

Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis

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Abstract. Salonen JT, Nyssönen K, Salonen R, Lakka H-M, Kaikkonen J, Porkkala-Sarataho E, Voutilainen S, Lakka TA, Rissanen T, Leskinen L, tuomainen T-P, Valkonen V-P, Ristonmaa U (University of Kuopio, Finland), Poulsen HE (University of Copenhagen, Copenhagen, Denmark). Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis. *J Intern Med* 2000; **248**: 377–386.

Objectives. To study the efficacy of vitamin E and C supplementation on the progression of carotid atherosclerosis, hypothesizing an enhanced preventive effect in men and in smokers and synergism between vitamins.

Design and subjects. Double-masked two-by-two factorial trial, randomization in four strata (by gender and smoking status) to receive twice daily either 91 mg (136 IU) of d- α -tocopherol, 250 mg of slow-release vitamin C, a combination of these or placebo for three years. A randomized sample of 520 smoking and nonsmoking men and postmenopausal women aged 45–69 years with serum cholesterol ≥ 5.0 mmol L⁻¹ were studied.

Setting. The population of the city of Kuopio in Eastern Finland.

Intervention. Twice daily either a special formulation of 91 mg of d- α -tocopherol, 250 mg of slow-release

vitamin C, a combination of these (CellaVie[®]) or placebo for three years.

Measurements. Atherosclerotic progression, defined as the linear regression slope of ultrasonographically assessed common carotid artery mean intima-media thickness (IMT), was calculated over semi-annual assessments.

Results. The average increase of the mean IMT was 0.020 mm year⁻¹ amongst men randomized to placebo and 0.018 mm year⁻¹ in vitamin E, 0.017 mm year⁻¹ in vitamin C and 0.011 mm year⁻¹ in the vitamin combination group ($P = 0.008$ for E + C vs. placebo). The respective means in women were 0.016, 0.015, 0.017 and 0.016 mm year⁻¹. The proportion of men with progression was reduced by 74% (95% CI 36–89%, $P = 0.003$) by supplementation with the formulation containing both vitamins, as compared with placebo.

Conclusions. Our study shows that a combined supplementation with reasonable doses of both vitamin E and slow-release vitamin C can retard the progression of common carotid atherosclerosis in men. This may imply benefits with regard to other atherosclerosis-based events.

Keywords: antioxidants, atherosclerosis, B-mode ultrasonography, clinical randomized trials, population studies.

Introduction

Evidence from both basic research and epidemiology

indicates that enhanced lipid peroxidation is associated with accelerated atherogenesis [1–6], whereas that from randomized clinical trials is very

limited and controversial [5–7]. Whilst epidemiological studies suggest that lipid peroxidation might be most relevant in early phases of atherosclerotic lesion development [1, 2, 4, 5] and that vitamin E may have a protective effect, if any, in clinically healthy persons [8, 9], there are no previous randomized trials testing the hypothetical preventive effect of either vitamin E or C on atherosclerotic progression in clinically healthy subjects.

Vitamin E and vitamin C are considered two of the most important dietary antioxidants [4, 5, 9, 10]. Vitamin E may also have other anti-atherogenic properties [11–15]. When vitamin E works as an antioxidant it is oxidized to harmful α -tocopheroxyl radicals, which need to be reduced back to α -tocopherol. Vitamin C can regenerate α -tocopheroxyl radical to α -tocopherol [16]. Theoretically, supplementing high-risk individuals with high doses of vitamin E alone could even promote rather than reduce lipid peroxidation [17]. Also, in our prospective population study, vitamin C deficiency was associated with increased risk of coronary events [18]. For these reasons we designed a randomized clinical trial in which not only vitamin E but also vitamin C was supplemented.

The ASAP study was designed to test the main study hypothesis that the supplementation of 45–69 year-old smoking and nonsmoking men and postmenopausal women twice daily with a formulation containing either 91 mg of d- α -tocopherol or 250 mg of vitamin C or both vitamins to retard the progression of common carotid atherosclerosis. As men and cigarette smokers have enhanced oxidative stress and lipid peroxidation [1, 5], a greater effect was hypothesized a priori in men and in smokers than in women and in nonsmokers. Because of the synergism between vitamins E and C in the human body, the greatest protective effect was hypothesized by the combined supplementation.

Methods

Protocol

ASAP is a clinical placebo-controlled two-by-two factorial trial. All subjects had hypercholesterolaemia on entry to the lead-in period, defined as a serum cholesterol of ≥ 5.0 mmol L⁻¹ at screening.

