INTERMITTENT CLAUDICATION AND ABDOMINAL AORTIC ANEURYSM

Effects of lifestyle and antioxidant supplementation

Markareetta E. Törnwall

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki, for public examination, in Auditorium XIV University Main Building, on December 1st, 2000, at 12 noon.

Department of Nutrition, National Public Health Institute, Helsinki, Finland

&

Department of Public Health, University of Helsinki, Finland

Helsinki 2000

Publications of the National Public Health Institute
KTL

Julkaisija-Utgivare-Published

Kansanterveyslaitos (KTL)
Mannerheimintie 166
00300 Helsinki
Puh. Vaihde (09) 474 41, telefax (09) 4744 8408

Folkhälsoinstitutet
Mannerheimvägen 166
00300 Helsingfors
Tel. Växel (09) 474 41, telefax (09) 4744 8408

National Public Health Institute
Mannerheimintie 166
00300 Helsinki, Finland
Telephone +358 9 474 41, telefax + 358 9 4744 8408

ISBN 951-740-190-6 (pdf)
ISBN 951-740-189-2 (printed)
ISSN 0359-3584

To Martti and our sons Mikael and Matias

Supervised by

Docent Jarmo Virtamo
Department of Nutrition
National Public Health Institute
Helsinki

Professor Antti Aro
Department of Nutrition
National Public Health Institute
Helsinki

Reviewed by

Docent Suvi M. Virtanen
Tampere School of Public Health
University of Tampere

Professor Markku Koskenvuo
Department of Public Health
University of Turku

CONTENTS

ABBREVIATIONS

ATBC Study = Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

BMI = body mass index

CHAOS = Cambridge Heart Antioxidant Study

CHD = coronary heart disease

CI = confidence interval

CVD = cardiovascular disease

GISSI-Prevenzione trial = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico Prevenzione trial

HDL = high-density lipoprotein

HOPE Study = Heart Outcomes Prevention Evaluation Study

ICD = International Classification of Diseases

LDL = low-density lipoprotein

RR = relative risk

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Markareetta E. Törnwall, Jarmo Virtamo, Jari K. Haukka, Antti Aro, Demetrius Albanes, Jussi K. Huttunen. Prospective study of diet, lifestyle, and intermittent claudication in male smokers. *Am J Epidemiol* 2000:151;892-901

II Markareetta E. Törnwall, Jarmo Virtamo, Jari K. Haukka, Antti Aro, Demetrius Albanes, Brenda K. Edwards, Jussi K. Huttunen. Effect of α-tocopherol (vitamin E) and β-carotene supplementation on the incidence of intermittent claudication in male smokers. *Arterioscler Thromb Vasc Biol* 1997:17;3475-3480. [Erratum, *Arterioscler Thromb Vasc Biol* 1998:18;1197]

III Markareetta E. Törnwall, Jarmo Virtamo, Jari K. Haukka, Antti Aro, Demetrius Albanes, Jussi K. Huttunen. The effect of alpha-tocopherol and beta-carotene supplementation on symptoms and progression of intermittent claudication in a controlled trial. *Atherosclerosis* 1999:147;193-197.

IV Markareetta E. Törnwall, Jarmo Virtamo, Jari K. Haukka, Demetrius Albanes, Jussi K. Huttunen. Life-style factors and risk for abdominal aortic aneurysm in a cohort of Finnish male smokers. *Epidemiology*, in press, January 2001

V Markareetta E. Törnwall, Jarmo Virtamo, Jari K. Haukka, Demetrius Albanes, Jussi K. Huttunen. Alpha-tocopherol (vitamin E) and beta-carotene supplementation does not affect the risk for large abdominal aortic aneurysm in a controlled trial. *Atherosclerosis*, in press

ABSTRACT

An imbalance between free radical production and antioxidant defence may contribute to atherogenesis. Prospective studies suggest that high intake of antioxidant vitamins – dietary or supplemental – may be protective against cardiovascular diseases.

The present work examined, first, the relation between classical atherosclerotic risk factors and diet and risk for two manifestations of peripheral atherosclerosis, i.e., intermittent claudication and abdominal aortic aneurysm; second, the effect of alpha-tocopherol and beta-carotene supplementation on the risk for the two endpoints; third, the effect of supplementation on the progression of claudication.

The present work is part of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, which was a randomized, double-blind, placebo-controlled trial primarily undertaken to examine the effect of alpha-tocopherol and beta-carotene supplementation on the development of lung cancer and secondary on the development of other cancers and cardiovascular diseases. Participants were 50- to 69-year-old male smokers living in southwestern Finland (n = 29 133). They were randomly assigned to receive either 50 mg of alpha-tocopherol, 20 mg of beta-carotene, or both, or placebo daily in a 2 x 2 factorial design. At study entry (1985-1988) several risk factors were assessed covering medical history, diet, serum lipids, and blood pressure. The follow-up (ending in spring 1993) consisted of regular follow-up visits and monitoring of national morbidity and mortality registers.

Intermittent claudication was evaluated through a structured questionnaire (Rose) at baseline and annually thereafter. Among the 26 872 subjects without intermittent claudication at study entry, 2 578 new cases with typical intermittent claudication were detected during a mean follow-up of 4 years. Serum total cholesterol, blood pressure, age, smoking, and history of diabetes were positively associated with risk for intermittent claudication, whereas HDL-cholesterol, moderate exercise, and basic education were inversely associated with the risk. Smoking cessation during follow-up reduced significantly the subsequent risk for claudication. Additionally, high intake of energy, carbohydrate, fiber, n-6 polyunsaturated fatty acids, vitamin C, gamma-tocopherol, and carotenoids was associated with decreased risk for intermittent claudication compared to low intake.

Among subjects free of intermittent claudication at study entry (n = 26 872), no beneficial effect on claudication incidence was observed with alpha-tocopherol supplementation compared to those not supplemented with alpha-tocopherol. Correspondingly, supplementation with beta-carotene showed no effect. Neither was there any effect of either supplementation on the recurrence of claudication among those 1 484 subjects who had intermittent claudication at study entry. Among the same symptomatic subjects, risk for peripheral arterial surgery was significantly increased with beta-carotene supplementation, but alpha-tocopherol supplementation showed no effect.

Abdominal aortic aneurysm cases were detected through registers, and the diagnoses were checked. Among the 29 133 ATBC Study subjects, 181 experienced rupture of an abdominal aortic aneurysm or underwent surgery with graft placement due to a large abdominal aortic aneurysm. Serum total cholesterol, blood pressure, age, and years of smoking were observed to be independent risk factors for large abdominal aortic aneurysm, whereas high HDL-cholesterol level was associated with decreased risk. No associations between nutrient intakes and risk for aortic aneurysm were observed. Supplementation with alpha-tocopherol compared to no alpha-tocopherol, or with beta-carotene compared to no beta-carotene showed no effect on the risk for large abdominal aortic aneurysm during a mean follow-up of 5.8 years.

In conclusion, classical risk factors for coronary heart disease seem to be independent risk factors both for intermittent claudication and for abdominal aortic aneurysm. A diet rich in vegetables, fruits, and vegetable oils may be protective against intermittent claudication among older male smokers. Smoking cessation seems to be effective in prevention of intermittent claudication. No evidence supports supplemental use of alpha-tocopherol or beta-carotene in order to prevent intermittent claudication or abdominal aortic aneurysm, nor for treatment of intermittent claudication among life-long male smokers.

1. INTRODUCTION

Atherosclerotic diseases are the major causes of morbidity and mortality in developed countries. Manifestations can be chronic, decreasing quality of life, or acute, causing sudden and often premature death or severe disablement. Thus, the burden of atherosclerotic diseases is considerable upon both diseased individuals and the whole of society. A huge amount of research has been focused on developing new drugs and treatment procedures, and solving the pathophysiology of atherosclerosis. Today, atherosclerosis is more than just accumulation of lipids: it is considered an inflammatory-related disease (Ross, 1999; Berliner et al., 1995).

Epidemiologic studies have revealed many risk factors for atherosclerosis, most of them dependent on our lifestyle or on our genome. Additionally, protective factors have been suggested, among them antioxidants. A diet rich in vegetables and fruits has been associated with lower risk for coronary heart disease. Similar associations have been reported for high intake of antioxidant vitamins and fiber, and for high serum levels of vitamins E and C, and carotenoids.

The link between antioxidant vitamins and atherosclerosis is the oxidation hypothesis. One key step in the atherogenic cascade is the oxidative modification of low-density lipoprotein (LDL) in the vessel wall. Oxidized LDL enhances inflammatory and thrombotic processes involved in atherogenesis (Kaplan and Aviram, 1999). It is taken up by macrophages through the scavenger receptor system, which lacks a feedback mechanism. The uncontrolled accumulation of lipids into the macrophages leads to the foam cell formation and eventually to fatty streaks. Lipoproteins carry antioxidants, most abundantly alpha-tocopherol (main constituent of vitamin E) and beta-carotene. During oxidative

conditions antioxidants are suggested to protect polyunsaturated fatty acids from oxidation. Alpha-tocopherol is considered the most efficient chain-breaking antioxidant, whereas beta-carotene acts as a singlet-oxygen quencher. In-vitro studies have consistently shown that alpha-tocopherol increases resistance of LDL to copper-induced oxidation (Princen et al., 1995; Dieber-Rotheneder et al., 1991), whereas data on beta-carotene is less consistent (Reaven et al., 1994; Levy et al., 1996). Although experimental studies indicate that natural antioxidants may reduce oxidative processes involved in atherogenesis, clinical trials conducted thus far have not confirmed beneficial effects of antioxidant supplementation on cardiovascular events (Virtamo et al., 1998; Hennekens et al., 1996).

In the clinical trials most attention has been paid to myocardial infarction or to cardiovascular mortality, with less interest focused on peripheral atherosclerosis. Atherosclerosis in pelvic or femoral arteries leads to intermittent claudication – exercise-induced pain in calves – which limits everyday activities. If blood flow is sufficiently impaired, vascular surgery is required. Atherosclerotic changes in the aorta may lead to aneurysm formation. Large abdominal aortic aneurysms are often asymptomatic until a life-threatening event of rupture.

The beneficial effect of vitamin E on intermittent claudication was suggested as early as in 1948 (Shute et al., 1948), although at that time, the antioxidant properties of vitamin E were unknown. During the next decades, a few small controlled studies were performed, some supporting the early clinical findings and some indicating no effect. However, an implication of possible beneficial effect remained, and use of vitamin E supplementation has been recommended to many patients suffering from intermittent claudication.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was designed not only to explore effects of alpha-tocopherol and beta-carotene supplementation on cancer incidence but also on cardiovascular diseases. The design's being randomized, double-blind, placebo-controlled trial, and the large number of participants offered a good opportunity to find answers to questions such as: whether antioxidant supplementation has any effect on incidence of intermittent claudication or abdominal aortic aneurysm, or whether it relieves claudication. Additionally, the ATBC Study design included measurements of many cardiovascular risk factors and an extensive dietary assessment. Hence the ATBC Study was suitable also for cohort analysis, and it was possible to obtain new information on life-style factors associated with risk for intermittent claudication and abdominal aortic aneurysms.

2. REVIEW OF THE LITERATURE

2.1 The oxidation hypothesis

Reactive oxygen species are continuously generated in the body both as a consequence of normal cellular respiration and by exogenous oxidants (Maxwell, 1995). Several lines of defense against these free radicals exist. These comprise endogenous enzymes (catalase, glutathione peroxidase, and superoxide dismutase), non-enzymatic endogenous compounds (urate, bilirubine, glutathione, and coenzyme Q) and antioxidant vitamins (beta-carotene, vitamin E, and C). Normally, the generation and

elimination of free radicals is in balance. It is suggested that oxidative damage is involved in the pathogenesis of many diseases, including atherogenesis, cancer, arthritis, and cataracts as well as in aging. Thus, it has been hypothesized that if endogenous antioxidant mechanisms are inadequate, a greater amount of diet-derived antioxidants may prove beneficial in prevention of degenerative diseases.

One major risk factor for atherosclerotic vascular disease is elevated levels of low-density lipoprotein (LDL). The earliest sign of atherosclerosis is fatty-streak formation. Fatty streaks contain foam cells, i.e., macrophages loaded with cholesterol. It has been shown that macrophages take up modified LDL but not native LDL by a scavenger receptor pathway which is not downregulated (Esterbauer et al., 1989). In-vitro studies indicate that the arterial wall cells: endothelial cells, smooth muscle cells, monocyte-macrophages, and neutrophils are able to oxidatively modify LDL (Gokce and Frei, 1996). Modification of LDL in the subendothelial space is suggested to occur in steps leading first in minimally oxidized LDL. Further oxidation leads to alterations in apolipoprotein B, which is needed before oxidized LDL is recognized by the macrophage scavenger receptor (Gokce and Frei, 1996). However, the latest evidence indicates that oxidative changes do not occur in apolipoprotein B, rather oxidized lipids are attached to it, leading to recognition by a scavenger receptor (Parthasarathy et al., 1999; Bird et al., 1999). Oxidized LDL has several atherogenic properties. LDL induces recruitment of monocytes by stimulating endothelial cells, which also liberate cytokines that are chemotactic for T-lymphocytes and neutrophils. It stimulates differentiation of monocytes into macrophages. Furthermore, oxidized LDL induces proliferation of smooth muscle cells, impairs endothelial function, and it is cytotoxic, inducing prothrombotic factors and stimulation of matrix-degrading enzymes (Kaplan and Aviram, 1999).

2.2 LDL and antioxidants

Lipoproteins transport cholesterol between the liver and tissues. They also carry lipid-soluble antioxidants: alpha-tocopherol, gamma-tocopherol, coenzyme-Q, beta-carotene, and other carotenoids. The antioxidants are suggested to protect the polyunsaturated fatty acids in LDL from peroxidation. Esterbauer and his co-workers demonstrated that, under Cu++ -stimulated oxidation, antioxidants are consumed in the sequence: tocopherols first and beta-carotene last before peroxidation occurs (Esterbauer et al., 1989). Several studies have shown that alpha-tocopherol supplementation increases resistance of LDL to oxidation (Princen et al., 1995; Jialal et al., 1995; Dieber-Rotheneder et al., 1991). Although supplementation with high-doses of beta-carotene increases its content in LDL, susceptibility of LDL to oxidation has not been consistently reduced (Princen et al., 1992; Reaven et al., 1994; Gaziano et al., 1995; Levy et al., 1996). Beta-carotene is suggested to act in the vessel wall, inhibiting LDL oxidation induced by intimal cells (Reaven et al., 1994; Dugas et al., 1999). One should bear in mind that the in-vitro studies of LDL oxidation and antioxidant protection may not reflect actual conditions in the arterial wall. There is evidence indicating the presence of oxidized LDL in vivo. LDL extracted from human atherosclerotic lesions has shown great resemblance to in-vitro oxidized LDL indicating, that atheromas in vivo contain oxidatively modified LDL (Ylä-Herttuala et al., 1989).

Additionally, auto-antibodies against oxidized LDL have been found in human serum (Palinski et al., 1988; Salonen et al., 1992), and titers were higher among patients with carotid atherosclerosis than among controls (Salonen et al., 1992).

2.3 Biology, sources, and function of antioxidant vitamins

Vitamin E

Vitamin E is a lipid-soluble vitamin present in cell membranes and lipoproteins. Vitamin E is a collective name for four different tocopherols and four tocotrienols occurring in nature all with vitamin E activity. Of them, alpha-tocopherol has the highest vitamin E activity, which is determined by the ability to overcome sterility in vitamin E-deficient rats. The biologic activity of vitamin E is expressed as alpha-tocopherol equivalents, one alpha-tocopherol equivalent being the activity of 1 mg of natural (RRR-) alpha-tocopherol. Chemically synthesized alpha-tocopherol contains eight different stereoisomers, only one of which, RRR-alpha-tocopherol, occurs naturally. The biologic activity of the synthetic stereoisomers varies, and 1 mg of synthetic D-l-alpha-tocopherol has 74% of the activity of natural alpha-tocopherol (NRC, 1989). Additionally, the amount of vitamin E can be expressed in international units (IU). One IU of vitamin E is equivalent to 1 mg of synthetic D-l-alpha-tocopheryl acetate. In the literature, vitamin E is often used as a synonym for alpha-tocopherol, and in reference to other studies, the term used there is also used here.

The most abundant tocopherol in our diet is alpha-tocopherol. The average daily intake of vitamin E in Finland is 11 mg for men and 8 mg for women (National Public Health Institute, 1998). The main sources are vegetable oils, wheat germ, green leafy vegetables, and nuts. The Institute of Medicine of the National Academies has recently provided revised dietary recommendations for antioxidants. The recommended daily intake of alpha-tocopherol is 15 mg for adults (Institute of Medicine, 2000). This exceeds the former recommendation of 10 mg per day by the RDA in 1989 (NRC, 1989). Vitamin E deficiency is rare in adults, associated with chronic fat malabsorption. Vitamin E is considered safe up to 1 000 mg per day, however; vitamin E supplementation may be contraindicated in the presence of a coagulation defect due to vitamin K deficiency or during anticoagulant therapy, as it reduces platelet adherence and aggregation (Steiner, 1993; Mabile et al., 1999).

