Clinical Investigation and Reports

Six-Year Effect of Combined Vitamin C and E Supplementation on Atherosclerotic Progression
The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study

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Background—Self-selected supplementation of vitamin E has been associated with reduced coronary events and atherosclerotic progression, but the evidence from clinical trials is controversial. In the first 3 years of the ASAP trial, the supplementation with 136 IU of vitamin E plus 250 mg of slow-release vitamin C twice daily slowed down the progression of carotid atherosclerosis in men but not women. This article examines the 6-year effect of supplementation on common carotid artery (CCA) intima-media thickness (IMT).

Methods and Results—The subjects were 520 smoking and nonsmoking men and postmenopausal women aged 45 to 69 years with serum cholesterol ≥ 5.0 mmol/L (193 mg/dL), 440 (84.6%) of whom completed the study. Atherosclerotic progression was assessed ultrasonographically. In covariance analysis in both sexes, supplementation reduced the main study outcome, the slope of mean CCA-IMT, by 26% (95% CI, 5 to 46, $P=0.014$), in men by 33% (95% CI, 4 to 62, $P=0.024$) and in women by 14% (not significant). In both sexes combined, the average annual increase of the mean CCA-IMT was 0.014 mm in the unsupplemented and 0.010 mm in the supplemented group (25% treatment effect, 95% CI, 2 to 49, $P=0.034$). In men, this treatment effect was 37% (95 CI, 4 to 69, $P=0.028$). The effect was larger in subjects with either low baseline plasma vitamin C levels or CCA plaques. Vitamin E had no effect on HDL cholesterol.

Conclusions—These data replicate our 3-year findings confirming that the supplementation with combination of vitamin E and slow-release vitamin C slows down atherosclerotic progression in hypercholesterolemic persons. (*Circulation.* 2003;107:947-953.)

Key Words: arteriosclerosis ■ trials ■ ultrasonics ■ antioxidants ■ lipids

Multidisciplinary evidence indicates that enhanced lipid peroxidation is associated with accelerated atherogenesis[1–4] and that self-selected use of antioxidant supplements is associated with reduced atherosclerosis.[5,6] However, contradictory findings have been observed in randomized clinical trials.[3,4,7–13] The reasons for this have been discussed extensively.[3,4,7–13] Briefly, differences in study populations, the supplements, and outcome measures seem to explain this variability.

Most clinical trials are in populations with high antioxidant intake, and therefore the supplementation would be expected to be less effective.[8] Vitamin E and vitamin C are the most important dietary antioxidants.[6,7,14] When vitamin E works as an antioxidant, it is oxidized to harmful radical, which needs to be reduced back to α-tocopherol, eg, by vitamin C.[15] Recently, supplementing smokers with high polyunsaturated fatty acid diet with high doses of vitamin E alone promoted rather than reduced lipid peroxidation.[16] In our prospective population study, vitamin C deficiency was associated with coronary events.[17] Thus, it is conceivable that in most completed and ongoing antioxidant supplementation trials, a wrong kind of supplement is given.

We designed a randomized clinical trial in which both vitamin E and a coantioxidant, slow-release vitamin C, were supplemented in a physiological ratio.[18] The main purpose of the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study was to test the effect of reasonable supplemented doses of vitamin E and vitamin C and their
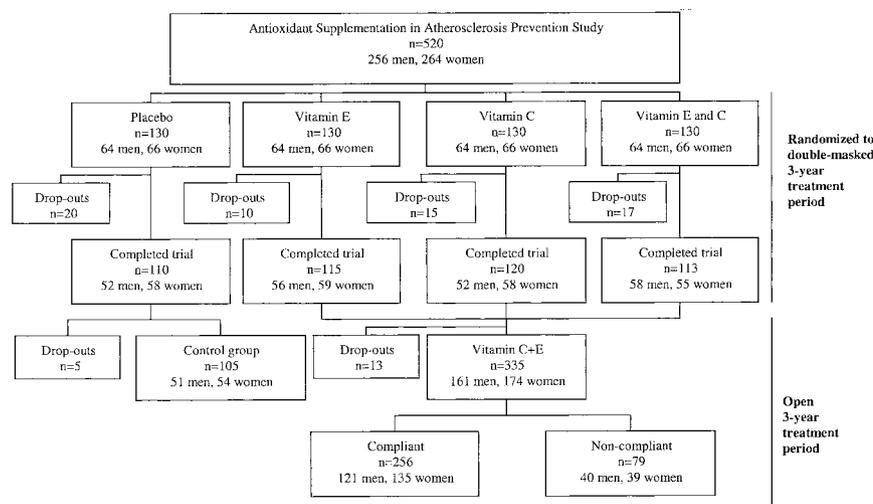
Figure 1. The study design and the flow of subjects.

