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Clinical Trials Testing Cardiovascular Benefits of Antioxidant Supplementation*

JUKKA T. SALONEN[a,b,c,†]

[a]*Department of Public Health and General Practice, The Research Institute of Public Health, University of Kuopio, 70211 Kuopio, Finland;* [b]*Oy Jurilab Ltd, Kuopio, Finland;* [c]*Inner Savo Health Centre, Suonenjoki, Finland*

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Self-selected supplementation of vitamin E has been associated with reduced coronary events and atherosclerotic progression, but the evidence from clinical trials is controversial. ASAP was a 6-year randomized trial to study the effect of supplementation with vitamin E plus slow-release vitamin C on carotid atherosclerotic progression in 520 hypercholesterolemic men and women aged 45–69 years. The supplementation reduced the progression of carotid atherosclerosis by 26% ($P = 0.014$), by 33% ($P = 0.024$) in men and 14% (not significant) in women. The effect was larger in subjects with low baseline vitamin C or atherosclerotic plaques. In the Harvard IVUS trial, the combined supplementation with vitamins E and C significantly inhibited the progression of coronary atherosclerosis in one year. These data confirm that the supplementation with a combination of vitamins E and C can retard atherosclerotic progression. The findings of completed trials testing the effect on cardiovascular events are less consistent. The major on-going clinical trials include the SU.VI.MAX, WHS, WACS and WAVE studies. These involve in total over 80,000 subjects, who are treated with antioxidative supplements for years. The results of these studies will become available during 2003–2006. They may provide the necessary additional information concerning the effect of antioxidants on cardiovascular events.

Keywords: α-Tocopherol; Ascorbic acid; Arteriosclerosis; Trials; Ultrasonics

INTRODUCTION

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.[3,4,7–22] The reasons for the inconsistency of the findings of vitamin E supplementation trials have been discussed extensively.[3,4,7–15] Briefly, differences in study populations, the supplements and outcome measures appear to explain this variability.

TRIALS OF ATHEROSCLEROTIC PROGRESSION

The ASAP Study

The ASAP study was designed to study whether that the supplementation of 45–69-year-old hypercholesterolemic men and postmenopausal women with a formulation containing either vitamin E or slow-release vitamin C or both vitamins will retard the progression of common carotid atherosclerosis.[22] Secondary hypotheses concerned lipid peroxidation, blood pressure and coronary events. ASAP is a randomized clinical trial. All subjects had hypercholesterolemia at entry to the lead-in period, defined as serum cholesterol of >5.0 mmol/l (193 mg/dl) at screening. Excluded were subjects with severe obesity, type 1 diabetes, uncontrolled hypertension and premenopausal women and those taking oral estrogen therapy, or antioxidants.

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†Address: The Research Institute of Public Health, University of Kuopio, Harjulantie 1, P.O. Box 1627, 70211 Kuopio, Finland. Fax: +358-17-162936. E-mail: jukka.salonen@uku.fi

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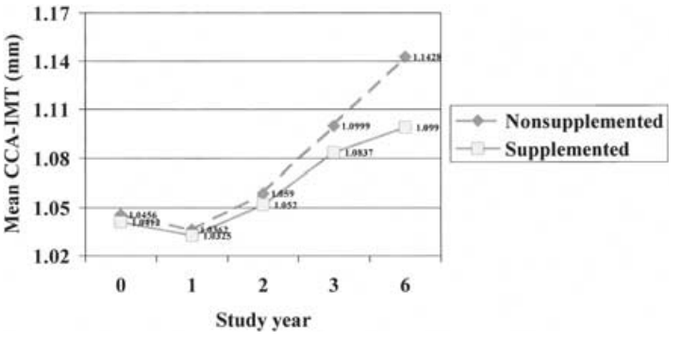

FIGURE 1 The mean CCA-IMT in the annual assessments in male participants of the ASAP study, randomized to supplementation and to no supplements.

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. After the lead-in the subjects were randomly allocated to receive twice daily with meal either (1) 91 mg of 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 three years as an open study, during which period all the supplemented subjects received the vitamin combination. The doses were chosen to keep the plasma ratio of vitamins C and E concentrations similar as in unsupplemented persons. This was tested in pilot and kinetic studies.[23–25] The pilot studies also established that a reasonably constant plasma level of vitamin C was achieved by the dosing of one slow-release tablet with the morning and evening meal. The subjects were randomized separately in four strata of approximately equal size: (1) smoking (>5 cigarettes/day) men, (2) non-smoking men, (3) smoking postmenopausal women, and (4) non-smoking postmenopausal women. All participants signed a written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

The subjects came to baseline visits and were randomized between October 1994 and October 1995. Follow-up visits were 6, 12, l8, 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) by high-resolution B-mode ultrasonography[22,26,27] was carried out at all these eight visits.