Subjects were not entered into the trial if they

were premenopausal or had regular oral oestrogen substitution therapy, regular intake of antioxidants, acetosalicylic acid or any other drug with antioxidative properties, severe obesity (BMI > 32 kg m⁻²), type 1 diabetes, uncontrolled hypertension (diastolic blood pressure > 105 mmHg), any condition limiting mobility, making study visits impossible, severe disease shortening life expectancy or other disease or condition worsening the adherence to the measurements or treatment.

The study consisted of an eight-week placebo lead-in phase and a 3-year double-masked treatment period.

Assignment

After the lead-in the subjects were randomly allocated to either (1) 91 mg of d- α -tocopherol twice daily (corresponding to 200 mg of d- α -tocopheryl acetate and 272 IU of vitamin E a day); (2) 250 mg slow-release ascorbic acid twice daily; (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 colour. The study was originally planned to be a 3-year double-blind trial. To enable the study of the effect of the supplementation, prior to opening the treatment codes, a decision was made to extend the study for another three years, supplementing all previously supplemented subjects with CellaVie[®].

The doses of the supplements were chosen to keep the plasma ratio of vitamin C and E concentrations similar to that of unsupplemented persons. This was tested in pilot and kinetic studies [19, 20]. The pilot studies also established that a reasonably constant plasma level of vitamin C was achieved by the dosing of one slow-release tablet in the morning and another in the evening. The subjects were randomized separately in four strata of approximately equal size: (1) smoking (≥ 5 cigarettes/day) men; (2) nonsmoking men; (3) smoking postmenopausal women; and (4) nonsmoking postmenopausal women. All subjects gave a written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

The subjects made baseline visits and were randomized in 1994–5. Follow-up visits were 6, 12, 18, 24, 30 and 36 months later. Supplements were given, returned tablets were counted and ultrasonographic assessment of common carotid

artery (CCA) intima-media thickness (IMT) [8, 21] was carried out at all these seven visits.

Masking

The study was double-masked. The masking was carried out by the provider of the supplements (Ferrosan A/S, Denmark) and delivered to the data centre of the Field Centre, Research Institute of Public Health, University of Kuopio, after the completion of reading of the videotapes of ultrasonographic examinations.

Participant recruitment and flow

The subjects for the study were recruited by multiple advertisements in the main local newspaper. After

screening of volunteers by telephone, 946 eligible persons were invited to screening, 803 were examined and 660 persons were entered into an eight-week lead-in phase (Fig. 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, 62 subjects (11.9%) dropped out of the trial by the end of three treatment years, and for 458 subjects (88.1%: 225 men, 233 women) the variable for atherosclerotic progression could be constructed.

