TIINA RISSANEN

Association of lycopene and dietary intake of fruits, berries and vegetables with atherosclerosis and cardiovascular diseases

Epidemiologic evidence

Doctoral dissertation

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Research Institute of Public Health
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ABSTRACT

Diets rich in fruits and vegetables have aroused interest because of their potential health benefits against chronic diseases, such as cardiovascular diseases (CVD) and cancer. There are likely to be multiple mechanisms through which a plant dominated diet can protect against CVD. The proposed beneficial substances include many components including carotenoids. Lycopene is one of the main carotenoids in the Western diet. Interest in carotenoids, particularly in lycopene is on the increase following the recent publication of epidemiological studies which have associated high lycopene levels with reductions in CVD incidence. The purpose of this thesis was to study the role of blood levels of lycopene with regard to cardiovascular health in women and men living in eastern Finland. We also estimate the role of dietary intake of fruits, berries and vegetables in the risk of CVD and total mortality.

We first examined the association between the plasma concentration of lycopene and the intima-media thickness of common carotid artery wall (CCA-IMT) in a cross-sectional analysis of Antioxidant Supplementation in the Atherosclerosis Prevention (ASAP) Study in 520 high risk men and women. Low plasma levels (lower than the median) of lycopene were associated with an 18% increased intima-media thickness in men, compared with men whose plasma levels of lycopene were higher. In women, the difference was not significant. Next we examined the association between serum levels of lycopene and CCA-IMT in 1028 middle-aged men in the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. We found that in men with low serum levels of lycopene (lowest quarter) the adjusted CCA-IMT had a significant increment in both mean CCA-IMT and maximal CCA-IMT as compared with the other men.

In the KIHD study we examined the role of serum levels of lycopene with regard to the risk of acute coronary events and ischemic strokes. The subjects were 725 middle-aged men free of coronary heart disease and stroke at the study baseline. We found that men with a low serum level of lycopene (the lowest quarter) had an over three-fold risk of an acute coronary event or stroke as compared with others.

We also studied the role of dietary intake of fruits, berries and vegetables as a risk lowering factor with regard to CVD-related and total mortality in the KIHD study (n=1950 and 2641, respectively) and found that a high intake of fruits, berries and vegetables (highest fifth, > 400g/day) was associated with a 41% decrease in CVD-related and a 34% decrease in overall mortality in middle-aged men.

On the basis of this work it is evident that dietary vegetables have a role to play in preserving cardiovascular health in Finland. The blood concentration of lycopene, a biomarker of tomato-rich food, may have a role in the early stages of atherogenesis and also on the risk of atherosclerotic vascular events and may thus have clinical and public health relevance. The findings of this work confirm those of previous studies that a diet dominated by plant-derived foods promotes good cardiovascular health.

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YHTEENVETO


SVST–tutkimuksen osana selvitimme myös seerumin lykopeenipitoisuuden yhteyttä sydäninfarkti- ja aivohalvausriskoihin 725 miehillä. Miehillä, joiden seerumin lykopeenipitoisuus oli matalin (alin neljännesc, oli yli kolminkertainen riski sairastaa sydäninfarktiin tai aivohalvaukseen verrattuna miehiin, joiden seerumin lykopeenipitoisuus oli kohtalainen tai korkea.

Lopuksi tutkimme kasvisten, hedelmien ja marjojen saannin yhteyttä kokonais- sekä sydän- ja verisuonituuttikiveliisuteen SVST-kohortissa. Tässä tutkimuksessa seurattiin 2641 (kokonaiskiveliisus) tai 1950 (sydäntautikiveliisus) keski-ikäistä itäsuomalaisista miestä yli 12 vuoden ajan. Marjojen ja hedelmien kohtalainen ja runsas käyttö oli yhteydessä alentuneeseen sydäntauti- ja kokonaiskiveliisuteen. Yli 400 g päivässä kasviksia, marjoja ja hedelmää syöneillä miehillä kokonaiskiveliisus oli 34% ja sydäntautikiveliisus 41% pienempi enintään 130 g syöneillä miehillä.

Tämän väitöskirjatyön perusteella voidaan sanoa, että veren lykopeenipitoisuudella näyttäisi olevan merkitystä myös suomalaisten sydän- ja verisuonitautiriskin kannalta. Tämän työn tulokset tukevat aikaisempiä tutkimuksia, joissa runsaasti kasviksia sisältävän ruokavalion on todettu olevan hyväksi sydänterveydelle.
To my maths teacher in the ninth grade
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Kuopio, May, 2003

Tiina Rissanen
ABBREVIATIONS

8-OHdG 8-hydroxy-2’-deoxyguanosine
ANOVA Analysis of variance
ARIC Atherosclerosis Risk in Communities Study
ASAP Antioxidant Supplementation in Atherosclerosis Prevention Study
ATBC Alpha-tocopherol, Beta-Carotene Cancer Prevention Study
AMI Acute myocardial infarction
BMI Body mass index
CARET β-Carotene and Retinol Efficacy Trial
CCA Common carotid arteries
CCA-IMT Intima media thickness of common carotid arteries
CHD Coronary heart disease
CI Confidence interval
CRP C-reactive protein
CUDAS Perth Carotid Ultrasound Disease Assessment Study
CV Coefficient of variation
CVD Cardiovascular disease
DNA Deoxyribonucleic acid
EURAMIC European Study of Antioxidants, Myocardial Infarction and Cancer of the Breast
EVA Etude sur le Vieillissement Artériel Study
F Female
FFQ Food frequency questionnaire
HDL High density lipoprotein
HPLC High-performance liquid chromatography
ICD International Classification of Diseases
IHD Ischemic heart disease
IMT Intima media thickness
KIHD Kuopio Ischaemic Heart Disease Risk Factor Study
LDL Low density lipoprotein
M Male
MI Myocardial infarction
MONICA Monitoring of Trends and Determinants of Cardiovascular Disease
mMortality
NHANES National Health and Nutrition Examination Survey
OR Odds ratio
PUFA Polynsaturated fatty acids
r Correlation coefficients
RDA Recommended dietary allowance
RR Relative risk
SVVT Sydän- ja verisuonilentuus vaaratekijätutkimus
TBARS Thiobarbituric acid reactive substances
tHcy Total homocysteine
UV Ultraviolet
VLDL Very low density lipoprotein
LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications referred to in the text by their Roman numerals I-IV:


In addition, some unpublished data is presented.
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1 INTRODUCTION

Cardiovascular diseases (CVD) are the main cause of death in the Western countries. However, even though CVD mortality has decreased during the last decades in Finland they still account for over 40% of the total mortality (http://statfin.stat.fi). The CVD prevalence rate varies greatly between national populations. In the Seven Countries Study (Menotti et al. 1999), low coronary heart disease (CHD) -related mortality rates have been found in Southern European countries and Japan in contrast to the high mortality rates that have been found in USA, the Netherlands and Finland. Diets also vary extensively between different populations. In the eastern part of Finland, the mortality rate was 10-times higher than in Crete where the population consume a Mediterranean diet rich in plant foods year-round and relatively poor in animal foods (Menotti et al. 1999). There are likely to be multiple mechanisms through which a plant dominated diet can promote health. There are many proposed beneficial substances including carotenoids.

Carotenoids are pigments responsible for the yellow to red colour of fruits and vegetables. They are also provide a source of natural colouring agents to the food industry. Carotenoid-like pigments produce the characteristic colours of some birds, fishes and crustaceans. These compounds were isolated for the first time in 1831 from carrots (Krinsky. 1993). Lycopene is a red colored plant pigment and in contrast to most other carotenoids which are widely distributed among a great variety of fruits and vegetables, it is restricted mainly to tomatoes and tomato products (Heinonen. 1991, Mangels et al. 1993). Nonetheless it is one of the major carotenoids in the Western diet.

Various biological effects have been attributed to carotenoids. One possible mechanism of action of carotenoids is via their antioxidant activity but other mechanisms may also contribute to their beneficial effects (Bendich and Olson. 1989). Ernester and co-workers reported the first observations on the biological activities of lycopene in 1959, showing that injected lycopene increased the survival rate of mice which were irradiated and it also increased the resistance of mice toward bacterial infections and the development of ascites tumors (reviewed by Stahl and Sies. 1996).

A few previous epidemiological studies have detected an association between lycopene and the risk of heart diseases or atherosclerosis. In these studies, subjects with lower lycopene levels in adipose tissue (Kohlmeier et al. 1997) or lower blood lycopene concentrations (Street et al. 1994, Klipstein-Grobusch et al. 2000, McQuillan et al. 2001, Gianetti et al. 2002) had an increased risk of coronary events or increased atherosclerosis compared with others, although this has not been noted in all studies (D’Odorico et al. 2000).

The purpose of this work was to study the role of blood lycopene with regard to atherosclerosis and cardiovascular health in middle-aged women and men living in eastern Finland. This work also focuses on the association of the consumption of fruits, berries and vegetables with mortality in men.
2 REVIEW OF THE LITERATURE

2.1 Carotenoids and lycopene

Of more than 600 carotenoid compounds identified, approximately 50 are consumed in the human diet (Krinsky. 1993, Crews et al. 2001). About a dozen carotenoids account for the majority of dietary intake and are found in observable concentrations in human blood and tissues (Stahl and Sies. 1996, Crews et al. 2001) with the most common of them being lycopene, α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin (Crews et al. 2001) (Figure 1). Since they act as antioxidants and are assumed to prevent the damage caused by free radicals, they are proposed to limit against chronic diseases. Like other carotenoids, lycopene is synthesized by plants and microorganisms but not by animals and thus is primarily found in plants (Gerster. 1997).


Figure 1. Chemical structures of common carotenoids and retinol.
2.1.1 Chemistry

Vitamin A is synthesized from β-ionone, thus carotenoids with no-substituted β-ionone cycles (α-carotene, β-carotene and β-cryptoxanthin) have provitamin A activity. The highest activity has been found for all-trans β-carotene. Lycopene lacks the β-ionone ring structure and is therefore devoid of provitamin A activity (Figure 1). Carotenoids rich in conjugated double bonds are divided into two groups, hydrocarbon carotenoids and oxy-carotenoids or xanthophylls. α-Carotene, β-carotene and lycopene belong to the class of hydrocarbon carotenoids and contain 11 conjugated double bonds and also two non-conjugated carbon-carbon double bonds. Astaxanthin, zeaxanthin, cantaxanthin, cryptoxanthin and lutein contain also 11 conjugated double bonds and at least one oxygen atom and thus belong the xanthophylls (Gerster. 1997, Clinton. 1998).

In addition to their provitamin A activity, carotenoids have been suggested to have many other biological functions. They are efficient scavengers of free radicals (Di Mascio et al. 1989). They also have been shown to protect low density lipoproteins (LDL) against oxidation in vitro. However, in vivo results are inconsistent (Krinsky. 1993, Stahl and Sies. 1996, Agarwal and Rao. 1998, Clinton. 1998, Rao and Agarwal. 1998a, Krinsky. 2001). Other functions include enhancement of gap junctions, tumor-suppressive activity, immunomodulation, carcinogenesis and protection of DNA against peroxidation (Stahl and Sies. 1996, Rao and Agarwal. 1998b, Rao and Agarwal. 2000, Rao. 2002).