Of a larger number of screened volunteers, 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 re-examination. Thus, the average annual drop-out rate was only 2.6%. Of the randomized men, 212 (82.8%) and of women, 228 (86.4%) completed the 6-year study.

Of the 390 subjects randomized to supplementation, 335 continued the study after three years and 256 (76.4%) took the supplements as instructed for six years, whereas 18 subjects stopped the supplements during the first three study years and further 61 subjects during the last three study years. The mean plasma α-tocopherol concentration changed in six years in the group randomized to supplementation ($n = 335$) from 33.0–47.4 μmol/l (43.6% increase), in those who took the supplements ($n = 256$) from 32.9–51.7 μmol/l (57.1% increase) and in the unsupplemented group from 32.3–30.7 μmol/l (62% treatment effect). The mean plasma ascorbate concentration changed in the group randomized to supplementation from 67.7–87.9 μmol/l (29.8% increase), in those who took the supplements from 68.8–94.7 μmol/l (37.7% increase) and in the unsupplemented group from 70.7–63.2 μmol/l (10.6% decline), 48% treatment effect.

The means of the mean CCA-IMT in the annual assessments are presented in Fig. 1 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 unadjustable 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 non-supplemented 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, $P = 0.004$). In men but not in women, the difference between treatment groups in the slope ($P = 0.008$) was statistically significant.

TABLE I The mean adjusted 6-year progression of carotid atherosclerosis in the ASAP study, estimated as the 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 ($n = 440$)

Indicator of change (mm/year)*	Mean (SE)		Difference between groups	95% Confidence interval of difference	*P*-value for difference
	Supplemented ($n = 335$)	No supplements ($n = 105$)			
Slope across study period	0.0117 (0.0008)	0.0158 (0.0014)	0.0041	0.0008 … 0.0074	0.014
Difference between end and baseline	0.0102 (0.001)	0.0137 (0.001)	0.0035	0.0003 … 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 gender, baseline mean CCA-IMT, classification of severity of the right and left CCA, baseline vitamin C concentration and three indicator variables for summer baseline examination months (July, August, September).

In a covariance analysis in both men and women combined, there was a 26% (95% CI 5–46%, $P = 0.014$) treatment effect in the main study outcome, the slope of mean CCA-IMT in all subjects (Table I) and a 30% (95% CI 10–51%, $P = 0.003$) treatment effect in those who took their supplements. The respective treatment effect was 33% (95% CI 4–62%, $P = 0.024$) in all men and 14% (95% CI 15–44%, not significant) in all women. Among the compliant men and women the treatment effect was 39% (95% CI 9–69%, $P = 0.010$) and 17% (95% CI 11–44%, $P = 0.235$), respectively.

The treatment effect was greater in the participants who already had plaques in the CCAs at baseline, as compared to those who had no plaques in the segments of CCAs examined (Fig. 2). 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 12–58%, $P = 0.003$) in all subjects. The treatment effect was 54% in the subjects who already had at least one plaque obstructing more than 20% of arterial lumen diameter at baseline. The treatment effect was also larger and principally confined in participants who had baseline plasma vitamin C levels below median ($<71.25\ \mu mol/l$).

The ASAP study demonstrates that the combination of supplemented reasonable doses of vitamin E and slow-release 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. As men had considerably lower baseline levels of both plasma α-tocopherol and ascorbate, it is possible that the greater observed benefit in this group could be simply due to the greater increase of these vitamins due to supplementation. The finding that the treatment effects were larger when only compliant participants were included into the analysis, speaks in favor of causality.

Both the vitamins 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 drop-out rate during the trial was exceptionally low.