Follow-up

Equipment

Two identical Biosound Phase 2 systems were used

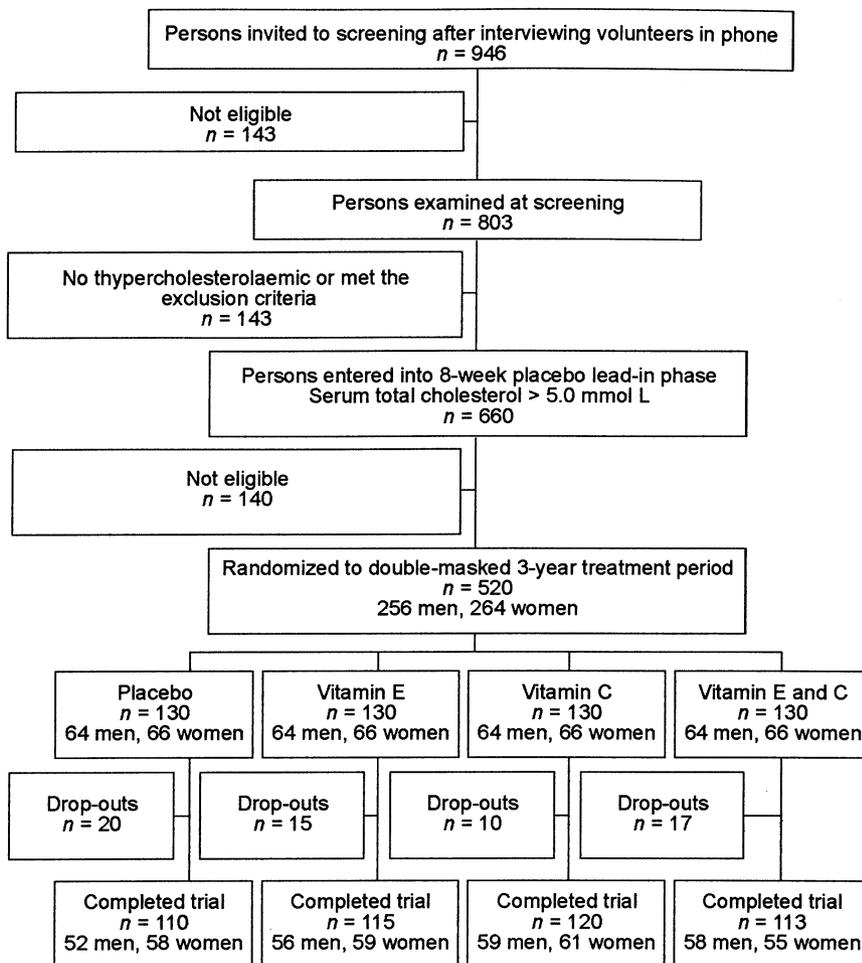


Fig. 1 Flow chart describing progress of participants through the ASAP trial.

(Biosound, Indianapolis, IN, USA) equipped with a 8–10 MHz annular array transducer. The scanings were videotaped with PAL S-VHS Panasonic AG 7330E VCR.

Observers

Four ultrasound technicians (AM, JT, PV, RP) trained in arterial scanning for between several months and a few years prior to the study, carried out the scanings. An experienced physician (RS) was the supervisor.

Scanning (imaging) procedure and videorecording

The ultrasonographic scanning was performed after a supine rest of 10 min, the subject in the supine position. First, a diagnostic examination of the entire accessible carotid tree was performed to find the most severe lesions. Secondly, the site of the greatest IMT at baseline in the CCA far wall was located and scanned thoroughly from three angles: anterolateral, lateral and posterolateral.

Measurement from videotapes

All IMT measurements (at both baseline and follow-up) from 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) that was clearly visible at baseline in the far wall of the CCA below the bulb. At this location IMT was measured in diastole for a length of 10 mm (or shorter, if not visible) at one angle for the far wall. Most often this was the distal centimetre of CCA. All IMT measurements were carried out after the 36-month examination by one very experienced technician (JT).

Ultrasound image analysis

Computer analysis of ultrasound images to measure IMT was performed with a reading station equipped with a Data Translation DT 2861 video frame grabber interfaced to a Panasonic AG 7355 VCR. We used the Prosound software, developed by Robert Selzer [22], based on automated boundary detection. IMT was determined as the average difference at on average 100 points between the intima–lumen and media–adventitia interfaces.

Measurement variability

Three technicians (AM, JT, PV) scanned 10 subjects twice at a weeks' interval in 1995. The videotapes from all scanings were read by one observer. The repeat correlations for the mean CCA-IMT were 0.988, 0.995 and 0.998, and pairwise interobserver correlations 0.975, 0.983 and 0.995.

Construction of the main outcome variable

Atherosclerotic progression was defined a priori as the linear regression slope of the mean common carotid IMT over six or seven points of follow-up time (0, 6, 12, 18, 24, 30 and 36 months). For 34 subjects, one follow-up was missing. First, the mean CCA-IMT from the right and the left sides was averaged, and then the slope was computed across time-specific means. To assess the consistency of results, the analyses were repeated by including all subjects for whom at least two time-specific IMT means were available. The findings were virtually unchanged.

Other measurements

Plasma ascorbic acid, dehydroascorbic acid and α -tocopherol were determined by liquid chromatographic methods [19, 20]. Cholesterol and triglycerides were determined with enzymatic methods [8]. Serum LDL cholesterol was measured based on precipitation using polyvinyl sulphate and HDL cholesterol after precipitation with magnesium chloride [8]. Serum ferritin was determined by an immunoradiometric assay (Bio Rad, Quantimune, Hercules, CA, USA) and plasma fibrinogen based on coagulation. Dietary intake of foods and nutrients was assessed at baseline by 4-day instructed food recording. Physical activity was assessed by a 12-month checked questionnaire [23]. Blood pressure was measured manually in sitting position after a rest of 10 min, three measurements at 3-min intervals. Concomitant diseases and medications were recorded annually by a structured interview. The study physicians also recorded all clinical findings and medications. Other measurements have been described [1, 2, 8, 18–20, 23].