combination on the progression of common carotid atherosclerosis in middle-aged high-risk men and women. Because men are at enhanced oxidative stress and lipid peroxidation, a greater atherosclerotic progression-retarding effect was hypothesized a priori in men than in women. Because of the synergism between vitamin E and vitamin C in the human body, the greatest protective effect was hypothesized to be attained by the combined supplementation.

Methods

Study Design, Inclusion and Exclusion Criteria, and Supplements

This report concerns the 6-year effect on atherosclerosis. All subjects had hypercholesterolemia at entry to the lead-in period, defined as serum cholesterol ≥ 5.0 mmol/L (193 mg/dL) at screening. Subjects were not entered into the trial if they had regular intake of antioxidants, acetosalicylate, or any other drug with antioxidative properties, severe obesity (body mass index >32 kg/m^2), type 1 diabetes, uncontrolled hypertension (sitting diastolic blood pressure >105 mm Hg), any condition limiting mobility, or severe disease shortening life expectancy. Premenopausal women and those taking oral estrogen therapy were also excluded.

The study consisted of 8-week dietary counseling and placebo lead-in phase, a 3-year double-masked treatment period, and a 3-year open treatment period (Figure 1). After the lead in, the subjects were randomly allocated to receive twice daily with meal one of the following: (1) 91 mg d-α-tocopherol (corresponding to 100 mg of d-α-tocopheryl acetate and 136 IU of vitamin E); (2) 250 mg slow-release ascorbic acid; (3) both d-α-tocopherol and slow-release ascorbic acid in a single tablet (CellaVie); or (4) placebo only. All tablets were identical in appearance, size, and color. After the double-blind 3-year period, the study was continued for another 3 years as an open study, during which period all the supplemented subjects received the vitamin combination and the placebo subjects continued without supplementation. The doses were chosen to keep the plasma ratio of vitamin C and E concentrations similar as in unsupplemented persons.[19,20] The pilot studies established that a reasonably constant plasma level of vitamin C was achieved. The subjects were randomized separately in the following 4 strata of approximately equal size: (1) smoking (≥ 5 cigarettes/d) men; (2) nonsmoking men; (3) smoking postmenopausal women; and (4) nonsmoking postmenopausal women. All participants signed a written informed consent. The Research Ethics Committee of the University of Kuopio approved the study protocol.

The subjects came to baseline visits and were randomized during 1994 to 1995. Follow-up visits were 6, 12, 18, 24, 30, 36, and 72 months later. Supplements were given, returned tablets were counted, and ultrasonographic assessment of common carotid artery (CCA) intima-media thickness (IMT)[18,21,22] was carried out at all of these 8 visits.

Study Participants

After screening volunteers over the phone, 946 eligible persons were invited, 803 were examined, and 660 were entered into a 8-week lead-in phase (Figure 1). Of these, 520 subjects (256 men and 264 women) were randomized into the trial. In each treatment group, 64 men and 66 women were randomized. Of the 520 participants randomized, 440 (84.6%) completed the study and underwent the 6-year reexamination. Of these, 212 were men and 228 women. Thus, the average annual dropout rate was only 2.6%.

Assessment of Atherosclerotic Progression

The progression of CCA atherosclerosis was carried out by high-resolution ultrasonography.[18] Briefly, the protocol included the scanning of CCA, the carotid bulbs, and the proximal internal carotid artery. After a diagnostic examination of entire accessible carotid tree, the site of the greatest IMT at baseline in the CCA far wall was located and scanned thoroughly from 3 fixed angles. The severity of atherosclerosis was classified using a 4-categorical scale separately for right and left CCA.[21] The 2 middle categories were combined in the statistical analysis.

