

α -Tocopherol (vitamin E) and β -carotene supplementation does not affect the risk for large abdominal aortic aneurysm in a controlled trial

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Abstract

Antioxidants may retard atherogenesis and limit inflammatory processes involved in aneurysm formation. We evaluated effects of α -tocopherol and β -carotene supplementation on incidence of large abdominal aortic aneurysm (AAA) in a randomised, double-blind, placebo-controlled trial. Subjects ($n = 29\,133$) were 50–69-years-old male smokers, participants in the Finnish α -Tocopherol, β -Carotene Cancer Prevention (ATBC) Study. They were randomised to receive either 50 mg/day of α -tocopherol, or 20 mg/day of β -carotene, or both, or placebo in a 2×2 design. Incidence of AAA was evaluated from mortality and hospital registers. During 5.8 years of follow-up, 181 men were diagnosed with either ruptured AAA ($n = 77$) or nonruptured large AAA treated with aneurysmectomy ($n = 104$). Relative risk (RR) for AAA was 0.83 (95% confidence interval [CI] 0.62–1.11) among men receiving α -tocopherol compared with those who did not, and 0.93 (95% CI 0.69–1.24) among men receiving β -carotene compared with those who did not. A modest though nonsignificant decrease in risk for nonruptured AAA was observed among α -tocopherol supplemented men (RR 0.71, 95% CI 0.48–1.04) compared with men not receiving α -tocopherol. For β -carotene, RR for nonruptured AAA was 0.86 (95% CI 0.59–1.27) compared with men not receiving β -carotene. Neither antioxidant affected risk for ruptured AAA. In conclusion, long-term supplementation with α -tocopherol or β -carotene had no preventive effect on large AAA among male smokers. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: α -Tocopherol; β -Carotene; Supplementation; Abdominal aortic aneurysm

1. Introduction

Abdominal aortic aneurysm (AAA) is a common and often a fatal condition of the human aorta with a clear male predominance. In the scope of public health, the importance of AAA has been growing, because its mortality rate has not shown a decreasing trend during the last decades [1–3], as has that of other cardiovascular diseases.

AAA is a degenerative disorder with a complex etiology. Atherosclerosis is considered the major cause, but lately evidence of the importance of other factors has emerged. There is evidence of familial clustering and involvement of hemodynamic factors [4]. In patients with aneurysm, histological studies have shown atherosclerosis, inflammation, and loss of elastin and collagen content in the aortic wall [5,6]. In the few prospective studies carried out, association between AAA and atherosclerotic risk factors such as smoking, hypertension, and elevated serum cholesterol has been evident [7,8].

Oxidative modification of low density lipoprotein (LDL)-cholesterol is considered one key factor in the atherogenic cascade. Oxidised LDL has shown immunogenic and chemotactic properties [9,10]. It impairs

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endothelial function, and stimulates platelet aggregation and growth of smooth muscle cells [11,12], and its unregulated uptake into macrophages leads to foam-cell formation [13]. Recent evidence indicates involvement of oxidised LDL also in matrix destruction by upregulating matrix metalloproteinase-9 expression and reducing expression of a tissue inhibitor of matrix metalloproteinase [14]. Antioxidants have improved the resistance of LDL-cholesterol to copper-induced oxidation *in vitro*. Evidence of α -tocopherol (the main constituent of vitamin E) has been consistent [15–17], unlike that of β -carotene [18–20]. It has been suggested that β -carotene acts in the vessel wall inhibiting intimal cell oxidation of LDL [21]. In addition, α -tocopherol has been shown to down-regulate scavenger receptor activity in human macrophages [22].

Current evidence indicates that atherosclerosis is an inflammatory disease with involvement of several oxidative processes in the artery wall [23]. Evidence also exists of free radical involvement and enhanced lipid peroxidation in the vessel wall of patients with AAA [24].

These findings raised our interest to study the effects of α -tocopherol and β -carotene supplementation on the risk for AAA. It was done within the α -Tocopherol, β -Carotene Cancer Prevention (ATBC) Study, which was primarily aimed at examining cancer preventive effects of α -tocopherol and β -carotene supplementation in male smokers [25]. However, the ATBC Study protocol included also the evaluation of cardiovascular diseases.

