male normolipidemic chronic smokers. The intervention consisted of 268 mg dl-alpha-tocopherol daily, containing 400 IU of vitamin E activity. Plasma TBARS decreased in smokers given either active or placebo intervention, while LDL lag time increased significantly in the active treatment group, suggesting reduced *in vitro* susceptibility to oxidation. However, supplementation was unable to decrease antibodies against oxidized LDL.[21] Since antibodies against oxidized LDL have been

Table 2. *Percent change in susceptibility of RBC to H_2O_2-induced lipid peroxidation* in vitro *as determined by MDA after 20 weeks of supplementation with varying doses of vitamin E[13]*

Dose of Vitamin E	Percent Change in MDA
*Placebo (no vitamin E)	18-percent decrease
*70 mg/day	61-percent decrease
*140 mg/day	73-percent decrease
*560 mg/day	63-percent decrease
*1,050 mg/day	64-percent decrease
1,050 mg/day to non-smokers	42-percent increase

* indicates smoker group only

reported to be associated with cardiovascular disease,[22,23] and since an increase in antibodies against oxidized LDL suggests increased physiological oxidative stress, it would seem this dose of alpha-tocopherol, even over two years, is unable to completely offset free radical-induced oxidative stress in smokers.

Several other biomarkers of *in vivo* functional oxidative stress have also been observed in smokers subsequent to alpha-tocopherol intervention. Breath pentane output (BPO) is assessed by collecting exhaled air and measuring the quantity of pentanes, which are formed in the body as a result of peroxidation of PUFAs. A portion of pentanes is volatile and eliminated in respiration; therefore, BPO provides a functional surrogate measure of oxidative stress to lipids. BPO would be expected to increase as oxidative stress increases. 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 8-iso-prostanglandin F2alpha (8-iso-PGF(2alpha)) 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]

Hoshino et al investigated the effect of two weeks of supplementation with 800 IU dl-alpha-tocopherol daily at bedtime on lipid peroxidation in 13 smokers (5 males and 8 females) with a median age of 29 years. In order to assess lipid peroxidation, BPO was used as a surrogate functional marker of lipid peroxidation. Baseline levels of BPO were substantially higher in smokers as opposed to 19 non-smokers used as controls (BPO of 16.3 versus 5.8 pMol/kg body weight/min in smokers versus non-smokers). The elevated BPO occurred despite comparable baseline plasma concentrations of vitamin E in both groups. Addition of supplemental vitamin E for two weeks resulted in a decrease in BPO; however, the reduced values remained higher among smokers than those found at baseline among non-smokers. Four weeks after cessation of vitamin E supplementation, BPO had rebounded to a higher level (>22) than that originally found among smokers prior to supplementation. In addition to this unwanted rebound effect, although supplementation increased alpha-tocopherol levels, it reduced plasma gamma- and beta-tocopherols significantly.[3]

Patrignani et al performed a randomized, double-blind, placebo-controlled study of the effects of increasing doses of vitamin E every three weeks on urinary F2-isoprostanes. Urinary 8-iso-PGF(2alpha) was used to assess urinary F2-isoprostanes. Forty-six cigarette smokers (minimum of 15-30 cigarettes daily for the previous two years) were started on a daily dose of 300 mg dl-alpha-tocopheryl acetate. After three weeks this dose was increased to 600 mg daily. Following week six, the dose was increased to 1,200 mg daily. Plasma levels of vitamin E increased dose-dependently up to a maximum twofold increase; however, after discontinuing supplementation they returned to pre-supplementation levels. Despite

doubling of plasma levels of vitamin E, no statistically significant changes in urinary F2-isoprostane excretion were observed.[26]

Reilly et al reported a similar negative result in a shorter duration trial of alpha-tocopherol supplementation. Participants had a mean daily intake of 25 cigarettes. Supplementation of alpha-tocopherol at doses of either 100 or 800 IU daily for five days had no effect on, or ability to suppress, urinary levels of F2-isoprostane in smokers.[24]

Several biomarker studies have examined the impact of vitamin E supplementation on biomarkers of DNA damage. Urinary excretion rate is measured for 8-hydroxydeoxyguanosine (8-OHdG), a repair product of DNA that increases subsequent to oxidative damage to DNA. It is used as a biomarker to evaluate risk of carcinogenesis and has physiological correspondence to free radical damage. Despite achieving a substantial increase in plasma concentrations of alpha-tocopherol, supplementation of 20 male smokers with 100 mg d-alpha-tocopheryl acetate twice daily for two months was unable to create a significant change in the urinary excretion rate of 8-OHdG.[27]

Sister chromatid exchange (SCE) in lymphocytes is used as a biomarker for *in vivo* cytogenic damage to DNA. Supplementation of 900 IU vitamin E daily for six weeks was unable to decrease the frequency of SCE in smokers.[28]

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.[29]