In most foods, lycopene and other carotenoids typically occur in the all-trans configuration, since this is the most stable form (Clinton. 1998). In human blood and tissues, cis isomers of lycopene make up to >50% of the total lycopene (Stahl et al. 1992, Clinton. 1998, Richelle et al. 2002). In tomatoes and tomato products all-trans lycopene account for 90-95% of the total lycopene (Clinton. 1998, Richelle et al. 2002). Due to the high number of double bonds, carotenoids can undergo trans to cis isomerization. Rotation of any of 11 double bonds of lycopene allows for the formation of a number of cis isomers (Liaaen-Jensen et al. 2001) (Figure 2). Exposure to light or heating can promote the isomerization and this reaction becomes increased with temperature and processing time (Gerster. 1997, Shi and Le Maguer. 2000). However, the processing accounts for only <10% of the increase in the amount of cis isomers (Boileau et al. 2002). In animal studies, a minor (<10%) effect on isomerization has been found due to exposure to the low pH present in the stomach (Boileau et al. 2002). Since there is a large of cis isomers in the human body, there is some evidence that tissue isomerases might be involved in trans-cis isomerization reactions in vivo. However, in the European multicentre supplementation study in 400 healthy participants, the isomer distribution of lycopene in serum after 20-week study period was the same as than present in the capsules (Olmedilla et al. 2002).
2.1.2 Bioavailability and metabolism

The bioavailability of dietary lycopene appears to be dependent upon several factors. In general, absorption of carotenoids depends on their bioavailability from the food matrix and their solubility in micelles (Krinsky, 1993, Boileau et al. 2002). Lycopene is absorbed better from heat processed foods than from unprocessed sources and also better in the presence of dietary fats (Stahl and Sies. 1992, Bohm and Bitsch. 1999). The trans isomers of lycopene are absorbed more poorly than the cis-isomers (Boileau et al. 1999). It is possible that cis isomers are more soluble in bile acid micelles and thus are well transferred into chylomicrons. It has also been shown that the cis-isomers of lycopene primarily accumulate in serum and tissues (Yeum et al. 1996, Gartner et al. 1997). The inter-individual variation of absolute absorption of lycopene is large (Parker et al. 1999). Since other carotenoids may utilize the same pathway, it would be anticipated that the presence may antagonize or perhaps can improve the bioavailability and absorption of lycopene (Gerster. 1997). Other factors that influence the absorption
of carotenoids include the presence of dietary fiber, the health status of an individual and the physical form of the carotenoid (van het Hof et al. 2000a).

Lycopene shares the same chylomicron pathway for its absorption as other lipid soluble compounds. Intestinal uptake of carotenoids occurs by passive diffusion (Figure 3). After absorption into intestinal mucosal cells, lycopene molecules are processed by the chylomicron system. Carotenoids are packaged into triglycerol-rich chylomicrons and secreted into the lymph for delivery to the bloodstream where they are rapidly degraded by lipoprotein lipase. The liver rapidly takes up the resulting chylomicron remnants. Carotenoids are secreted from the bloodstream in very low density lipoproteins (VLDL) by the liver (Boileau et al. 2002). Newly absorbed carotenoids are found in the chylomicron fraction. However, chylomicron catabolism and hepatic uptake of chylomicron remnants are rapid processes (half life 10-15 min) and thus fasting and even postprandial state plasma carotenoids are not found in chylomicrons but instead are located in high density lipoproteins (HDL) and LDL (Parker et al. 1999).

![Figure 3. Absorption of lycopene. Abbreviation: LYC = lycopene](image)

The majority of the hydrocarbon carotenoids, such as β-carotene and lycopene are found in the LDL, with the remainder in the HDL (Stahl and Sies. 1996). However, very little is known about the metabolism of lycopene specifically.

Lycopene accumulates in relatively few tissues with the highest concentrations being found in testis, adrenal and liver (Stahl and Sies. 1996, Gerster. 1997). Other tissues where lycopene has been determined are skin, ovary, adipose, lung and kidney. It is a highly lipophilic compound and is mostly commonly located within cell membranes (Clinton. 1998).

The blood concentration of carotenoid varies between individuals. In an American study by Brady and co-workers with 400 male and female participants, low serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, and lutein+zeaxanthin generally were associated with male gender, smoking, young age, low non-HDL cholesterol, high alcohol consumption and high body mass index (BMI) (Brady et al. 1996). Low serum lycopene levels were associated with old age and low non-HDL cholesterol. In part of the two-center cancer preventing
trial from Florida and Connecticut by Mayne et al., the researchers examined
determinants of plasma lycopene levels in a cross-sectional analysis from 111
male and female participants (Mayne et al. 1999). They found that plasma
cholesterol, marital status and lycopene intake (measured by the food frequency
method) were the most important determinants of the plasma concentration of
lycopene. Women had a slightly but nonsignificantly higher plasma concentration
of lycopene than men, while it did not differ between smokers and non-smokers.
In the lycopene capsule supplementation study, non-dietary lycopene
consumption of 15 mg/day resulted in 2-fold increases in the concentration of
serum lycopene (Olmedilla et al. 2002).

2.1.3 Dietary sources and intake of lycopene

Lycopene is found mainly in tomatoes and tomato products. Other sources of
lycopene are watermelon, rosehips, pink grapefruit and guava (Table 1).
Processing of tomatoes increases the bioavailability of lycopene. Cellular matrix
disruption of raw tomatoes by heating or mechanical homogenization enhances
the lycopene content of triglyceride-rich lipoproteins by 22-38% (Gartner et al.
Tomatoes and olive oil are two well known and major components of the
Mediterranean diet. In addition, most of the tomatoes used in the Southern Europe
are cooked.

Table 1. Lycopene content of selected foods1 (µg/100 g).

<table>
<thead>
<tr>
<th>Food</th>
<th>Lycopene Content (µg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato, fresh</td>
<td>3000-3100</td>
</tr>
<tr>
<td>Tomato, canned</td>
<td>9700</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>8600-9300</td>
</tr>
<tr>
<td>Tomato ketchup</td>
<td>9900-17000</td>
</tr>
<tr>
<td>Watermelon</td>
<td>4100-4900</td>
</tr>
<tr>
<td>Pink grapefruit</td>
<td>1500-3400</td>
</tr>
<tr>
<td>Guava</td>
<td>5400</td>
</tr>
<tr>
<td>Rosehip, canned</td>
<td>780</td>
</tr>
</tbody>
</table>

1 Data taken from (Heinonen. 1991, Mangels et al. 1993, Holden et al. 1999)

There are no recommended dietary allowances (RDA) in the USA or in the
Nordic Nutrition Recommendations for carotenoids. The amount of carotenoids in
the diet is difficult to estimate, partly because methods used for the establishment
of food composition tables are not specific and sensitive enough.

There are major differences in the daily intake of lycopene between populations.
In the Finnish Mobile Clinic Health Examination Survey (Järvinen. 1995) the
daily intake of lycopene was 0.9 mg for women and 0.7 mg for men, whereas the
intake of lycopene was found to be 1.3 mg in Spain (Granado et al. 1996) and as
high as 6.6 mg in the USA (Slattery et al. 2000). Tomatoes account for over 80%
of the lycopene in the American diets. In Finland, the proportion of tomatoes may
be even greater.
2.1.4 Role in human health

Although carotenoids are not essential for human health, they have biological actions that may be important in maintaining health and preventing disease. There are a number of prospective studies, which have investigated the role of lycopene or tomato products in cancer prevention (Weisburger. 1998, Giovannucci. 1999, Sengupta and Das. 1999). The evidence for a benefit is strongest against the cancers of the lung, stomach and particularly prostate gland (Clinton. 1998, Giovannucci. 1999, Cohen. 2002, Giovannucci. 2002, Hadley et al. 2002). Giovannucci has reviewed 61 epidemiological studies examining the association between tomato intake or concentration of lycopene and cancers (Giovannucci. 1999). Almost half (49%) of the studies found relative risks (RR) of 0.6 or less and about two thirds (67%) with RRs less than 0.8. In the recent findings from two large cohort studies from USA and Finland, a high intake of lycopene (the highest fifth) was associated with decreased risk of prostate cancer among 47,000 men in the Health Professionals Follow-Up Study (Giovannucci et al. 2002) and lung cancer among 27,000 male smokers in the Alpha-tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (Holick et al. 2002). In a Japanese study (Ito et al. 2002) the serum concentration of lycopene was inversely associated with cancer mortality among 2,444 participants, and in an American case-control study (Vogt et al. 2002) the serum lycopene level was inversely associated with prostate cancer in 209 cases and 288 controls. However, in the Netherlands Cohort study (Schuurman et al. 2002) lycopene intake was not associated with prostate cancer among 58,000 middle-aged Dutch men. In 26 patients with newly diagnosed prostate cancer, a three-week daily supplement with tomato oleoresin containing 30 mg lycopene seemed to be associated with non-significantly smaller tumor size and lower prostate specific antigen levels (Kucuk et al. 2002). In a Canadian case-control study among 24 participants, there were 44% lower serum and 78% lower prostate tissue lycopene levels found in the prostate cancer patients than in the controls (Rao et al. 1999). The protective effect of blood or dietary lycopene or tomato consumption against lung cancer has been found mainly in case-control studies, whereas data from cohort studies is inconsistent (Giovannucci. 1999, Arab et al. 2002).

Dietary intake of β-carotene has been found to be positively associated with pulmonary function in case-control data (Hu and Cassano. 2000, Chen et al. 2001), although the information for lycopene and other carotenoids is more limited. Lycopene has been measured in reasonable amounts in lung tissue and high serum concentrations of lycopene have been associated with a significantly better lung function, measured as forced expiratory volume, among elderly Dutch participants (Grieveink et al. 2000). A non significant association between plasma concentration and intake of lycopene and forced expiratory volume was observed in American study (Schunemann et al. 2001, Schunemann et al. 2002). In a small supplementation study where a daily dose of 30 mg of lycopene was administered to 20 patients with exercise-induced asthma, 55% of the patients were significantly protected against exercise-induced asthma after one week intervention (Neuman et al. 2000).
It has been proposed that oxidative damage may have a role in the degenerative central nervous system illnesses and cognitive performance (Halliwell. 1994). The brain is an ideal target for free radical damage because of its high lipid composition. Some studies have found a protective effect of blood lycopene on nervous diseases but the results are inconsistent (Jimenez-Jimenez et al. 1993, Snowdon et al. 1996, Schmidt et al. 1998, Foy et al. 1999). In the Nun Study (Snowdon et al. 1996), low plasma levels of lycopene were associated with dependence in self-care among 77-98 years old women. In the Austrian Stroke Prevention Study (Schmidt et al. 1998) no association was found between plasma levels of lycopene and cognitive function among middle-aged and elderly (50-75 years old) men and women. In a case-control study from Ireland (Foy et al. 1999) Parkinson disease patients had significantly lower levels of plasma lycopene than controls, but no association was found in a Spanish case-control study (Jimenez-Jimenez et al. 1993).

There is considerable evidence that carotenoids, particularly the xanthophylls, are negatively associated with the formation of cataract and also age related macular degeneration, diseases which are very common among the elderly (Jacques. 1999, Moeller et al. 2000, Taylor and Hobbs. 2001, Mares-Perlman et al. 2002). The prevalence of cataracts is approximately 45-50% in people over 75 years of age (Taylor and Hobbs. 2001, Mares-Perlman et al. 2002). Lutein and zeaxanthin are carotenoids that have been associated with a decreased risk of cataract since they are the only carotenoids accumulated in the retina and other ocular tissues (Wooten and Hammond. 2002). Oxidative stress is high in the eye due to the intense light exposure and the high rate of oxidative metabolism in the retina. The antioxidative effects of carotenoids may decrease the emergence of cataract and age related macular degeneration. However, the association between specific carotenoids and the risk of cataract is unclear and the results concerning the protective effect of lycopene are conflicting (Brown et al. 1999, Gale et al. 2001, Taylor et al. 2002, Valero et al. 2002).

Carotenoids, particularly β-carotene, have been suggested to contribute to ultraviolet (UV) photosensitivity and protection from UV-induced erythema (Stahl et al. 2000, Alaluf et al. 2002, Stahl and Sies. 2002). In a 12-week supplementation study, both β-carotene supplementation of 24 mg/day and the combination of lycopene, lutein and β-carotene (8 mg/day each) significantly and equally protected against UV-induced erythema (Heinrich et al. 2003). After a 10-week intervention, 40g of tomato paste with 10 g of olive oil per day, reduced by 40% the formation of erythema when compared to the controls who received olive oil only (Stahl et al. 2001).