All IMT measurements (both baseline and follow-up) from S-VHS videotapes were made at the same site and angle at all examinations of each subject, which was the site with the greatest IMT (in any angle), clearly visible at baseline. At this location, IMT was measured in diastole for a length of 10 mm (or shorter, if not visible) in one angle for the far wall. Most often this was the distal centimeter of CCA. All IMT measurements were carried out by one very experienced technician (J.T.), who was blinded to the supplementation status of the subjects. For the image analysis, the ProWin (CALTECH) software using automated boundary detection was used. The correlation between the baseline and 72-month mean CCA-IMT was 0.95 (Figure 2).

The linear regression slope of the mean common carotid IMT over all points of follow-up time was used as the primary outcome measure.[18] Confirmatory analyses using second-order polynomial regression slopes and linear slopes started from study months 6 or 12, and those based on polynomial repeated measures modeling gave similar results (not presented). The differences in the length of period between reexaminations were taken into account in the computation of the regression slope. The mean CCA-IMT from the right and the left side was averaged, and then the slope was computed across time-specific means. For confirmation, we also used the simple difference between the final mean IMT at 72 months and the baseline mean IMT.
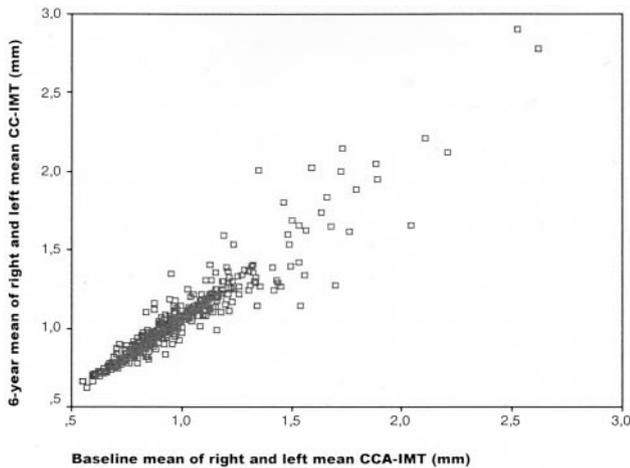
Figure 2. The 72-month mean of right and left mean CCA-IMT plotted against the baseline mean of right and left CCA-IMT.

Other Measurements

Ascorbic acid was stabilized in heparin plasma with metaphosphoric acid on plasma separation, frozen at −80°C, and determined with high-pressure liquid chromatography.[19] Heparin plasma for α-tocopherol was extracted with ethanol and hexane and measured by reversed phase high-pressure liquid chromatography.[20] Plasma F2-isoprostane concentrations were measured by a gas chromatography–mass spectrometry method.[23]

Concomitant diseases and medications were recorded at study years 0, 1, 3, and 6 by a structured interview. The study physicians also recorded all clinical findings and medications.

Statistical Methods

All of the 440 participants for whom the ultrasound examination was available at the end were included in the primary intention-to-treat statistical analysis. Because the subjects were randomized in strata and the a priori power calculations were based on analysis in men, the statistical analyses were also done in men and women separately. Because the outcome variables were not perfectly normally distributed, we used the nonparametric Mann-Whitney test to examine differences between the 2 treatment groups. For comparisons taking into account covariates, general linear modeling of the SPSS 10.0 for Windows was used. Covariates in the models were sex, baseline mean CCA-IMT, classification of severity of the right and left CCA, baseline vitamin C concentration, and 3 indicator variables for summer baseline examination months (July, August, and September). These are the months in Finland when vitamin C intake and plasma levels are notably higher than in other times.[24] Despite the one-sided hypotheses, probability values are reported as two-sided.