2. Methods

2.1. The α -tocopherol, β -carotene cancer prevention study

The ATBC Study was a randomised, double-blind, placebo-controlled trial with a 2×2 factorial design [26]. The participants were recruited by postal questionnaire from among the total population of men 50–69-years old, living in southwestern Finland ($n = 290\,406$) from 1985 through 1988 (Fig. 1). Men who were current smokers (at least five cigarettes per day) and willing to participate in the study ($n = 42\,957$) were invited to undergo baseline examinations. Subjects with prior cancer, any serious disease limiting long-term participation, or use of anticoagulants, vitamin E (over 20 mg/day), β -carotene (over 6 mg/day), or vitamin A (over 20 000 IU/day) supplements were excluded. The final number of participants in the ATBC Study was 29 133. The men were randomly assigned in blocks of eight within the 14 local study centers into four intervention groups: 50 mg of α -tocopherol (DL- α -tocopheryl acetate) daily; 20 mg of β -carotene daily; both; or placebo. All subjects provided written informed consent before randomisation. The ATBC Study was approved by the institutional review boards of the National Public Health Institute, Finland, and the National Cancer Institute, USA. The investigation conforms with the principles outlined in the Declaration of Helsinki. A data and safety monitoring committee was

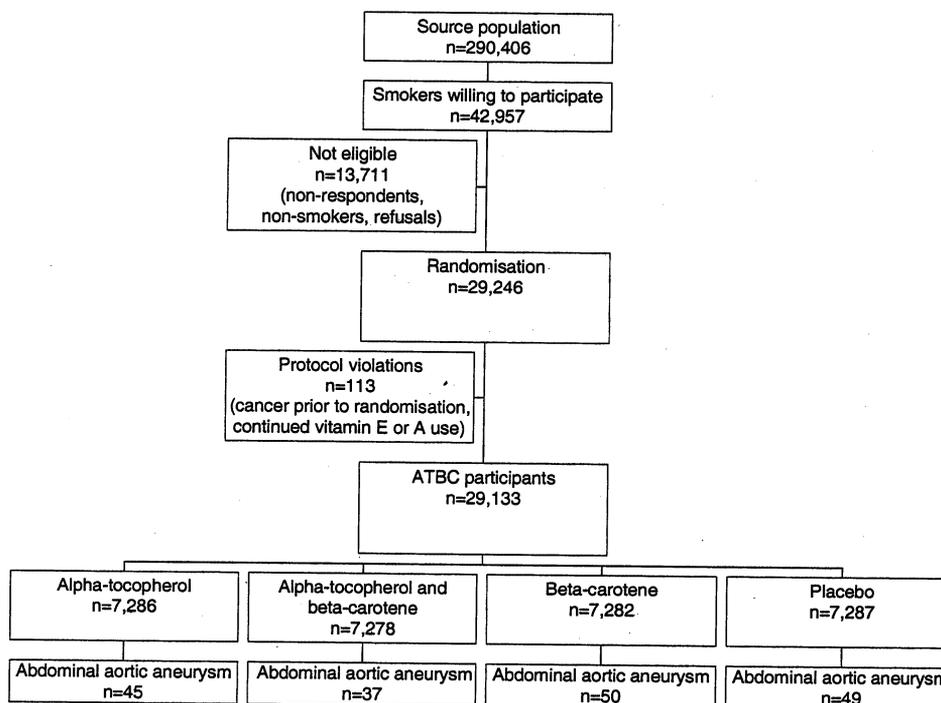


Fig. 1. Trial profile.

Table 1
Medians of baseline characteristics of the ATBC Study population

Characteristic	α -Tocopherol	α -Tocopherol and β -carotene	β -Carotene	Placebo
Number of subjects	7286	7278	7282	7287
Age (Year)	57	57	57	57
Years of smoking (Year)	36	36	37	36
Number of cigarettes per day	20	20	20	20
Serum cholesterol (mmol/l, mg/dl)	6.15 (237.5)	6.18 (238.6)	6.14 (237.1)	6.15 (237.5)
Serum HDL-cholesterol (mmol/l, mg/dl) ^a	1.11 (42.9)	1.11 (42.9)	1.12 (43.2)	1.12 (43.2)
Blood pressure systolic (mmHg)	140	140	140	140
Blood pressure diastolic (mmHg)	88	88	88	88

^a HDL, high-density lipoprotein.

convened twice annually throughout the study to evaluate unblinded data relevant to safety and efficacy.

2.2. Baseline measurements

At baseline, background characteristics such as medical history and smoking habits were collected through questionnaires. Symptoms of intermittent claudication were evaluated in an interview with the Rose questionnaire [27]. Blood pressure, height and weight were measured, and a blood sample was drawn and serum stored at -70°C . Serum total and high-density lipoprotein (HDL)-cholesterol concentrations were determined enzymatically (CHOD-PAP method, Boehringer Mannheim) [28]. HDL cholesterol was measured after precipitation with dextran sulphate and magnesium chloride [29].