No toxic actions of lycopene have been reported. However, a prolonged excessive consumption of tomato juice may induce lycopenemia with coloration of skin and the liver (Stahl and Sies. 1996, La Placa et al. 2000).
2.1.4.1 Antioxidant activity

The oxidative modification of LDL particles may play a role in the formation of foam cells, atherosclerotic lesions and CHD (Salonen et al. 1992, Witzum and Hörkkö. 1997). The lipid peroxidation can be divided into three separate stages; initiation, propagation and termination. During initiation, conjugated dienes are formed by removal of a hydrogen atom from the polyunsaturated fatty acids (PUFA). In the propagation phase, molecular oxygen reacts with the carbon-centered radicals formed from PUFA, resulting in highly reactive peroxide radicals. The radical-forming chain reaction continues resulting in production of peroxyl radicals and new fatty acid radicals. In the termination phase, the peroxyl radicals are converted to stable products. The chemical structures of end products vary and can include ketones, aldehydes and alcohols. Antioxidants preventing lipid peroxidation can be divided into two groups: preventative antioxidants and chain breaking antioxidants (Halliwell and Gutteridge. 1989).

Oxidative damage to DNA could be the most important contributor to the risk of cancer development. Attacks on DNA by reactive oxygen, chlorine and nitrogen species could result in oxidative DNA damage. The sequela of this process are numerous oxidation and modification products. Though offer the damage can be repaired by a complex system of enzymes. It is also possible that the enzymes involved in DNA repair are themselves damaged by these reactive species. (Halliwell. 2000)

Very little is known about the specific biological mechanisms through which carotenoids can protect human health. However, many of the biological effects and health benefits of carotenoids are hypothesized to occur via protection against oxidative damage (Di Mascio et al. 1989, Halliwell. 1994, Stahl and Sies. 1996, Gerster. 1997). Research into the antioxidant activity of carotenoids was initiated by the description of their singlet oxygen quenching properties and their ability to trap peroxyl radicals (Stahl and Sies. 1996, Clinton. 1998, Krinsky. 1998). The quenching activity of carotenoids mainly depends on the number of conjugated double bonds. Lycopene consists of 11 conjugated and two non-conjugated double bonds and among the common carotenoids it is known to be the most efficient quencher of singlet oxygen (Di Mascio et al. 1989). A two week period of consumption of a lycopene free diet has been shown to decrease serum lycopene by 50% and in addition to increase lipid peroxidation measured by thiobarbituric acid-reactive substances (TBARS) by 25% (Rao and Agarwal. 1998b). In an intervention study, the daily consumption of tomato products (tomato sauce, tomato juice and tomato oleoresin one week each) reduced the LDL oxidation measured by TBARS and the formation of conjugated dienes (average 25% and 13% over placebo) (Agarwal and Rao. 1998). Consumption of 25 g of tomato puree per day for two weeks reduced the lymphocyte DNA damage by approximately 50% (Porini and Riso. 2000) but did not affect the total antioxidant capacity of plasma in women (Pellegrini et al. 2000). Two weeks of tomato juice supplementation (330 g/day) reduced TBARS by 12% and increased LDL resistance against in vitro oxidation (lag time) by 18% (Bub et al. 2000). Four weeks consumption of tomato juice (500 mL/day) increased the lag time by 42% in patients with type 2 diabetes (Uritchard et al. 2000). In an Italian case-
control study, a significant inverse association between lymphocyte DNA damage, measured by 8-hydroxy-2'-deoxyguanosine (8-OHdG), and plasma concentrations of lycopene, lutein, α- and β-carotene was observed among patients with Alzheimer disease (Mecocci et al. 2002).

Results from two randomized trials, the ATBC study (The ATBC Cancer Prevention Study Group. 1994) and the β-Carotene and Retinol Efficacy Trial (CARET) (Omenn et al. 1996) showed that supplemental β-carotene increased the risk of lung cancer in high risk groups of male smokers. These unexpected findings led to a re-examination of the antioxidant hypothesis of β-carotene and other carotenoids which have been shown to act as prooxidants at very high, non-physiological, supplement doses in contrast to their physiological levels (Krinsky. 2001).

2.1.4.2 Other biological activities

It has also been suggested that lycopene could decrease morbidity of CVD by reducing the serum LDL cholesterol concentration (Fuhrman et al. 1997). In cell cultures, addition of lycopene to macrophage cell lines increased the activity of the LDL receptors. Dietary supplementation with 60 mg of lycopene for three months reduced the plasma LDL cholesterol concentration by 14% (Fuhrman et al. 1997). However, this trial was very small with only 6 participants and without any control group. Thus the reduction of the LDL cholesterol concentration is not a feasible mechanism.

The association with the acute phase response in atherosclerosis might be one mechanism for the protective effect of lycopene. It is the only carotenoid which can reduce both the monocyte adhesion of the human aortic endothelial cell and the expression of adhesion molecules on the cell surface (Martin et al. 2000). Carotenoids have also been shown to enhance immune responses (Bendich. 1989). C-reactive protein (CRP), fibrinogen, and white blood cell count, the markers of inflammation, have been linked to clinically manifested heart disease (Danesh et al. 1998, Ridker et al. 1998). In the National Health and Nutrition Examination Survey III (NHANES III) the serum concentrations of five carotenoids (lycopene, α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin) were significantly lower in those with high levels of serum C-reactive protein among nonsmoking participants of 25-55 years old (Kritchevsky et al. 2000). However, there was no association between individual carotenoids and CRP. Nevertheless in the same NHANES III Survey, the CRP concentrations were inversely related to serum concentrations of lycopene, β-carotene and lutein/zeaxanthin among children and adolescents aged 6-16 years (Ford et al. 2002). In the Nun Study, the serum CRP was associated with decreased plasma levels of lycopene, α-carotene, and β-carotene (Boossalis et al. 1996).

The most commonly proposed mechanisms to explain how lycopene and other carotenoids could protect against cancer are induction of gap-junctional communication, antioxidant activity, induction of detoxifying enzymes and growth control of cancer cells (Stahl and Sies. 1996, Cooper et al. 1999). Lycopene has been shown to inhibit the growth of human oral, lung, endometrial and mammary cancer cells in cultures and to be more effective than α- or β-
carotene in enhancing gap-junctional communication between cancer cells (Levy et al. 1995, Livny et al. 2002).

2.1.4.3 Lycopene and subclinical atherosclerosis

The association between blood concentration or dietary intake of lycopene and carotid atherosclerosis has been studied earlier only in a few studies (Table 2). In a case-control study performed in the participants in the Atherosclerosis Risk in Communities (ARIC) study (Iribarren et al. 1997), an increase in serum concentration of lycopene was associated with nonsignificantly lower odds of being a case (the 90th percentile of IMT) after adjusting for other CVD risk factors. In the same ARIC study, high dietary intake of carotenoids with provitamin A activity was associated with lower prevalence of carotid plaques and lower thickness of the artery wall, although these associations were not statistically significant (Kritchevsky et al. 1998). In that study, the dietary intake of lycopene was not assessed. In the Rotterdam study (Klipstein-Grobusch et al. 2000), serum lycopene was the only carotenoid which associated with decreased risk of aortic atherosclerosis in current and former smokers after adjusting for age and sex. Although the results concerning the whole study population were nonsignificant, the authors concluded that there was evidence for a modest inverse association between serum levels of lycopene and aortic atherosclerosis in the study population. In the Perth Carotid Ultrasound Disease Assessment (CUDAS) Study, an inverse association between plasma lycopene and CC-IMT was found in women. Among men the association was weaker and nonsignificant. In the Bruneck Study, high plasma levels of α- and β-carotene but not lycopene were associated with a decreased 5-year incidence of atherosclerotic lesions in the carotid arteries (D’Odorico et al. 2000). In a recent Italian study (Gianetti et al. 2002), plasma levels of lycopene showed a trend towards lower values in participants with essential hypertension and peripheral vascular disease compared with healthy subjects or participants with uncomplicated hypertension. Serum lycopene also showed a statistical significantly negative correlation with maximal IMT.

In the Etude sur le Vieillissement Artériel (EVA) study, plasma total carotenoid levels were inversely associated with the CCA-IMT in men, but after adjustment the associations were not significant (Bonithon-Kopp et al. 1997). In men, the prevalence of carotid plaques decreased linearly across the quarters of plasma concentration of carotenoids (P <0.016). In that study plasma concentrations of lycopene were not measured.

2.1.4.4 Epidemiological evidence about lycopene or other carotenoids and cardiovascular diseases

A number of epidemiological studies have shown an association between low β-carotene and the risk of CVD. However, the results of previous studies concerning the association between β-carotene and the risk of CVD are inconsistent (Kardinal et al. 1993, Street et al. 1994, Greenberg et al. 1996). Although an
increased plasma β-carotene concentration has been associated with a reduced risk of an acute myocardial infarction (AMI), no effect of β-carotene supplementation was found in the cancer prevention trials (Greenberg et al. 1996, Hennekens et al. 1996). β-Carotene supplementation has even been associated with increased CHD mortality in two trials, the ATBC study and in the Physician Health Study (The ATBC Cancer Prevention Study Group. 1994, Omenn et al. 1996). Isomers of β-carotene exist in varying forms in nature (cis-trans isomeric mixtures) instead of the all-trans forms which are found in synthetic β-carotene supplements (Patrick. 2000). In the supplementation studies, synthetic β-carotene was used, which may have different effects than natural β-carotene. On the other hand, β-carotene and other carotenoids are often found in the same foods, and it is possible that serum β-carotene is only an indicator for the consumption of other carotenoids. Thus, β-carotene could be a marker for favourable dietary or lifestyle factors associated with a reduced risk of CVD. In his recent review, Krinsky hypothesized than high doses of carotenoids have a prooxidant effect (Krinsky. 2001). It is possible that at physiological levels carotenoids could prevent cellular damage, but at the higher doses used in supplementation studies, the ability to protect against cell damage is lost.

The association between blood or tissue lycopene concentration and CHD has been studied earlier in a few studies (Table 2). The multicenter case control European Study of Antioxidants, Myocardial Infarction and Cancer of the Breast (EURAMIC) examined the association between the antioxidant concentration in fat tissue and the risk of myocardial infarction (MI) in ten countries (Kohliemeier et al. 1997). The study found that men with the highest concentrations of lycopene in their adipose tissue had a 48% reduction in the risk for developing CVD when compared with men with the lowest lycopene levels. The model was adjusted for CVD risk factors and adipose tissue levels of α- and β-carotene. In a part of the EURAMIC study from the Malaga center (Gomez-Aracena et al. 1997), there was 60% lower risk of MI among those participants in the highest fifth of adipose tissue lycopene concentration as compared with the participants in the lowest fifth. In a nested case-control study from Washington County (Street et al. 1994), low serum levels of β-carotene, lycopene, lutein and zeaxanthin were associated with an increased risk of subsequent MI in smokers, but not in nonsmokers.