Neunteufl et al supplemented 600 IU dl-alpha-tocopherol to 11 male smokers (mean age 28 years; average of 23 cigarettes per day) for four weeks in order to determine whether short-term supplementation could attenuate the transient impairment of endothelial function resulting from an episode of acute smoking and reverse the chronic endothelial dysfunction. Eleven smokers were given placebo and 11 non-smokers were used as a control group. While four weeks of supplementation was able to modestly attenuate endothelial

dysfunction subsequent to an acute episode of smoking a single cigarette, it had no ability to reverse the chronic endothelial dysfunction found in these smokers.[29]

Green et al compared the impact of supplemental vitamin E on endothelial function as assessed by aspects of the nitric oxide dilator system in long-term smokers and non-smokers. Nine male non-smoking subjects and eight male smokers (average of 36 cigarettes per day for more than seven years) were given 1,000 IU d-alpha-tocopherol daily for a four-week period. Five additional non-smokers received placebo as a further control group. Vitamin E supplementation had no apparent effect on basal endothelial function in any of the studied groups.[30]

Heitzer et al investigated the relationship between vitamin E supplementation and endothelium-dependent relaxation as it interfaced with hypercholesterolemia and chronic smoking. In order to assess relationships they utilized 13 non-smoking subjects with hypercholesterolemia, 15 smoking subjects with hypercholesterolemia, and 14 smokers with lipid levels falling within normal limits. Subjects within each group were randomly assigned in a 1:2 ratio to receive either placebo or 544 IU d-alpha-tocopheryl acetate daily for four months. While supplementation with vitamin E augmented endothelium-dependent relaxation among the subset of hypercholesterolemic smokers, no significant benefit was found among either hypercholesterolemic non-smokers or normocholesterolemic chronic smokers. The hypercholesterolemic smokers also had significantly higher levels of antibodies against oxidized LDL. Supplementation of vitamin E resulted in no significant change in antibody titers in the hypercholesterolemic and smoking normocholesterolemic subjects, but was able to reduce antibody titers in the hypercholesterolemic smokers to levels comparable to those found in the other groups.[31]

While more research is needed, this study suggests, at least with respect to biomarkers of endothelial function and oxidized LDL, alpha-tocopherol supplementation might prove beneficial in smokers.

Interactions between alpha-Tocopherol and Essential Fatty Acids (EFAs)

Since the quantity and need for vitamin E varies based on the types and quantity of fats consumed in the diet, several researchers have monitored biomarkers of lipid peroxidation in an attempt to investigate the interactions between alpha-tocopherol supplementation of smokers and either varying dietary fats or supplementing with fish oil.

Weinberg et al investigated the impact of varying the type of dietary fat in combination with supplementing the diet with 800 IU vitamin E daily among cigarette smokers. Ten subjects who each smoked more than 20 cigarettes daily were recruited. A baseline diet was fed to all subjects for three weeks during which the major source of dietary fat was olive oil. The phase one diet consisted of 45-percent carbohydrates, 20-percent protein, and 35-percent fat. During this part of the study olive oil served as the primary dietary source of fat. The fat breakdown was saturated fat as 7.5 percent of calories; monounsaturated fatty acids (MUFAs) as 20 percent of calories; and PUFAs as 7.5 percent of calories.

Values were obtained for LDL oxidation lag time and rate as well as for plasma total F(2)-isoprostanes and PGF(2alpha) following three weeks on this diet. The diet was then modified by replacing olive oil with by high-linoleic safflower oil. The resultant fat distribution during this phase of the study was saturated fat as 7.5 percent of calories; MUFAs as 7.5 percent of calories; and PUFAs as 20 percent of calories.

After three weeks on the high-linoleic safflower oil diet, F(2)-isoprostanes increased from 53.0 to 116.2 nMol/L and PGF(2alpha) increased from 3.5 to 5.5 nMol/L. No changes in LDL oxidation parameters (lag time or rate) were observed. The observed changes suggest the higher PUFA diet resulted in an increase of *in vivo* lipid peroxidation. The high-PUFA diet was continued for an additional three weeks, during which time subjects received 400 IU dl-alpha-tocopheryl acetate twice daily.[32]

While supplementation increased plasma and LDL concentrations of vitamin E and resulted in an increase in LDL oxidation lag time (theoretically indicating a decreased susceptibility of LDL to oxidative stress and, hence, an increased functional supply of antioxidants within the LDL), there was no change in oxidation rate, and supplementation paradoxically further increased F(2)-isoprostanes to 188.2 nMol/L and PGF(2alpha) to 7.8 nMol/L.[32]

The increase in F(2)-isoprostanes and PGF(2alpha) subsequent to supplementation of a diet high in PUFAs with this dose of vitamin E suggests, at least under these circumstances, vitamin E given in the dl-alpha-tocopheryl form might exert pro-oxidant effects in cigarette smokers.