2.3. Follow-up

During the follow-up, participants visited their local study center three times per year. At every follow-up visit, men returned the pack with the remaining study capsules and received a new supply. Overall capsule compliance was estimated by dividing the number of capsules taken by the number of days in the trial. Median capsule compliance was 99% among active participants in all supplementation groups. Follow-up continued until diagnosis of AAA, death, or end of the trial, 30 April, 1993, with a total of 169 408 person years.

2.4. Ascertainment of end points

The endpoint of this study was AAA, either nonruptured that was electively or urgently operated on, or ruptured. Information on aortic aneurysm came from the National Register of Causes of Death, and also from the National Hospital Discharge Register in which operation codes are recorded. Both registers use the codes of the International Classification of Diseases (ICD). We searched for ICD-8 codes 44 100–44 199

(used until 1986), ICD-9 codes 4410A–4419X (used after 1986), and operation codes for aneurysmectomy with graft placement. Altogether, there were 202 cases. We collected hospital and autopsy records to identify those with ruptured infrarenal AAA or nonruptured infrarenal AAA (minimum width 3.5 cm) with prosthetic graft placement. Of the 202 cases, 181 fulfilled these criteria. The use of registers made it possible to obtain information both from active participants and from dropouts.

2.5. Statistical analysis

The incidence of AAA was assessed per 10 000 person years of follow-up in the four supplementation groups. All analyses were by intention to treat. Kaplan–Meier curves and two-sided *P*-value derived from the unweighted log-rank statistics were assessed for the four supplementation groups. Cox proportional hazards regression was used for crude and multivariate relative risk (RR) estimations, with the supplementation groups as explanatory variables. Continuous variables (blood pressure, total and HDL-cholesterol and years of smoking) were divided into tertiles in the regression model. Age (<55, 55–59, 60–64, >64 years) and number of cigarettes per day (5–14, 15–24, >24) were categorised. The proportional hazards assumption was tested and not rejected. Interaction between α -tocopherol and β -carotene, as well as between supplementations and baseline variables was tested by the likelihood ratio test. There were no significant interactions. The results are mostly presented in the 2×2 factorial design; α -tocopherol compared with no α -tocopherol and β -carotene compared with no β -carotene.

3. Results

At study entry, median age of the participants was 57, they smoked 20 cigarettes daily, and had smoked for 36 years. There were no differences in baseline

characteristics between supplementation groups (Table 1).

Among the 29 133 participants, 181 men were diagnosed with a large AAA during a mean of 5.8 years of follow-up. Of the aneurysms, 77 were spontaneously ruptured, and 104 were not ruptured but operated on either electively or urgently. Over half of the men with a ruptured aneurysm died within 28 days (45 cases, evenly distributed among the four treatment groups), whereas only nine men of the 104 with nonruptured aneurysm died. The median width of ruptured aneurysms was 7 cm, and that of nonruptured 6 cm at the time of surgery. Data in autopsy and hospital records were insufficient to ascertain the width of the aneurysm in 16% of the cases. The median age of men with ruptured aneurysm was slightly higher (66 years) than that of men with nonruptured aneurysm (65).

Of the baseline variables, age, smoking years, total cholesterol, and diastolic blood pressure were positively associated with the risk for abdominal aneurysm, whereas HDL-cholesterol was inversely associated. Occlusive atherosclerosis of the iliac arteries can predispose to abdominal aneurysmal formation. We used baseline history or symptoms of intermittent claudication (evaluated by a questionnaire) as an indicator of atherosclerosis of the iliac arteries ($n = 2261$). Because there was no independent effect on risk nor modification of the effect of α -tocopherol or β -carotene supplementation on risk for AAA, baseline claudication was thus not included as a confounder in the multivariate models.

Incidence of AAAs per 10 000 person years in each of the four intervention groups was as follows, 10.6 for α -tocopherol alone, 8.6 for α -tocopherol and β -carotene, 11.8 for β -carotene alone; and 11.5 for placebo. The differences between the intervention groups were not significant (log-rank test, $P = 0.52$, Fig. 2). Crude RRs for AAA compared with placebo were 0.97 (95% confidence interval [CI] 0.61–1.38) for those who received α -tocopherol, 0.76 (95% CI 0.50–1.15) for those who received α -tocopherol and β -carotene, and 1.03 (95% CI 0.69–1.52) for those who received β -carotene. Adjustment with the baseline variables affected the results only slightly.