Results

Compliance and Adverse Events

Of the 390 subjects randomized to supplementation, 335 continued the study after 3 years and 256 (76.4%) took the supplements as instructed for 6 years, whereas 62 subjects stopped the supplements during the first 3 study years and an additional 18 subjects during the last 3 study years. The mean plasma α-tocopherol concentration changed in 6 years in the group randomized to supplementation (n=335) from 33.0 to 47.4 μmol/L (43.6% increase), in those who took the supplements (n=256) from 32.9 to 51.7 μmol/L (57.1% increase), and in the unsupplemented group (n=105) from 32.3 to 30.7 μmol/L (5.0% decrease, 62% treatment effect). The mean plasma ascorbate concentration changed in the group randomized to supplementation from 67.7 to 87.9 μmol/L (29.8% increase), in those who took the supplements from 68.8 to 94.7 μmol/L (37.7% increase), and in the unsupplemented group from 70.7 to 63.2 μmol/L (10.6% decline), 48% treatment effect.

The causes of dropouts in the supplemented and unsupplemented men and women are shown in Table 1. There were no significant differences either in the proportions of the reasons for dropouts or in the cumulative incidence of all adverse events between the treatment groups.

Atherosclerotic Progression

The means of the mean CCA-IMT in the annual assessments are presented in Figure 3 for the unsupplemented and supplemented male participants. There was a small reduction in the mean CCA-IMT during the first study year, after which the CCA-IMT started to progress approximately linearly. The lines started to diverge after the first study year.

The mean annual increase of the mean CCA-IMT, estimated as the linear slope across all time points, was 0.0156 mm/year (SD 0.0182) in the 105 nonsupplemented participants and 25% less, 0.0118 mm/year (SD 0.0136), in the 335 supplemented participants (P=0.007 for difference). Among the 256 participants who took their supplements, the CCA-IMT slope was 0.0111 mm/year (29% treatment effect, P=0.004).

When estimated as the simple difference between the last (72-month) and the first (baseline) assessments, the mean annual increase of the mean CCA-IMT was 0.0134 mm/year (SD 0.016) in the nonsupplemented subjects, 0.0103 mm/year (SD 0.014) in the subjects randomized to supplements, and

TABLE 1.   Causes of Dropouts During 6 Study Years in the Randomized Treatment Groups for Men and Women

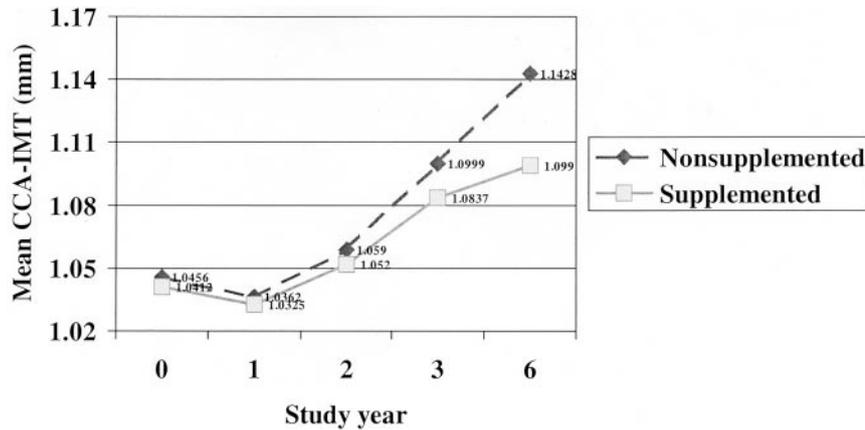| Cause of Dropout	Men		Women	
	Supplemented (n=192)	No Supplements (n=64)	Supplemented (n=198)	No Supplements (n=66)
Death	12	3	7	0
Severe adverse event	11	3	6	1
Other adverse event	1	2	2	1
Refusal or other reason	7	5	9	10
Total	31	13	24	12

Figure 3. The mean CCA-IMT in the annual assessments in male participants randomized to supplementation and to no supplements.

0.0095 mm/year (SD 0.012) in those who took the supplements ($P=0.007$ and $P=0.002$ for difference). In men, the difference between treatment groups both in the slope ($P=0.008$) and the difference ($P=0.002$) was statistically significant. In women, neither difference was significant.