Howard and co-workers speculated that the lower incidence of CHD found in Toulouse compared to Belfast could be partly due to the significantly higher plasma α-carotene, lutein and β-cryptoxanthin concentrations in the inhabitants of Toulouse (Howard et al. 1996). In that study there were no significant differences in plasma lycopene levels between the populations. In the cross sectional Linköping-Vilnus Coronary Risk Assessment Study there were lower plasma levels of lycopene, β-carotene and α-tocopherol and a higher CHD mortality in Lithuania as compared to Sweden (Kristenson et al. 1997). In addition, the authors found a difference in oxidation of LDL between these two populations and speculated that the increase in CHD in Lithuania may be related to the lower antioxidant status of that population. In the prospective Massachusetts Health Care Panel Study (Gaziano et al. 1995) with 1300 participants, a high dietary intake of total carotenoids was associated with a reduced risk of fatal MI and CVD death.
There are no earlier studies of the association between circulating levels of lycopene and the risk of stroke. In the prospective Health Professionals Study (Ascherio et al. 1999), a high dietary intake of lutein was associated with a reduced risk for ischemic stroke, whereas the dietary intake of lycopene or α- or β-carotene measured by food-frequency questionnaire had no association with the stroke risk.
<table>
<thead>
<tr>
<th>Study, nationality of subjects publication year</th>
<th>Type of study Follow-up (years)</th>
<th>Sex</th>
<th>n</th>
<th>Outcome</th>
<th>Sample of lycopene</th>
<th>Mean levels</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>American (Street et al. 1994)</td>
<td>Nested case-control (14)</td>
<td>F, M</td>
<td>369</td>
<td>MI</td>
<td>Serum</td>
<td>0.74 μmol/L</td>
<td>Adjusted odds ratio 0.27 (0.14-0.53) in smokers with serum level of lycopene lower than median. Adjusted odds ratio 0.52 (0.33-0.82) in the highest 10th compared to the lowest 90th percentile.</td>
</tr>
<tr>
<td>EURAMIC Study Multicenter (Kohlmeier et al. 1997)</td>
<td>Case-control</td>
<td>M</td>
<td>1,379</td>
<td>MI</td>
<td>Adipose tissue</td>
<td>0.21-0.36 μg/g</td>
<td>Lower plasma levels of lycopene and higher risk of CHD mortality in Vilnius than in Linköping.</td>
</tr>
<tr>
<td>The Linköping-Vilnius Coronary Disease Risk Assessment Study Swedish, Lithuanian (Kristenson et al. 1997)</td>
<td>Cross sectional</td>
<td>M</td>
<td>210</td>
<td>CHD mortality</td>
<td>Plasma</td>
<td>0.33-0.62 μmol/L</td>
<td>Nonsignificantly lower odds of being a case with increases in lycopene.</td>
</tr>
<tr>
<td>ARIC Study American (Iribarren et al. 1997)</td>
<td>Case-control</td>
<td>F, M</td>
<td>462</td>
<td>IMT</td>
<td>Serum</td>
<td>0.91 μmol/L</td>
<td>Adjusted odds ratio 0.35 (0.13-0.94) in the highest quarter compared to the lowest in smokers.</td>
</tr>
<tr>
<td>The Rotterdam Study Dutch (Klipstein-Grobusch et al. 2000)</td>
<td>Case-control</td>
<td>F, M</td>
<td>216</td>
<td>Plaques of the abdominal aorta</td>
<td>Serum</td>
<td>0.13 μmol/L</td>
<td>Lycopene did not significantly predict against the risk of atherosclerosis.</td>
</tr>
<tr>
<td>Bruneck Study Italian (D’Odorico et al. 2000)</td>
<td>Cross sectional and prospective (5)</td>
<td>F, M</td>
<td>392</td>
<td>Prevalence and incidence of carotid plaques</td>
<td>Serum</td>
<td>0.53-0.76 μmol/L</td>
<td>An inverse association between plasma lycopene and CCA-IMT in women.</td>
</tr>
<tr>
<td>The CUDAS Study Australian (McQuillan et al. 2001)</td>
<td>Cross sectional</td>
<td>F, M</td>
<td>1,111</td>
<td>IMT</td>
<td>Plasma</td>
<td>0.39-0.41 μmol/L</td>
<td>An inverse association between lycopene and IMT in patients with essential hypertension and peripheral vascular diseases compared with healthy controls and patients with uncomplicated hypertension.</td>
</tr>
<tr>
<td>Italian (Gianotti et al. 2002)</td>
<td>Case-control</td>
<td>F, M</td>
<td>33</td>
<td>IMT</td>
<td>Plasma</td>
<td>1.19 μmol/L</td>
<td></td>
</tr>
</tbody>
</table>

CHD = coronary heart disease, CVD = cardiovascular diseases, F = female, M = male, MI = myocardial infarction, IMT = intima media thickness, 1 Healthy controls, 2 Whole study population
2.2 Fruits, berries and vegetables

2.2.1 Dietary intake and recommendations

The Second Joint Task Force of European and other Societies on Coronary Prevention recommended that if an individual wishes to avoid CVD he/she should consume at least 400 grams of fruits and vegetables each day (Wood et al. 1998). The American Heart Associations Eating Plan for Healthy Americans recommends five or more servings per day of a variety of fruits and vegetables (Krauss et al. 2000). There was a campaign by the organization Kotimaiset Kasvikset to promote the consumption of at least 0.5 kg fruits, berries and vegetables per day in Finland in 2002. Recently, it has been suggested that more emphasis should be placed on the daily consumption of fruits and vegetables from diverse sources (Heber and Bowerman. 2001).

In the Finnish Mobile Clinic Study among middle-aged Finnish men, consumption of fruits and vegetables (without potatoes) was 194 g/d (Knek et al. 1994). In a recent Finnish cross-sectional study of 95 young adults, the daily intake of fruits, berries and vegetables was approximately 325 g in men and 375 g in women (Kleemola et al. 2002). In the Seven Countries Study, the lowest average intake of vegetables was in Finland (105 g) and the highest average intake of vegetables was in Italy (260 g) measured around the year 1970 (Mulder et al. 2000). Furthermore, the average fruit intake in Finland was 40 g and the highest intake of fruits was in Crete (460 g). In the Nutrition Status Survey in Massachusetts, middle-aged men consumed fruits and vegetables approximately 510 g/d (Sahyoun et al. 1996). In the Finnish Nutrition Report, the intake of fruits and berries and vegetables was 389 g in 1991 an 428 g in 1999 (Lahti-Koski and Kilkkinen. 2001). In the Health Professionals' Follow-Up Study, and in the Nurses’ Health Study (Joshipura et al. 2001) and in the Framingham Heart Study (Gillman et al. 1995), the intakes of fruits and vegetables were approximately five servings/day and in the Women’s Health Study six servings/day (Liu et al. 2000). The standard portion size of fruits and vegetables was for example one banana or a small glass of juice. In the Framingham Heart Study, potatoes were also included in the total amount of fruits and vegetables, which was not done in the other studies.

The total intake of vegetables and fruits was observed to be the most significant determinant of plasma carotenoids except for lycopene and thus blood carotenoid levels are useful biomarkers of vegetables and fruits intake (Campbell et al. 1994). In a cross-sectional study using data from the NHANES III (Ford et al. 2002), researchers studied the association between serum levels of lycopene, α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin and intakes of vegetables and fruits among children and adolescents aged 12-16 years. They found a significant positive association between the intake of fruits and vegetables and the concentrations of carotenoids, except lycopene. The correlations between blood lycopene and the total intake of vegetables and fruits have been negative, -0.02--0.14, while the correlation between blood lycopene and intake of lycopene-rich foods was in the range 0.11-0.12 (Campbell et al. 1994, Vogt et al. 2002).
2.2.2 Epidemiological evidence about the intake of fruits, berries and vegetables and morbidity and mortality from CVD

Several prospective studies have investigated the association between intake of fruits and vegetables and the risk of CVD and mortality (Table 3). However, even though the beneficial effect of a plant dominated diet has been long suspected, most of the epidemiological data have been published during recent years. In general, these studies have evaluated separately the association of fruits and vegetables instead of both together. Hu and Willett have recently evaluated 147 original investigations and reviews of metabolic studies, epidemiologic studies and dietary intervention trials of diet and CHD. They concluded that a diet with plenty of fruits, vegetables and whole grain and in addition, an adequate intake of unsaturated fats and fish oils is optimal against CHD (Hu and Willett. 2002).

In the Women’s Health Study (Liu et al. 2000) after five-years of follow-up, a high intake of fruits and vegetables was associated with a decreased risk of CVD and MI. After further adjustment for CVD risk factors (age, BMI, smoking, alcohol intake, physical activity, history of high cholesterol, hypertension or diabetes) and use of multivitamins or vitamin C supplements, an inverse trend was still observed, although it was not statistically significant. The association was stronger among participants who had no history of diabetes, hypertension or high cholesterol at baseline. In the Nurses’ Health Study and in the Health Professionals’ Follow-up Study (Joshipura et al. 2001) persons in the top 80% of fruit and vegetable intake had a 20% decreased risk of CHD compared with the lowest fifth during 12 years of follow-up. In that same study (Joshipura et al. 1999) after eight years of follow-up, the risk of ischemic stroke was 31% lower among participants in the highest fifth of fruit and vegetable intake as compared with those in the lowest fifth. In the Physicians’ Health Study (Liu et al. 2001), the association was investigated between intake of carotenoid-rich vegetables and the risk of CHD. The risk was 23% lower among men who consumed at least 2.5 servings/day of vegetables compared with men who consumed less than one serving/day. Among smokers the representative risk was 59% lower.

In the recently published NHANES I (Bazzano et al. 2002), high intake of fruits and vegetables were associated with 24% lower ischemic heart disease (IHD) mortality, 27% lower CHD mortality and 15% lower all-cause mortality as well as 27% lower stroke incidence and 42% lower stroke mortality compared to participants whose average consumption of fruits and vegetables were less than once per day. Although the results concerning IHD-related, stroke-related and overall mortality were not statistically significant, the authors concluded that the frequency of fruit and vegetable intake was inversely associated with stroke incidence, stroke mortality, IHD mortality, CVD mortality and all-cause mortality. In the Study of Men Born in 1913 (Strandhagen et al. 2000) from the west coast of Sweden, lower overall mortality was found among men with high (6-7 times/week) fruit intake during the follow-up of 16 years (P=0.042) and during 26 years (P=0.051) of follow-up compared to men with low fruit intake. The authors did not find any association between fruit or vegetable intake and CVD. The meta-analysis by Law and Morris studied the association between the markers of intake of fruits and berries and the risk of IHD in 20 studies (Law and
Morris. 1998). The corresponding markers were dietary intake of fruits and vegetables, carotenoids, vitamin C, fruit fiber and vegetable fiber, and serum concentrations of carotenoids and vitamin C. The authors concluded that the risk of IHD is about 15% lower at the 90th than it is in the 10% centile of consumption of fruits and berries.

It has also been shown that changes in dietary patterns can have an impact on morbidity. The North Karelia project can claim responsibility for many changes in dietary patterns between 1972 and 1992 in Finland (Pietinen et al. 1988, Vartiainen et al. 2000). The main changes were increased consumption of fruits and vegetables, with also changes towards the use of vegetable margarine and vegetable oils instead of butter and low fat milk replacing of whole milk. In addition, the mean intake of sodium was reduced. During these 20 years, the serum total cholesterol concentrations and blood pressure were decreased by 13% and 9% in men and 18% and 13% in women, respectively. These changes contributed to a large extent to the 55% decline in age standardized CHD mortality in men and to the 68% decline in women.
Table 3. Prospective studies on the association of dietary intake of fruits, berries and vegetables with the risk of morbidity and mortality from CVD, CHD and overall mortality.