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 6–12 months of the study, possibly due to early drop-outs of the least healthy subjects, an effect of

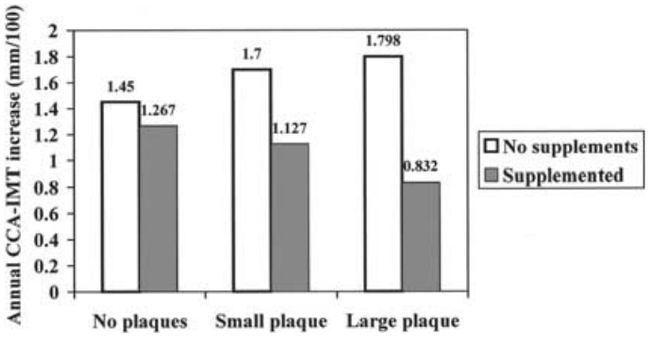

FIGURE 2 The mean adjusted annual increase (slope) of the mean CCA-IMT in the non-supplemented and supplemented ASAP study participants according to the severity of CCA atherosclerosis at baseline. Covariates in the linear model were gender, baseline vitamin C concentration and three indicator variables for summer baseline examination months (July, August, September). $P = 0.003$ for the statistical significance of the treatment effect. Large plaques are those which obstruct at least 20% of the lumen diameter.

the recruitment on health habits, or even vasodilating effects of the supplements.

The SECURE Study

After the 3-year results of the ASAP study, two other studies concerning the effect of vitamin E on atherosclerotic progression have been reported. In both studies, a large dose of vitamin E alone has been supplemented. In the SECURE, a substudy of the HOPE trial, 400 IU of vitamin E daily had no detectable effect on carotid IMT change in 4–5.5 years in 637 elderly high-risk men and women.[28] In the SECURE, maximal IMT values, which involve large random variability, were used instead of the mean of a large number of IMT measurements, as in the ASAP study. Also, upper carotid segments were included in the outcome variables. The inclusion of these also enhances the random error, thus compromising the study power further. This is reflected in the very low 3-week repeat correlation ($r = 0.87$), as compared to the 6-year repeat correlation of 0.95 in the ASAP study.

In the 2-year Dutch study in 189 smoking men, there was a similar IMT growth reducing effect as in the ASAP study, but non-significant due to the small sample size.[29] Neither changes in plasma vitamin E levels nor in lipid peroxidation have been reported from either study. In both these 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 than in Eastern Finland.

The Harvard IVUS Study

The study was conducted in cardiac transplantation patients, as this condition is associated with oxidant stress, which may contribute to the development of accelerated coronary arteriosclerosis.[l6] The authors postulated that treatment with antioxidant vitamins C and E would retard the progression of transplant-associated arteriosclerosis. The study was a double-blind placebo-controlled trial, in which 40 (35 males) patients (0–2 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 one year. The primary outcome was the change in average intimal index (plaque area divided by vessel area) measured by intravascular ultrasonography (IVUS). IVUS and vitamins C and E plasma concentrations were assessed at baseline and at one-year follow-up. All patients received pravastatin. Analyses were by intention to treat.

Vitamins C and E concentrations increased in the vitamin group (vitamin C 43 [SD 21] to 103 [43] μmol/l; vitamin E 24 [14] to 65 [27] μmol/l) but did not change in the placebo group (vitamin C 45 [15] vs 43 [16] mmol/l; vitamin E 27 [14] vs 27 [9] mmol/l; $P < 0.0001$ for difference between groups). During one 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% [1]; $P = 0.008$). The authors concluded that the supplementation with antioxidant vitamins C and E retards the early progression of transplant-associated coronary arteriosclerosis.[16]

Conclusion

Taken together, the ASAP and the Harvard IVU study show that long-term supplementation of high-risk persons with both vitamins E and C combined can retard the progression of human atherosclerosis, at least in men.

TRIALS CONCERNING CARDIOVASCULAR EVENTS

Four larger randomized clinical trials have tested the ability of vitamin E supplementation to prevent cardiovascular events in different populations. The Alpha-Tocopherol Beta-Carotene (ATBC) Study,[17] the Cambridge Heart Antioxidant Study (CHAOS),[18] the Gruppo Italiano per lo Studio della Supervienza nell'Infarto miocardico (GISSI) Study,[19] and the Heart Outcomes Prevention Evaluation (HOPE) Study.[20] In addition, at least one smaller secondary prevention trial has been completed, the Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE).[21]

In the ATBC study 50 mg *all rac*-α-tocopheryl acetate daily for 5–8 years was associated with a modest trend towards a decreased incidence of angina pectoris and no reduction in cardiovascular mortality in male smokers. There was an unexpected excess of hemorrhagic strokes in the treatment group. The ATBC study was, however, not designed to investigate cardiovascular disease development but was a cancer prevention study.