Vitamin E functions as an antioxidant, protecting cellular membranes from oxidative destruction. Alpha-tocopherol inhibits free radical-induced lipid peroxidation and breaks the chain-reaction of oxidation. During this action, alpha-tocopherol is oxidized to an alpha-tocopheroxyl radical, which can be regenerated to alpha-tocopherol by vitamin C, glutathione, and coenzyme-Q (Kamal-Eldin and Appelqvist, 1996; Wang and Quinn, 1999). Tocopherols act also as a singlet oxygen quencher (Kamal-Eldin and Appelqvist, 1996). Beyond its antioxidant activity, alpha-tocopherol is suggested to inhibit protein kinase C activity, which may be one mechanism through which alpha-tocopherol can inhibit

smooth muscle-cell proliferation, and preserve nitric oxide-mediated arterial relaxation (Keaney Jr. et al., 1999).

Beta-carotene

Carotenoids are naturally occurring pigments in green leafy vegetables and yellow fruits and vegetables. Over 600 different carotenoids exist, and approximately 50 of them show vitamin A activity (Bendich and Olson, 1989). Beta-carotene is one of them. One beta-carotene molecule is enzymatically cleaved into two molecules of vitamin A - a fat-soluble vitamin - in the intestinal mucosa. Not all dietary beta-carotene is converted to vitamin A; some is absorbed unchanged (Thompson, JN et al., 1985). High doses of beta-carotene do not lead to high vitamin A levels, because of a feedback mechanism. The bioavailability of beta-carotene appears to be better from supplements than from vegetables (Brown et al., 1989). No actual recommendation for beta-carotene intake exists other than equivalents used for recommendations for vitamin A intake: 900 retinol equivalents (Sandström et al., 1996) or 1000 retinol equivalents (NRC, 1989), which correspond to 5.4 or 6 mg of beta-carotene, respectively. Beta-carotene is considered an antioxidant. It is efficient singlet oxygen quencher in vitro. It may be able to regenerate the alpha-tocopheroxyl radical into alpha-tocopherol (Böhm et al., 1997). However, there is no convincing evidence that beta-carotene functions as an antioxidant in vivo. That it functions, instead, as a pro-oxidant was suggested after the first controlled trials indicated that beta-carotene supplementation increases risk for lung cancer among smokers (The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994; Omenn et al., 1996).

Vitamin C

Vitamin C, ascorbic acid, is a water-soluble vitamin; deficiency in C leads to scurvy. The best sources of vitamin C are citrus fruits, green vegetables, and berries. The average daily intake in Finland is 106 mg among men and 111 mg among women (National Public Health Institute, 1998). The recommended dietary intake is 90 mg per day for men and 75 mg for women, and for smokers an additional 35 mg per day (Institute of Medicine, 2000). Vitamin C is considered safe in doses 20-fold higher than the average intake. Vitamin C acts as an antioxidant in biological fluids, scavenging reactive oxygen and nitrogen species. It also regenerates other antioxidants: alpha-tocopherol, beta-carotene, urate, and glutathione (Halliwell, 1996).

Several other dietary compounds also show antioxidant capacity, such as other carotenoids (lutein, lycopene), flavonoids (polyphenolic substances) and the micronutrient selenium that forms an integral part of the antioxidant enzyme glutathione peroxidase. The potential beneficial effect of these substances on cardiovascular diseases (CVD) and cancer is under investigation in experimental and epidemiological studies.

2.4 Antioxidants and cardiovascular diseases

Ecological studies in western populations indicate that high consumption of vegetables and fruits, or dietary antioxidant vitamins is related to lower cardiovascular mortality (Belizzi et al., 1994; Acheson and Williams, 1983; Verlangieri et al., 1985; Ginter, 1979).

Serum and tissue levels of antioxidants

In a cross-cultural study, low serum alpha-tocopherol, vitamin C, and beta-carotene levels were associated with higher mortality from CHD (Gey et al., 1991). However, another cross-cultural study concluded that neither serum vitamin E nor C was associated with regional differences in CHD mortality (Riemersma et al., 1989). In several case-control studies, low serum vitamin E levels have been detected among patients with CHD (Sklodowska et al., 1991; Riemersma et al., 1991; Regnström et al., 1996), whereas in three nested case-control studies, no association appeared (Hense et al., 1993; Salonen et al., 1985; Kok et al., 1987). High serum beta-carotene levels have, in prospective studies, been more consistently associated with lower risk for CHD (Street et al., 1994; Morris et al., 1994; Greenberg et al., 1996). In a European multicenter case-control study, the association between adipose tissue antioxidants and myocardial infarction was assessed. Alpha-carotene, beta-carotene, and lycopene were all inversely associated with risk for myocardial infarction when examined separately, but upon simultaneous analyses, only lycopene retained a significant association with risk for myocardial infarction (Kohlmeier et al., 1997). Adipose tissue alpha-tocopherol was not associated with risk for myocardial infarction (Kardinaal et al., 1993). Additionally, in a Dutch case-control study, in which serum concentrations of carotenoids were compared between cases with aortic atherosclerosis and healthy controls, serum lycopene showed an inverse association with aortic atherosclerosis among non-smokers, whereas other carotenoids (alpha- and beta-carotene, lutein, zeaxanthin and beta-cryptoxanthin) were not associated (Klipstein-Grobusch et al., 2000).

Prospective studies of antioxidant intake

Several large cohort studies have assessed associations between antioxidant intake and risk for CHD. The largest cohort comprised over 87 000 middle-aged U.S. female nurses. That study showed an inverse association between vitamin E intake and the risk for CHD, but this association was limited to vitamin E-supplement users with a median intake of 208 IU/d (Stampfer et al., 1993). Correspondingly, intake of vitamin E supplements was inversely associated with risk for CHD events or CHD mortality in two other U.S. cohorts comprising either male health professionals or elderly men and women (Rimm et al., 1993; Losonczy et al., 1996). Interestingly, dietary but not supplemental intake of vitamin E showed a significant inverse association with risk for fatal CHD in a cohort of postmenopausal U.S. women and among women and men in the Finnish Mobile Clinic cohort (Kushi et al., 1996; Knekt et al., 1994). The Rotterdam Study and the Scottish Heart Health Study cohorts revealed no association (Table 1) (Klipstein-Grobusch et al., 1999; Todd et al., 1999).

Table 1. Association between vitamin E intake and risk for coronary heart disease (CHD) in cohort studies.

Reference	Population	Years of follow-up	RR (95% CI) for CHD
Stampfer et al., 1993	87 245 women in USA	8	0.63 (0.45-0.88), supplement users vs. nonusers
Rimm et al., 1993	39 910 men in USA	4	0.64 (0.49-0.83), 419 vs. 6.4 mg*
Knekt et al., 1994	2 385 women &	14	0.35 (0.14-0.88), >7.1 vs. ≤5.3 mg*
	2 748 men in Finland		0.68 (0.42-1.11), >8.9 vs. ≤6.8 mg*
Kushi et al., 1996	34 486 women in USA	7	0.38 (0.18-0.80), ≥9.6 vs. <4.9 mg*
Losonczy et al., 1996	11 178 women & men in USA	10	0.59 (0.37-0.93), supplement users vs. nonusers
Klipstein-Grobusch et al., 1999	4 802 women & men in Netherlands	4	1.07 (0.67-1.73), >14.2 vs. 10.2 mg*
Todd et al., 1999	5 875 women &	10	1.17 (0.65-2.11), >3.7 vs. 2.3 mg/4.18MJ*
	5 754 men in Scotland		0.95 (0.68-1.31), >3.3 vs. 1.9 mg/4.18 MJ*

RR = relative risk
CI= confidence interval
*highest vs. lowest intake

High intake of beta-carotene was associated with decreased risk for CHD among men in the Scottish Heart Health Study, but in the Health Professional Study and the Rotterdam Study the inverse association was limited to smokers (Todd et al., 1999; Rimm et al., 1993; Klipstein-Grobusch et al., 1999). In three other cohorts, no association occurred (Table 2) (Kushi et al., 1996; Pandley et al., 1995; Knekt et al., 1994).

Table 2. Association between beta-carotene intake and risk for coronary heart disease (CHD) in cohort studies.

Reference	Population	Years of follow-up	RR (95% CI) for CHD
Rimm et al., 1993	39 910 men in USA	4	0.30 (0.11-0.82) for smokers 0.60 (0.38-0.94) for former smokers ≥14 388 vs. <5 030 IU*
Knekt et al., 1994	2 385 women & 2 748 men in Finland	14	0.62 (0.30-1.29), >383 vs. ≤182 ug* 1.02 (0.70-1.38), >258 vs. ≤147 ug
Pandley et al., 1995	1 556 men in USA	24	0.84, p for trend 0.09, ≥410 vs. ≤290 ug*
Kushi et al., 1996	34 486 women in USA	7	1.19 (0.67-2.12) ≥13 027 vs. ≤4 349 IU*
Klipstein-Grobusch et al., 1999	4 802 women & men in Netherlands	4	0.55 (0.34-0.83) for current and former smokers, >1.57 vs. <1.1 mg energy-adjusted*
Todd et al, 1999	5 875 women & 5 754 men in Scotland	10	0.75 (0.40-1.40), >2705 vs. <978 ug / 4.18MJ* 0.68 (0.48-0.96), >1 934 vs. <693 ug / 4.18MJ*

RR = relative risk
CI= confidence interval
*highest vs. lowest intake

High vitamin C intake was associated with decreased risk for CHD events or mortality among men in the Scottish Heart Health Study and among both genders in the Epidemiologic Follow-up Study of the National Health and Nutrition Examination Survey (NHANES) from the USA (Todd et al., 1999; Enstrom et al., 1992). In the Western Electric Study cohort, an inverse association between vitamin C intake and risk for CHD mortality was observed only among male nonsmokers (Pandley et al., 1995), and in the Finnish Mobile Clinic cohort, the inverse association was limited to women (Table 3) (Knekt et al., 1994).

Table 3. Association between vitamin C intake and risk for coronary heart disease (CHD) in cohort studies.

Reference	Population	Years of follow-up	RR (95% CI) for CHD
Enstrom et al., 1992	6 869 women & 4 479 men in USA	10	#0.75 (0.55-0.99) #0.66 (0.53-0.82) >50 mg+supplements vs. <49 mg*
Rimm et al., 1993	39 910 men in USA	4	0.89 (0.68-1.16), 1 162 vs. 92 mg*
Knekt, et al., 1994	2 385 women & 2 748 men in Finland	14	0.49 (0.24-0.98), >91 vs. ≤61 mg* 1.00 (0.68-1.45), >85 vs. ≤60 mg*
Pandley et al., 1995	1 556 men in USA	24	0.58, p for trend 0.04 for nonsmokers, ≥113 vs. ≤82 mg*
Kushi et al., 1996	34 486 women in USA	7	1.43 (0.75-2.70), ≥196 vs. ≤87 mg*
Losconczy et al., 1996	11 178 women & men in USA	10	0.99 (0.75-1.33), supplement users vs. nonusers
Klipstein-Grobusch et al., 1999	4 802 women & men in Netherlands	4	0.88 (0.56-1.38), >126 vs. <87 mg*
Todd et al., 1999	5 875 women & 5754 men in Scotland	10	0.63 (0.31-1.02), >40 vs. <19 mg / 4.18MJ* 0.57 (0.40-0.83), >29 vs. <16 mg / 4.18MJ*

#Standardized mortality ratio
RR = relative risk
CI= confidence interval
*highest vs. lowest intake

In conclusion, associations have been somewhat inconsistent, and in none of the studies did all three antioxidants show any significant association. Different cohorts are not directly comparable because of variation in study populations, in end-point definition, and in dietary methods. In cohorts, supplement users may differ profoundly from those who do not voluntarily take supplements. They may be more health-conscious, with a lower risk-factor profile. Most likely all factors associated with such behavior are not measured and adjusted for in the analysis. Thus, the intake of antioxidant supplements may be a surrogate for other factors associated with lower disease risk.

Trials of antioxidant supplementation

Up till now, several controlled trials of antioxidant supplementation have been conducted aimed at both primary and secondary prevention of CVD. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blind, placebo-controlled trial primarily designed to evaluate preventive effects of alpha-tocopherol and beta-carotene supplementation on lung cancer and secondly on other cancers and cardiovascular diseases among over 29 000 Finnish male smokers (The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994). They were randomized to receive

50 mg of alpha-tocopherol, or 20 mg of beta-carotene, or both, or placebo in one capsule per day. Neither supplement affected significantly the incidence of angina pectoris, myocardial infarction or CHD mortality during a median follow-up of 6 years (Virtamo et al., 1998; Rapola et al., 1996). However, beta-carotene supplementation increased risk for fatal CHD (RR 1.43, 95% CI 1.08-1.88) among men with previous myocardial infarction (Rapola et al., 1997). Risk for cerebral infarction was significantly lower among men receiving alpha-tocopherol than among men not receiving it (RR 0.86, 95% CI 0.75-0.99), but risk for fatal subarachnoid hemorrhage was significantly higher among those receiving alpha-tocopherol (RR 2.81, 95% CI 1.37-5.79) (Leppälä et al., 2000). Beta-carotene supplementation was linked with higher risk for intracerebral hemorrhage (RR 1.62, 95% CI 1.10-2.36) compared to risk in those receiving non (Leppälä et al., 2000).

In the Physicians' Health Study over 22 000 U.S. male physicians were randomized to receive 50 mg of beta-carotene or placebo every other day for 12 years, with no effect observed on cardiovascular mortality or myocardial infarction incidence (Hennekens et al., 1996). Similarly, no benefit of beta-carotene was observed among nearly 40 000 U.S. women randomized to receive 50 mg of beta-carotene, or 600 IU of alpha-tocopherol, or 100 mg of aspirin, or placebo every other day (Lee et al., 1999). Because the beta-carotene part of this Women's' Health Study was terminated after two years the number of cardiovascular events was small. In these two studies only 13% and 11% of the participants were smokers. In a fourth large trial, namely the Beta-Carotene and Retinol Efficacy Trial (CARET), the effect of a combination of 30 mg of beta-carotene and 25 000 IU of vitamin A supplementation on lung cancer and CVD diseases was assessed among 18 000 current or former smokers or workers exposed to asbestos. A suggestion of increased risk for cardiovascular mortality (RR 1.26, 95% CI 0.99-1.61) was observed among those who received beta-carotene and vitamin A compared to risk in the placebo group during a mean follow-up of 4 years (Omenn et al., 1996).

The other trials were aimed at secondary prevention of cardiovascular diseases. The Heart Outcomes Prevention Evaluation (HOPE) Study was a randomized trial testing ramipril and alpha-tocopherol (400 IU/d) among subjects who had experienced myocardial infarction or were otherwise at high risk for cardiovascular events. Supplementation with alpha-tocopherol for 4.5 years showed no effect on myocardial infarction, stroke or cardiovascular mortality (RR 1.05, 95% 0.90-1.22) among nearly 10 000 participants (The Heart Outcome Prevention Evaluation Study Investigators, 2000). In the unblinded Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial 11 000 patients with CHD were randomly assigned to receive 300 mg of alpha-tocopherol or 1 g of fish-derived polyunsaturated fatty acids in a 2x2 factorial design. Alpha-tocopherol supplementation showed no effect on secondary prevention of fatal or non-fatal cardiovascular events (RR 0.98, 95% 0.87-1.10) during 3.5 years of follow-up (GISSI-Prevenzione Investigators, 1999). Instead, among 2 002 patients with CHD in the Cambridge Heart Antioxidant Study (CHAOS) supplementation with 400 IU and 800 IU of alpha-tocopherol reduced significantly the incidence of non-fatal myocardial infarction (RR 0.23, 95% CI 0.11-0.47) (Stephens et al., 1996). This surprisingly

high risk-reduction was not, however, reflected in cardiovascular mortality (RR 1.18, 95% CI 0.62-2.27). In this study, the median follow-up time was only 510 days, and the number of events was small.