Among those who received α -tocopherol, RR for aortic aneurysm was 0.83 (95% CI 0.62–1.11) compared with those who did not receive α -tocopherol, and among β -carotene supplemented 0.93 (95% CI 0.69–1.24) compared with those who did not receive it. Adjustment for baseline variables did not alter these results (Table 2). In addition, none of the baseline variables modified the effect of supplements on the incidence of aortic aneurysm.

Among men who underwent surgery due to large nonruptured AAA, RR among the α -tocopherol supplemented was 0.71 (95% CI 0.48–1.04) compared with

men who did not receive α -tocopherol, and among the β -carotene supplemented 0.86 (95% CI 0.59–1.27) compared with men who did not receive it. Adjustment with the baseline variables affected results only slightly (Table 3). Elective surgery was performed on 86 men. The effect of α -tocopherol and β -carotene supplementation on incidence of these cases was similar to that among all nonruptured cases.

No difference existed in RR for ruptured aortic aneurysm among the α -tocopherol supplemented compared with those who received no α -tocopherol and among the β -carotene supplemented compared with those who received no β -carotene (RR 1.03, 95% CI 0.66–1.61 for both). Adjustment with baseline variables did not markedly alter these results (Table 3).

4. Discussion

In this study of the effect of supplementation with two antioxidants, α -tocopherol and β -carotene, on the incidence of large AAA, we observed no significant effect of either antioxidant. However, a 30% nonsignificant risk reduction was found in incidence of nonruptured AAA among those who received α -tocopherol. Although this may be a chance finding, it can be hypothesised that α -tocopherol supplementation may retard the growth of small aneurysms, whereas it has no effect on large ones prone to rupture. Moreover, the low frequency of large nonruptured AAAs limited our possibility to achieve statistically significant effect despite the large size of the trial.

From the ATBC Study data, we were able to find men who had been hospitalised due to surgically

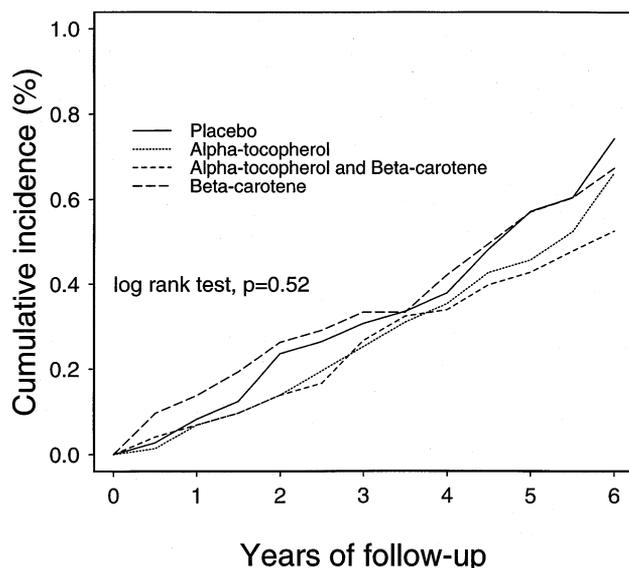


Fig. 2. Kaplan-Meier estimates of incidence of AAA in the four supplementation groups.

Table 2
Incidence of AAA per 10 000 person years and RR and 95% CI among men with and without α -tocopherol or β -carotene supplementation

	α -Tocopherol	No α -tocopherol	β -Carotene	No β -carotene
Number of cases	82	99	87	94
Incidence	9.7	11.7	10.3	11.1
Crude RR (95% CI)	0.83 (0.62–1.11)	1.00	0.93 (0.69–1.24)	1.00
Multivariate RR (95% CI) ^a	0.82 (0.61–1.10)	1.00	0.93 (0.69–1.25)	1.00

^a Adjusted for age, years of smoking, number of daily cigarettes, serum total and HDL-cholesterol, systolic and diastolic blood pressure.

treated AAA or had it listed as their cause of death. We evaluated all hospital and autopsy records to exclude false-positive cases. No screening for aneurysm was performed at the study entry or during follow-up, thus no information of small aneurysms or asymptomatic and uncomplicated large ones was available. In this study, 1.2% of men over 60-years old at baseline were diagnosed with large AAA. In previous screening studies, an AAA was found in some 2% of men 60–75 years of age [30,31]. The sizes of the aneurysms were from 4 to 6 cm, whereas in our data the mean diameter was over 6 cm, as our endpoint was ruptured aneurysm or a large one that was prone to rupture and electively operated on. In the Rotterdam study, the prevalence of AAA over 5 cm in diameter was 0.8% in men aged over 55 years [32]. Additionally, it is conceivable that a few fatal cases were lost through sudden death with no autopsy, although autopsies were performed on 64% of the ATBC Study participants who had cardiovascular disease (ICD-9 codes 390–459) as their underlying cause of death. These unknown false negatives attenuate our possibilities of detecting the effect of α -tocopherol and β -carotene on incidence of large AAA.