In a covariance analysis in both men and women combined, there was a 26% (95% CI, 5 to 46; $P=0.014$) treatment effect in the main study outcome, the slope of mean CCA-IMT in all subjects (Table 2), and a 30% (95% CI, 10 to 51; $P=0.003$) treatment effect in those who took their supplements. The respective treatment effect was 33% (95% CI, 4 to 62; $P=0.024$) in all men and 14% (95% CI, −15 to 44, not significant) in all women (Table 3). Among the compliant men and women, the treatment effect was 39% (95% CI, 9 to 69, $P=0.010$) and 17% (95% CI, −11 to 44, $P=0.235$), respectively. There was no significant difference in the treatment effect between smokers and nonsmokers. In a repeated-measures model analyzing the CCA-IMT at study years 0, 1, 2, 3, and 6, the within-subject effect for the time-supplementation interaction was significant for men ($P=0.019$) but not for women. Only the linear component of the trend difference was significant ($P=0.013$), with a trend in the quadratic component ($P=0.097$), nonsignificant cubic ($P=0.544$), and fourth order ($P=0.915$) components.

When computed as the difference between the last and the first mean IMT measure in both men and women combined, the average annual adjusted increase of the mean CCA-IMT was 0.0137 mm in the placebo group and 0.0102 mm in the supplemented group, a 25% treatment effect (95% CI, 2 to 49; $P=0.034$, Table 2). In men, the average annual increase

of CCA-IMT was 0.0162 mm in the placebo group and 0.0103 mm in the supplemented group (37% treatment effect; 95% CI, 4 to 69; $P=0.028$; Table 3). In women, the respective changes were 0.0111 and 0.0102 mm (difference not significant). When the first and second 3-year periods were analyzed separately, the treatment effect in men was significant only during the second period ($P=0.029$).

The treatment effect was greater in the participants who already had plaques in CCAs at baseline compared with those who had no plaques in the segments of CCAs examined (Figure 4). In the covariance model, allowing for the baseline atherosclerosis severity (but not baseline CCA-IMT), the treatment effect on the slope was 35% (95% CI, 12 to 58; $P=0.003$) in all subjects. The treatment effect was 54% in the subjects who already had at least one plaque obstructing >20% of arterial lumen diameter at baseline. The treatment effect was also larger and principally confined to participants who had baseline plasma vitamin C levels below median (<71.25 μmol/L).

The 6-year change of the mean CCA-IMT correlated inversely with the change of plasma α-tocopherol concentration in men ($r=-0.137$, $P=0.047$) but not in women ($r=0.030$, $P=0.650$).

Change in Plasma Vitamins and F_2-Isoprostanes

Changes in plasma vitamin E and C concentrations were similar in men and women during both the first 1 and 3 years of supplementation (Table 4). In men, the plasma F_2-isoprostane concentration was reduced in the vitamin E group significantly, whereas there was an increase in the placebo and vitamin C groups (Table 4). In women, F_2-isoprostanes declined more in

TABLE 2.   Mean Adjusted 6-Year Change* of the Mean Common Carotid Artery Intima-Media Thickness in Participants Who Were Randomized to Vitamin C Plus E Supplements and in the Control Group in Multivariate General Linear Models (n=440)

	Mean (SEM)				
Indicator of Change, mm/y†	Supplemented (n=335)	No Supplements (n=105)	Difference Between Groups	95% CI of Difference	P for Difference
Slope across study period	0.0117 (0.0008)	0.0158 (0.0014)	0.0041	0.0008 to 0.0074	0.014
Difference between end and baseline	0.0102 (0.001)	0.0137 (0.001)	0.0035	0.0003 to 0.0067	0.034

*The linear slope over repetitive assessments of mean IMT. Difference is the difference between the end (72-month) and baseline mean IMT divided by the value 6.

†Covariates in the model are sex, baseline mean CCA-IMT, classification of severity of the right and left CCA, baseline vitamin C concentration, and 3 indicator variables for summer baseline examination months (July, August, and September).