<table>
<thead>
<tr>
<th>Study, nationality of subjects, publication year</th>
<th>Years of follow-up</th>
<th>n</th>
<th>Sex Age, y</th>
<th>Outcome</th>
<th>Variable Intake</th>
<th>Dietary method</th>
<th>Main results adjusted relative risks (95% Confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnish Mobile Clinic Study</td>
<td>14</td>
<td>5,133</td>
<td>F, M</td>
<td>CVD mort.</td>
<td>Fruit and vegetables</td>
<td>FFQ</td>
<td>Men: 0.66 (0.46, 0.96) for highest vs. the lowest third of vegetable intake</td>
</tr>
<tr>
<td>(Knekt et al. 1994)</td>
<td></td>
<td></td>
<td>30-69</td>
<td></td>
<td></td>
<td></td>
<td>Women: 0.77 (0.52, 1.12) fruit intake</td>
</tr>
<tr>
<td>The Nutrition Status Survey</td>
<td>9-12</td>
<td>725</td>
<td>F, M</td>
<td>CHD and overall mort.</td>
<td>Vegetables</td>
<td>3-day food record</td>
<td>0.51 (0.27, 0.95) for highest vs. the lowest third (CHD mortality)</td>
</tr>
<tr>
<td>American (Sahyoun et al. 1996)</td>
<td></td>
<td></td>
<td>≥ 60</td>
<td></td>
<td></td>
<td></td>
<td>0.49 (0.31, 0.78) (overall mortality)</td>
</tr>
<tr>
<td>Women’s Health Study</td>
<td>5</td>
<td>39,876</td>
<td>F</td>
<td>CVD and MI</td>
<td>Fruit and vegetables</td>
<td>FFQ</td>
<td>0.85 (0.61, 1.17) for highest vs. the lowest fifth (CVD)</td>
</tr>
<tr>
<td>American (Liu et al. 2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.45 (0.22, 0.91) (CVD) after exclusions</td>
</tr>
<tr>
<td>The Study of Men Born in 1913</td>
<td>16, 26</td>
<td>792</td>
<td>M</td>
<td>Overall mort.</td>
<td>Fruit</td>
<td>FFQ</td>
<td>0.85 (0.76, 0.97), 16 years of follow-up, for 6-7 times per week vs. 0-1 times per week</td>
</tr>
<tr>
<td>Swedish (Strandhagen et al. 2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.90 (0.84, 1.00), 26 years of follow-up</td>
</tr>
<tr>
<td>Alpha-Tocopherol, Beta-carotene Cancer</td>
<td>6.1</td>
<td>25,372</td>
<td>M</td>
<td>MI and CHD mort.</td>
<td>Fruit and vegetables</td>
<td>FFQ</td>
<td>0.87 (0.72, 1.05) for highest vs. the lowest fifth of fruit intake (MI)</td>
</tr>
<tr>
<td>Prevention Study</td>
<td>Finnish (Hirvonen et al. 2000)</td>
<td></td>
<td>50-69</td>
<td></td>
<td></td>
<td></td>
<td>0.77 (0.63, 0.94) vegetable intake (MI)</td>
</tr>
<tr>
<td>The Physicians’ Health Study</td>
<td>12</td>
<td>15,220</td>
<td>M</td>
<td>CHD</td>
<td>Carotenoid rich vegetables</td>
<td>FFQ</td>
<td>0.87 (0.70, 1.08) fruit intake (CHD mortality)</td>
</tr>
<tr>
<td>American (Liu et al. 2001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68 (0.50, 0.95) vegetable intake (CHD mortality)</td>
</tr>
<tr>
<td>The Nurses Health Study &amp; Health Professionals’ Follow-up Study, American (Joshipura et al. 2001)</td>
<td>8-14</td>
<td>42,148</td>
<td>M</td>
<td>CHD</td>
<td>Fruit and vegetables</td>
<td>FFQ</td>
<td>0.77 (0.60, 0.98) for men who consumed at least 2.5 servings/day vs. men who consumed &lt;1 servings/day</td>
</tr>
<tr>
<td>NHANES I</td>
<td>19</td>
<td>9,608</td>
<td>F, M</td>
<td>CVD, CHD and overall mort.</td>
<td>Fruit and vegetables</td>
<td>FFQ</td>
<td>0.73 (0.58, 0.92) for ≥ 3 times/day vs. &lt;1 time/day (CVD mortality)</td>
</tr>
<tr>
<td>American (Bazzano et al. 2002)</td>
<td></td>
<td></td>
<td>25-74</td>
<td></td>
<td></td>
<td></td>
<td>0.76 (0.56, 1.03) (CHD mortality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85 (0.72, 1.00) (overall mortality)</td>
</tr>
</tbody>
</table>

F=Female, M=Male, MI= Myocardial Infarction, CHD=coronary heart disease, CVD=cardiovascular disease, mort.=mortality, NHANES I = the First National Health and Nutrition Examination Survey, FFQ= Food frequency questionnaire
2.2.3 Biological mechanisms

Since fruits, berries and vegetables are chemically complex foods, it is difficult to pinpoint the single nutrients contributing most to the cardioprotective effects. There are probably several potential components and multiple mechanisms in fruits, berries and vegetables that may have protective effects against CVD. The proposed beneficial substances include antioxidant vitamins, folate, fiber and potassium. The antioxidant compounds in fruits and vegetables, such as vitamin C, β-carotene and other carotenoids and flavonoids may have an effect on the risk of CHD by preventing the oxidation of cholesterol in the arteries (Ness and Powles. 1997). However, results from the epidemiological studies concerning antioxidant vitamins and flavonoids and risk of CHD are inconsistent (Law and Morris. 1998, Hollman and Katan. 1999).

Folate, which is found especially in green leafy vegetables, helps to lower blood homocysteine, a proposed risk factor for CVD (Kuller and Evans. 1998). In the Finnish dietary intervention study, a high folate diet (600 μg/day) was associated with a 78% higher serum concentration of folate and a 13% lower plasma concentration of homocysteine when compare with low folate diet (200 μg/day) during five weeks of the diet period (Silaste et al. 2001). In the Dutch study, a high intake of fruits and vegetables (500 g/day) was associated with 11% lower plasma levels of homocysteine and 15% higher plasma folate as compared with a lower intake (100 g/day) (Broekmans et al. 2000). A dietary folate intake of approximately 500 μg/day from vegetables and citrus fruits decreased the plasma homocysteine concentration by 14% during four weeks (Brouwer et al. 1999).

Fruits and vegetables are rich in soluble fiber, and in many epidemiological studies, dietary fiber has been shown to be associated with a decreased risk of CVD (Jacobs et al. 1998, Liu et al. 1999, Ludwig et al. 1999, Todd et al. 1999, Wolk et al. 1999). The main protective properties of fiber may be attributable to their hypocholesterolemic effects, which is a consequence mainly from the alterations they cause in hepatic cholesterol and lipoprotein metabolism (Fernandez. 2001).

There is increasing evidence from epidemiological and clinical studies that potassium intake has an important role in regulating blood pressure (He and MacGregor. 2001). High potassium intake may also have other beneficial effects independent of its effect on blood pressure. However, there are data from a few prospective studies available concerning the effects of potassium intake on CHD. In the NHANES I, a low intake of potassium (the lowest quarter) was associated with 28% higher hazard ratio of stroke as compared with a higher intake (Bazzano et al. 2001). In the Cardiovascular Health Study, subjects in the lowest fifth of potassium intake had an increased risk of stroke (RR 1.76, 95% confidence interval (CI) 1.21 to 2.57) when compared with the highest fifth among participants who were not taking diuretics (Green et al. 2002).

Since dietary recommendations recommend the intake of whole foods instead of nutrients, the effects of these constituents might be best evaluated by investigating the intake of fruits and vegetables. Furthermore, confirmation of the associations between foods and disease risk will help and simplify advice for a heart healthy diet.
3 AIMS OF THE STUDY

The aims of the present work were:

1. To study the association between blood lycopene concentration and ultrasonographically assessed common carotid artery intima-media thickness in both men and women.

2. To study the association between the serum concentration of lycopene and the risk of acute coronary events and stroke in men.

3. To test the hypothesis that a high intake of fruits, berries and vegetables is associated with a decreased mortality in men.
4 MATERIALS AND METHODS

4.1 Study populations

4.1.1 Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study

The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study was a 2x2 factorial placebo-controlled randomized trial to evaluate the effects of a special formulation of vitamin E and slow-released vitamin C supplementation on oxidative stress, lipid peroxidation, atherosclerotic progression, and the incidence of upper respiratory infections in high-risk men and women (Salonen et al. 2003).

The subjects were regularly smoking (>5 cigarettes/day) or nonsmoking men and postmenopausal women aged 45-69 years with a serum cholesterol concentration >5.0 mmol/L at a screening visit. The exclusions included uncontrolled hypertension and severe diseases. Smoking and nonsmoking men and women were separately randomized (in 4 strata) for 3 years into one of the following treatments: 200 mg of RRR-α-tocopherol acetate (272 IU vitamin E) daily, 500 mg of slow release ascorbic acid daily, both antioxidants, or placebo. A total of 520 subjects (256 men, 264 women) were randomized into the trial. Subjects came to the baseline visits at the Research Institute of Public Health, University of Kuopio, between October 1994 and October 1995. The Research Ethics Committee, Hospital District of Northern Savo approved the study protocol. For work I, blood for lycopene and other chemical measurements was drawn before antioxidant supplementation.

4.1.2 Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study

The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study is an on-going population-based study of risk factors for cardiovascular diseases, atherosclerosis, and related outcomes in men from Eastern Finland (Salonen. 1988). The baseline examinations were carried out between March 1984 and December 1989. The study sample was composed of 3235 men aged 42, 48, 54, or 60 years at baseline examination with 2682 (82.9%) actually participating. In original work IV, which was concerned with CVD-related mortality, subjects with a history of CVD at the baseline (n=677) were excluded as these conditions might have affect their dietary choices. Prevalent CVD was defined as either a history of strokes, acute coronary event or angina pectoris or positive angina pectoris on effort in the Rose interview or the use of nitroglycerine tablets once a week or more frequently. After the exclusions, complete data were available for 1950 men. Risk of all-cause and non-CVD-related deaths were estimated without exclusions, thus the analyses included 2641 men whose food record data were available.

The baseline examinations for original work II were carried out between March 1991 and December 1993. These examinations were at the same time the 4-year follow-up survey of the KIHD cohort. Out of a total of 1229 men eligible for the follow-up study, 52 had died, were suffering from severe illness, or had migrated away from the region, and 139 could not be contacted or refused to participate.
Thus, 1038 men were examined in the follow-up study. As previous disease can affect the diet, those men with a prevalent CHD or stroke (n=306) were excluded from the present analyses. Of the remaining 732 men, data on serum lycopene concentrations were available for 725 men.

4.2 Ascertainment of acute follow-up events

Acute coronary events and strokes
The collection of data on and the diagnostic classification of acute coronary events and strokes by the end of 1992 were carried out as a part of the multinational WHO MONICA (MONitoring of Trends and Determinants in CArdiovascular Diseases) project, in which detailed information of all non-fatal or fatal coronary events and strokes were collected prospectively (Tuinastall-Pedoe et al. 1994). At baseline, all KIHD study participants lived in the province of Kuopio, one of the monitoring areas of the Finnish part of the WHO MONICA project (FINMONICA) (Tuomilehto et al. 1992, Tuomilehto et al. 1996). In the FINMONICA study, regional coronary and stroke register teams collected data on coronary events and strokes from hospitals and wards of health centers and classified the events, as described in detail previously (Tuomilehto et al. 1992, Tuomilehto et al. 1996, Salomaa et al. 1997). Data on coronary events and strokes from the beginning of 1993 were obtained by computer linkage to the national hospital discharge and death registers. Coronary events were collected and classified using the same procedures as in the FINMONICA study (Tuomilehto et al. 1992, Tuomilehto et al. 1996, Salomaa et al. 1997).

In original work III based on the 4-year follow-up survey, definite AMIs, probable AMIs, prolonged chest pain episodes and definite ischemic strokes were used as outcome events. If a subject had multiple non-fatal coronary events or strokes during the follow-up, the first event was considered as the end point. The average follow-up time to the first coronary event or stroke was 5.3 years. Of all 41 outcome events, 19 were definite AMIs, 10 probable AMIs, 4 typical acute chest pain episodes and 8 ischemic strokes. The analyses were repeated after 7.6 years follow-up. Among 64 outcome events which occurred, 29 were definite AMIs, 15 probable AMIs, 7 typical acute chest pain episodes and 13 were ischemic strokes.