The ongoing trials

Several trials are currently in progress, two of them aimed at primary and secondary prevention of CVD among women (Rexrode et al., 2000; Manson et al., 1995). In the Women's' Health Study, the effect of 600 IU of alpha-tocopherol, or 100 mg of aspirin, or placebo every other day is being assessed among nearly 40 000 postmenopausal female nurses. In the Women's' Antioxidant and Cardiovascular Study, the effect of 600 IU of alpha-tocopherol, or 500 mg of vitamin C, or 50 mg of beta-carotene, or placebo on alternate days is being evaluated among 8 000 women with CVD in a 2x2x2-factorial design. The Physicians' Health Study is continuing with 7 500 voluntary former participants and 7 500 new physicians. The supplementation includes 50 mg of beta-carotene every other day, or 400 IU of vitamin E every other day, or 500 mg of vitamin C daily, or multivitamin daily, or placebo in a 2x2x2x2 factorial design (Christen et al., 2000). In France, a primary prevention study (Supplementation en Vitamines et Minéraux Antioxidants, SU.VI.M.AX) is being conducted among the general population (over 12 000 men and women) with daily supplementation of combination of vitamins (C and E and beta-carotene) and minerals (selenium and zinc) at nutritional doses testing their effect on cancer and CVD mortality (Hercberg et al., 1998). In the U.K., the Heart Protection Study is testing the effect of a combination of vitamins E (600 mg) and C (250 mg), and beta-carotene (20 mg) daily on CHD among 20 000 patients with CVD or at high risk for it (Jha et al., 1995). In addition, in autumn 2000, a new large-scale trial has begun in the USA, aimed primary at prevention of prostate cancer and secondarily at prevention of other cancers and CHD with 400 mg of vitamin E and 200 µg of selenium administered to over 30 000 men.

In summary, current evidence indicates that beta-carotene supplementation does not prevent CHD events and may increase risk for fatal CHD, at least among smokers. Because the effect of alpha-tocopherol supplementation on primary prevention of CVD has been assessed only in the ATBC Study, one must wait for the ongoing trials to end before drawing conclusions. In considering vitamin E in secondary prevention of CVD, the findings have been inconsistent. Hopefully, the ongoing trials will clarify this matter. Vitamin C is under study; no data are yet available. Overall, the field is still open and attracting the interest of many research groups.

2.5 Other nutrients and cardiovascular diseases

In addition to antioxidants, other nutrients have been studied in relation to CVD. The Seven Countries Study showed that in populations with high intake of saturated fat and low intake of monounsaturated fat, mortality for CVD was elevated (Keys et al., 1986). Two large U.S. cohorts showed similar associations (Shekelle et al., 1981; Kushi et al., 1985). High intake of polyunsaturated fat has shown an inverse association with risk for CHD in some cohorts such as the Nurses' Health Study and among the control group of the Multiple Risk factor Intervention Trial (Hu et al., 1997; Dolecek, 1992). In

contrast, in other cohort studies, no association has been evident (Kushi et al., 1985; Posner et al., 1991; Ascherio et al., 1996). High intake of trans-fatty acids was associated with increased risk for CHD in the Nurses' Health Study and in the ATBC Study cohort (Willet et al., 1993; Pietinen et al., 1997), but not in the Health Professionals cohort (Ascherio et al., 1996). Altogether, some inconsistency in associations of different fatty acid groups and risk for CHD has been evident within diverse cohorts (Posner et al., 1991; Ascherio et al., 1996; Hu et al., 1997; Pietinen et al., 1997). In favor of current dietary recommendations that emphasize use of plant-derived food is the increasing evidence of protective association between intake of fiber and risk for CHD (Kushi et al., 1985; Rimm et al., 1996a; Pietinen et al., 1996; Todd et al., 1999; Wolk et al., 1999). However, such an association has not been evident in all cohorts (Fehily et al., 1993; Kromhout et al., 1982). Furthermore, the relation between alcohol consumption and risk for CHD has been extensively investigated in observational studies. In a recent review by Rimm and his colleagues, a strong association between moderate alcohol consumption and decreased risk for CHD was evident (Rimm et al., 1996b). The authors concluded that the benefit is likely to derive from alcohol instead of other components of different drink types.

2.6 Intermittent claudication

Etiology and prevalence

Atherosclerosis in the arteries of the lower extremities can result in insufficient blood flow during exercise, leading to intermittent claudication, which is defined as pain in the calves induced on exertion, which is relieved by short rest. Intermittent claudication is by definition a symptom. Thus its diagnosis is subjective. Fluctuation of symptoms is typical. The degree of atherosclerotic stenosis in pelvic and leg arteries correlates only partly with the severity of symptoms, because of differences in collateral circulation. The prevalence of intermittent claudication among middle-aged or older men has varied between 2.2% and 5.8% in Europe and the USA (Schroll and Munck, 1981; Criqui et al., 1985).

Risk factors for intermittent claudication

Intermittent claudication is more common among men than women and among individuals with other manifestations of atherosclerosis (Reunanen et al., 1982; Criqui et al., 1992). The major risk factor is smoking (Kannel and McGee, 1985; Ingolfsson et al., 1994; Bowlin et al., 1994). High serum total cholesterol level, diabetes mellitus, and elevated blood pressure have been noted in cohort studies as other risk factors (Kannel and McGee, 1985; Bainton et al., 1994; Dagenais et al., 1991). Higher education was protective in one cohort, but not in another (Bowlin et al., 1994; Dagenais et al., 1991). Relative weight has shown inconsistent associations with risk for intermittent claudication (Kannel and McGee, 1985; Dagenais et al., 1991). Physical activity has not been assessed in these previous cohort studies. The relation between diet and peripheral occlusive arterial disease has been assessed but not extensively. In a cross-sectional study of 1 592 men and women from Edinburgh intake of fiber and alcohol was positively associated with ankle brachial pressure index among men only, intake of alpha-

tocopherol showed a similar positive association for both genders, and intake of vitamin C only among nonsmokers (Donnan et al., 1993). In a hospital-based case-control study from Greece, high intake of vitamin C, fiber, and polyunsaturated fatty acids suggested a protective association, whereas high intake of saturated fatty acids, cholesterol, and protein increased risk for peripheral arterial occlusive disease (Katsouyanni et al., 1991).

Vitamin E and intermittent claudication

Several decades ago, alpha-tocopherol supplementation was reported to relieve symptoms among patients with angina pectoris and intermittent claudication (Shute et al., 1948). Since that observation, several clinical studies with alpha-tocopherol supplementation have been performed, most of them, although not all, suggesting an improvement in walking distance (Boyd et al., 1949; Ratcliffe, 1949; Boyd and Marks, 1963; Haeger, 1982), (Hutchison and Williams, 1978) (Table 4). In two double-blind, placebo-controlled studies, improvement in patients' walking distance was noticeable among those receiving alpha-tocopherol compared to results for those receiving placebo (Livingstone and Jones, 1958; Williams et al., 1962), but no such effect was evident in two other double-blind studies (Table 4) (Hamilton et al., 1953; Westheim et al., 1975). Altogether, the body of evidence has been inconsistent, and the opinion of the Cochrane Database is that not enough data exists to recommend treating claudication patients with vitamin E supplements (Kleijnen and Mackerras, 1998). This year, new evidence of the effect of alpha-tocopherol supplementation on the incidence of intermittent claudication came from the HOPE Study. Alpha-tocopherol supplementation (400 IU/day) showed no preventive effect (RR 1.02, 95% CI 0.92-1.13) among those who received alpha-tocopherol compared to placebo during 4.5 years of follow-up (The Heart Outcome Prevention Evaluation Study Investigators, 2000).

Table 4. Studies of alpha-tocopherol (AT) supplementation as treatment for intermittent claudication.

Reference	Study type	Dose of AT per day	No. of patients	Duration	Findings
Boyd et al., 1949	Clinical	400 mg	81	Several months	Subjective improvement
Hutchison & Williams, 1978	Clinical	1600 IU	9	5 years	No effect on calf blood flow
Ratcliffe, 1949	Controlled, open, nonrandomized	400 mg other drugs	41 25	3 months	Improvement in walking test
Haeger, 1982	Controlled, open, nonrandomized	300 mg other drugs	122 36	1-16 years	Improvement in walking test
Boyd and Marks, 1963	Controlled, single-blind, Nonrandomized	400 mg placebo	17 16	3 months	Improvement in walking test
Livingstone & Jones, 1958	Controlled, double-blind, randomized	600 mg placebo	20 20	10 months	Improvement in walking test
Hamilton, 1953	Controlled, double-blind, randomized	450 IU placebo	20 21	3 months	No difference in walking test between groups
Williams et al., 1962	Controlled, double-blind, randomized	1600 mg placebo	16 17	1 year	Suggestion of benefit in walking test
Westheim et al., 1975	Controlled, double-blind, randomized	300 mg placebo	40 40	8 months	No difference in walking test between groups

2.7 Abdominal aortic aneurysm

Etiology and prevalence

Abdominal aortic aneurysm is a degenerative disorder of a multifactorial etiology. Atherosclerosis is considered the major cause. Lately, more emphasis has been given to genetic and environmental factors affecting the production and breakdown of elastin and collagen (Thompson, RW, 1996; Freestone et al., 1995). The destruction of elastin unavoidably exposes collagen to a greater amount of biomechanical stress, thus promoting the life-threatening event of rupture (Thompson, RW, 1996). In patients with aneurysm, histological studies have shown atherosclerosis, inflammation, and loss of elastin and collagen content in the aortic wall (Thompson, RW, 1996; Freestone et al., 1995). Based on screening studies, the prevalence of abdominal aortic aneurysm has ranged from 4.1% to 8.5% among men over 55 years old in Western populations (Simoni et al., 1995; Pleumeekers et al., 1995; Scott et al., 1995). Male predominance has been observed, with a male/female ratio of 6 to 1 (Simoni et al., 1995; Semmens et al., 1998).

Risk factors for abdominal aortic aneurysm

In the few prospective studies conducted, associations have been detected between abdominal aortic aneurysm and smoking, hypertension, and elevated serum cholesterol level (Strachan, 1991; Reed et al., 1992). Physical activity has shown an inverse association in one cohort but no association in another, whereas height and relative weight were each associated with increased risk (Reed et al., 1992; Hammond and Garfinkel, 1969). Data on diet and abdominal aortic aneurysm is almost absent. One report comes from the Honolulu Heart Program cohort of 8 006 men of Japanese ancestry with 151 aneurysms during 20 years of follow-up. No association was observed between alcohol intake or nutrients measured in a 24-hour dietary recall and the risk for aortic aneurysm (Reed et al., 1992).

3. AIMS OF THE STUDY

The main aim of this work was to assess the relevance of antioxidants –both dietary and supplementary - on peripheral atherosclerosis, i.e., intermittent claudication and abdominal aortic aneurysm.

The specific aims of is study were to assess:

1. The associations between diet, classical risk factors for atherosclerosis, concentrations of serum alpha-tocopherol and beta-carotene and risk for intermittent claudication and abdominal aortic aneurysm among male smokers. (I, IV)

2. The efficacy of alpha-tocopherol and beta-carotene supplementation on incidence of intermittent claudication in a randomized double-blind placebo-controlled trial design among men free of this symptom at study entry. (II)

3. Whether alpha-tocopherol and beta-carotene supplementation affect the recurrence of claudication and risk for peripheral vascular surgery among subjects who reported intermittent claudication at study entry. (III)

4. The effect of alpha-tocopherol and beta-carotene supplementation on the incidence of abdominal aortic aneurysms that were either ruptured or electively operated on in a randomized double-blind placebo-controlled trial design. (V)

4. SUBJECTS AND METHODS

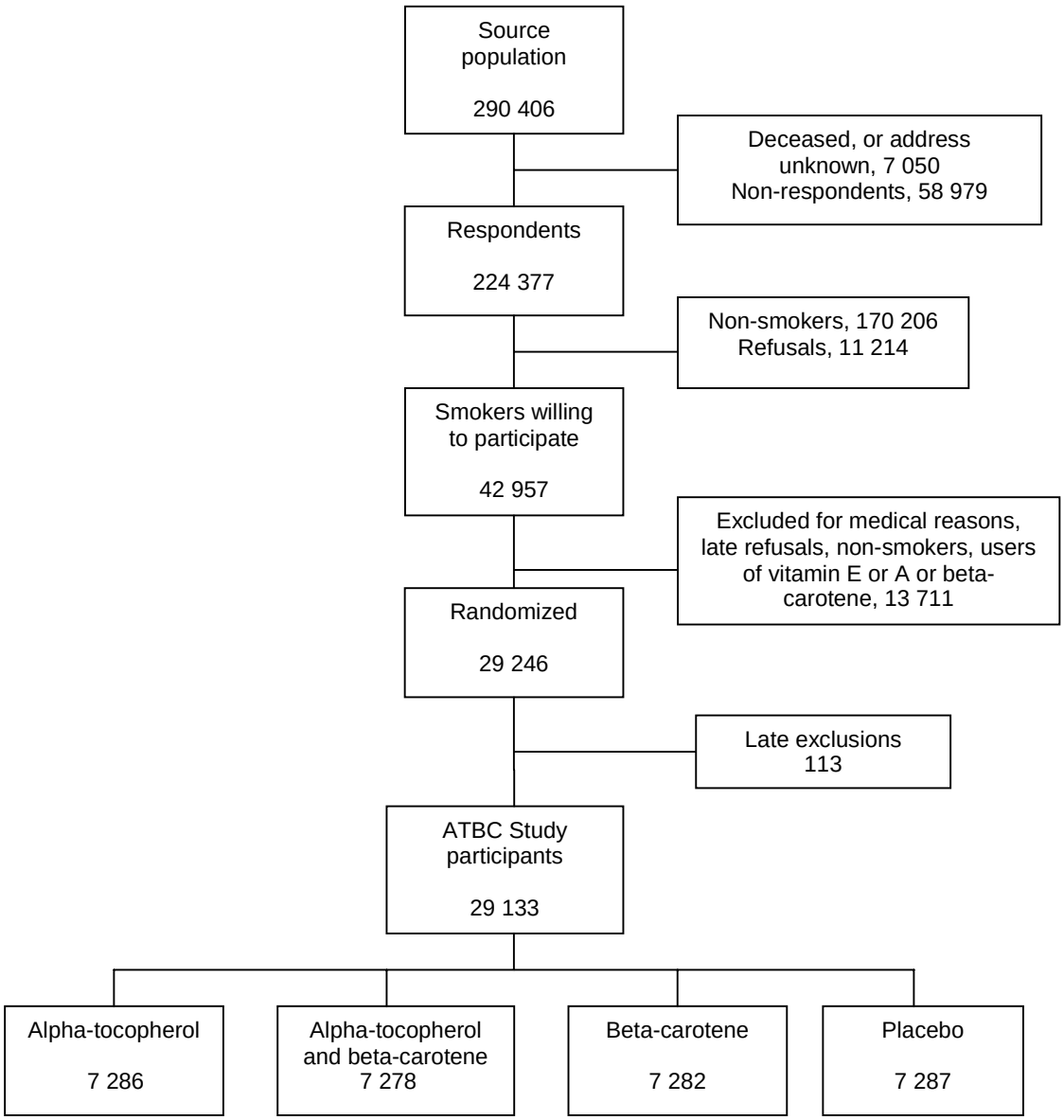
4.1 The ATBC Study

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blind, placebo-controlled trial. The primary aim was to test the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of lung cancer and secondly to evaluate its effect on other cancers, all-cause mortality, and incidence of other diseases including atherosclerotic disorders. The ATBC study was a joint project between the National Public Health Institute of Finland and the National Cancer Institute of the USA.

Eligibility, recruitment, and exclusions

Participants were screened by a postal questionnaire from among a total population of 50- to 69-year-old men living in 14 adjoining areas of southwestern Finland (n = 290 406). Men who were current smokers (smoked five or more cigarettes per day) and willing to participate in the study (n = 42 957) were invited to undergo baseline examinations. Exclusion criteria were previous malignancy other than non-melanoma skin cancer, severe angina on exertion (Rose criteria, Grade 2) (Rose, 1962), chronic renal insufficiency, cirrhosis of the liver, chronic alcoholism, anticoagulant therapy, current use of supplements containing vitamin E (over 20 mg per day), beta-carotene (over 6 mg per day) or vitamin A (over 20 000 IU per day), and other medical problems such as psychiatric disorders or physical disability that might limit long-term participation. (Figure 1)

Figure 1. Flow-chart of the ATBC Study



Baseline examinations

Before randomization, the men visited their local study center twice. They completed a questionnaire covering their medical history, smoking habits, education and physical activity. Study nurses interviewed the men with a structured questionnaire concerning intermittent claudication and angina pectoris symptoms. Height, weight, and heart rate were measured. Blood pressure was measured by mercury sphygmomanometry from the right arm while the subject was seated. The lower of two measurements taken at least 1 minute apart was recorded. A blood sample was drawn and serum stored at -70°C. The men were informed about the trial and signed the consent form. They received a diet-history questionnaire to be completed at home. The questionnaire was checked at the second visit.