Statistical analysis

Based on our previous KAPS study [8] we expected

that men receiving placebo would have an average CCA-IMT increase of $0.03 \text{ mm year}^{-1}$. As our previous data were taken from men, the power analysis and the primary study hypothesis concerned men. The goal for the sample size was set at 250 men and 250 women (125 in each stratum), which was expected to result in 215 men and 215 women at the end of the 3-year period at an annual drop-out rate of 5%. A 25% treatment effect was assumed, detectable at $\alpha = 0.05$ with power of > 0.80 in men in the vitamin E plus C group compared with the double-placebo group and with all other groups.

All study participants for whom the main outcome variable was available were included in the statistical analysis according to the intention-to-treat principle. As the subjects were randomized separately in four strata (smoking men, nonsmoking men, smoking women, nonsmoking women), this stratification was maintained also in the statistical analysis. As the a priori power calculations were based on analysis in men, the primary statistical analysis was performed in men and women separately.

To test the consistency of results, the outcome variable and the slope of the mean CCA-IMT over all available follow-up assessments was used both as a continuous variable in general linear models and as a dichotomous variable in logistic models. The cut-off for the dichotomization was the median amongst all 225 men. Odds ratios were estimated as antilogarithms of coefficients and their confidence intervals (CI) based on normality assumption of SPSS 8.0 for Windows.

As the distribution of the slope of mean CCA-IMT was not perfectly normally distributed, we used

nonparametric methods to test the significance of the heterogeneity (Kruskal–Wallis variance analysis) of outcome between the four treatment groups and the difference between the groups randomized to the combination and others (Mann–Whitney test). In spite of one-sided hypotheses, two-sided *P*-values are reported.

Results

Adverse events, compliance and adherence to treatment

Six study participants died during the first three study years. All of these were men. In the placebo group, there was one death due to cardiac dysrhythmia. In the vitamin E group there were three deaths, of which one was accidental, one due to alcohol intoxication and one sudden coronary death. One man in the vitamin C group died of subarachnoid haemorrhage and one man in the vitamin combination group due to complications of carotid endarterectomy.

The distribution of the 62 drop-outs according to the cause of drop-out and treatment group is presented in Table 1 separately for men and women in the randomized groups. There were no differences between the randomized groups (Fig. 1).

On the basis of count of returned tablets, during the whole trial on average 94.9% of tablets were used, with almost no differences between either strata or treatment groups.

Concomitant diseases and medications

The distributions of major cardiovascular diseases

Table 1 The causes of drop-outs in the four treatment groups for men and women

Cause for drop-out	Men				Women			
	Placebo	Vitamin E	Vitamin C	Both vitamins	Placebo	Vitamin E	Vitamin C	Both vitamins
Death	1	3	1	1	0	0	0	0
Severe adverse event	2	1	1	0	1	2	1	5
Adverse event	4	1	2	2	1	3	2	0
Refusal or other reason	5	3	1	1	6	3	3	6
Total	12	8	5	4	8	8	6	11

Table 2 Concomitant cardiovascular diseases and medications at baseline (randomization)

	Men				Women			
	Placebo	Vitamin E	Vitamin C	Both vitamins	Placebo	Vitamin E	Vitamin C	Both vitamins
Myocardial infarction	2	5	3	14	0	0	1	0
Other coronary heart disease	4	5	12	6	10	3	6	6
Hypertension	16	23	19	17	17	13	14	13
Lipid lowering drugs*	0	1	1	1	1	4	1	2
Antihypertensive drugs	12	15	16	16	17	11	13	12
Angina pectoris drugs	3	3	10	1	6	2	4	5
Any cardiovascular medication	15	16	22	16	22	11	16	15

*All were HMG Co-A reductase inhibiting agents.

and medications in the treatment groups are shown in Table 2. At baseline, 14 men (6.2%) and one woman had a history of myocardial infarction and 27 men and 25 women a history of other coronary disease. Fewer men in the placebo group had CHD than in the other treatment groups. Thirty-one per cent of men and 27% of women took some type of cardiovascular drug. Men randomized to take vitamin C took more angina pectoris drugs and cardiovascular drugs than men in other groups. There were no other significant differences between the randomized treatment groups.