Deaths
Deaths were ascertained via a computer linkage to the national death registry using the Finnish social security number. There were no losses to follow-up. All deaths that occurred from the study entry to December 31, 1999, were included. Deaths were coded with the International Classification of Diseases, Ninth Revision (World Health Organization 1977). All deaths and deaths caused by CVD (ICD-9 codes 390-459) were chosen as end points. Average follow-up time in original work IV based on KIHD study baseline examinations for the cohort was 12.8 years. During the follow-up time, there were 485 overall deaths, of which 240 resulted from CVD-related causes and 245 from non-CVD-related causes.
4.3 Ultrasonographic assessment of intima-media thickness of common carotid artery wall

Intima-media thickness of carotid artery wall CCA-IMT was assessed by high-resolution B-mode ultrasonography of the right and left common carotid arteries (CCA) at the distal end of the CCA, proximal to the carotid bulb. The ultrasound equipment (Biosound Phase 2, Biosound Inc., Indianapolis, USA) was equipped with an 8 or 10 MHz annular array transducer. In original work II, three distinct angles of interrogation (projections) were used: the anterolateral, the lateral and the posterolateral. Images in original work II were focused on the posterior wall of the right and left CCA. The whole examination was video-recorded. The ultrasonographic scanning of CCA, the carotid bulbs and the proximal internal carotid artery were performed after a supine rest period of 10 minutes (original work I) or 15 minutes (original work II). The scanning was started with a diagnostic examination of an entire accessible carotid tree, to find the site and angle of the greatest IMT at the baseline in the CCA far wall focusing separately on the far and on the near wall. All measurements were performed in diastole, assessed as the phase with the smallest lumen diameter.

Thicknesses of intima-media measurements were made via computerized analysis of the videotaped ultrasound images using Prosound software (University of Southern California, Los Angeles, California) developed by Robert Selzer. The software is based on a three-step edge detection process, which deals with variable densities and discontinuities in the intima-media boundary (Blankenhorn et al. 1993, Selzer et al. 1994). IMT was determined as the average difference at 100 points between the intima/lumen and media/adventitia interface per 1 cm of edge measured (Salonen et al. 2000). The original work I used a “mean IMT” and for original work II, two measures, “mean” and “maximal IMT” were used. The “mean IMT” was computed as the mean of approximately 100 IMT measurements in the right CCA and another 100 measurements in the left CCA. The “maximal IMT” was computed as the average of the points of maximum thickness from the right and left CCAs, indicative of the depth of intrusion of IMT into lumen in this part of the CCA.

In original work I, the assessment of variability of IMT measurement were used as follows: Three technicians scanned 10 subjects twice at a one week interval in 1995. The videotapes from all scannings were analyzed by one observer. The repeat correlations for the mean CCA-IMT were 0.988, 0.995 and 0.998 and pairwise inter-observer correlations 0.975, 0.983 and 0.995.

In original work II, a corresponding variability measurements was used. A separate study concerning the intra- and inter-observer variability of intima-media thickness measurements was carried out in 10 randomly chosen middle-aged eastern Finnish men, who had been participated in the KIHD study. The between-observer coefficient of variation was 10.5% for the first assessments by four observers for both the right and left CCA. The correlation coefficients ranged from 0.90 to 0.99. The intra-observer variability (reproducibility) was described by the absolute value of the difference between the first and the third measurement by each observer. The mean absolute difference was 0.087 mm, which is 8.1% of the mean of all measurements. (Salonen R. et al. 1991)
4.4 Dietary assessment

Dietary intake of nutrients and energy was assessed by four day food recording. Instructions were given and a nutritionist checked the completed food records. Intake of nutrients was estimated using the NUTRICA® software. The data bank of NUTRICA® is compiled using mainly Finnish values of nutrient composition of foods. Food records were calculated for work I and IV in 2000 using NUTRICA® version 2.5, which takes into account losses of vitamins occurring in food preparation. For the assessment of dietary fatty acids in original work IV, the earlier NUTRICA® version 1.0 (1990) was used because of the changes of fatty acid contents of margarines in Finland occurring during the last fifteen years.

For original work IV, all nutrients were adjusted for dietary energy intake using the residual method (Willett and Stampfer. 1998a) Energy adjustment is based on the concept that a larger or more physically active person requires a higher caloric intake, which is associated with a higher absolute intake of all nutrients. Therefore energy adjustment takes into account differences in energy requirements among individuals. The residuals were standardized by the mean nutrient intake of a subject consuming 10 MJ/d, the approximate average total energy intake in this study population. In this work, the intake of fruits, berries and vegetables also included jams, nectars and juices but did not include potatoes.

4.5 Laboratory measurements

In the KIHD study (original work II-IV), at the baseline and the re-examinations, the subjects came to give venous blood samples between 8 and 10 a.m. They were instructed to abstain from ingesting alcohol for three days and from smoking and eating for 12 hours. After the subject had rested in the supine position for 30 minutes, blood samples were obtained by venipuncture and collected into vacuum tubes (Venoject; Terumo, Leuven, Belgium). No tourniquet was used. In the four-year re-examination visit blood for folate and cholesterol determination, for lipoprotein separation and for α-tocopherol, lycopene, β-carotene and retinol measurements was drawn into serum tubes. The baseline and re-examination visits conducted during several years (see 4.1.2).

In the ASAP study (original work I) the subjects came to give venous blood samples between 8 and 10 a.m. They were instructed to abstain from eating for 12 hours and from ingesting alcohol for a week before examinations. After the subject had rested in the sitting position for 5 min, blood was drawn into vacuum tubes (Venoject; Terumo, Leuven, Belgium). On the day before blood drawing, a 24-hour urine was collected. The visits divided according different parts of the year.
4.5.1 Measurement of blood lycopene and \( \beta \)-carotene concentration

Plasma (original work I) and serum (original works II and III) for lycopene and \( \beta \)-carotene determination was extracted with ethanol and hexane, the measurements were carried out by a reversed phase high performance liquid chromatography (HPLC) method in samples that had been kept at -80\(^\circ\)C for three to 15 months (original work I) and four to 36 months (original works II and III) (Porkkala-Sarataho et al. 1996). The concentrations of \( \alpha \)-tocopherol and retinol can be measured in the same run. Briefly, 200 \( \mu \)L of heparinized plasma was extracted with 5 mL of hexane and 1 mL of ethanol containing \( \alpha \)-tocopherol acetate as an internal standard. The hexane layer was separated and evaporated to dryness. The residue was dissolved in mobile phase. The mobile phase consisted of acetonitrile-methanol-chloroform, 47+47+6 by volume. A reverse phase C18 column was used, and peaks were detected at wavelengths of 470 nm for lycopene and 454 nm for \( \beta \)-carotene by a diode array detector (Model 168, Beckman Instruments, USA). Pure analytes from Sigma (St Louis, USA) were used as primary standards and their concentrations were determined spectrophotometrically according to Thurnham et al. (Thurnham et al. 1988). As the stability of the pure carotenoids is poor, a frozen plasma pool was used as the secondary standard with the analysis batches. The extraction efficiency was tested by adding known amounts of lycopene standard (Sigma) and calculating the recovery. The recoveries were 80\% for 0.45 \( \mu \)mol/L and 75\% for 1.35 \( \mu \)mol/L of lycopene added. For evaluation of the stability of lycopene, we have calculated the means of the first 56 samples stored for 36 months (0.113 \( \mu \)mol/L) and the last 56 samples (0.110 \( \mu \)mol/L) stored only for four months. Serum and plasma lycopene samples were well preserved until the measurement. The detection limit for each carotenoid with this method was 0.03-0.07 \( \mu \)mol/L. The values below the detection limits of the assay batch were marked as 0.00 in the statistical analysis. The coefficients of variation (CV) were determined with a serum pool analyzed in 25 separate batches. The CV was 11.0\% for lycopene and 16.2\% for \( \beta \)-carotene.

4.5.2 Other laboratory measurements

In the baseline of the KIHD study, the methods used for determination of serum total, LDL and HDL cholesterol and triglycerides (Salonen J.T. et al. 1991) and 24-hour urinary excretion of nicotine metabolites (Puhakainen et al. 1987) have been described previously. Diabetes was defined as either a previous diagnosis of diabetes with oral antidiabetic drug or insulin treatment, or fasting whole blood glucose concentration \( \geq \)6.7 mmol/L. Serum insulin level was determined by a radioimmunoassay, serum haptoglobin and plasma fibrinogen level by a coagulometer. Plasma vitamin C was measured by HPLC (Nyyssönen et al. 1997).

In the four-year follow-up survey of the KIHD study (original works II and III), serum for \( \alpha \)-tocopherol determinations was stored at -80\(^\circ\)C until extracted with ethanol and hexane and measured by the HPLC method as described in 4.5.1. Lipoproteins were separated from fresh serum samples by combined ultracentrifugation and precipitation (Salonen et al. 1995). Serum total, LDL and
HDL cholesterol (Kone Instruments, Espoo, Finland) and triglyceride (Boehringer Mannheim, Mannheim, Germany) concentrations were determined enzymatically with an automatic analyzer (Kone Specific, Kone Instruments, Finland). Serum folate was determined by a radioimmunoassay (Bio-Rad, Hercules, California, USA).

In the ASAP study, plasma α-tocopherol was determined by HPLC (see 4.5.1). Serum total, LDL and HDL cholesterol were determined from fresh samples with an enzymatic colorimetric method (Boehringer Mannheim, Mannheim, Germany). Serum LDL cholesterol was measured after precipitation with polyvinyl sulphate (Boehringer Mannheim, Mannheim, Germany) and HDL cholesterol from supernatant after magnesium chloride dextran sulphate precipitation. Serum triglycerides were measured colorimetrically (Boehringer Mannheim, Mannheim, Germany). The 24-hour urinary excretion of nicotine metabolites was measured by a colorimetric method (Erlab, Kuopio, Finland). To separate the effect of α-tocopherol from that of serum lipids, values for lipid standardized α-tocopherol were used in the statistical analysis (Salonen et al. 1995). Plasma total homocysteine (tHcy) concentration was determined with an HPLC method (Voutilainen et al. 1998).

4.6 Other measurements

In the KIHD study (original works II-IV) the daily number of cigarettes, cigars, and pipefuls of tobacco, duration of regular smoking in years, history of AMI, angina pectoris, and medication were recorded with a self-administered questionnaire, which was checked by an interviewer. A subject was defined as a smoker if he had ever smoked on a regular basis and had smoked cigarettes, cigars, or pipe within the past 30 days. Repeat interviews to obtain medical history of CHD was conducted by a physician. The family history of CHD was defined as positive if the father, mother, sister, or brother of the subject had a history of CHD. The measurement of maximal oxygen uptake has been described previously (Lakka et al. 1994).

Two trained nurses measured resting blood pressure with a random-zero mercury sphygmomanometer (Hawksley, United Kingdom). The measuring protocol included, after five minutes of supine rest, three measurements in the supine, one in the standing and two in the sitting positions at five minutes’ intervals. The mean of all six measurements was used as the systolic and diastolic blood pressure. Subjects with systolic blood pressure >160 mm Hg or diastolic blood pressure >95 mm Hg or who used antihypertensive drugs were classified as hypertensive.

In the ASAP study (original work I) blood pressure was measured manually with the subject in a sitting position after a rest period of 10 minutes, three measurements at 3-minute intervals, and the means were used.

The BMI was computed as the ratio of weight to the square of height (kg/m²). The consumption of alcohol in the previous 12 months was assessed with the Nordic Alcohol Consumption Inventory, which contains 15 items (Simpura. 1981).
4.7 Statistical analysis

For original work I, the subjects were divided into two categories according to the concentration of plasma lycopene. The higher-than-median level was compared to the lower level. The statistical significance of differences between these two lycopene groups in the main characteristics of the subjects was assessed by the Student’s t-test. The association between plasma lycopene and ultrasonographically assessed CCA-IMT was tested for statistical significance also by using covariance analysis, adjusting for age, smoking, serum triglycerides, serum HDL and LDL cholesterol, plasma concentration of tHcy and systolic blood pressure, ultrasound observer and intake of saturated fatty acids, vitamin C, vitamin E and fiber.

For original works II and III, the subjects were classified into quarters according to their serum levels of lycopene. In original work II, the association between serum levels of lycopene and ultrasonographically assessed CCA-IMT was tested for statistical significance by using covariance analysis. Three different sets of covariates were used: model 1) age, ultrasound observer, examination years; model 2) model 1 and also systolic blood pressure, serum HDL and LDL cholesterol, and smoking; model 3) model 2 and serum triglycerides, BMI, and three nutritional factors, serum folate, α-carotene and β-tocopherol. The statistical significance of differences between these lycopene groups in the main characteristics of the subjects was studied by using the analysis of variance (one-way ANOVA). The correlation between CCA-IMT and nutritional factors and cardiovascular risk factors were estimated by Spearman’s correlation coefficients.