Randomization

A total of 29 246 men were randomized into one of four intervention regimens. Randomization was performed in blocks of eight, stratified by the 14 study areas. Randomization strata varied in size from 456 to 8 499 participants. Of the participants, 113 were subsequently found to be ineligible and were excluded from the follow-up. Reasons for this were pre-existing malignancy, presence of undiagnosed lung cancer at baseline, continued spontaneous use of vitamin supplements, and nonsmoker status. These late-excluded participants were evenly distributed in the trial regimen groups: 24 in the alpha-tocopherol group, 30 in the alpha-tocopherol and beta-carotene group, 28 in the beta-carotene group, and 31 in the placebo group. Of these men, 98 were free of claudication at study entry. During follow-up, ten of them developed intermittent claudication; three had been randomized to the alpha-tocopherol group, two to the beta-carotene group, three to receive both supplements, and two to the placebo group. Additionally, one of the late-excluded subjects underwent surgery for ruptured abdominal aortic aneurysm. He had originally been randomized to the placebo group.

Intervention

Intervention regimens were 50 mg of alpha-tocopherol (synthetic dl-alpha-tocopheryl acetate, 50% powder), 20 mg of beta-carotene (synthetic beta-carotene, 10% water-soluble beadlets), or both, or placebo in one capsule per day. This design allowed investigation of two agents in a single trial setting, provided there was no interaction between them. The four randomized groups were of equal size; 7 286 received alpha-tocopherol, 7 278 alpha-tocopherol and beta-carotene, 7 282 beta-carotene, and 7 287 placebo. Thus, according to the 2x2 design, about half received alpha-tocopherol (alpha-tocopherol alone and alpha-tocopherol plus beta-carotene groups, n = 14 564) and the other half did not (beta-carotene and placebo groups, n = 14 569); similarly, about half received beta-carotene (beta-carotene alone and alpha-tocopherol plus beta-carotene groups, n = 14 560) and the other half did not (alpha-tocopherol and placebo groups, n = 14 573). The study capsules were one-size hard gelatin capsules. The formulations were colored with quinoline yellow. Capsules were provided in calendar blister packs, each with a four-month supply.

Time-table

Recruitment began in April 1985 and continued until June 1988. Active intervention continued until April 30, 1993 and ranged from 5 to 8 years (median 6.1 years).

Capsule compliance and drop-outs

During follow-up visits, the capsule pack from the previous period was returned, residual capsules were counted and recorded, and a new pack was dispensed. The overall capsule compliance was calculated by dividing the number of capsules taken by the number of days in the trial. It was 93% with no significant differences between the supplementation groups (range 93.1-93.4%). Only 4% were poor compliers (i.e., took less than 50% of their capsules). Practically all poor compliers dropped out of the study during their first trial year.

When a subject, for any reason, failed to attend further follow-up visits, he was considered a drop-out. The overall drop-out rate during the trial varied only slightly across the four regimen groups (30.1% in the alpha-tocopherol group, 31.0% in the alpha-tocopherol and beta-carotene group, 31.3% in the beta-carotene group, and 30.7% in the placebo group). The most common reasons for dropping out were illness (21%) and death (22%) (The ATBC Cancer Prevention Study Group, 1994).

Ethics and monitoring

The ATBC Study was approved by the institutional review boards of the National Public Health Institute, Helsinki, Finland, and the National Cancer Institute, Bethesda, MD, USA. A four-member Data and Safety Monitoring Committee was convened twice annually to review study progress and scrutinize any side-effects and unexpected toxicity.

Laboratory measurements

Serum total and high-density lipoprotein (HDL) cholesterol concentrations were analyzed enzymatically (CHOD-PAP method, Boehringer Mannheim) (Kattermann et al., 1984). HDL cholesterol was measured after precipitation of low-density and very low-density cholesterol with dextran sulphate and magnesium chloride (Kostner, 1976). Serum alpha-tocopherol and beta-carotene concentrations were measured by high-performance liquid chromatography (Milne and Botnen, 1986).

Dietary assessment

The usual diet over the previous 12 months, including alcohol consumption, was assessed with a self-administered diet-history questionnaire at baseline. This questionnaire covered the consumption of about 200 food items and 70 mixed dishes. A picture booklet with 126 color photographs of foods, each with 3 to 5 different portion sizes, helped the subjects to estimate average portions (Haapa et al., 1985). The reproducibility and validity of this questionnaire has been assessed in a pilot study carried

out among 190 men before the ATBC Study. The intraclass correlations for most nutrients ranged from 0.6 to 0.7, being highest for alcohol (0.88) in the reproducibility study (Pietinen et al., 1988). In the validity study, Pearson correlation coefficients between nutrient intake values from food records and food use questionnaires ranged from 0.40 for selenium to 0.80 for alcohol (Pietinen et al., 1988). Food consumption and nutrient intakes were computed by use of the database of the National Public Health Institute (Ovaskainen et al., 1996).

The follow-up

During the ATBC Study, participants visited their local study center every 4 months. They were asked about their health (symptoms, visits to physicians), use of nontrial vitamin supplementation, and smoking habits since their last visit. A subject was considered to have stopped smoking if he reported not to have smoked at two consecutive follow-up visits (covering 8 months). During the study, 6 131 (21%) of the participants stopped smoking, with no differences across the supplementation groups. Once a year, the questionnaire concerning cardiovascular symptoms was re-administered by interview. Blood sampling was repeated for all participants in the third follow-up year. In addition to scheduled study visits, the men were followed up by use of information in the Hospital Discharge Register and the Register of Causes of Death.

Serum alpha-tocopherol level increased by 50% among the supplemented subjects (baseline median alpha-tocopherol 26.7 μmol/l vs. 40.3 μmol/l at 3 years with alpha-tocopherol supplementation), whereas serum beta-carotene level increased 17-fold (baseline median beta-carotene 0.32 μmol/l vs. 5.51 μmol/l at 3 years with beta-carotene supplementation). Among nonsupplemented subjects, serum levels of alpha-tocopherol and beta-carotene remained close to the baseline level (28.8 μmol/l for alpha-tocopherol and 0.34 μmol/l for beta-carotene at 3 years).

Baseline characteristics

The median age of the participants was 57, they had smoked for 36 years, and they currently smoked 20 cigarettes per day. Their median blood pressure was 140/88 mmHg, and their median serum total cholesterol concentration was 6.15 mmol/l, and HDL-cholesterol 1.12 mmol/l. Educational status was low; 85% of the participants reported only elementary school as their basic education. Baseline characteristics did not differ between the four treatment groups (Table 5).

Table 5. Baseline characteristics (medians and proportions) of the ATBC Study participants by supplementation group.

Baseline characteristics	Alpha-tocopherol *n = 7 286*	Alpha-tocopherol and beta-carotene *n = 7 278*	Beta-carotene *n = 7 282*	Placebo *n = 7 287*
Age, yr	57	57	57	57
Years of smoking	36	36	37	36
No. of cigarettes /day	20	20	20	20
Serum total cholesterol, mmol/L	6.15	6.18	6.14	6.15
Serum HDL-cholesterol*, mmol/L	1.11	1.11	1.12	1.12
Systolic blood pressure, mmHg	140	140	140	140
Diastolic blood pressure, mmHg	88	88	88	88
Body mass index, kg/m^2	26	26	26	26
History of diabetes, %	4	5	5	4
Exercise in leisure-time#, %	59	59	59	58
Education beyond elementary school, %	15	16	15	15

*HDL = high-density cholesterol
#slight to moderate exercise

4.2 Subjects

Intermittent claudication (Studies I-III)

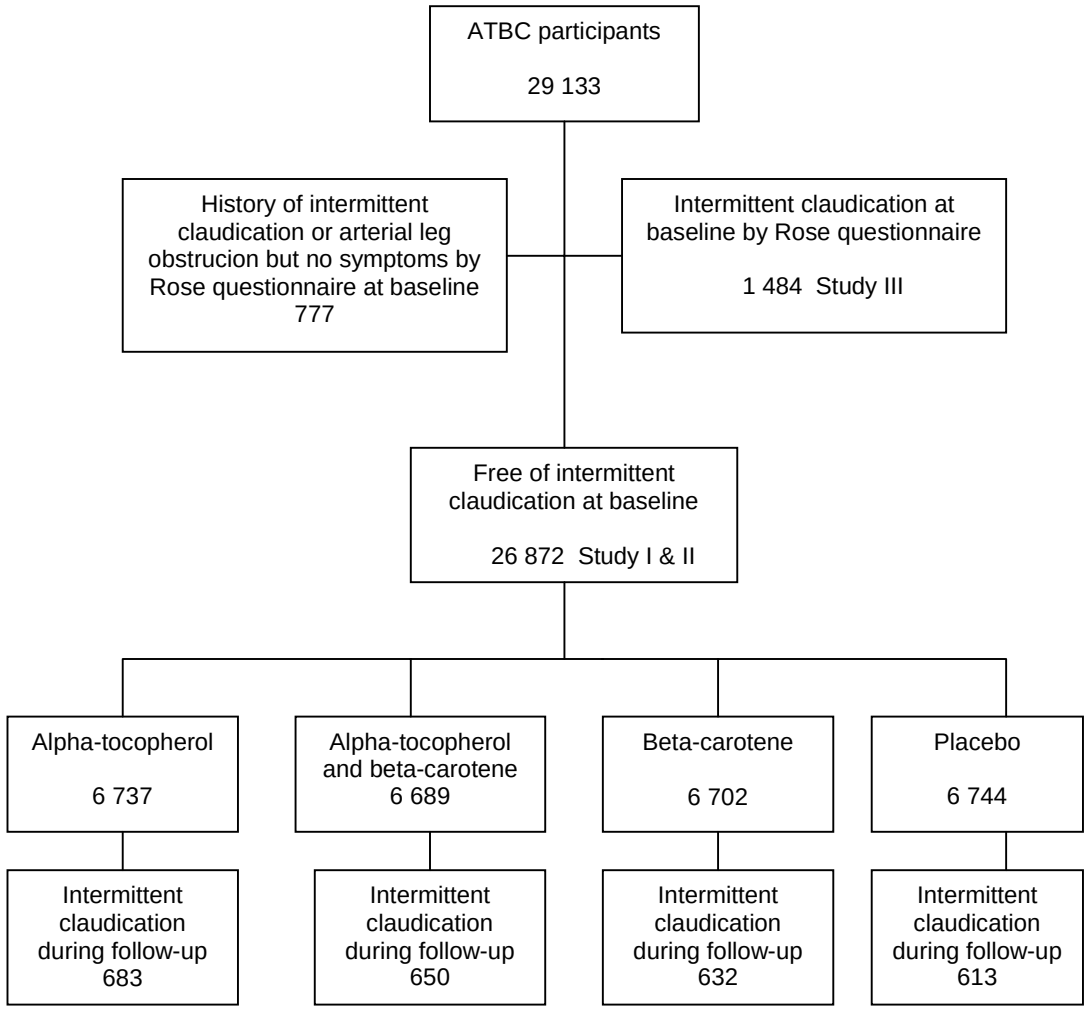
In order to study risk factors for intermittent claudication, and the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of claudication, 2 261 participants with history of intermittent claudication at baseline were excluded (Study I and II) (Figure 2). Such a history of intermittent claudication was based on an interview with questionnaire (Rose) concerning intermittent claudication (n = 1 484) (Rose, 1962) or a self-reported history of claudication or arterial leg obstruction diagnosed by a physician (n = 777). Median values for baseline characteristics among the 26 872 participants free of claudication at study entry are presented in Table 6. Median values were similar in the four supplementation groups (II: Table 1, p. 3 477).

Table 6. Baseline characteristics (medians, interquartile range, and proportions) among subjects without intermittent claudication at study entry.

Characteristic	Median (interquartile range) *n=26 872*
Age, yr	57 (53-61)
Years of smoking	36 (30-41)
No. of cigarettes / day	20 (15-25)
Serum total cholesterol, mmol/L	6.13 (5.43-6.92)
Serum HDL-cholesterol*, mmol/L	1.13 (0.96-1.34)
Systolic blood pressure, mmHg	140 (128-152)
Diastolic blood pressure, mmHg	88 (80-94)
Body mass index, kg/m^2	26.0 (23.7-28.5)
Serum alpha-tocopherol, mg/L	11.5 (9.8-13.6)
Serum beta-carotene, μg/L	172 (110-262)
History of diabetes, %	4
Exercise in leisure-time#, %	59
Education beyond elementary school, %	16
Total energy intake, kcal/d	2733 (2269-3271)
Alcohol consumption, g/d	11.2 (2.6-25.7)

*HDL = high-density lipoprotein
#slight to moderate exercise

Figure 2. Baseline data on intermittent claudication among ATBC Study participants.



Study III assessed the effect of alpha-tocopherol and beta-carotene supplementation on recurrence of claudication and on risk for peripheral vascular surgery. All participants who at baseline had been detected by the Rose questionnaire as symptomatic (n = 1 484) were selected as subjects. Of these, 49% had severe and 51% mild typical intermittent claudication at study entry. They were evenly distributed in the four supplementation groups (344 in the alpha-tocopherol, 390 in the alpha-tocopherol and beta-carotene, 377 in the beta-carotene, and 373 in the placebo group). They were in balance for all baseline factors (III: Table 1, p. 195).

Abdominal aortic aneurysm (Studies IV, V)

No selection was made among the ATBC Study participants for the analysis of abdominal aortic aneurysm. All participants were followed through registers for rupture of an aneurysm or elective surgery with graft placement (Study IV and V).

4.3 Endpoints

Intermittent claudication (Studies I-III)

Claudication was assessed by the Rose questionnaire, a structured questionnaire designed for epidemiological field surveys (Rose, 1962). Typical intermittent claudication was defined as pain in one or both calves induced on exertion and relieved at resting for ten minutes or less. This definition includes both severe (pain occurs even while walking at an ordinary pace on the level) and mild (pain occurs while walking uphill or hurrying) typical intermittent claudication. The questionnaire was re-administered by interview once a year throughout the study. The first occurrence of typical intermittent claudication was recorded as the end-point for Study I and for Study II.

In the analysis of the effect of antioxidant supplementation on the recurrence of intermittent claudication (Study III), we used the annually reinterviewed questionnaires. In total, the subjects reported 2 683 occurrences of typical claudication during 5 633 person-years. Information on peripheral arterial surgery such as endarterectomy and any arterial bypass operation on the lower extremities was collected from the National Hospital Discharge Register, which, in Finland, contains records of all surgical operations. The first operation occurring during the trial was considered in the analysis.

Abdominal aortic aneurysm (Studies IV, V)

The endpoint for studies concerning aortic aneurysm was based on two registers: the National Register of Causes of Death, and the National Hospital Discharge Register. Both registers use the codes of the International Classification of Diseases (ICD). We searched for ICD-8 codes 44100 to 44199 (used until 1986), ICD-9 codes 4410A to 4419X (after 1986), and operation codes for aneurysmectomy with graft placement. Altogether, we found 202 cases, of which 96 had one of these ICD codes for the cause of death. We collected hospital and autopsy records to identify those with ruptured abdominal aortic

aneurysm or nonruptured abdominal aortic aneurysm with prosthetic graft placement. Of the 202 cases, 181 fulfilled these criteria. Of the aneurysms, 77 were spontaneously ruptured and 104 did not rupture but were operated upon either electively or urgently. The use of registers made it possible to obtain information both from active participants and from dropouts.

4.4 Statistics

Cohort setting (Studies I and IV)

The association of risk factors with risk for intermittent claudication and abdominal aortic aneurysm were analyzed by the Cox proportional hazards model (Breslow and Day, 1987). Risk factors were divided into clinically relevant categories: age in 5-year age-groups; systolic and diastolic blood pressure of ≤130, >130-160, >160, and ≤85, >85-100, >100 mmHg; serum total and HDL-cholesterol of ≤5.0, >5.0-6.5, >6.5 and ≤0.9, >0.9-1.5, >1.5 mmol/L; body mass index (BMI) of ≤25, >25-27, >27 kg/m^2; years of smoking numbering ≤32, >32-40, >40, and number of cigarettes smoked daily as ≤14, 15-24, >24. Three risk factors were dichotomous: leisure-time exercise (no exercise versus slight to moderate exercise), education (elementary school versus junior high school or above) and history of diabetes mellitus (yes, no). In the multivariate model, all these risk factors and alpha-tocopherol and beta-carotene supplementation as main effects (alpha-tocopherol supplementation versus no alpha-tocopherol supplementation and beta-carotene supplementation versus no beta-carotene supplementation) were modelled simultaneously. Significance was tested by the likelihood ratio test in Study I and by the Wald test in Study IV, where an ordinal variable with quartile scores was refitted and its coefficient was tested. In Study I, cessation of smoking was used as a time-dependent variable and was analyzed by the Cox proportional hazards model with simultaneous adjustment for the other risk factors.

In the analysis of diet, all nutrient intakes except alcohol consumption were log-transformed and energy-corrected by the residual method (Willet, 1990), and divided into quartiles, with the lowest quartile as the reference group. Alcohol intake was divided into five categories: nondrinkers, ≤15, >15-30, >30-60, >60 g/day. The Cox proportional hazards model was used to estimate relative risks with simultaneous adjustment for age, smoking, and energy intake. Each nutrient was added to the model separately. In further analyses, the other risk factors were included in the model (for more details see original publication I p. 894 and IV p. 6). Linearity of the trend was tested by the Wald test. For the analyses of nutrients, 2 407 incident cases of claudication and 169 cases of abdominal aortic aneurysm had completed the diet-history questionnaire at baseline.