Other baseline characteristics

The distributions of the main baseline characteristics of male and female study participants are shown in Table 3. The smoking men had lower serum total, LDL and HDL cholesterol, plasma total ascorbate, α -tocopherol and β -carotene concentrations and greater baseline mean IMT and increase of mean IMT in three years (not shown) than the other groups. Both smoking men and smoking women had lower dietary vitamin C intake and higher dietary saturated fat intake and plasma fibrinogen than the nonsmokers. Of smoking men 20.2% but of smoking women only 12.1% had plasma total ascorbate $< 25 \mu\text{mol L}^{-1}$. Amongst both smokers and nonsmokers, men had lower plasma total ascorbate, α -tocopherol and β -carotene levels and higher dietary intake of saturated fats, serum homocysteine levels and baseline CCA-IMT than women. Amongst men, but not women, smokers had a greater mean baseline CCA-IMT than nonsmokers ($P < 0.001$ for all differences).

There were no significant differences between the

randomized treatment groups within any stratum. For all 225 men, the mean baseline CCA-IMT was 1.12, 1.04, 1.08 and 1.05 mm in the four randomized groups (vitamin E, vitamin C, CellaVie[®] and placebo). For all 233 women, the respective baseline means were 0.95, 0.86, 0.93 and 0.94 mm.

Change in plasma vitamin E and C levels

In men, the mean plasma α -tocopherol concentration increased in the placebo group from 31.0 to 33.2 $\mu\text{mol L}^{-1}$ (by 7.2%), in the vitamin E group from 31.7 to 60.1 $\mu\text{mol L}^{-1}$ (by 89.2%), in the vitamin C group from 32.3 to 33.9 $\mu\text{mol L}^{-1}$ (by 5.1%) and in the group randomized to both vitamins from 32.1 to 55.2 $\mu\text{mol L}^{-1}$ (by 71.9%). The respective changes of plasma total ascorbate concentration were -5.0 , 3.8, 71.5 and 59.9%. In women, plasma α -tocopherol concentration increased in placebo, vitamin E, vitamin C and double vitamin groups by 5.6, 82.0, 4.0 and 75.4%, and plasma total ascorbate by -1.1 , 2.5, 47.1 and 46.1%, respectively ($P < 0.001$ for heterogeneity for all comparisons).

Atherosclerotic progression

The average unadjusted increase (slope) of the mean CCA-IMT was 0.020 mm year⁻¹ amongst men who were randomized to only placebo, 0.018 mm year⁻¹ in those who received only vitamin E, 0.017 mm year⁻¹ in men who received only vitamin C and 0.011 mm year⁻¹ in those who received both vitamins ($P = 0.043$ for heterogeneity). The IMT progression was significantly less in

Table 3 Distributions of other main baseline characteristics of participants in the four randomization strata