In work III, the lowest quarter was compared with the three higher quarters. The relationship of serum lycopene with the risk of acute coronary events and strokes was analyzed using Cox’ proportional hazards’ models. Relative hazards (risks), adjusted for other risk factors, were estimated as antilogarithms of coefficients for independent variables. The CIs were estimated based on the assumption of asymptotic normality of estimates.

In work IV, the subjects were divided into fifths according to the intake of fruits, berries and vegetables. Risk ratios (RR) for CVD-related and non-CVD-related deaths likewise a total mortality, adjusted for risk factors were estimated by Cox’ proportional hazards’ model. Five different sets of covariates were used: 1) age and examination years; 2) model 1 and smoking (measured as urinary excretion of nicotine metabolites), and alcohol consumption; 3) model 2 and systolic and diastolic blood pressure, BMI, diabetes, serum HDL and LDL cholesterol and triglyceride levels; 4) model 3 and maximal oxygen uptake in exercise test; 5) model 4 and energy adjusted intakes of vitamin C and E, β-carotene, lycopene, fibre and folate. Relative hazards (risks), adjusted for covariates, were estimated as antilogarithms of coefficients in multivariate models.

The CIs were estimated under the assumption of asymptotic normality of the estimates. Baseline characteristics of the cohort members in fifths of intake of fruits, berries and vegetables were expressed as means and compared with ANOVA. The correlations between CVD risk factors, mean and maximal CCA-
IMT and dietary intake of fruits, berries and vegetables were estimated by Pearson’s correlation coefficients.

In original works I and III, the statistical analyses were carried out using SPSS 9.01 for Windows software and in works II and IV SPSS 10.0 for Windows. All statistical tests were two-tailed and a p-value less than .05 was considered as significant.
5 RESULTS

5.1 Blood lycopene and the intima-media thickness of the carotid artery wall (I, II)

ASAP Study
In original work I, the mean concentration of plasma lycopene in the ASAP study population was 0.16 (SD 0.11) μmol/L, ranging from below the detection limit to 0.62 μmol/L in men and to 0.64 μmol/L in women. The mean plasma concentrations of lycopene were higher in women (0.17, SD 0.11 μmol/L) than in men (0.14, SD 0.12 μmol/L) (P=0.007 for difference).

The subjects were divided into two categories according to the concentration of plasma lycopene. The higher-than-median level was compared to the lower level. For men, age, the plasma concentration of tHcy and dietary vitamin C differed statistically significantly between the men who had plasma level of lycopene lower than the median (<0.12 μmol/L) compared to those who had a value higher than the median (≥0.12 μmol/L). Those women with a lower level of plasma lycopene (<0.15 μmol/L) differed significantly with regard to age, HDL cholesterol, systolic blood pressure, plasma concentration of tHcy and dietary vitamin C intake from those with the higher level of plasma lycopene (≥0.15 μmol/L).

Mean IMT of the right and left CCA was 1.18 mm in men and 0.95 mm in women with low plasma lycopene level and 0.97 mm in men (P<0.001 for difference) and 0.89 mm in women (P=0.012 for difference) with higher plasma levels of lycopene. In men in the highest quarter of plasma lycopene levels, the CCA-IMT was 17.5% and in women 5.6% lower than in those men and women in the lowest quarter of plasma lycopene level (Figure 4).

In a covariance analysis, adjusting for age, smoking, serum triglycerides, serum HDL and LDL cholesterol, plasma tHcy and systolic blood pressure, ultrasound observer and intake of four nutrients (proportion of saturated fatty acids of total energy, vitamin C, vitamin E and fiber), a low plasma lycopene level was associated with a 17.8% increased CCA-IMT in men, compared with men whose plasma levels of lycopene were higher than the median (P=0.003 for difference). In women, the difference did not remain significant after the adjustments. We repeated analysis in smokers and non-smokers. The association between plasma level of lycopene and CCA-IMT seems to be stronger among non-smokers than among smokers. However the interaction was nonsignificant (P=0.472 for women, P=0.732 for men).

We also studied the intake of lycopene and found that 31% of men and 18% of women did not consume any lycopene at all during the four-day food recording and the intake of lycopene differed by as much as 52% between the examination months.
Figure 4. Mean intima-media thickness (IMT) of the carotid artery wall in the quarters of plasma lycopene concentration in middle-aged Finnish men and women in the ASAP study. The plasma lycopene concentration quarters for men were <0.06, 0.06-0.11, 0.12-0.18 and >0.18 μmol/L and for women <0.09, 0.09-0.15, 0.16-0.22 and >0.22 μmol/L.

KIHD Study
In the KIHD study (original work II) the mean concentration of serum lycopene was 0.15 (SD 0.14) μmol/L, ranging from below the detection limit to 1.02 μmol/L. The subjects were classified into quarters according to their serum levels of lycopene. Serum β-carotene, folate, and α-tocopherol, age, systolic blood pressure, BMI and smoking differed statistically significantly between the quarters of serum concentration of lycopene.

Mean and maximal CCA-IMT differed significantly between the quarters of serum concentration of lycopene (P<0.001 for both). In a covariance analysis we observed a significant inverse association between serum levels of lycopene and mean and maximal CCA-IMT. In model 1 after adjustment for age, examination years and ultrasound observer, men in the lowest quarter of serum concentration of lycopene exhibited a significant increment in both mean CCA-IMT (P<0.001 for difference) and maximal CCA-IMT (P<0.001 for difference) as compared to the other men. Additional adjustment for covariates (model 2: model 1 and systolic blood pressure, serum LDL and HDL cholesterol and smoking) did not change the observed results (P=0.001 and P<0.001 for difference). Furthermore, in model 3, with adjustment for model 2 and serum triglycerides, BMI, serum folate, β-carotene and α-tocopherol concentration, a similar inverse trend
remained (P=0.005 and P=0.001 for difference, respectively). The increments of the mean and maximal CCA-IMT were linear across the quarters of the serum concentration of lycopene. The P-values for a linear trend for mean and maximal CCA-IMT in model 1 were 0.001 and <0.001. In all models, the mean and maximal CCA-IMT decreased linearly across the quarters of serum lycopene increments (Table 4). The association between serum level of lycopene and CCA-IMT seems to be stronger in smokers than in non-smokers. However, the interaction was nonsignificant (P=0.068).
Table 4. Adjusted (mean and maximal) thickness of intima-media of common carotid artery (CCA-IMT) (95% confidence intervals) in quarters of serum lycopene concentration (<0.04, 0.04-0.13, 0.14-0.22, >0.22 μmol/L) in the three different models in the KIHD study.

<table>
<thead>
<tr>
<th>Serum lycopene</th>
<th>Lowest quarter</th>
<th>Second lowest quarter</th>
<th>Second highest quarter</th>
<th>Highest quarter</th>
<th>P for linear trend(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CCA-IMT (mm)</td>
<td>0.92 (0.89, 0.95)</td>
<td>0.88 (0.85, 0.90)</td>
<td>0.87 (0.85, 0.89)</td>
<td>0.85 (0.83, 0.88)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maximal CCA-IMT(mm)</td>
<td>1.28 (1.25, 1.32)</td>
<td>1.21 (1.18, 1.24)</td>
<td>1.20 (1.16, 1.23)</td>
<td>1.18 (1.15, 1.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Model 2:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CCA-IMT (mm)</td>
<td>0.92 (0.89, 0.94)</td>
<td>0.88 (0.85, 0.90)</td>
<td>0.87 (0.85, 0.89)</td>
<td>0.86 (0.83, 0.88)</td>
<td>0.012</td>
</tr>
<tr>
<td>Maximal CCA-IMT(mm)</td>
<td>1.27 (1.24, 1.31)</td>
<td>1.21 (1.18, 1.25)</td>
<td>1.20 (1.17, 1.23)</td>
<td>1.19 (1.15, 1.22)</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Model 3:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CCA-IMT (mm)</td>
<td>0.91 (0.89, 0.93)</td>
<td>0.87 (0.85, 0.90)</td>
<td>0.87 (0.85, 0.90)</td>
<td>0.86 (0.84, 0.89)</td>
<td>0.039</td>
</tr>
<tr>
<td>Maximal CCA-IMT(mm)</td>
<td>1.27 (1.23, 1.30)</td>
<td>1.21 (1.18, 1.24)</td>
<td>1.20 (1.17, 1.23)</td>
<td>1.19 (1.16, 1.23)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age, examination years and ultrasound observer
Model 2: Model 1 + smoking, systolic blood pressure, serum concentrations of HDL and LDL cholesterol
Model 3: Model 2 + serum triglycerides, BMI, serum concentrations of α-tocopherol, folate and β-carotene
\(^1\) Covariance analysis
5.2 Serum lycopene and the incidence of acute coronary events and ischaemic stroke (III)

Men who developed an acute coronary event or an ischaemic stroke had a 39% lower (P=0.003) serum levels of lycopene, and also a significantly lower plasma level of vitamin C, higher systolic blood pressure and age than the other men. We categorised the subjects into quarters of the serum level of lycopene (≤0.07, 0.08 - 0.14, 0.15 - 0.24 and >0.24 μmol/L) and compared the lowest to the other quarters. Men in the lowest quarter of serum concentration of lycopene differed statistically significantly from men with higher lycopene concentrations with regard to systolic blood pressure and age and with regard to nutritional factors including plasma vitamin C, serum folate and β-carotene.

Twenty-three men (12.6%) in the lowest quarter and 18 men (3.3%) in other quarters suffered an acute coronary event or stroke (P for difference <0.001) during the average follow-up time of five years and three months. In a Cox’ proportional hazards’ model adjusting for age, examination years, systolic blood pressure and three nutritional factors (plasma vitamin C and serum β-carotene and folate), men in the lowest quarter of serum concentration of lycopene had a 3.3-fold (95% CI 1.7 to 6.4) risk of an acute coronary event or stroke as compared with the others. We repeated a similar analysis for acute coronary events alone. Adjusted RR for acute coronary events among men in the lowest quarter of serum level of lycopene was a 2.8 (95% CI 1.4 to 5.7). The risk of acute coronary events or stroke decreased on the average by 4% for each increment in serum lycopene concentration of 0.01 μmol/L.

We repeated the analysis after 7 years and 7 months (up to 9 years 10 months) of follow-up. Thirty-one men (16.8%) in the lowest quarter and 33 men (6.0%) in the three other quarters developed an acute coronary event or stroke (P for difference <0.001) during the follow-up. In an adjusted Cox’ model (adjusting for age, examination years, systolic blood pressure, serum β-carotene, folate and plasma vitamin C), men in the lowest quarter of serum concentration of lycopene had a 2.4-fold (95% CI 1.4 to 4.0) risk of an acute coronary event or stroke as compared with others. The corresponding adjusted RR for acute coronary events alone among men in the lowest quarter of serum level of lycopene was a 2.3 fold risk (95% CI 1.3 to 4.1). In Figure 5, the cumulative increase of morbidity in quarters of serum concentration of lycopene is presented to illustrate the earlier occurrence of acute coronary event or stroke among men in the lowest quarter as compared to the others. The association seems to be stronger in young (46-55 years) than in old (>55 years) men (adjusted RRs 3.3, 95% CI 1.4 to 7.7 and 2.0, 95% CI 1.0 to 3.9, respectively). We also repeated the analysis among smokers and non-smokers. The association between low serum concentration of lycopene and the risk of acute coronary events or stroke appeared to be at the same level in smokers and non-smokers.

We also studied the dietary intake of lycopene in the baseline of the KIHD study and found that over 40% of the population did not consume lycopene at all during the four-day food recording. We repeated the measurement in the eleven-year follow-up examinations, and found that 20% were still not consuming lycopene at all during the period of the food recording.
Figure 5. Cumulative morbidity of an acute coronary event or a stroke up to 9 years and 10 months (mean 7 years 7 months) of follow-up according to the quarters of serum concentration of lycopene in middle-aged Finnish men in the KIHD study.