In addition, associations between baseline serum alpha-tocopherol and beta-carotene concentrations and risk for intermittent claudication and abdominal aortic aneurysm were assessed by the Cox proportional hazards model. First, serum levels were adjusted for age, smoking, and total and HDL-cholesterol, and second, a multivariate model was created including the other risk factors. Linearity of the trend was tested by the Wald test.

Trial setting (Studies II and V)

The incidence of intermittent claudication or abdominal aortic aneurysm was calculated by dividing number of new events by cumulative person-years of follow-up. The Kaplan-Meier method was used for calculation of cumulative incidence, and two-sided P-values derived from the unweighted log-rank statistics were assessed for each of the four supplementation groups (Breslow and Day, 1987). Incidences were also calculated for alpha-tocopherol compared to no alpha-tocopherol and for beta-carotene compared to no beta-carotene, which allowed us to analyze the effects of alpha-tocopherol and beta-carotene separately. All analyses were done according to the intention-to-treat principle. Cox proportional hazards regression served for the crude and multivariate relative risk estimations, with supplementation groups as explanatory variables. Baseline factors included in the multivariate models were: age, smoking years, number of cigarettes per day, systolic and diastolic blood pressure, total and HDL-cholesterol; and, additionally, in the analysis of claudication, also BMI, strenousness and frequency of leisure-time exercise, alcohol consumption, and history of diabetes. The proportional hazards assumption was tested and not rejected. Interaction between alpha-tocopherol and beta-carotene, as well as between supplementations and baseline variables was tested by the likelihood ratio test.

Treatment effect (Study III)

The effect of alpha-tocopherol and beta-carotene supplementation on recurrence of intermittent claudication during follow-up was analyzed by the Generalized Estimating Equations (GEE model) for dependent data, which takes into account the intraindividual correlation in repeated measurements of claudication (Zeger and Liang, 1986). The analysis used the 2x2 - factorial design. Baseline variables considered as risk factors for intermittent claudication were included in the model: age, years of smoking, number of cigarettes smoked per day, BMI, consumption of alcohol, systolic and diastolic blood pressure, total and HDL-cholesterol, and history of diabetes. Additionally we included in the GEE-model cessation of smoking as a time-dependent variable. The effect of recurrence of intermittent claudication on dropping out was modelled by the Poisson regression.

The effect of alpha-tocopherol and beta-carotene supplementation on risk for peripheral vascular surgery was analyzed by the Cox proportional hazards model. All risk ratios reported were calculated by stratified study area, due to possible areal variation in operation frequencies. Analysis of risk for surgery was done according to the intention-to-treat principle.

5. RESULTS

5.1 Intermittent claudication

Risk factors for intermittent claudication (Study I)

Classical risk factors

Risk for claudication increased with increasing age, number of cigarettes smoked per day, years of smoking, systolic blood pressure, serum total cholesterol, and with baseline history of diabetes. Risk was lower with high levels of serum HDL-cholesterol, education above elementary school, and more leisure-time exercise. (Table 7)

Diet

Intake of energy was inversely associated with the risk for intermittent claudication. Of the macronutrients, high energy-adjusted intakes of carbohydrate and fiber were significantly associated with decreased risk for intermittent claudication. The energy-adjusted intakes of total fat and protein showed no association with the risk for claudication, nor did alcohol consumption. (Table 8)

Of the different fatty-acid groups, energy-adjusted intake of polyunsaturated fatty acids showed a significant inverse association with intermittent claudication in the age- and smoking-adjusted but not in the multivariate model. Intake of n-6 polyunsaturated fatty acids was inversely associated with claudication in both models (highest quartile vs. lowest, multivariate relative risk 0.91, 95% CI 0.81-1.02; p for linear trend 0.048), whereas n-3 polyunsaturated fatty acids showed no association. Neither dietary cholesterol, saturated fatty acids, nor *cis*- or *trans*-monounsaturated fatty acids showed any effect on risk for claudication. (I: Table 2, p. 897)

Table 7. Relative risks and 95% confidence intervals (CI) of life-style factors for intermittent claudication among subjects without claudication at study entry.

Risk factor	Cut-off points	Multivariate relative risk*, (95% CI)	P for trend
Age, yr	≤55	1.00	
	>55-60	1.10 (0.99-1.24)	
	>60-65	1.32 (1.15-1.50)	
	>65	1.34 (1.14-1.57)	
			<0.01
Cigarettes /day	≤14	1.00	
	15-24	1.22 (1.09-1.36)	
	>24	1.36 (1.20-1.53)	
			<0.001
Years of smoking	≤32	1.00	
	>32-40	1.28 (1.15-1.42)	
	>40	1.57 (1.38-1.78)	
			<0.001
Body mass index, kg/m^2	≤25	1.00	
	>25-27	0.98 (0.88-1.09)	
	>27	0.98 (0.89-1.08)	0.9
Systolic BP#, mmHg	≤130	1.00	
	>130-160	1.24 (1.12-1.38)	
	>160	1.85 (1.61-2.13)	<0.001
Diastolic BP, mmHg	≤85	1.00	
	>85-100	0.93 (0.85-1.02)	
	>100	0.88 (0.76-1.03)	0.2
Total cholesterol, mmol/L	≤5.0	1.00	
	>5.0-6.5	1.15 (1.01-1.31)	
	>6.5	1.33 (1.17-1.52)	<0.001
HDL-cholesterol†, mmol/L	≤0.9	1.00	
	>0.9-1.5	0.75 (0.68-0.82)	
	>1.5	0.59 (0.51-0.69)	<0.001
History of diabetes	No	1.00	
	Yes	2.03 (1.75-2.35)	<0.001
Education	Elementary school	1.00	
	Additional education	0.60 (0.53-0.68)	<0.001
Leisure-time exercise	No	1.00	
	Yes	0.79 (0.73-0.85)	<0.001

*Multivariate model with simultaneous adjustment for all the risk factors and, additionally, alpha-tocopherol and beta-carotene supplementation group by Cox proportional hazards model
#BP = blood pressure
†HDL = high-density lipoprotein

Table 8. Relative risks and 95% confidence intervals (CI) for intermittent claudication by quartiles of daily intake of energy, macronutrients, and alcohol, among subjects without claudication at study entry.

Macronutrient	Quartile median	Relative risk, 95% CI Age- and smoking-adjusted*	p for trend†	Relative risk, 95% CI Multivariate‡	p for trend
Total energy, kcal	1987	1.00		1.00	
	2497	0.87 (0.78-0.97)		0.84 (0.74-0.96)	
	2967	0.92 (0.82-1.03)		0.85 (0.72-1.01)	
	3695	0.91 (0.81-1.02)	0.03	0.79 (0.61-1.02)	0.03
Fat, g	80	1.00		1.00	
	106	1.00 (0.89-1.13)		1.00 (0.89-1.13)	
	130	0.99 (0.88-1.11)		0.98 (0.87-1.10)	
	169	1.15 (1.03-1.28)	0.17	1.12 (1.00-1.25)	0.33
Protein, g	72	1.00		1.00	
	91	1.03 (0.92-1.15)		1.01 (0.91-1.14)	
	109	1.03 (0.92-1.15)		1.00 (0.89-1.12)	
	136	1.10 (0.98-1.23)	0.18	1.03 (0.92-1.16)	0.73
Carbohydrate, g	203	1.00		1.00	
	266	0.93 (0.83-1.04)		0.93 (0.83-1.04)	
	323	0.88 (0.78-0.98)		0.87 (0.78-0.98)	
	411	0.86 (0.77-0.97)	0.006	0.86 (0.76-0.96)	0.005
Fiber, g	15	1.00		1.00	
	21	0.86 (0.77-0.96)		0.84 (0.75-0.94)	
	27	0.88 (0.79-0.98)		0.87 (0.77-0.97)	
	37	0.91 (0.81-1.01)	0.01	0.87 (0.77-0.97)	0.002
Alcohol, g	0	1.00		1.00	
	5	0.96 (0.84-1.10)		1.02 (0.89-1.17)	
	23	1.01 (0.87-1.17)		1.10 (0.95-1.27)	
	40	0.93 (0.79-1.10)		1.01 (0.86-1.19)	
	78	0.96 (0.76-1.21)	0.59	1.08 (0.85-1.36)	0.48

All nutrients except alcohol were energy-corrected by the residual method for the analyses
Each nutrient was separately analyzed by the Cox model
*adjusted for baseline age, years of smoking, number of cigarettes smoked per day, and energy intake
†p for linear trend by Wald test
‡adjusted for baseline age, years of smoking, number of cigarettes smoked per day, energy intake, systolic blood pressure, serum total and HDL-cholesterol, basic education, leisure-time exercise, history of diabetes, alpha-tocopherol and beta-carotene supplementation, and smoking cessation
Intake of alcohol was divided into five categories: nondrinkers, ≤15, >15-30, >30-60, and >60 g/d

High intake of carotenoids (sum of beta-carotene, alpha-carotene, gamma-carotene, lycopene, capsanthin, cryptoxanthin, canthaxanthin, lutein and zeaxanthin) was significantly associated with lower risk for claudication (highest quartile vs. lowest, multivariate relative risk 0.82, 95% CI 0.73-0.92; p for linear trend < 0.001). Of the individual carotenoids, beta-carotene, lycopene, and lutein + zeaxanthin showed a significantly lower risk for claudication with increasing levels of intake, whereas intake of alpha-carotene showed no association. Intakes of vitamin E and its main constituents alpha-tocopherol and gamma-tocopherol were significantly inversely associated with risk for claudication in the age- and smoking-adjusted model, but after multivariate adjustment only gamma-tocopherol maintained a significant association (highest quartile vs. lowest, relative risk 0.89, 95% CI 0.79-1.00; p for linear trend 0.02). Intake of vitamin C showed a significant inverse association with risk for claudication (highest quartile vs. lowest, multivariate relative risk 0.86, 95% CI 0.77-0.97; p for linear trend 0.004). Dietary selenium was not associated with claudication. (I: Table 3, p. 898)

In further analysis, intakes of vitamin C and carotenoids were added to the multivariate model simultaneously. Intake of carotenoids retained its significant inverse associations, but intake of vitamin C was no longer associated with risk for claudication. Similarly, intakes of different carotenoids were analyzed simultaneously in the multivariate model. Lycopene and lutein + zeaxanthin retained their protective effect, whereas beta-carotene was not significantly associated with claudication. (I: p. 895)

Serum

Relative risk for claudication was significantly lower with higher levels of serum alpha-tocopherol or beta-carotene (highest quartile vs. lowest, relative risk 0.88, 95% CI 0.77-1.00; p for linear trend 0.006, and relative risk 0.77, 95% CI 0.68-0.86; p for linear trend <0.001, respectively) (I, Table 4, p. 899). Correlations between serum and dietary alpha-tocopherol and beta-carotene were 0.21 and 0.23.

Effect of antioxidant supplementation on incidence of intermittent claudication (Study II)

Compliance and drop-out rate

Overall capsule compliance was 93% in all intervention groups. The overall drop-out rate was 30%, similar in all intervention groups over time.

Effect of supplementation on intermittent claudication

During a mean follow-up of 4 years (107 370 person years), 2 578 new cases of typical intermittent claudication were detected by the Rose questionnaire among the 26 872 men without claudication at baseline. Incidence of intermittent claudication per 1 000 person-years was 22.7 in the placebo group, 25.4 in the alpha-tocopherol group, 24.3 in the alpha-tocopherol plus beta-carotene group, and 23.7 in the beta-carotene alone group. The Kaplan-Meier curves of cumulative incidence are presented in Figure 3. The overall difference between supplementation groups was not statistically significant (log-rank test p = 0.13). An 11% increase in risk for developing intermittent claudication was observable

among those who received alpha-tocopherol alone compared to those receiving placebo. Beta-carotene supplementation or supplementation with both alpha-tocopherol and beta-carotene had no statistically significant effect on incidence of claudication (Table 9). Interaction between alpha-tocopherol and beta-carotene supplementation in the effect on intermittent claudication did not reach statistical significance ($p = 0.06$).

A nonsignificant increase in risk for intermittent claudication occurred among those who received alpha-tocopherol compared to those who did not (relative risk 1.07, 95% CI 0.99-1.15). Beta-carotene supplementation showed no effect (relative risk 0.99, 95% CI 0.92-1.07 among those who received beta-carotene compared to those not receiving it). Adjustment for baseline variables did not change these results.

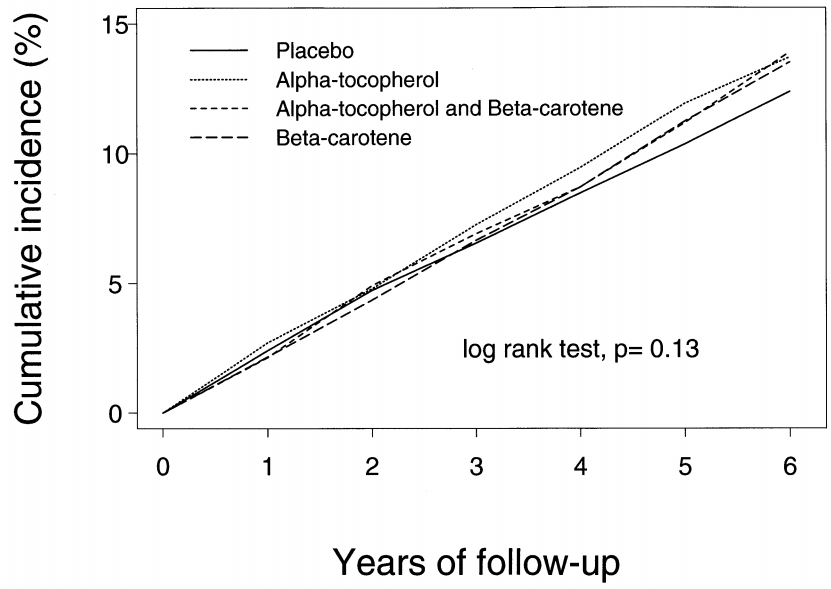
An interaction was found with one of the baseline variables – history of diabetes. Among those who had diabetes at baseline, alpha-tocopherol supplementation significantly increased risk for claudication compared to those not supplemented with alpha-tocopherol, whereas among those with no history of diabetes, alpha-tocopherol supplementation did not affect risk for claudication. (II: p. 3477)

Table 9. Incidence and relative risk for intermittent claudication (IC) by supplementation group among subjects without claudication at study entry.

Supplementation	Number of cases with IC	Incidence / 1000 person years	Crude relative risk, 95% CI*	Multivariate relative risk†, 95% CI
Placebo	613	22.7	1.00	1.00
Alpha-tocopherol	683	25.4	1.11 (1.00-1.24)	1.11 (0.99-1.23)
Alpha-tocopherol plus beta-carotene	650	24.3	1.06 (0.95-1.18)	1.04 (0.93-1.16)
Beta-carotene	632	23.7	1.04 (0.93-1.16)	1.01 (0.90-1.13)

Relative risks calculated by Cox proportional hazards model
*CI = confidence interval
†adjusted for baseline age, smoking years, daily number of cigarettes, serum total and HDL cholesterol, systolic and diastolic blood pressure, BMI, alcohol consumption, physical activity, and history of diabetes

Figure 3. Cumulative incidence of intermittent claudication in the four supplementation groups.



Treatment effect of antioxidant supplementation on intermittent claudication (Study III)

Compliance and drop-out rate

Overall capsule compliance was 91%. The drop-out rate was 44%, with deaths (n = 386) included. Risk for dropping out was similar in all intervention groups, and recurrence of typical intermittent claudication did not affect drop-out risk.

Recurrence of symptoms

At the annual interviews, the 1 484 men with claudication at baseline reported typical intermittent claudication 2 683 times during 3.8 years of follow-up (5 444 person years). The average annual prevalence of intermittent claudication was 48% throughout the study. The crude odds ratio for recurrence of intermittent claudication by the GEE model was 0.97 (95% CI 0.81-1.15) among those who received alpha-tocopherol compared to those who did not and 1.02 (95% CI 0.86-1.21) among those who received beta-carotene compared to those who did not. Adjustment for baseline factors did not change these results (III: Table 2, p. 196). The level of symptoms at baseline (severe or mild typical symptoms) did not affect the results. No interaction was evident between alpha-tocopherol and beta-carotene supplementation in their effects on claudication (p = 0.71).