The disparity between an increased *in vitro* lag time and increased *in vivo* F2-isoprostane levels suggests a problem and possible limitation in the varying measures used to assess susceptibility or resistance to oxidative stress. Weinberg et al suggest the disparity likely indicates the *ex vivo* kinetic measurements of LDL oxidation are likely to be less physiologically relevant when seeking to determine *in vivo* global oxidative stress.[32]

Data from the Honolulu Heart Program suggests fish intake might protect smokers against lung cancer and cardiovascular disease.[33,34] While this association is encouraging, the impact of fish oil supplementation on these clinical endpoints in smokers is currently not established. Currently no data exists to indicate whether supplementation of fish oil to smokers would definitively modify clinical endpoints. However, two studies have looked at the short-term effect on biomarkers of lipid peroxidation subsequent to providing smokers with supplemental fish oil. In one case, changes in biomarkers of oxidative stress subsequent to fish oil administration suggest the possibility of increased lipid peroxidation,[35] while in the other, the overall effect of supplementation resulted in no significant changes in lipid peroxidation.[2]

In the study that indicated no net change[35] the observed population was hyperlipidemic smokers, the fish oil contained 30 mg coenzyme Q10 (CoQ10), and results were compared to a control group receiving a fatty acid supplement containing approximately 20-percent omega-6 and -3 fatty acids.[2] One or more of these differences in study design might explain the differences in observed lipid peroxidation. Regardless of these differences, providing alpha-tocopherol, either exclusively or in conjunction with other antioxidants, appeared to have a beneficial interaction with fish oil supplementation.

When 400 mg vitamin E was given with 10 g fish oil it partially countered the increase in plasma TBARS associated with the fish oil supplementation.[35] When an antioxidant combination consisting of 75 mg vitamin E, 150 mg ascorbic acid, and 15 mg beta-carotene was provided with the fish oil supplement containing 5 g each of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) along with 30 mg CoQ10, resistance to measured parameters of LDL oxidation approximated values found in non-smoking controls.[2]

Discussion

Supplementation of alpha-tocopherol to smokers consistently increases serum and tissue levels of alpha-tocopherol; however, it might have unwanted *in vivo* effects on levels of other nutritional compounds, such as ascorbic acid, beta-carotene, lycopene, and gamma-and beta-tocopherol. More research is required to clarify the pharmacokinetic interactions between alpha-tocopherol administration and these compounds and to identify whether additional nutrient-nutrient interactions exist when alpha-tocopherol is administered in pharmacological doses to smokers.

The results of the ATBC Study suggest supplementation of a smoker's diet with alpha-tocopherol might be beneficial, have no effect, or be potentially harmful depending on which clinical endpoint is studied. The statistically significant difference found between hemorrhagic and thrombotic stroke incidence and/or mortality subsequent to alpha-tocopherol supplementation indicates a potential need to pre-identify candidates at risk for these various types of strokes and modify alpha-tocopherol recommendations accordingly.

The differential benefits obtained with respect to the common cold (observed in the ATBC Study depending upon whether a person lived in a city or not, and smoked fewer then 15 cigarettes or not), and endothelial function and antibodies against oxidized LDL in hypercholesterolemic but not normocholesterolemic smokers, suggests a strong need for increased stratification when designing trials to measure the benefits of alpha-tocopherol in smokers.

The impact of alpha-tocopherol on various biomarkers assessed to date seems to suggest vitamin E, at least as alpha-tocopherol, is incapable of mitigating the unwanted physiological effects caused by cigarette smoking. Supplementation, while capable of positively modifying some markers of *in vitro* lipid peroxidation, is far less capable of impacting *in vivo* biomarkers. While, over the course of two weeks, supplementation slightly decreased BPO, it was unable to lower this biomarker to levels even remotely similar to non-smokers. Of potential concern is the rebound increase above pre-supplementation levels once a smoker discontinues alpha-tocopherol supplementation, since adherence to long-term vitamin supplementation can be challenging to maintain in some individuals. Alpha-tocopherol appears to exert no positive impact on F2-isopentanes nor did it alter DNA damage as assessed by 8OHdG or SCE. Alpha-tocopherol appears to be unable to positively modify endothelial function with the possible exception of smokers with hypercholesterolemia.

The interactions with alpha-tocopherol and dietary fats require further elaboration. Current available evidence, which is presently limited, suggests dietary guidance should favor monounsaturated fats such as olive oil rather than polyunsaturated omega-6 vegetable oils in smokers. More extensive research is required to verify this recommendation and to determine whether other factors such as genetic polymorphisms might require modifying the type of dietary fats recommended to active smokers. The tocopheryl form of vitamin E in this study of dietary fats and vitamin E supplementation might also possibly be less effective than other forms of vitamin E and requires further clarification. Until more information is available, it might be prudent to preferentially utilize the tocopherol, rather than the tocopheryl, form of vitamin E in smokers.

Until more information is available, it might be prudent to recommend a low dose of several antioxidants including alpha-tocopherol, ascorbic acid, and CoQ10 to smokers taking high doses of supplementary fish oil.

There is currently no clear consensus on the most appropriate dose of vitamin E for smokers. In all likelihood, the best dose would vary among individuals and would depend on number of cigarettes smoked, diet, lifestyle, genetics, and other factors. Although vitamin E is an antioxidant and generally considered to be non-toxic,[36] it appears to be capable of functioning as a pro-oxidant under certain conditions and at high or prolonged doses.[37,38] Because of this and the interactions with other antioxidant substances as the dose increased, until more information is available it might be better to err on the side of lower doses in combination with an array of other antioxidant nutrients and foods, rather than relying on doses that fall within the pharmacological range.

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