In CHAOS, 2002 individuals with clinical and angiographic evidence of CVD received RRR-α-tocopherol from "natural sources", the first 546 subjects were given 800 IU/day, the remaining ones received 400 IU/day, but the two groups were combined for statistical analyses. α-Tocopherol supplementation resulted in a significant (77%) reduction of non-fatal myocardial infarction and a non-significant increase in early deaths from CVD and in total mortality. An increased risk of hemorrhagic stroke was not observed.

In the GISSI trial, 11,324 patients with previous myocardial infarction were given 300 mg *all rac*-α-tocopherol/day alone or in combination with 1 g

polyunsaturated fatty acids. There was no significant effect on the pre-defined combined primary outcome, all-cause death, non-fatal CV death, non-fatal MI, non-fatal stroke. In a later four-way re-analysis, considering the individual outcomes separately, a significant 20% reduction in CV death in the α-tocopherol group was reported.

In the HOPE study, 2545 women and 6996 men at high risk of CVD were randomized to groups receiving either 400 IU/day of vitamin E from "natural sources" or ramipril (an angiotensin-converting enzyme inhibitor) and matching placebos. Primary outcomes were non-fatal MI, stroke, and CV deaths; secondary outcomes unstable angina, congestive heart failure, revascularization or amputation, death from any cause, complications of diabetes and cancer. No effect of vitamin E on any of the parameters related to atherosclerosis was observed.

In the smaller SPACE study 196 hemodialysis patients with pre-existing cardiovascular disease received 800 IU/day RRR-α-tocopherol or placebo. A composite variable consisting of fatal and non-fatal MI, ischemic stroke, peripheral vascular disease and unstable angina was defined as primary endpoint. Secondary endpoints were total mortality and CVD mortality. A significant (54%) reduction in composite CVD end-points was observed.

Two large studies that have been recently completed have reported only preliminary results. These are the British Heart Protection Study (HPS) and the American Physicians' Health Study (PHS) II. The HPS was a multi-centre, randomized, double-blind, placebo-controlled trial with a 2 × 2 factorial design. About 20,536 patients aged 55–75 at increased 5-year risk of myocardial infarction were enrolled. Eligible patients at high risk were randomly assigned to receive either antioxidant vitamins (the combination of 600 IU of vitamin E, 250 mg vitamin C and 20 mg beta-carotene) or simvastatin or both or neither (placebo). The primary study outcome was death from CHD and fatal and non-fatal myocardial infarction. Secondary outcomes were death from any cause, fatal and non-fatal stroke, peripheral vascular disease, major vascular procedure and cancer. According to a congress abstract, the vitamins did not produce any beneficial or adverse effects on vascular or non-vascular morbidity or mortality in this population.

The Physicians' Health Study II was a randomized, double-blind, placebo-controlled trial enrolling 15,000 willing and eligible physicians aged 55 years and older.[30] The study design was 2 × 2 × 2 × 2 factorial to test beta-carotene, vitamin E (400 IU synthetic on alternate days), vitamin C (500 mg/day) and multivitamin ("RDA of most vitamins and minerals") in the prevention of total and prostate cancer, CVD, and the age-related eye diseases (cataract and macular degeneration). Recruitment started in 1999. Willing PHS I participants were included, those who received beta-carotene in PHS I trial continued on beta-carotene. Preliminary findings were reported at the AHA Scientific sessions in November 2001. According to these, there was no significant effect by any of the vitamins on cardiovascular outcomes.

Conclusion

The findings of the completed clinical trials testing the effect of antioxidant supplements on cardiovascular events are inconsistent. While they show no benefit in healthy persons, they suggest a possible benefit in high-risk individuals.

ON-GOING TRIALS CONCERNING CARDIOVASCULAR EVENTS

The major on-going clinical trials of cardiovascular events include the SU.VI.MAX, WHS, WACS and WAVE studies. The studies are described shortly below.

The SU.VI.MAX study is 8-year controlled trial in 13,000 French volunteers: 60% women aged 35–60 years and 40% men aged 45–60, randomized to an intervention or a placebo group.[31] The intervention group is supplemented daily with all of the following: 120 mg of vitamin C, 30 mg of vitamin E, 6 mg of beta-carotene, 100 μg of selenium, and 20 mg of zinc. The main outcomes studied are cancer and CHD incidence. Other indicators, like overall mortality and morbidity, as well as health care consumption, are also recorded. Participants were enrolled between October 1994 and June 1995 and are expected to continue treatment for 8 years. Final analysis will be conducted by the end of spring 2003.