Risk for surgery

Peripheral arterial surgery was performed on 101 (7%) subjects with claudication at baseline (23 in the alpha-tocopherol group, 27 in the alpha-tocopherol plus beta-carotene group, 34 in the beta-carotene

group, and 17 in the placebo group) during a mean follow-up time of 5.3 years (7 849 person years). Relative risk for peripheral arterial surgery was 1.02 (95% CI 0.69-1.51) among those who received alpha-tocopherol compared to risk of those who did not, and 1.51 (95% CI 1.01-2.26) among those who received beta-carotene compared to those who did not. Adjustment for risk factors changed only slightly these relative risks (Table 10). Among those who reported mild intermittent claudication at baseline, beta-carotene supplementation significantly increased risk for surgery (odds ratio 2.44, 95% CI 1.08-5.53) compared to risk of those not supplemented with beta-carotene. The corresponding risk for surgery was only moderately increased (odds ratio 1.15, 95% CI 0.72-1.83) among those who reported severe claudication at baseline. No interaction occurred between alpha-tocopherol and beta-carotene supplementation in their effect on risk for surgery ($p = 0.23$).

Table 10. Relative risk and 95% confidence interval (CI) for peripheral arterial surgery by supplementation with either alpha-tocopherol or beta-carotene among subjects with intermittent claudication at study entry.

Supplementation	Number of cases with surgery	Crude relative risk, 95% CI	Adjusted* relative risk, 95% CI
No alpha-tocopherol	51	1.00	1.00
Alpha-tocopherol	50	1.02 (0.69-1.51)	0.88 (0.58-1.33)
No beta-carotene	40	1.00	1.00
Beta-carotene	61	1.51 (1.01-2.26)	1.60 (1.05-2.44)

Relative risks calculated by Cox proportional hazards model and stratified by study area.
*Adjusted for baseline age, smoking years, daily number of cigarettes, serum total and HDL cholesterol, systolic and diastolic blood pressure, BMI, alcohol consumption, and history of diabetes

Effect of smoking cessation

Incidence of intermittent claudication

The effect of smoking cessation on risk for claudication was assessed among men who were free of claudication at study entry (n = 26 872). During follow-up, 4 538 (17% in all intervention groups) subjects stopped smoking. Of these men, 271 reported claudication after smoking cessation during a follow-up of 2.4 years. In a multivariate model (baseline variables and supplementation groups included), smoking cessation reduced significantly the risk for claudication; the subsequent relative risk was 0.86 (95% CI 0.75-0.99).

Recurrence of symptoms

Among the 1 484 men with intermittent claudication at study entry, as detected by the Rose Questionnaire, 283 (19%) stopped smoking. Of these 66 were in the alpha-tocopherol group, 70 in the alpha-tocopherol plus beta-carotene group, 76 in the beta-carotene group, and 71 in the placebo group. Cessation of smoking decreased slightly but not significantly their subsequent risk for recurrence of intermittent claudication (odds ratio 0.82, 95% CI 0.67-1.01).

5.2 Abdominal aortic aneurysm

Risk factors for abdominal aortic aneurysm (Study IV)

Classical risk factors

Risk for abdominal aortic aneurysm rose with increasing age, years of smoking, blood pressure, and serum total cholesterol (Table 11). HDL-cholesterol showed a strong inverse association with risk. The association was of similar magnitude whether HDL-cholesterol level was modelled alone, or along with all other risk factors, including alcohol consumption.

Diet and serum

Intake of total energy was inversely associated with risk for abdominal aortic aneurysm (highest quartile vs. lowest, relative risk 0.59, 95% CI 0.38-0.94; p for trend <0.01) (Table 12). The association between energy intake and the risk for aortic aneurysm remained similar when energy derived from alcohol was excluded. Neither energy-adjusted intake of macronutrients (fat, carbohydrate, protein, and fiber) nor alcohol consumption was associated with risk (Table 12). When association of the different fatty acid groups (saturated, polyunsaturated, trans-, and cis- monounsaturated fatty acids) was evaluated, there was no influence on risk. Additionally, analysis of dietary antioxidants (vitamin C and E, carotenoids, and selenium) showed no association. Neither alpha-tocopherol nor beta-carotene concentrations in serum were associated with risk for aortic aneurysm.

Table 11. Relative risks and 95% confidence intervals (CI) of life-style factors for abdominal aortic aneurysm among ATBC Study participants.

Risk factor	Cut-off points	Multivariate relative risk*, (95% CI)	P for trend
Age, yr	≤55	1.00	
	>55-60	1.81 (1.05-3.14)	
	>60-65	3.15 (1.76-5.65)	
	>65	4.60 (2.44-8.69)	<0.001
Cigarettes /day	≤14	1.00	
	15-24	1.01 (0.70-1.46)	
	>24	0.81 (0.52-1.27)	0.32
Years of smoking	≤32	1.00	
	>32-40	1.45 (0.88-2.39)	
	>40	2.25 (1.33-3.81)	<0.01
Body mass index, kg/m^2	≤25	1.00	
	>25-27	0.84 (0.57-1.26)	
	>27	0.78 (0.55-1.11)	0.13
Systolic BP†, mmHg	≤130	1.00	
	131-160	1.34 (0.87-2.07)	
	>160	1.95 (1.15-3.30)	<0.05
Diastolic BP, mmHg	≤85	1.00	
	86-100	1.35 (0.93-1.94)	
	>100	1.78 (1.03-3.05)	<0.05
Total cholesterol, mmol/L	≤5.0	1.00	
	>5.0-6.5	1.30 (0.77-2.20)	
	>6.5	1.81 (1.07-3.05)	<0.01
HDL-cholesterol‡, mmol/L	≤0.9	1.00	
	>0.9-1.5	0.40 (0.29-0.56)	
	>1.5	0.15 (0.07-0.31)	<0.001
History of diabetes	No	1.00	
	Yes	0.43 (0.16-1.15)	0.09
Education	Elementary school	1.00	
	Additional education	1.01 (0.67-1.54)	0.97
Leisure-time exercise	Yes	1.00	
	No	1.29 (0.95-1.73)	0.10

*Multivariate model with simultaneous adjustment for all the risk factors and, additionally, alpha-tocopherol and beta-carotene supplementation group by Cox proportional hazards model.
† BP = blood pressure
‡HDL = high-density lipoprotein

Table 12. Relative risks and 95% confidence intervals (CI) for abdominal aortic aneurysm by quartiles of daily intake of energy, macronutrients, and alcohol among ATBC Study participants.

Macronutrient	Quartile median	Relative risk, 95% CI Age- and smoking-adjusted*	P for trend†	Relative risk, 95% CI Multivariate‡	P for trend
Total energy, kcal	1 984	1.00		1.00	
	2 495	0.87 (0.60-1.27)		0.92 (0.63-1.35)	
	2 969	0.60 (0.39-0.93)		0.63 (0.41-0.93)	
	3 695	0.56 (0.35-0.88)	<0.01	0.59 (0.38-0.94)	<0.01
Fat, g	80	1.00		1.00	
	106	1.08 (0.71-1.64)		1.14 (0.74-1.74)	
	130	0.89 (0.57-1.39)		0.92 (0.59-1.44)	
	169	0.91 (0.60-1.40)	0.51	0.95 (0.62-1.46)	0.60
Protein, g	72	1.00		1.00	
	91	0.86 (0.55-1.34)		0.82 (0.53-1.27)	
	109	0.91 (0.59-1.41)		0.87 (0.56-1.35)	
	136	1.26 (0.84-1.90)	0.25	1.13 (0.75-1.70)	0.51
Carbohydrate, g	203	1.00		1.00	
	266	0.73 (0.46-1.60)		0.71 (0.45-1.13)	
	322	0.84 (0.54-1.30)		0.78 (0.50-1.21)	
	411	1.14 (0.76-1.70)	0.39	1.02 (0.68-1.54)	0.76
Fiber, g	15	1.00		1.00	
	21	0.89 (0.58-1.36)		0.83 (0.54-1.27)	
	27	0.79 (0.51-1.23)		0.74 (0.48-1.15)	
	37	1.00 (0.66-1.51)	0.87	0.90 (0.59-1.36)	0.55
Alcohol, g	0	1.00		1.00	
	5	0.76 (0.50-1.16)		0.81 (0.53-1.24)	
	23	0.60 (0.35-1.01)		0.70 (0.41-1.19)	
	40	0.77 (0.44-1.36)		0.91 (0.51-1.63)	
	78	0.75 (0.31-1.83)	0.29	0.93 (0.38-2.29)	0.74

All nutrients except alcohol were energy-corrected by the residual method for the analyses
Each nutrient was separately analysed by the Cox model
*Adjusted for baseline age, years of smoking and total energy intake
†P for linear trend by Wald test
‡Adjusted for age, years of smoking, total energy intake, serum total and HDL-cholesterol, systolic and diastolic blood pressure, and alpha-tocopherol and beta-carotene supplementation group.
Intake of alcohol divided into five categories: nondrinkers, ≤15, >15-30, >30-60, and >60 g/d

Effect of antioxidant supplementation on incidence of abdominal aortic aneurysm (Study V)

Effect of supplementation

During a mean follow-up of 5.8 years (169 408 person years) 181 cases of abdominal aortic aneurysm were detected. The incidence of abdominal aortic aneurysm per 10 000 person years was 10.6 for alpha-tocopherol supplementation, 8.6 for alpha-tocopherol plus beta-carotene supplementation, 11.8 for beta-carotene supplementation, and 11.5 for placebo (Table 13). No differences between the intervention groups were statistically significant (Figure 4). Crude relative risks for abdominal aortic aneurysm compared to placebo were 0.92 (95% CI 0.61-1.38) for those who received alpha-tocopherol, 0.76 (95% CI 0.50-1.15) for those who received alpha-tocopherol plus beta-carotene, and 1.03 (95% CI 0.69-1.52) for those who received beta-carotene. Adjustment for the baseline variables affected these results only slightly (Table 13). There was no interaction between alpha-tocopherol and beta-carotene supplementations ($p = 0.06$). None of the baseline variables modified the effect of supplements on the incidence of aortic aneurysm. The crude relative risk for aortic aneurysm was 0.83 (95% CI 0.62-1.11)

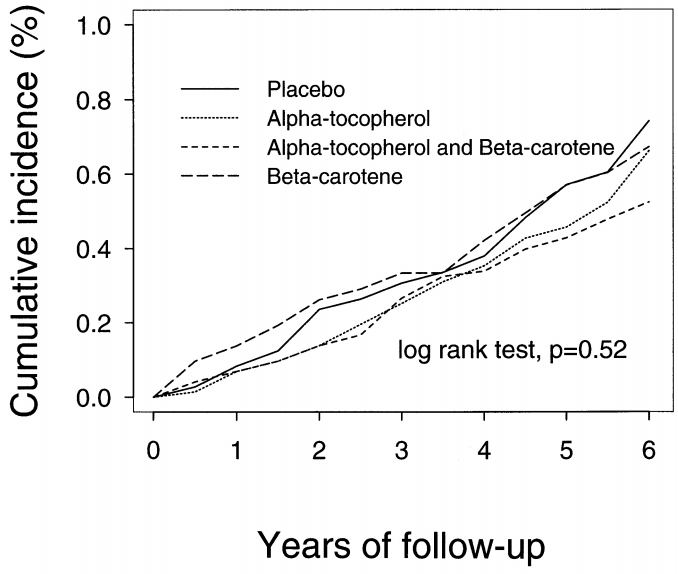
among those who received alpha-tocopherol compared to those who did not, and 0.93 (95% CI 0.69-1.24) among those who received beta-carotene compared to those who did not (V: Table 2, p. 16).

Table 13. Incidence and relative risk for abdominal aortic aneurysm by supplementation group among ATBC Study participants.

Supplementation	Number of cases with aneurysm	Incidence / 10 000 person-years	Crude relative risk, (95% CI)*	Multivariate relative risk†, (95% CI)
Placebo	49	11.5	1.00	1.00
Alpha-tocopherol	45	10.6	0.92 (0.61-1.38)	0.87 (0.58-1.31)
Alpha-tocopherol plus beta-carotene	37	8.6	0.76 (0.50-1.16)	0.75 (0.49-1.16)
Beta-carotene	50	11.8	1.03 (0.69-1.52)	0.99 (0.66-1.47)

Relative risks calculated by Cox proportional hazards model
*CI = confidence interval
†adjusted for age, years of smoking, number of daily cigarettes, serum total and HDL-cholesterol, systolic and diastolic blood pressure

Figure 4. Cumulative incidence of large abdominal aortic aneurysm in the four supplementation groups.



Aneurysms were divided into two subgroups: nonruptured and ruptured. Risk for nonruptured abdominal aortic aneurysm tended to be 30% lower among those who received alpha-tocopherol than among those who did not. This difference did not, however, reach statistical significance. Beta-carotene supplementation showed no effect. There was no difference in relative risk for ruptured aortic aneurysm among those who received alpha-tocopherol and those who did not, or among those who received beta-carotene and those who did not. (V: Table 3, p. 17)

6. DISCUSSION

6.1 Methodological considerations

The ATBC Study

The ATBC Study was primarily aimed at evaluating the effect of alpha-tocopherol and beta-carotene supplementation on lung cancer incidence. However, the secondary aim was to evaluate the effect of antioxidants on the development of other cancers and cardiovascular diseases. The study protocol included at baseline structured questionnaires concerning intermittent claudication and angina pectoris in addition to questions on medical history. Several measurements were performed to assess risk factors for atherosclerosis. During follow-up, structured questionnaires were re-administered, current health was evaluated, and diagnoses of illnesses were obtained through the National Hospital Discharge and Mortality Registers.

Subjects

All subjects were men with a long smoking history, since the primary interest was in preventing lung cancer. They were, however, at high risk for cardiovascular diseases as well, because smoking is one of the major risk factors for atherosclerosis. Strictly, findings of he present study can be generalized only to male smokers. Because all subjects were of late middle-age, it is obvious that to some extent atherosclerotic changes were already present at baseline. During enrollment some selection occurred due to previous and current diseases, and due to impaired ability to participate in a long-term study. This should be kept in mind when interpreting the results, because it is generally hypothesized that subjects who are not reachable or are considered ineligible for follow-up studies, are at the highest risk for disease.

Validity of definition of intermittent claudication

The definition of intermittent claudication was based on the Rose questionnaire. In the original study by Rose, the sensitivity and specificity of this questionnaire were reported to be high, 92% and 100%, respectively, based on 37 patients with intermittent claudication and 18 patients with other leg pain (Rose, 1962). In a larger sample of 586 claudicants and 61 patients with other leg pain, sensitivity was 60% and specificity 91% (Leng and Fowkes, 1992). It is likely that misclassification has led to a slight underestimation of the effect of supplementation (Fleiss, 1973). There is, however, no reason to assume that such misclassification would vary by supplementation group. In the cohort analyses, misclassification may have attenuated the associations between risk factors and endpoint. In assessment of intermittent claudication, it should be kept in mind that by definition claudication is a symptom. It reflects peripheral atherosclerosis, but the severity of atherosclerotic lesions may vary between individuals. Fluctuation of symptoms and spontaneous recovery are typical for intermittent claudication; this variability in its natural course partly explains the modest constancy of questionnaire responses (48%).

Compliance and drop-out

The overall capsule compliance in the ATBC Study was high. Compliance was assessed by calculating the number of capsules used per days in the trial. The rise in serum alpha-tocopherol and beta-carotene levels measured in the third follow-up year confirms the good compliance. One obvious problem in the analysis of intermittent claudication is the lack of information from dropouts. It was possible to detect cases only among active participants. Whether incidence of intermittent claudication would differ among dropouts and those who participated, was impossible to assess. No significant differences existed in dropout incidences from the four intervention groups; neither did occurrence of intermittent claudication significantly modify dropout incidence. Respectively, in the cohort setting, endpoint information was lost due to dropping out. Risk factors may have affected not only risk for intermittent claudication but also risk for dropping out. How the lack of endpoint data of dropouts affected the observed risk estimates was impossible to ascertain.

Validity of register-based endpoints

Two endpoints in this work were based on registers: surgery on arteries of the lower extremities and abdominal aortic aneurysm. Operation codes for arterial bypass or endarterectomy came from the Hospital Discharge Register, and register data was used as such. In a Finnish evaluation, codes for any operation from the Hospital Discharge Register matched hospital records in 85% of cases (Aro et al., 1990). Based on such results, register data seem to be sufficiently valid for analysis. Hospital and autopsy records of aortic aneurysms were collected and diagnoses checked. The number of ATBC Study subjects with asymptomatic and uncomplicated abdominal aortic aneurysm is unknown. However, because the aneurysm endpoint comprised ruptured aneurysms and those electively operated on, one can expect to have identified most subjects with large, clinically relevant abdominal aortic aneurysms. Sudden-death cases that were not autopsied must be considered potentially a group of missing cases, although autopsies were performed on 64% of the ATBC Study participants who had CVD as their underlying cause of death.

Validity of the dietary questionnaire

The dietary questionnaire used in the ATBC Study was validated and its reproducibility and validity were found to be satisfactory (Pietinen et al., 1988). Pearson correlation coefficients between food records and food-use questionnaires were 0.69 for vitamin C, 0.70 for vitamin E, and 0.73 for fiber. Dietary assessment always involves some degree of measurement error, and misclassification leads to attenuation of associations. Over-reporting of healthy foods and under-reporting of unhealthy ones is one problem. Energy adjustment, as introduced by Willett, corrects some of this problem in reporting (Willett, 1990).