Baseline characteristic	Smoking men (n = 100)			Non-smoking men (n = 125)			Smoking women (n = 110)			Non-smoking women (n = 123)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Age (years)	59.5	46.0	70.0	60.4	45.4	70.0	58.1	47.1	69.6	60.9	46.8	70.4
Serum total cholesterol (mmol L ⁻¹)	6.05	3.41	8.32	6.53	4.39	9.92	6.22	4.42	8.86	6.73	4.42	11.57
Serum LDL cholesterol (mmol L ⁻¹)	4.33	1.42	6.36	4.73	2.45	8.14	4.25	2.57	6.91	4.67	2.03	9.05
Serum HDL cholesterol (mmol L ⁻¹)	1.12	0.55	1.83	1.14	0.68	2.21	1.35	0.69	2.75	1.43	0.68	2.55
Serum triglycerides (mmol L ⁻¹)	1.55	0.38	4.40	1.73	0.51	7.51	1.47	0.54	4.59	1.63	0.45	21.60
Plasma fibrinogen (g L ⁻¹)	3.79	2.1	5.5	3.47	2.1	5.4	3.83	2.4	5.6	3.59	2.2	5.4
Plasma total ascorbate (μmol L ⁻¹)	57.4	5.3	138.5	68.1	12.3	131.8	69.8	11.2	138.1	82.5	21.2	127.9
Plasma α-tocopherol (μmol L ⁻¹)	29.7	14.7	48.0	33.5	19.4	60.7	31.2	19.2	52.8	35.4	19.9	54.3
Plasma β-carotene (μmol L ⁻¹)	0.28	0.02	0.95	0.39	0.02	2.47	0.44	0.08	1.97	0.59	0.03	2.03
Plasma homocysteine (μmol L ⁻¹)	10.8	5.9	25.1	10.5	6.1	19.2	9.5	4.5	16.9	9.3	4.7	16.3
Serum ferritin (μg L ⁻¹)	120	12	376	142.3	9	1235	88.7	8	1090	66.7	5	414
Smoking (cigarettes per day)	17.3	0	60	0.2	0	4	12.9	0	28	0.1	0	4
Intake of saturated fat (% of energy)	17.2	9.2	29.0	15.0	7.1	27.0	16.9	9.0	28.2	14.3	6.9	22.7
Dietary vitamin E (mg/1000 kcal day ⁻¹)	5.0	2.2	11.3	5.3	2.5	12.4	5.1	2.2	9.1	5.5	2.8	9.3
Dietary vitamin C (mg/1000 kcal day ⁻¹)	42.6	3.3	211.0	49.5	6.1	191.0	56.5	13.5	322.0	75.1	8.7	325.0
Alcohol intake (g per week)	111	0	491	77	0	440	39	0	225	15	0	161
Total physical activity (min per week)	193	15	600	215	20	655	205	30	640	232	20	905
Weight (kg)	77.2	51.4	103.1	79.6	53.2	96.3	66.6	45.4	90.0	66.8	46.5	91.9
Waist-to-hip circumference ratio	0.95	0.81	1.04	0.95	0.80	1.03	0.84	0.69	0.97	0.81	0.72	0.95
Systolic blood pressure (mmHg)	132.3	97.7	188.3	131.2	97.3	171.7	130.3	97.0	190.0	130.1	93.3	184.7
Diastolic blood pressure (mmHg)	78.8	55.7	99.3	81.4	60.7	99.3	76.2	51.3	99.3	78.5	58.7	99.3
Mean CCA-IMT (mm)	1.10	0.62	2.04	1.04	0.55	2.53	0.92	0.60	2.23	0.92	0.59	1.49

Table 4 The mean adjusted 3-year change* of the mean carotid artery intima–media thickness in participants who received vitamins E and C supplements in a multivariate general linear model

Supplement	Men (<i>n</i> = 225)					Women (<i>n</i> = 233)				
	Yes		No		<i>P</i>	Yes		No		<i>P</i>
	Mean (<i>n</i>)	SE	Mean (<i>n</i>)	SE		Mean (<i>n</i>)	SE	Mean (<i>n</i>)	SE	
Vitamin E (<i>n</i> = 115)	0.0118 (56)	0.005	0.0143 (169)	0.002	0.571	0.0165 (59)	0.005	0.0170 (174)	0.002	0.904
Vitamin C (<i>n</i> = 120)	0.0119 (59)	0.005	0.0142 (166)	0.002	0.600	0.0174 (61)	0.005	0.0160 (172)	0.002	0.732
Both vitamins (<i>n</i> = 113)	0.0086 (58)	0.005	0.0175 (167)	0.002	0.049	0.0170 (55)	0.005	0.0164 (178)	0.002	0.895

CI denotes confidence interval.

*Change estimated as the linear slope over 6-monthly assessments of mean IMT (mm year⁻¹).

†Statistical significance of contrasts to the double-placebo group.

Covariates in the model for both men and women are serum cholesterol and ferritin concentrations, and three indicator variables for baseline examination months.

men who were randomized to both vitamins, compared with either all other men ($P = 0.009$) or compared with the double placebo men ($P = 0.008$). The respective means in women were 0.016, 0.015, 0.017 and 0.016 mm year⁻¹ (not significant).

Of all baseline measurements, serum ferritin and total cholesterol concentrations were most predictive of IMT progression in a step-up linear regression model in men. These, and indicator variables for predictive baseline examination months, were entered as covariates in linear covariance models predicting IMT progression (Table 4). The covariate-adjusted IMT increase was 50.9% less (0.009 vs. 0.018 mm year⁻¹) in men who received both vitamins E and C, compared with other men ($P = 0.044$), and 45.0% less (0.011 vs. 0.020 mm year⁻¹) compared with the placebo men ($P = 0.049$). Differences between other supplementation groups were not statistically significant. The treatment effect of the vitamin C + E combination was larger amongst smoking men than nonsmoking men (Fig. 2). In smoking men, the covariate-adjusted IMT increase was reduced by 64% and in nonsmoking men, by 30%. None of the treatment effects were significant in women.