TABLE 3.   Mean Adjusted 6-Year Change of the Mean Common Carotid Artery Intima-Media Thickness in Male and Female Participants Who Were Randomized to Vitamin C Plus E Supplements and in the Control Group in Multivariate General Linear Models

	Men (n=212)				Women (n=228)			
	Mean (SEM)				Mean (SEM)			
Indicator of Change, mm/y*	Supplemented	No Supplements	Difference Between Groups (95% CI)	P	Supplemented	No Supplements	Difference Between Groups (95% CI)	P
Slope across study period†	0.0122 (0.0013)	0.0183 (0.0023)	0.0061 (0.0008 to 0.0114)	0.024	0.0113 (0.0010)	0.0132 (0.0017)	0.0019 (−0.0020 to 0.0058)	0.346
Difference between end and baseline	0.0103 (0.001)	0.0162 (0.002)	0.0059 (0.0006 to 0.0112)	0.028	0.0102 (0.001)	0.0111 (0.002)	0.0009 (−0.0028 to 0.0046)	0.625

*Linear slope over repetitive assessments of mean IMT. Difference is the difference between the end (72-month) and baseline mean IMT divided by the value 6.

†Covariates in the models for both men and women are baseline mean CCA-IMT, classification of severity of the right and left CCA, baseline vitamin C concentration, and 3 indicator variables for summer baseline examination months (July, August, and September).

the placebo group than in those who received either vitamin E or C supplements. The treatment effect of vitamin E (mean −6.2 ng/L; 95% CI, −2.2 to −10.2) was statistically significant ($P=0.003$, n=100) in men in a linear variance model allowing for interaction. In women, the respective treatment effect was not significant ($P=0.906$, n=47). The change in plasma F_2-isoprostanes correlated with the change of both plasma α-to-copherol ($r=-0.284$, $P=0.004$) and plasma lipid-standardized α-tocopherol ($r=-0.323$, $P=0.001$) in men, whereas these correlations were absent in women ($r=0.057$, $r=-0.020$, respectively).

Change in Serum HDL Cholesterol

The mean HDL cholesterol increased in 3 years significantly more among men who received vitamin C supplement than in men who received placebo ($P=0.025$, Table 4), whereas vitamin E had no effect on serum HDL cholesterol in men. In women, neither vitamin C nor vitamin E had any significant effect on serum HDL cholesterol in 36 months. Findings for 12 and 24 months were similar. The adjustment for baseline HDL cholesterol had no major effect on the findings.

Discussion

The present 6-year data provide a confirmation for our 3-year findings, demonstrating that the combination of supplemented reasonable doses of vitamin E and slow-release

Figure 4. The mean adjusted annual increase (slope) of the mean CCA-IMT in the nonsupplemented and supplemented study participants according to the severity of CCA atherosclerosis at baseline. Covariates in the linear model were sex, baseline vitamin C concentration, and 3 indicator variables for summer baseline examination months (July, August, and September). $P=0.003$ for the statistical significance of the treatment effect. Large plaques are those that obstruct at least 20% of the lumen diameter.

vitamin C, taken with meal, may slow down the progression of carotid atherosclerosis in healthy hypercholesterolemic persons. Our study suggests that the benefit is greatest in men and may even be limited to men only. The observed effect modification by sex needs to be retested in additional clinical trials, because we cannot fully rule out the possibility that the chance would have caused the sex difference. Because men had considerably lower baseline levels of both plasma α-to-copherol and ascorbate, it is possible that the greater observed benefit in this group could be simply attributable to the greater increase of these vitamins. However, we also observed a lipid peroxidation–reducing effect of vitamin E only in men, whereas in women vitamin E had little or no effect on lipid peroxidation, as measured as plasma F_2-isoprostane concentration. This sex difference is a novel finding that urgently needs to be retested in other controlled clinical trials.