6.2 Risk factors

Classical risk factors

Risk factors under study may be divided into those affected by lifestyle (smoking, exercise, diet) and those that cannot (age). However, many of the risk factors for atherosclerosis are combinations; diet and genome both affect serum cholesterol level, with much variation between individuals. In this work, several risk factors commonly referred to as classical risk factors for atherosclerotic diseases were clearly associated with risk for both intermittent claudication and large abdominal aortic aneurysms. Increasing age, elevated blood pressure, high serum total cholesterol concentration, and long history of smoking were significant risk factors. Similar associations have been observed in prospective studies of risk factors for intermittent claudication (Kannel and McGee, 1985; Bainton et al., 1994; Dagenais et al., 1991) and abdominal aortic aneurysm (Strachan, 1991; Reed et al., 1992). That high serum HDL-cholesterol showed a strong inverse association with intermittent claudication and especially with abdominal aortic aneurysm adds to the increasing amount of evidence of the importance of HDL-cholesterol as a protective factor. Other mechanisms of HDL beyond reverse cholesterol transport have been suggested, such as its antioxidative effect (Francis and Perry, 1999). Although all subjects were long-term smokers, cessation of smoking was beneficial. It lowered risk for subsequent claudication among those free of this symptom at study entry and suggested a benefit for those subjects with claudication at baseline. These findings place further emphasis on preventive work in modifying the lipid profile and encouraging nonsmoking to improve public health.

High intake of energy showed a protective association with risk for claudication and aortic aneurysm, though it is unlikely that energy intake itself is a protective factor. Need for energy depends on level of daily exercise, body mass, and individual metabolism. A high amount of exercise is associated with an increased need for energy; thus energy intake can be interpreted as a crude measure of physical activity, especially after controlling for BMI, age, and sex (Willett and Stampfer, 1998). In this study, leisure-time exercise was inversely associated with risk only for claudication. Measurement of exercise through one question in a questionnaire naturally limits validity, and may attenuate the possibility of detecting true differences in the level of daily exercise. On the other hand, a strong protective association between HDL-cholesterol level and both end-points gives further support for this explanation, since regular exercise is reported to increase HDL-cholesterol concentration (Sagiv and Goldbourt, 1994).

Diet

Diet may be considered healthy or unhealthy. Several countries have offered recommendations on how people should construct their diets. Generally, vegetable oils, vegetables, and fruits are favored, with advice to reduce intake of animal fat and meat. In the present work, the associations observed between nutrient intake and risk for claudication are in agreement with the general consensus as to a healthy diet. High intake of fiber, carbohydrates, n-6 polyunsaturated fatty acids, and antioxidant vitamins was

inversely associated with risk for intermittent claudication. However, none of the nutrients were associated with risk for large abdominal aortic aneurysm. This may indicate that, in the pathogenesis of aneurysm formation, genetic factors and the toxic effect of smoking play a greater role than do dietary factors.

Serum

Findings for serum alpha-tocopherol and beta-carotene levels were coherent with dietary findings. High levels of cholesterol-adjusted serum alpha-tocopherol and beta-carotene were associated with low risk for intermittent claudication, but not for abdominal aortic aneurysm. Serum alpha-tocopherol and beta-carotene correlated with dietary intake, although the correlation coefficient was quite weak. In prospective studies, inverse associations between serum antioxidant levels and cardiovascular disease risk have been more consistent for beta-carotene (Greenberg et al., 1996) than for alpha-tocopherol (Salonen et al., 1985), although neither has shown an association in all studies (Sahyoun et al., 1996).

6.3 Supplementation

Prevention

Neither alpha-tocopherol nor beta-carotene supplementation showed any preventive effect on incidence of intermittent claudication or large abdominal aortic aneurysm. An unexpected subgroup result appeared. Among subjects with a history of diabetes mellitus, the incidence of intermittent claudication was higher in those on alpha-tocopherol supplementation compared to no alpha-tocopherol supplementation. No plausible hypothesis exists through which alpha-tocopherol supplementation could enhance development of claudication among subjects with diabetes. This observation thus may be a chance finding resulting from multiple comparisons.

These findings of no benefit are not surprising, since the first trials of primary prevention of cardiovascular diseases with antioxidant supplements have not revealed such favorable effects as were expected based on the observational studies (Stampfer et al., 1993; Rimm et al., 1993; Klipstein-Grobusch et al., 1999; Todd et al., 1999). In the ATBC Study, no preventive effect on coronary heart disease was evident with either antioxidant (Virtamo et al., 1998; Rapola et al., 1996), whereas risk for cerebral infarction was significantly reduced with alpha-tocopherol supplementation (Leppälä et al., 2000). The effect of alpha-tocopherol supplementation on mortality of cerebral infarction did not reach statistical significance. However, risk for fatal subarachnoid hemorrhage was significantly increased with alpha-tocopherol supplementation (Leppälä et al., 2000). In two other large-scale trials, no benefit was observed from beta-carotene supplementation against cardiovascular diseases or mortality (Omenn et al., 1996; Hennekens et al., 1996). In addition, no effect of alpha-tocopherol supplementation on claudication incidence was evident among subjects at high risk for cardiovascular disease in the HOPE Study (The Heart Outcome Prevention Evaluation Study Investigators, 2000). Neither was there any effect on secondary prevention of coronary heart disease with alpha-tocopherol supplementation in the

HOPE and the GISSI-Prevenzione studies (The Heart Outcome Prevention Evaluation Study Investigators, 2000; GISSI-Prevenzione Investigators, 1999). In contrast, in the CHAOS trial the risk for non-fatal myocardial infarction was significantly reduced among CHD patients who received alpha-tocopherol, however, a nonsignificant 30% increase in CHD mortality occurred during supplementation lasting less than one and a half years (Stephens et al., 1996).

This work seems to include a discrepancy between findings of a relation between dietary and supplemented antioxidants and incidence of claudication. Several explanations can be given. The antioxidant hypothesis may hold at physiological concentrations of antioxidants but not at pharmacological ones. Thus, supplementation with high doses would not be beneficial to a well-nourished population. Second, the duration of supplementation may have been too short or occurred at a too-advanced age. However, because atherogenesis is a continuous process, antioxidant supplementation would be expected to be beneficial at any time. Dietary intake, on the other hand, is suggested to reflect life-long exposure. Third, high intakes of specific antioxidants are markers of high consumption of vegetables and fruits. These contain many additional compounds – both known and unknown – that may be responsible for the beneficial association detected in epidemiological studies. Finally, these nutritional factors may, merely be indicators of a more healthy life-style.

Treatment effect

For decades patients with intermittent claudication have been treated with vitamin E supplements. The early studies were mainly clinical observations, and the subsequent few controlled, double-blind studies were of small size and with a short duration of supplementation (Livingstone and Jones, 1958; Williams et al., 1962; Hamilton et al., 1953). Indeed, the Cochrane database, in which current evidence is reviewed, gives no recommendation for treatment of intermittent claudication with alpha-tocopherol (Kleijnen and Mackerras, 1998). In the present work, neither alpha-tocopherol nor beta-carotene supplementation showed any effect on recurrence of symptoms among subjects with intermittent claudication. Instead, beta-carotene significantly increased risk for vascular surgery among those subjects with mild typical intermittent claudication at study entry. The mechanism behind this finding is unclear. Some evidence exists that beta-carotene is incorporated into atherosclerotic plaques (Prince et al., 1988). Whether beta-carotene may favor smooth, developing plaques that are prone to rupture instead of being incorporated into calcified plaques can only be hypothesized. It is thus concluded that alpha-tocopherol supplementation did not relieve intermittent claudication, and beta-carotene supplementation even appeared harmful.

7. CONCLUSIONS

1. A diet rich in antioxidants, carbohydrate, fiber, and polyunsaturated fatty acids was associated with decreased risk for intermittent claudication; additionally, physical activity, amount of education, and smoking cessation showed a protective association. Nutrient intake had no association with risk for abdominal aortic aneurysm. However, the classic risk factors for atherosclerotic diseases were associated both with the risk for intermittent claudication and for abdominal aortic aneurysm.

2. Alpha-tocopherol or beta-carotene supplementation in doses several-fold higher than the average dietary intake showed no preventive effect on incidence of intermittent claudication.

3. Alpha-tocopherol supplementation did not affect recurrence of claudication or risk for surgery among subjects with claudication at study entry. Beta-carotene supplementation increased slightly the risk for vascular surgery, but showed no effect on the recurrence of symptoms.

4. Long-term supplementation with alpha-tocopherol or beta-carotene showed no effect on risk for large abdominal aortic aneurysm.

Thus, among long-term male smokers, supplemental antioxidants seem to have no beneficial effect on intermittent claudication or abdominal aortic aneurysm. Instead, a lifestyle aiming at non-smoking, improving the lipid profile, lowering blood pressure, and increasing physical activity seems to offer advantages against development both of intermittent claudication and of abdominal aortic aneurysm.

8. REFERENCES

Acheson RM, Williams DRR. Does consumption of fruit and vegetables protect against stroke? Lancet 1:1191-1193, 1983.

Aro S, Koskinen R, Keskimäki I. Sairaalastapoistorekisterin diagnoosi-, toimenpide- ja tapaturmatietojen luotettavuus. Duodecim 106:1443-1450, 1990.

Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willet WC. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. Br Med J 313:84-90, 1996.

Bainton D, Sweetnam P, Baker I, Elwood P. Peripheral vascular disease: consequence for survival and association with risk factors in the Speedwell Prospective Heart Disease Study. Br Heart J 72:128-132, 1994.

Belizzi MC, Franklin MF, Duthie GG, James WPT. Vitamin E and coronary heart disease: the European paradox. Eur J Clin Nutr 48:822-831, 1994.

Bendich A, Olson JA. Biological actions of carotenoids. FASEB J 3:1927-1932, 1989.

Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis:Basic Mechanisms. Oxidaion, Inflammation, and Genetics. Circulation 91:2488-2496, 1995.

Bird DA, Gillotte KL, Hörkkö S, Friedman P, Dennis EA, Witztum JL, Steinberg D. Reseptors for oxidized low-density lipoprotein on elicited mouse peritoneal macrophages can recognize both the mofified lipid moieties and the modified protein moieties: Implications with respect to macrophage recognition of apoptotic cells. Proc Natl Acad Sci U S A 96:6347-6352, 1999.

Bowlin SJ, Medalie JH, Flocke SA, Zyzanski SJ, Goldbourt U. Epidemiology of Intermittent Claudication in Middle-aged Men. Am J Epidemiol 140:418-430, 1994.

Boyd AM, Ratcliffe AH, Jepson RP, James WH. Intermittent claudication. A clinical study. J Bone Joint Surg 31:325-355, 1949.

Boyd AM, Marks J. Treatment of intermittent claudication. A reappraisal of the value of alpha-tocopherol. Angiology 14:198-208, 1963.

Breslow NE, Day NE. Statistical Methods in Cancer Research. v. II. Lyon: IARC, 1987.

Brown ED, Micozzi MS, Craft JG, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, Smith Jr JC. Plasma carotenoids in nomral men after a single ingestion of vegetables or purified beta-carotene. Am J Clin Nutr 49:1258-1265, 1989.

Böhm F, Edge R, Land EJ, McGarvey DJ, Truscott TG. Carotenoids enhance vitamin E antioxidant efficiency. J Am Chem Soc 119:621-622, 1997.

Christen WG, Gaziano JM, Hennekens CH. Design of Physicians' Health Study II - A randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. Ann Epidemiol 10:125-134, 2000.

Criqui MH, Fronek A, Barrett-Conner E, Klauber MR, Gabriel S, Goodman D. The prevalence of peripheral arterial disease in a defined population. Circulation 71:510-515, 1985.

Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. N Engl J Med 326:381-386, 1992.

Dagenais GR, Maurice S, Robitaille N-M, Gingras S, Lupien PJ. Intermittent Claudication in Quebec men from 1974-1986: the Quebec Cardiovascular Study. Clin Invest Med 14:93-100, 1991.

Dieber-Rotheneder M, Puhl H, Waeg G, Striegl G, Esterbauer H. Effect of oral supplementation with D-alpha-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. J Lipid Res 32:1325-1332, 1991.

Dolecek TA. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. Proc Soc Exp Biol Med 200:177-182, 1992.

Donnan PT, Thomson M, Fowkes FGR, Prescott RJ, Housley E. Diet as a risk factor for peripheral arterial disease in the general population:The Edinburgh Artery Study. Am J Clin Nutr 57:917-921, 1993.

Dugas TR, Morel DW, Harrison EH. Dietary supplementation with beta-carotene, but not with lycopene, inhibits endothelial cell-mediated oxidation of low-density lipoprotein. Free Radic Biol Med 26:1238-1244, 1999.

Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. Epidemiology 3:194-202, 1992.

Esterbauer H, Rotheneder M, Striegl G, Waeg G, Ashy A, Sattler W, Jurgens G. Vitamin E and other lipophilic antioxidants protect LDL against oxidation. Fat Sci Technol 91:316-324, 1989.

Fehily AM, Yarnell JWG, Sweetnam PM, Elwood PC. Diet and incident ischaemic heart disease: the Caerphilly Study. Br J Nutr 69:303-314, 1993.

Fleiss JL. Statistical Methods for Rates and Proportions. New York: John Wiley & Sons, 1973.

Francis GA, Perry RJ. Targeting HDL-mediated cellular cholesterol efflux for the treatment and prevention of atherosclerosis. Clin Chim Acta 286:219-230, 1999.

Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 15:1145-1151, 1995.

Gaziano JM, Hatta A, Flynn M, Johnson EJ, Krinsky NI, Ridker PM, Hennekens CH, Frei B. Supplementation with ß-carotene in vivo and in vitro does not inhibit low density lipoprotein oxidation. Atherosclerosis 112:187-195, 1995.

Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. Am J Clin Nutr 53:326S-334S, 1991.

Ginter E. Decline of coronary mortality in United States and vitamin C. Am J Clin Nutr 32:511-512, 1979.

GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 354:447-455, 1999.

Gokce N, Frei B. Basic research in antioxidant inhibition of steps in atherogenesis. J Cardiovasc Risk 3:352-357, 1996.

Greenberg ER, Baron JA, Karagas MR, Stukel TA, Nierenberg DW, Stevens MM, Mandel JS, Halle RW. Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation. JAMA 275:699-703, 1996.

Haapa E, Toponen T, Pietinen P, Räsänen L. Annoskuvakirja. Helsinki: Kansanterveyslaitos. Helsingin Yliopiston Ravitsemustieteen laitos, 1985.

Haeger K. Long-term study of alpha-tocopherol in intermittent claudication. Ann N Y Acad Sci 393:369-375, 1982.

Halliwell B. Vitamin C: Antioxidant or pro-oxidant in vivo? Free Rad Res 25:439-454, 1996.

Hamilton M, Wilson GM, Armitage P, Boyd JT. The treatment of intermittent claudication with vitamin E. Lancet 1:367-370, 1953.

Hammond EC, Garfinkel L. Coronary heart disease, stroke, and aortic aneurysm. Factors in the etiology. Arch Environ Health 19:167-182, 1969.

Hennekens CH, Buring JE, Manson JoAE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridger PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 334:1145-1149, 1996.

Hense HW, Stender M, Bors W, Keil U. Lack of association between serum vitamin E and myocardial infarction in a population with high vitamin E levels. Atherosclerosis 103:21-28, 1993.

Hercberg S, Galan P, Preziosi P, Roussel A-M, Arnaud J, Richard M-J, Malvy D, Paul-Dauphin A, Briancon S, Favier A. Background and rationale behind the SU.VI.MAX. Study, a prevention trial using nutritional doses of a combination of antioxidant vitamins and minerals to reduce cardiovascular diseases and cancers. Int J Vitam Nutr Res 68:3-20, 1998.

Hu FB, Stampfer MJ, Manson JoAE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willet WC. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med 337:1491-1499, 1997.

Hutchison KJ, Williams HTG. Calf blood flow and ankle systolic blood pressure in intermittent claudication monitored over five years. Angiology 29:719-727, 1978.

Ingolfsson IÖ, Sigurdsson G, Sigvaldson H, Thorgeirsson G, Sigfusson N. A marked decline in the prevalence and incidence of intermittent claudication in Icelandic men 1968-1986: A strong relationship to smoking and serum cholesterol - the Reykjavik Study. J Clin Epidemiol 47:1237-1243, 1994.

Institue of Medicine F and NB. Dietary reference intakes: applications in dietary assessment. Available at: http://www.nap.edu/catalog/9956.html. Accessed September 25, 2000.

Jha P, Flather M, Lonn E, Farkouh M, Yusuf S. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. Ann Int Med 123:860-872, 1995.