It has been suggested that atherosclerotic plaques in the intima of the abdominal aorta impair oxygen and nutrient supply to the inner layers of aortic wall [4]. This is particularly important in the lower parts of the aorta where the vasa vasorum is rare [33]. Deficiency of oxygen and nutrients may enhance degradative and inflammatory processes in the media and adventitia. Current experimental evidence indicates that α -tocopherol may have several ways of action in the vessel wall. It may increase resistance of LDL to oxidation [15] and thus prevent atherogenic effects of oxidatively modified LDL [13]. α -Tocopherol may inhibit proliferation of smooth muscle cells by inhibiting activation of protein kinase C [34]. Additionally, experimental studies indicate that α -tocopherol may reduce monocyte–endothelial cell adhesion and decrease production of several cytokines [35]. The mechanism behind these actions is yet unclear. It is suggested that transmigration of inflammatory cells and subsequent elaboration of cytokines and matrix metalloproteinases are associated with formation of AAA [4–6]. We can hypothesise that as a consequence of possible reduction of transmi-

gration of inflammatory cells by α -tocopherol, cytokine and proteinase production also could be reduced, eventually leading to reduced aneurysm formation.

It is unclear what dose of α -tocopherol would be optimal in primary prevention of cardiovascular diseases. Evidence from cohort studies suggests a beneficial association between intake of high supplemental doses of α -tocopherol (≥ 100 IU of vitamin E) and cardiovascular mortality [36,37], but also beneficial effects from diet alone (vitamin E intake highest quintile ≥ 9.6 IU) [38]. Thus far, no other trial results than those of the ATBC Study are available as to any effect of α -tocopherol on primary prevention of cardiovascular diseases; in our previous works no benefit was evident on coronary heart disease or intermittent claudication [39–41]. Risk for cerebral infarction was significantly lower among men receiving α -tocopherol compared with men who did not receive it, but risk for fatal haemorrhagic stroke was significantly increased in the ATBC Study [42]. In the Hope Study, α -tocopherol supplementation (400 IU/day) for 4.5 years showed no effect on cardiovascular endpoints [43]. Half of the subjects had previous myocardial infarction or were otherwise at high risk for cardiovascular events. In GISSI-Prevenzione trial, α -tocopherol supplementation (300 mg/day) showed no effect on secondary prevention of fatal or non-fatal cardiovascular events during 3.5 years of follow-up [44]. However, supplementation with 400 and 800 IU of α -tocopherol significantly reduced incidence of nonfatal myocardial infarction among patients with coronary heart disease in the CHAOS trial [45]. This finding was based on a moderate number of cases (14 myocardial infarctions in the α -tocopherol and 41 in the placebo group) during only 510 days of follow-up. Hopefully, the ongoing trials will shed more light on this issue.

Thus far all trials with β -carotene supplementation have pointed in the same direction. There has been no benefit [39,46,47], rather a suggestion of a harmful effect [48,49] from high-dose supplementation with β -carotene on cardiovascular mortality or risk for acute myocardial infarction.

In conclusion, no significant preventive effect of long-term supplementation with α -tocopherol and β -carotene on incidence of large AAA was evident. How-

Table 3
Incidence of nonruptured and ruptured AAA per 10 000 person years and RR and 95% CI among men with and without α -tocopherol or β -carotene supplementation

	α -Tocopherol	No α -tocopherol	β -Carotene	No β -carotene
<i>Nonruptured AAA</i>				
Number of cases	43	61	48	56
Incidence	5.1	7.2	5.7	6.6
Crude RR (95% CI)	0.71 (0.48–1.04)	1.00	0.86 (0.59–1.27)	1.00
Multivariate RR (95% CI) ^a	0.70 (0.47–1.03)	1.00	0.84 (0.57–1.24)	1.00
<i>Ruptured AAA</i>				
Number of cases	39	38	39	38
Incidence	4.6	4.5	4.6	4.5
Crude RR (95% CI)	1.03 (0.66–1.61)	1.00	1.03 (0.66–1.61)	1.00
Multivariate RR (95% CI) ^a	1.02 (0.65–1.60)	1.00	1.06 (0.68–1.67)	1.00

^a Adjusted for age, years of smoking, number of daily cigarettes, serum total and HDL-cholesterol, systolic and diastolic blood pressure.

ever, a beneficial effect cannot be ruled out before reports from other antioxidant trials are available.

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