The size of the treatment effect is not trivial. We observed in all men a 30% treatment effect and in both sexes combined a 25% treatment effect. The treatment effect among participants who had carotid plaques at baseline was >50%. These effects are comparable with those of the most effective cholesterol-lowering medications but with fewer adverse effects and lower cost. We have shown previously that the supplement used in the ASAP study elevated plasma vitamin C and E levels by 50% and reduced lipid peroxidation in vivo in men also by ≈50%.[23,25] Our present data suggest that vitamin E supplementation might not affect lipid peroxidation in vivo in women. The magnitude of the deceleration of arterial IMT growth parallels these numbers. In two subgroup analyses performed, the treatment effect was larger in participants who had low baseline plasma vitamin C levels and already plaques in the CCA. There was also a trend toward a greater effect in smokers than in nonsmokers. These 3 groups overlap. The finding that the treatment effects were larger when only compliant participants were included into the analysis speaks in favor of causality.

Both the vitamin E and C supplements were safe. There were neither excess deaths nor excess of other adverse events in the groups randomized to supplements, although the sample size was not designed to detect effects on either deaths or other disease events. Both the adherence to treatment and the bioavailability of the supplements were good, judged based on increases of plasma vitamin levels. The dropout rate during the trial was exceptionally low.

In theory, it is possible that the mechanisms responsible for the antiatherogenic effect of the vitamin C plus E combination
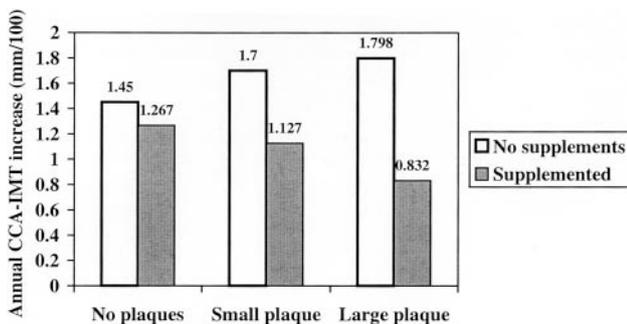
TABLE 4. Thirty-Six–Month Change [mean (SEM)] of Plasma Vitamin C and E (μmol/L), Plasma F2-Isoprostane (ng/L), and Serum HDL Cholesterol (mmol/L) Concentrations in the Randomized Treatment Groups for Men and Women

Change in Plasma Concentration of	Treatment Group				P for Heterogeneity
	Vitamin E	Vitamin C	Vitamin C+E	Placebo	
Men (n=197)					
Vitamin E	28.0 (3.0)	1.4 (0.9)	22.7 (1.4)	2.6 (1.1)	<0.001
Vitamin C	3.7 (3.5)	41.2 (4.8)	37.5 (4.0)	−4.8 (2.8)	<0.001
F_2-Isoprostanes (n=100)	−5.3 (2.0)	2.2 (2.1)	−2.0 (2.0)	2.9 (1.9)	0.020
HDL cholesterol	0.07 (0.02)	0.12 (0.02)*	0.04 (0.02)	0.04 (0.03)	0.060
Women (n=212)					
Vitamin E	27.5 (2.0)	1.3 (1.1)	24.8 (1.8)	1.2 (0.8)	<0.001
Vitamin C	3.0 (3.5)	34.4 (3.8)	34.0 (3.9)	−0.4 (3.4)	<0.001
F_2-Isoprostanes (n=47)	−1.8 (4.9)	0.3 (3.8)	−2.9 (3.5)	−4.0 (5.8)	0.912
HDL cholesterol	0.10 (0.03)	0.06 (0.03)	0.07 (0.03)	0.11 (0.03)	0.538

*Significantly (P=0.025) different from the change in the placebo group.

would be entirely or in part other than the inhibition of lipid peroxidation. After the discovery of the human tocopherol binding protein with possible receptor function, it has become evident that vitamin E exerts more functions in the human body than the antioxidative action and these other properties may be antiatherogenic.[8,26–31] α-Tocopherol increases protein phosphatase 2_{A1} activity and inhibits protein kinase C and smooth muscle cell proliferation, cell adhesion, and platelet aggregation and enhances nitric oxide bioavailability. It also counteracts inflammation and improves endothelium-dependent vasodilator function.[29,30] Finally, some evidence suggests that α-tocopherol might improve impaired insulin-mediated glucose uptake into cells and insulin sensitivity[31] and that vitamin E deficiency might be associated with an increased risk of type 2 diabetes.[32] α-Tocopherol exerts these pleiotropic effects only in its reduced form. Thus, a coantioxidant such as vitamin C is a necessary prerequisite for the antiatherogenic effects of vitamin E.