Jialal I, Fuller CJ, Huet BA. The effect of alpha-tocopherol supplementation on LDl oxidation. A dose-response study. Arterioscler Thromb Vasc Biol 15:190-198, 1995.

Kamal-Eldin A, Appelqvist L-Å. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671-701, 1996.

Kannel WB, McGee DL. Update on some epidemiologic features of intermittent claudication: The Framingham Study. J Am Geriatr Soc 33:13-18, 1985.

Kaplan M, Aviram M. Oxidized low-density lipoprotein: Atherogenic and proinflammatory characteristics during macrophage foam cell formation. An inhibitory role of nutritional antioxidants and serum paraoxonase. Clin Chem Lab Med 37:777-787, 1999.

Kardinaal AFM, Kok FJ, Ringstad J, Gomez-Aracena J, Mazaev VP, Kohlmeier L, Martin BC, Aro A, Kark JD, Delgado-Rodriguez M, Riemersma RA, van't Veer P, Huttunen JK, Martin-Moreno JM. Antioxidants in adipose tissue and risk of myocardial infarction: the EURAMIC study. Lancet 342:1379-1384, 1993.

Katsouyanni K, Skalkidis Y, Petridou E, Polychronopoulou-Trichopoulou A, Willett W, Trichopoulos D. Diet and peripheral arterial occlusive disease: the role of poly-, mono-, and saturated fatty acids. Am J Epidemiol 133:24-31, 1991.

Kattermann R, Jaworek D, Möller G, Assmann G, Björkheim I, Svensson L, Borner K, Boerma G, Lejinse B, Desager JP, Harwengt C, Kupke I, Tinder P. Multicentre study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 22:245-251, 1984.

Keaney Jr. JF, Simon DI, Freedman JE. Vitamin E and vascular homeostasis: implications for atherosclerosis. FASEB J 13:965-976, 1999.

Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH, Kromhout D, Nedeljkovic S, Punsar S, Seccareccia F, Toshima H. The diet and 15-year death rate in the Seven Countries Study. Am J Epidemiol 124:903-915, 1986.

Kleijnen J, Mackerras D. Vitamin E for treatment of intemittent claudication. The Cochrane Library Issue 3, 1998.

Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH, Boeing H, Hofman A, Grobbee DE, Witteman JCM. Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr 69:261-266, 1999.

Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witteman JCM. Serum carotenoids and atherosclerosis The Rotterdam Study. Atherosclerosis 148:49-56, 2000.

Knekt P, Reunanen A, Järvinen R, Seppänen R, Heliövaara M, Aromaa A. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. Am J Epidemiol 139:1180-1189, 1994.

Kohlmeier L, Kark JD, Gomez-Gracia E, Martin BC, Steck SE, Kardinaal AFM, Ringstad J, Thamm M, Masaev V, Riemersma R, Martin-Moreno JM, Huttunen JK, Kok FJ. Lycopene and myocardial infarction risk in the EURAMIC Study. Am J Epidemiol 146:618-626, 1997.

Kok FJ, de Bruijin AM, Vermeeren R, Hofman A, van Laar A, de Bruin M, Hermus RJJ, Valkenburg HA. Serum selenium, vitamin antioxidants, and cardiovascular mortality: a 9-year follow-up study in the Netherlands. Am J Clin Nutr 45:462-468, 1987.

Kostner GM. Enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. Clin Chem 22:695, 1976.

Kromhout D, Bosschieter EB, de Lezenne Coulander C. Dietary fiber and 10-year mortality from coronary heart disease, cancer, and all causes. The Zutphen study. Lancet 2:518-522, 1982.

Kushi LH, Lew RA, Stare FJ, Ellison CR, El Lozy M, Bourke G, Daly L, Graham I, Hickey N, Mulcahy R, Kevaney J. Diet and 20-year mortality from coronary heart disease.The Ireland-Boston Diet-Heart Study. N Engl J Med 312:811-818, 1985.

Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N Engl J Med 334:1156-1162, 1996.

Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 91:2102-2106, 1999.

Leng GC, Fowkes FGR. The Edinburgh claudication questionnaire: an improved version of the WHO/Rose questionnaire for use in epidemiological surveys. J Clin Epidemiol 45:1101-1109, 1992.

Leppälä JM, Virtamo J, Fogelholm R, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Controlled trial of alpha-tocopherol and beta-carotene supplementation on stroke incidence and mortality in male smokers. Arterioscler Thromb Vasc Biol 20:230-235, 2000.

Levy Y, Kaplan M, Ben-Amotz A, Aviram M. Effect of dietary supplementation of beta-carotene on human monocyte-macrophage-mediated oxidation of low-density lipoprotein. Isr J Med Sci 32:473-478, 1996.

Livingstone PD, Jones C. Treatment of intermittent claudication with vitamin E. Lancet 2:602-604, 1958.

Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. Am J Clin Nutr 64:190-196, 1996.

Mabile L, Bruckdorfer KR, Rice-Evans C. Moderate supplementation with natural alpha-tocopherol decreases platelet aggregation and low-density lipoprotein oxidation. Atherosclerosis 147:177-185, 1999.

Manson JoAE, Gaziano JM, Spelsberg A, Ridker PM, Cook NR, Buring JE, Willett WC, Hennekens CH. A secondary prevention trial of antioxidant vitamins and cardiovascular disease in women. Ann Epidemiol 5:261-269, 1995.

Maxwell SR. Prospects for the use of antioxidant therapies. Drugs 49:345-361, 1995.

Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene, and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin Chem 32:874-876, 1986.

Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. JAMA 272:1439-1441, 1994.

National Public Health Institute. The 1997 dietary survey of Finnish adults. B8/1998 ed. Helsinki: Publications of National Public Health Institute, 1998.

NRC Subcommittee on the Tenth Edition of the RDAs. Recommended Dietary Allowances. 10th ed. Waschington, D.C.: National Academy Press, 1989.

Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams J James H, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334:1150-1155, 1996.

Ovaskainen M-L, Valsta LM, Lauronen J. The compilation of food analysis values as a database for dietary studies - the Finnish experience. Food Chem 57:133-136, 1996.

Palinski W, Rosenfeld ME, Ylä-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL. Low density lipoprotein undergoes oxidative modification in vivo. Proc Natl Acad Sci U S A 86:1372-1376, 1988.

Pandley DK, Shekelle R, Selwyn BJ, Tangney C, Stamler J. Dietary vitamin C and beta-carotene and risk of death in middle-aged men. The Western Electric Study. Am J Epidemiol 142:1269-1278, 1995.

Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Oxidants and antioxidants in atherogenesis: an appraisal. J Lipid Res 40:2143-2157, 1999.

Pietinen P, Hartman AM, Haapa E, Räsänen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J, Huttunen JK. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. Am J Epidemiol 128:655-666, 1988.

Pietinen P, Rimm EB, Korhonen P, Hartman AM, Willett W c, Albanes D, Virtamo J. Intake of dietary fiber and risk for coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Circulation 94:2720-2727, 1996.

Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willet WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. Circulation 145:876-887, 1997.

Pleumeekers HJCM, Hoes AW, van der Does E, van Urk H, Hofman A, de Jong PTVM, Grobbee DE. Aneurysms of the abdominal aorta in older adults. The Rotterdam Study. Am J Epidemiol 142:1291-1299, 1995.

Posner BM, Cobb JL, Belanger AJ, Cupples LA, D'Agostino RB, Stokes III J. Dietary lipid predictors of coronary heart disease in men. The Framingham Study. Arch Intern Med 151:1181-1187, 1991.

Prince MR, LaMuraglia GM, MacNichol Jr. EF. Increased preferential absorption in human atherosclerotic plaque with oral beta carotene. Implications for laser endarterectomy. Circulation 78:338-344, 1988.

Princen HM, van Poppel G, Vogelezang C, Buytenhek R, Kok FJ. Supplementation with vitamin E but not beta-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. Arterioscler Thromb 12:554-562, 1992.

Princen HMG, van Duyvenvoorde W, Buytenhk R, van der Laarse A, van Poppel G, Leuven JAG, van Hinsbergh VWM. Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. Arterioscler Thromb Vasc Biol 15:325-333, 1995.

Rapola JM, Virtamo J, Haukka JK, Heinonen OP, Albanes D, Taylor PR, Huttunen JK. Effect of vitamin E and beta carotene on the incidence of angina pectoris. JAMA 275:693-698 (Erratum, JAMA 1998;279:1528), 1996.

Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet 349:1715-1720, 1997.

Ratcliffe AH. Vitamin E in intermittent claudication. Lancet 2:1128-1130, 1949.

Reaven PD, Ferguson E, Navab M, Powell FL. Susceptibility of human LDL to oxidative modification. Effects of variations in â-carotene concentration and oxygen tension. Arterioscler Thromb 14:1162-1169, 1994.

Reed D, Reed C, Stemmermann G, Hayashi T. Are aortic aneurysms caused by atherosclerosis? Circulation 85:205-211, 1992.

Regnström J, Nilsson J, Moldeus P, Ström K, Båvenholm P, Tornvall P, Hamsten A. Inverse relation between the concentration of low-density-lipoprotein vitamin E and severity of coronary artery disease. Am J Clin Nutr 63:377-385, 1996.

Reunanen A, Takkunen H, Aromaa A. Prevalence of intermittent claudication and its effect on mortality. Acta Med Scan 211:249-256, 1982.

Rexrode KM, Lee I-M, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the Women's Health Study. J Women Helath Gen-B 9:19-27, 2000.

Riemersma RA, Oliver M, Elton RA, Alfthan G, Vartiainen E, Salo M, Rubba P, Mancini M, Georgi H, Vuilleumier J-P, Gey KF. Plasma antioxidants and coronary heart disease: Vitamins C and E, and selenium. Eur J Clin Nutr 44:143-150, 1989.

Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF. Risk of angina pectoris and plasma concentrations of vitamins A, C and E and carotene. Lancet 337:1-5, 1991.

Rimm EB, Stampfer MJ, Ascherio A, Giovannucci EL, Colditz GA, Willet WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 328:1450-1456, 1993.

Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willet WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. JAMA 275:447-451, 1996a.

Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits? Br Med J 312:731-736, 1996b.

Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 27:645-658, 1962.

Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med 340:115-126, 1999.

Sagiv M, Goldbourt U. Influence of physical work on high density lipoprotein cholesterol: implications for the risk of coronary heart disease. Int J Sports Med 15:261-266, 1994.

Sahyoun NR, Jacques PF, Russell RM. Carotenoids,vitamins C and E, mortality in an elderly population. Am J Epidemiol 144:501-511, 1996.

Salonen JT, Salonen R, Penttilä I, Herranen J, Jauhiainen M, Kantola M, Lappeteläinen R, Mäenpää PH, Alfthan G, Puska P. Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and the risk of death from coronary artery disease. Am J Cardiol 56:226-231, 1985.

Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssönen K, Palinski W, Witztum JL. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet 339:883-887, 1992.

Sandström B, Aro A, Becker W, Lyhne N, Pedersen JI, Porsdottir I. Nordiska näringsrekommendationer. v. 28. Köpenhamn: Nordiska Ministerrådet, 1996.

Schroll M, Munck O. Estimation of peripheral arteriosclerotic disease by ankle blood pressure measurements in a population study of 60-year-old men and women. J Chron Dis 34:261-269, 1981.

Scott RAP, Wilson NM, Ashton HA, Kay DN. Influence of screening on the incidence of ruptured abdominal aortic aneurysm: 5-year results of a randomized controlled study. Br J Surg 82:1066-1070, 1995.

Semmens JB, Norman PE, Lawrence-Brown MM, Bass AJ, Holman CD. Population-based record linkage study of the incidence of abdominal aortic aneurysm in Western Australia in 1985-1994. Br J Surg 85:648-652, 1998.

Shekelle RB, Shryock AM, Paul O, Lepper M, Stamler J, Liu S, Raynor Jr WJ. Diet, serum cholesterol, and death from coronary heart disease. N Engl J Med 304:65-70, 1981.

Shute EV, Vogelsang AB, Skelton FR, Shute WE. The influence of vitamin E on vascular disease. Surg Gynec Obst 86:1-8, 1948.

Simoni G, Pastorino C, Perrone R, Ardia A, Gianrossi R, Decian F, Cittadini GJ, Baiardi A, Bachi V. Screening for abdominal aortic aneurysm and associated risk factors in a general population. Eur J Vasc Endovasc Surg 10:207-210, 1995.

Sklodowska M, Wasowicz W, Gromadzinska J, Miroslaw W, Strzelczyk M, Malczyk J, Goch JH. Selenium and vitamin E concentrations in plasma and erythrocytes of angina pectoris patients. Trace Elements Med 8:113-117, 1991.

Stampfer MJ, Hennekens CH, Manson JoAE, Colditz GA, Rosner B, Willet WC. Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328:1444-1449, 1993.

Steiner M. Vitamin E: more than an antioxidant. Clin Cardiol 16 (Suppl.I):16-18, 1993.

Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ, Brown MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 347:781-786, 1996.

Strachan DP. Predictors of death from aortic aneurysm among middle-aged men: the Whitehall study. Br J Surg 78:401-404, 1991.

Street DA, Comstock GW, Salkeld RM, Schüep W, Klag MJ. Serum antioxidants and myocardial infarction. Are low levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? Circulation 90:1154-1161, 1994.

The ATBC Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics and compliance. Ann Epidemiol 4:1-9, 1994.

The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330:1029-1035, 1994.

The Heart Outcome Prevention Evaluation Study Investigators. Vitamin E supplmentation and cardiovascular events in high-risk patients. N Engl J Med 342:154-160, 2000.

Thompson JN, Duval S, Verdier P. Investigation of carotenoids in human blood using high-performance liquid chromatography. J Micronutr Anal 1:81-91, 1985.

Thompson RW. Basic science of abdominal aortic aneurysm: emerging therapeutic strategies for an unresolved clinical problem. Curr Opin Cardiol 11:504-518, 1996.

Todd S, Woodward M, Tunstall-Pedoe H, Bolton-Smith C. Dietary antioxidant vitamins and fiber in the etiology of cardiovascular disease and all-causes mortality: Results from the Scottish Heart Health Study. Am J Epidemiol 150:1073-1080, 1999.

Verlangieri AJ, Kapeghian JC, Bush M. Fruit and vegetable consumption and cardiovascular mortality. Med Hypotheses 16:7-15, 1985.

Virtamo J, Rapola JM, Ripatti S, Heinonen OP, Taylor PR, Albanes D, Huttune JK. Effect of vitamin E and Beta Carotene on the incidence of primary nonfatal myocardial infarction and fatal coronary heart disease. Arch Intern Med 158:668-675, 1998.

Wang X, Quinn PJ. Vitamin E and its function in membranes. Prog Lipid Res 38:309-336, 1999.

Westheim AS, Brox D, Selvaag OAW. D-alpha-tocopherol ved claudicatio intermittents. Tidsskr Nor Laegerforen 95:13-15, 1975.

Willett W. Nutritional epidemiology. New York/Oxford: Oxford University Press, 1990.

Willet WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341:581-585, 1993.

Williams HTG, Clein LJ, Macbeth RA. Alpha-tocopherol in the treatment of intermittent claudication. Canad Med Ass J 87:538-541, 1962.

Wolk A, Manson JoA, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. JAMA 281:1998-2004, 1999.

Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. Clin Invest Med 84:1086-1095, 1989.

Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42:121-130, 1986.

9. APPENDICES

The Rose questionnaire (Rose, 1962).

9. Katkokävelyoireisto kysymyssarja

Mikäli rastittamassasi vastausruudussa on *, siirry suoraan kohtaan 10.

9.1. Tuleeko Teille kipua alaraajoihin kävellessänne?	[*] 1 ei [ ] 2 kyllä
9.2. Tuleeko tämä kipu, kun seisotte paikallanne tai istutte?	[ ] 1 ei [*] 2 kyllä
9.3. Missä alaraajojen osissa tunnette sen?	[ ] 1 kipu esiintyy pohkeessa/pohkeissa [*] 2 kipu ei esiinny pohkeissa
9.4. Tuleeko se, kun kävelette ylämäkeä tai kiiruhdatte tasamaata?	[*] 1 ei [ ] 2 kyllä [ ] 3 ei kiiruhda koskaan, eikä kävele ylämäkeä
9.5. Tuleeko se, kun kävelette tavallista vauhtia tasaisella maalla?	[ ] 1 ei [ ] 2 kyllä
9.6. Häviääkö kipu koskaan, jos jatkatte edelleen kävelyä?	[ ] 1 ei [*] 2 kyllä
9.7. Mitä teette, jos se tulee kävellessänne?	[ ] 1 pysähdytte tai hidastatte kävelynopeutta [*] 2 jatkatte matkaa
9.8. Jos pysähdytte, mitä kivulle tapahtuu?	[ ] 1 helpottuu [*] 2 ei helpotu
9.9. Kuinka pian?	[ ] 1 10 minuutissa tai nopeammin [ ] 2 yli 10 minuutissa