5.3 Intake of fruits, berries and vegetables and mortality (IV)

The mean daily intake of fruits, berries and vegetables was 284 g (2-1538 g). The subjects were divided into fifths of the mean daily intake (<133, 133-214, 215-293, 294-408 and >408 g). Men in the highest fifth of intake were younger, smoked less and drank less alcohol. Also blood pressure, plasma levels of fibrinogen, serum total and LDL cholesterol were lower among these men compared with the others.

The intake of fruits, berries and vegetables was 41% lower in men who died of CVD during the first five years of follow-up and 20% lower when including all CVD deaths during the whole follow-up time of 12.8 years as compared to the men who survived. The respective estimates concerning non-CVD-related and all-cause deaths were 21% versus 12% and 13% versus 16%, (P<0.001 for all differences). During the average follow-up time, the occurrences of CVD death, non-CVD death, as well as total mortality were highest in those men in the lowest fifth of consumption of fruits, berries and vegetables.

In a Cox’ proportional hazards’ model we observed a significant inverse association between the intake of fruits, berries and vegetables and all-cause,
CVD- and non-CVD-related mortality. In model 1 after adjustment for age and examination years, men in the highest intake fifth had a RR of overall death of 0.65 (95% CI 0.49-0.86), CVD-related death of 0.43 (95% CI 0.24-0.76) and non-CVD-related death of 0.56 (95% CI 0.38-0.83) as compared with men in the lowest intake fifth. Additional adjustments (model 2: model 1 + smoking and alcohol consumption and model 3: model 2 + systolic and diastolic blood pressure, BMI, diabetes, serum HDL and LDL cholesterol and triglyceride levels) did not notably change the observed results. Also in model 4 (model 3 + maximal oxygen uptake in exercise test) an inverse trend was found between intake of fruits, berries and vegetables and all-cause, CVD- and also non-CVD-related mortality. In Figure 6 the cumulative increase of all-cause and CVD-related mortality (adjusted for age and examination years) in fifths of fruit, berry and vegetable intake is presented.
Figure 6. Cumulative all-cause and cardiovascular disease (CVD)-related mortality up to 15.75 years of follow-up according to the fifths of intake of fruits, berries and vegetables in middle aged Finnish men in the KIHD study.
6. DISCUSSION

6.1 Lycopene, atherosclerosis and the risk of coronary events and stroke

Our main finding in original works I-III was that high blood concentration of lycopene has a protective role against CHD in middle-aged men living in eastern Finland. This association was noticed both in the follow-up study and in the cross-sectional data. The association was strong and other CVD risk factors, including the major plant derived protective dietary factors, did not explain this association.

The mean blood concentration of lycopene has varied extensively in population-based studies from European countries and USA (Street et al. 1994, Kristenson et al. 1997, D’Odorico et al. 2000, Klipstein-Grobusch et al. 2000, McQuillan et al. 2001, Gianetti et al. 2002). There is only one earlier study, the Rotterdam Study (Klipstein-Grobusch et al. 1999), in which the mean circulating levels of lycopene were as low as in our study. The most likely explanation for these low levels is the low dietary intake of tomatoes or tomato products in Finland. Since in our study subjects also had very low levels of lycopene, the protective effect of lycopene was stronger than in the results from other countries. In these other countries the lack of subjects with low circulating levels of lycopene could be one explanation for the weak association found in these studies.

It is possible that the serum levels of lycopene could be only an indicator for some other beneficial dietary or lifestyle factors. However, the association of lycopene with atherosclerosis was statistically significant in both ASAP and KIHD studies, and adjustment for other plant-derived nutrients did not weaken the observed results. In addition, the correlation between blood lycopene concentrations and total intake of vegetables and fruits has been previously shown to be very low (Campbell et al. 1994), thus blood lycopene may not simply be a reflection of a healthy diet.

The effect of plasma lycopene on CCA-IMT in the ASAP study was gender specific. The low concentrations of plasma lycopene were associated with increased CCA-IMT in middle-aged men, but in women, the association was weaker and statistically non-significant. There are some possible explanations for this difference. One plausible explanation could be that women have a most effective endogenous antioxidative system. Another explanation could be that in women the incidence of CVD and the increase of CCA-IMT occurs at an older age than in men.

The association between blood concentration of lycopene and CCA-IMT in the male participants in the ASAP study was stronger among non-smokers than smokers whereas in the KIHD study we noted a corresponding stronger association among the smokers. The association between serum concentration of lycopene and coronary events and strokes was at the same level among smokers and non-smokers. It has been shown earlier that smokers have lower plasma concentrations of most carotenoids, but results concerning blood levels of lycopene according to the smoking status are confusing (Arab and Steck. 2000). The relation between lycopene and smoking is incompletely understood and more knowledge is needed to clarify this association.
6.2 Intake of fruits, berries and vegetables and mortality

We found in the prospective KIHD study that a moderate-to-high consumption of fruits, berries and vegetables is associated with a reduced risk of CVD-related, non-CVD related and overall mortality. Although it is possible that this finding is partly explained by the healthy lifestyles associated with a high fruit, berry and vegetable intake, adjustment for traditional CVD risk factors or lifestyle habits did not fully account for the observed beneficial effect of higher consumption. When this was being evaluated we used five different sets of covariates. The association remained after the adjustment for the potential confounding variables. The adjustment for protective dietary factors of plants weakened the association. However, the change in RR was fairly low, and thus there might be further dietary mediators of fruits, berries and vegetables other than intakes of vitamin C and E, β-carotene, lycopene, folate and fiber. We also found that maximal oxygen uptake in exercise test correlated positively with the intakes of fruits, berries and vegetables. It seems that men who consume more plant products have also a healthier lifestyle than men who consume less of these foods. Therefore, when studying associations between nutrients and diseases, it is important to include not only the traditional risk factors to the statistical models, but also other factors which are a part of a healthy lifestyle, such as exercise.

6.3 Dietary intake of lycopene and fruits, berries and vegetables

In original work IV, the dietary method we used was a four-day food record. The food record method has some merits when compared to other methods, e.g. dietary history method, food frequency questionnaire or a 24-hour recall. A major advantage of a food record method compared with recalls is that it does not rely on memory. Another strength is that portion sizes can be directly measured rather than estimated afterwards (Willett and Stampfer. 1998b) However, there are also some disadvantages of the food record method. The effect of season is not taken into account and it is also possible that the study participants make changes to their diet to make the record look better. However, a picture book with pictures of different size of portions was given to the participants to help them in evaluating portion size and the food records were checked by nutritionist together with participant. In addition, the dietary data has been complemented by food frequency data and biochemical markers. The food records have also been validated by repeating them among 50 participants after 12 months from the baseline measurements.

The daily intake of lycopene in Finland is very low. In other published Finnish studies, for example, the ATBC study (Hirvonen et al. 2000) and the Finnish Mobile Clinic Health Examination Survey (Järvinen. 1995) the daily intake of lycopene has been clearly under one mg/day. The intake of lycopene has been found to be as much as seven to ten times higher, in the USA (Forman et al. 1993). In the Finnish Balance Sheet for Food Commodities, the mean daily intake of tomatoes was 24 g in 1991 and 29 g in 1999 (Balance Sheet for Food Commodities 2000 and 2001. 2002). In the USA, tomato consumption in the 1990’s was 42 g/day (Lucier et al. 2000). In the baseline of the KIHD study
over, 40% of the population did not consume any lycopene during four-day food recording, in the eleven-year follow-up examinations still 20% were not consuming lycopene at all during food recording.

Dietary intake of lycopene was assessed in the KIHD and ASAP studies by the four-day food recording. However, four days is too short a time to estimate the intake of lycopene, at least in Finland since there is such a large day to day variation in the intake of lycopene. In the ASAP study the intake of lycopene differed by as much as 52% between the examination months. Thus the association between intake of lycopene and the disease risk should be evaluated using an appropriate food frequency questionnaire method.

The daily intake of fruits, berries and vegetables in our studies is at a comparable level with other European and American studies. In the KIHD study, the mean daily intake of fruits, berries and vegetables was 284 g. In the ASAP study, the corresponding values were 289 g in women and 346 g in men. In the Finnish Mobile Clinic Study, in the Finnish centres of the Seven Countries Study and in the ATBC Study, the consumptions of fruits and vegetables were lower than in our studies, in the Finnish Nutrition Report which represent whole population and in a recent Finnish study with younger participants, the corresponding intake was higher than in our studies (Knekt et al. 1994, Mulder et al. 2000, Lahti-Koski and Kilkkinnen. 2001, Kleemola et al. 2002, Michaud et al. 2002). However, the baseline measurements of the Finnish Mobile Clinic Study and the Seven Countries Study were carried out around 1960 and 1970. All participants of the ATBC study were smokers, whose consumption of fruits and vegetables could be different than that of the general male population. The intake of fruits and vegetables varies among European populations in men from approximately 100 g (former Yugoslavia) to almost 700 g (Crete) (Mulder et al. 2000, Voorrips et al. 2000). The corresponding values in American studies have been under 300g to over half kilogram (Sahyoun et al. 1996, Bazzano et al. 2002).

6.4 Methods and study design

When evaluating our results, some limitations have to be taken into account. First, we cannot rule out the possibility that plasma and serum lycopene samples had deteriorated during the storage time of four to 36 months at -80°C. However, the lycopene values from samples stored for 36 months were rather similar to those from assays of plasma stored only four months (see 4.5.1). Secondly, the follow-up period of original work III was quite short (5.3 years) and we had only a limited number of outcome events. During a longer follow-up period, diet could have changed and attenuated the association between dietary biomarkers and diseases. However, the association was similar when using a follow-up time of 7.6 years. There are also other prospective studies in which the follow-up times have been equal or even shorter than that used in our study (Klipstein-Grobusch et al. 1999, Gaziano et al. 1995).

Thirdly, we cannot fully exclude the possibility that part of the association between blood lycopene and atherosclerosis, coronary events and strokes may be secondary to confounding factors such as other dietary and lifestyle factors associated with the risk of CVD. Other plant-derived nutrients such as folate,
vitamin C or flavonoids of healthier quality of diet might contribute to the apparent benefit. However, other markers of a healthy diet, such as dietary intake or blood concentrations of vitamin C, vitamin E, folate or β-carotene did not attenuate the association between lycopene and atherosclerosis, coronary events or strokes in our study. However, there was an inverse association between blood lycopene and total intake of vegetables and fruits (Campbell et al. 1994, Ford et al. 2002, Vogt et al. 2002). Subjects who exercise more, eat a healthier diet and consume more energy, and they are likely to be the same subjects whose dietary intake of lycopene is the highest. Whatever the reason, it is possible that the influence of a healthy lifestyle cannot be fully controlled for by the multivariate models. It is also evident we should stress the benefits of increasing our consumption of vegetables, fruits and whole cereals at the expense of other foods considered less healthy, such as fatty meat, sweet desserts and pastries.
7 SUMMARY AND CONCLUSIONS

The results of this work can be summarized as follows:
1. Low blood levels of lycopene were associated with early atherosclerosis as measured in CCA-IMT in middle-aged men from eastern Finland,
2. Low blood levels of lycopene were associated with increased risk of CVD in men,
3. High or moderate intake of fruits, berries and vegetables was associated with a reduced risk of CVD-related, non-CVD related and overall mortality in middle-aged men living in eastern Finland.

On the basis of this work we can say that the blood concentration of lycopene, a biomarker of tomato-rich food, may play a role in delaying early stages of atherogenesis and also may decrease the risk of atherosclerotic vascular events and thus may have clinical and public health relevance, at least in men. Our findings provide additional evidence for the view that a higher intake of fruit and vegetable can help prevent heart diseases and mortality.

More information is needed to clarify the association between lycopene and CVD. In addition, clinical trials are warranted to evaluate the antioxidative effects and other possible benefits of lycopene and tomato products.
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