Because a coadministration of antioxidants attenuated the HDL cholesterol–increasing effect of a statin and niacin in 2 recent clinical trials,[36,37] we analyzed the long-term effect of vitamin E and C supplements on serum HDL cholesterol. Although vitamin E had no effect on HDL cholesterol levels, vitamin C tended to elevate HDL cholesterol in men but not in women. Although previous studies are inconsistent, there is some prior evidence supporting a possible HDL-elevating effect of vitamin C supplementation.[38,39]

After the 3-year results of the ASAP study, 3 other studies concerning the effect of vitamin E on atherosclerotic progression have been reported. The Harvard IVUS study was conducted in cardiac transplantation patients, because this condition is associated with oxidant stress, which may contribute to accelerated coronary arteriosclerosis.[33] The study was a double-blind placebo-controlled trial, in which 40 patients (35 males) (0 to 2 years after cardiac transplantation) were randomly assigned vitamin C 500 mg plus vitamin E 400 IU, each twice daily (n=19), or placebo (n=21) for 1 year. The primary outcome was the change in average intimal index (plaque area divided by vessel area) assessed by intravascular ultrasonography (IVUS). IVUS and vitamin C and E plasma concentrations were assessed

at baseline and at 1-year follow-up. Vitamin C and E concentrations increased in the vitamin group (vitamin C, 43 to 103 mmol/L; vitamin E, 24 to 65 mmol/L) but did not change in the placebo group (vitamin C, 45 versus 43 mmol/L; vitamin E, 27 versus 27 mmol/L; P<0.0001 for difference between groups). During 1 year of treatment, the intimal index increased in the placebo group by 8% (SE 2) but did not change significantly in the treatment group (0.8% [SE 1]; P=0.008 for difference). The authors concluded that the supplementation with the combination of antioxidant vitamins C and E retards the early progression of transplant-associated coronary arteriosclerosis.[33]

In two other studies, a large dose of vitamin E alone without a coantioxidant has been supplemented. In SECURE, a substudy of the HOPE trial, 400 IU of vitamin E daily had no detectable effect on carotid IMT change in 4 to 5.5 years in 637 elderly high-risk men and women.[34] In SECURE, maximal IMT values, which involve large random variability, were used instead of the mean of several IMT measurements, as in our study. Also, upper carotid segments were included in the outcome variables. The inclusion of these also enhances the random error,[21] thus compromising the study power additionally. This is reflected in the low 3-week repeat correlation (r=0.87), as compared with the 6-year repeat correlation of 0.95 in our study. In the short-term, 2-year Dutch study in 189 smoking men, there was a similar IMT growth-reducing effect as in our study, but it was nonsignificant because of the small sample size.[35] Neither changes in plasma vitamin E levels nor in lipid peroxidation have been reported from either study. In both studies, the subjects were normocholesterolemic. Both studies were conducted in countries where the use of antioxidative supplements is common and plasma levels of vitamins C and E higher are than in Eastern Finland.

In the Heart Protection Study, the combination of 600 mg of synthetic vitamin E, 250 mg of vitamin C, and 20 mg of β-carotene daily had no effect on the incidence of cardiovascular events in a mixed group of 15 454 men and 5082 women. Unless a beneficial effect of vitamin E and C was attenuated by β-carotene, these findings indicate that although the vitamin E plus C combination

might have a role in the prevention of early atherosclerotic lesions, it does not prevent clinical cardiovascular events.

Our study shows that trials testing antiatherogenic interventions need to be long enough. In most similar trials, IMT has also tended to decline in the first 6 to 12 months of the study, possibly because of early dropouts of the least healthy subjects, an effect of the recruitment on health habits or even vasodilating effects of the drug. This should be taken into account in the study protocols.

Conclusions

This randomized clinical trial shows that long-term supplementation of hypercholesterolemic persons with reasonable doses of both vitamin E and slow-release vitamin C combined can retard the progression of common carotid atherosclerosis, especially in men.

Acknowledgments

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