In men, the proportion of those who experienced progression was reduced by 74% (95% CI 36–89%, $P = 0.003$, Table 5) in the group randomized to receive both vitamins, as compared with those who received only placebo. The respective treatment effect was nonsignificant in groups that received only vitamin E or vitamin C, although there were trends towards protection (Table 5). These results were unaffected by the choice of covariates. In

women, the probability of atherosclerotic progression was similar in all four randomized groups.

Discussion

The present findings are the first demonstration in a population-based study of an atherosclerotic disease preventing effect of supplementation with antioxidative vitamins. Our study suggests that the benefit may be limited to men, and possibly to men who are at increased oxidative stress such as smokers or those who have insufficient status of dietary or

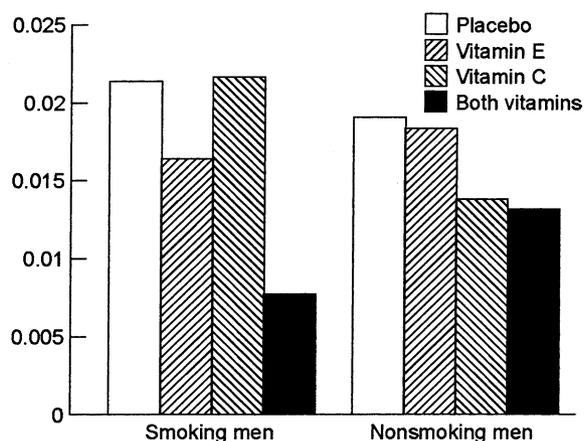


Fig. 2 Risk-factor-adjusted annual increase of the mean common carotid intima–media thickness in treatment groups amongst smoking and nonsmoking male participants. The covariates are listed in the footnote of Table 4.

Table 5 The effect of vitamin E and C supplements on the probability of atherosclerotic progression* in multivariate logistic models

Supplement	Men (<i>n</i> = 225)			Women (<i>n</i> = 233)		
	OR	95%CI	<i>P</i>	OR	95%CI	<i>P</i>
Vitamin E (<i>n</i> = 115)	0.56	0.23, 1.36	0.200	1.05	0.48, 2.32	0.903
Vitamin C (<i>n</i> = 120)	0.44	0.19, 1.06	0.066	1.08	0.49, 2.36	0.857
Both vitamins (<i>n</i> = 113)	0.26	0.11, 0.64	0.003	1.36	0.60, 3.04	0.461

*The slope of the mean IMT dichotomized at men's median (0.016 mm year⁻¹) for both genders.

OR denotes odds ratio and CI confidence interval. Three indicator variables for the three supplementation groups (double placebo as the reference group, *n* = 106) were entered with age, serum cholesterol and ferritin concentrations, systolic blood pressure, and 11 indicator variables for baseline examination months.

endogenous antioxidants. The observed effect modification by gender and smoking status needs to be retested in further clinical trials.

As smoking men had considerably lowered baseline levels of both plasma α -tocopherol and ascorbate, it is possible that the confinement of the observed benefit in this group could be simply due to the greater increase of these vitamins due to supplementation. The progression rate in smoking men who received vitamin E and C supplements was lower than in nonsmoking men receiving placebo. Thus, in this study the preventive effect of the supplementation was at least equal to the atherosclerosis promoting effect of smoking. This is not a trivial effect from the public health point of view.

The vitamin E and C supplements were safe. There were neither excess deaths nor excess 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 drop-out rate during the trial was exceptionally low. The observed atherosclerotic progression in the placebo group was of the expected magnitude, suggesting that a potential 'healthy participant effect' was small if any. However, the baseline vitamin E and C levels were higher than expected, especially vitamin C in women. This attenuated the achieved percentage increase in plasma vitamin levels in women and is the most likely explanation for the lack of effect on atherosclerotic progression in women. Another possible reason for the lack of observed effect in women may be insufficient statistical power in women, who had smaller baseline carotid wall

thickness and less atherosclerotic progression during the study.

Conclusions

In conclusion, our study shows that a formulation providing combined supplementation with reasonable doses of both vitamin E and slow-release vitamin C for at least three years can retard the progression of common carotid atherosclerosis substantially in regularly smoking men. This preventive effect may be generalizable to all men. However, this study does not provide evidence for any substantial preventive effect in postmenopausal women, although a small benefit cannot be ruled out. As common carotid plaques and increased intima-media thickness have been shown to predict coronary events [21, 24], this observation may imply benefits with regard to atherosclerosis-related events.

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