The Women's Health Study (WHS) is a randomized, double-blind, placebo-controlled trial using a 2 × 2 factorial design.[32] Participants were randomly assigned to either vitamin E (600 IU every other day) or placebo; and to aspirin (100 mg every other day) or placebo. Randomization began in February 1993 and ended in January 1996. The primary endpoint is the reduction of the risk of all important vascular events (a combined endpoint of non-fatal myocardial infarction, non-fatal stroke, and total cardiovascular death) and a decrease in the incidence of total malignant neoplasms of epithelial cell origin. Beginning in September 1996, the trial was extended for five years through August 2001 to allow for additional follow-up and data analysis.

Subjects in the Women's Antioxidant and Cardiovascular Study (WACS) were randomized in a 2 × 2 × 2 factorial design to 500 mg of vitamin C or

placebo daily, 600 mg of vitamin E or placebo on alternate days and/or 50 mg of beta-carotene or placebo on alternate days.[33] Endpoints are followed by mail for four years and include all major cardiovascular events such as non-fatal myocardial infarction, non-fatal stroke, coronary revascularization procedure, and cardiovascular mortality. The trial is conducted as a companion to the Women's Health Study (WHS), a large randomized trial assessing the efficacy of low-dose aspirin and vitamin E in primary prevention of cardiovascular disease and cancer among apparently healthy women. The trial is currently in treatment and follow-up phase which continues till February 2006. Starting in May 1998 the protocol was modified to include an arm treated with both folate and vitamins B6 and B12.

Subjects in the Women's Angiographic Vitamin and Estrogen Trial (WAVE) study are women, randomized into a 2×2 factorial trial of hormone replacement therapy and antioxidant therapy, into four treatment groups: both active hormone replacement and antioxidant; active hormone replacement therapy and antioxidant placebo; active antioxidant therapy and hormone replacement placebo; double placebo plus usual care. Antioxidants consist of a combination of vitamins E and C. Angiographic change is the primary endpoint of this trial. The study is double-blinded with respect to outcome variables. Recruitment ended in August 1999. The mean duration of follow-up will be approximately three years.

The on-going trials of cardiovascular events involve altogether over 80,000 subjects, who are treated with antioxidative supplements for years. The results of these studies will become available during 2003–2006. They may provide the necessary information concerning the effect of antioxidants on cardiovascular events. The subjects in all three on-going American studies are female, and in the SUVIMAX study 60% of the subjects are women, implicating a limited statistical power for men. Thus, it is possible, that the definite answer concerning the ability of antioxidative supplements to prevent CVD in men remains open. The findings of the ASAP study suggest that at least in some populations, the benefit of antioxidants, if any, will be present only for men.

NON-ANTIOXIDANT EFFECTS OF VITAMIN E

In theory, it is possible that the mechanisms responsible for the antiatherogenic effect of the vitamin C plus E combination 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. Also these other properties can be expected to be antiatherogenic.[8,34–40] Alpha-tocopherol increases protein phosphatase 2_{A1} activity and consequently inhibits protein kinase C and smooth muscle cell proliferation, cell adhesion and platelet aggregation and enhances the bioactivity of nitric oxide. It has also been shown to counteract inflammation and to improve endothelium-dependent vasodilator function.[36–38] Finally, there is some evidence suggesting that α-tocopherol might improve impaired insulin-mediated glucose uptake into cells and insulin sensitivity[39] and that vitamin E deficiency might be associated with an increased risk of type 2 diabetes.[40] Alpha-tocopherol exerts these pleiotrophic effects only in its reduced form. Thus a co-antioxidant such as vitamin C is a necessary prerequisite for the antiatherogenic effects of vitamin E.

SYNERGISM BETWEEN VITAMINS E AND C

Most clinical trials have been conducted in populations with high dietary intake and blood levels of antioxidants, and in these conditions the supplementation would be expected to be less effective.[8] Vitamins E and C are the most important dietary antioxidants.[6,7,41] When vitamin E works as an antioxidant it is oxidized to harmful α-tocopheroxyl radical, which needs to be reduced back to α-tocopherol. Vitamin C can regenerate α-tocopheroxyl radical to α-tocopherol.[42] Recently, supplementing smokers with high PUFA diet with high doses of vitamin E alone promoted rather than reduced lipid peroxidation.[43] In our prospective population study, vitamin C deficiency was associated with increased risk of coronary events.[44] Thus, it is conceivable that in the majority of completed antioxidant supplementation trials a wrong kind of supplement is given, whereas the combination is used in all on-going trials.

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