Low Plasma Lycopene Concentration Is Associated With Increased Intima-Media Thickness of the Carotid Artery Wall

Tiina Rissanen, Sari Voutilainen, Kristiina Nyyssönen, Riitta Salonen, Jukka T. Salonen

Abstract—Although a number of epidemiological studies have evaluated the association between β-carotene and the risk of cardiovascular diseases, there has been little research on the role of lycopene, an acyclic form of β-carotene, with regard to the risk of cardiovascular disease. We investigated the relationship between plasma concentrations of lycopene and intima-media thickness of the common carotid artery wall (CCA-IMT) in 520 middle-aged men and women (aged 45 to 69 years) in eastern Finland. They were examined from 1994 to 1995 at the baseline of the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study, a randomized trial concerning the effect of vitamin E and C supplementation on atherosclerotic progression. The subjects were classified into 2 categories according to the median concentration of plasma lycopene (0.12 μmol/L in men and 0.15 μmol/L in women). Mean CCA-IMT of the right and left common carotid arteries was 1.18 mm in men and 0.95 mm in women with plasma lycopene levels lower than the median and 0.97 mm in men (P<0.001 for difference) and 0.89 mm in women (P=0.027 for difference) with higher levels of plasma lycopene. In ANCOVA adjusting for cardiovascular risk factors and intake of nutrients, in men, low levels of plasma lycopene were associated with a 17.8% increment in CCA-IMT (P=0.003 for difference). In women, the difference did not remain significant after the adjustments. We conclude that low plasma lycopene concentrations are associated with early atherosclerosis, manifested as increased CCA-IMT, in middle-aged men living in eastern Finland. (Arterioscler Thromb Vasc Biol. 2000;20:2677-2681.)

Key Words: lycopene ■ atherosclerosis ■ carotid arteries ■ nutrition ■ carotenoids

Low circulating levels of carotenoids have been presumed to play a role in atherogenesis. There is some supporting data from studies within populations that have indicated that high levels of carotenoids in blood1,2 or adipose tissue3,4 are associated with decreased risk of cardiovascular disease (CVD). A few studies have evaluated the association between β-carotene and the risk of CVD, and the results have been inconsistent.4,5 Moreover, there has been little interest in the role of lycopene, an acyclic form of β-carotene, with regard to CVD risk. Like other carotenoids, lycopene is a natural pigment synthesized by plants and microorganisms but not by animals.6 Lycopene gives the familiar red color to tomato products and is one of the major carotenoids in the diet of Europeans and North Americans. Lycopene, which is an antioxidant carotenoid without provitamin A activity, has been shown to be a more potent antioxidant than α- or β-carotene.7 In the test tube, lycopene in LDL is used before β-carotene, lutein, zeaxanthin, or cryptoxanthin in copper-induced LDL oxidation reaction.8 Men who have high titers of autoantibodies against oxidatively modified LDL and those with elevated serum 7β-hydroxycholesterol levels have accelerated progression of carotid atherosclerosis.9,10 A decreased oxidative modification of LDL11 may be one of the mechanisms by which lycopene could reduce the risk of coronary heart disease (CHD) and atherosclerotic progression.

Very few previous studies have dealt with the association between a low concentration of plasma lycopene and early atherosclerosis, as manifested by increased intima-media thickness of the common carotid artery wall (CCA-IMT). The purpose of the present study was to test the hypothesis that healthy men and women with decreased levels of serum lycopene have increased thickness of the CCA-IMT.

Methods

Study Design and Population

The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study is a randomized, double-masked, placebo-controlled, 2×2 factorial trial concerning the effect of vitamin E and C supplementation in the prevention of atherosclerotic progression in smoking and nonsmoking men and postmenopausal women.12 The study was approved by the Research Ethics Committee of the University of Kuopio. All study subjects gave their written informed consent.
The subjects were regularly smoking (≥5 cigarettes/d) or non-smoking men and postmenopausal women aged 45 to 69 years with a serum cholesterol concentration of ≥5.0 mmol/L at a screening visit. Subjects were not entered into the trial if they had premenopause or regular oral estrogen substitution therapy, regular intake of antioxidants, acetylsalicylic acid or any other drug with antioxidative properties, severe obesity (body mass index >32 kg/m²), type 1 diabetes, uncontrolled hypertension (diastolic blood pressure >105 mm Hg), any condition limiting mobility (making study visits impossible), severe disease that could shorten life expectancy, or other disease or condition worsening the adherence to the measurements or treatment. The recruitment of the subjects was carried out in 1993 and the spring of 1994. For the present study, blood for lycopene and other chemical measurements was drawn before antioxidant supplementation. Subjects came to the baseline visits at the Research Institute of Public Health, University of Kuopio, between October 1994 and October 1995.

### Ultrasonographic Assessment of CCA-IMT

Two identical Biosound Phase 2 systems were used (Biosound) equipped with a 8- to 10-MHz annular array transducer, with a measurement precision of 0.03 mm. The scans were videotaped with a PAL S-VHS Panasonic AG 7330E VCR. The ultrasonographic examinations were carried out by 3 well-trained ultrasound technicians. The ultrasonographic scanning of the common carotid arteries (CCAs), the carotid bulbs, and the proximal internal carotid artery was performed after a supine rest of 10 minutes, the subject in the supine position. First, a diagnostic examination of the entire accessible carotid tree was performed to find the most severe lesions. Second, the site of the greatest IMT at baseline in the CCA far wall was located and scanned thoroughly from 3 angles: anterolateral, lateral, and posterolateral. IMT measurements from videotapes were made at the same site and angle at all examinations of each subject, which was the site with the greatest IMT (in any angle) that was clearly visible at baseline in the far wall of the CCA below the bulb. At this location, IMT was measured in diastole for a length of 10 mm (or shorter, if not visible) in 1 angle for the far wall. Most often, this was the distal centimeter of the CCA.

Computer analysis of ultrasound images to measure IMT was performed with a reading station equipped with Data Translation DT 2861 video frame grabber interfaced to a Panasonic AG 7355 VCR. Prosound software, developed by Robert Selzer (University of Southern California, Los Angeles), with automated boundary detection was used. IMT was determined as the average difference at, on average, 100 points between the intima-lumen and media-adventitia interfaces. The mean IMT was computed as the mean of ~100 IMT measurements in the right CCA and another 100 measurements in the left CCA.

For measurement variability, 3 technicians scanned 10 subjects twice during a week in 1995. The videotapes from all scans were read by 1 observer. The repeat correlations for the mean CCA-IMT were 0.998, 0.995, and 0.998, and pairwise interobserver correlations were 0.975, 0.983, and 0.995. Computer analysis of ultrasound images to measure IMT was performed by use of a PC with a Data Translation DT 2861 video frame grabber interfaced to a Panasonic AG 7335 VCR. The Prosound software developed by Robert Selzer was used.

### Chemical and Dietary Measurements

EDTA blood samples were obtained from subjects between 8:00 and 10:00 AM after an overnight fast. Subjects were instructed to abstain from alcohol for at least 1 week before the visit. The subjects were also instructed to avoid strenuous exercise during the previous 24 hours. Plasma and serum was separated within 60 minutes and stored at −80°C until analyzed. Plasma for lycopene determination was extracted with ethanol and hexane; the measurements were conducted by reversed-phase high-performance liquid chromatography (HPLC) with diode-array UV detection in samples that had been kept at −80°C for 3 to 15 months. With this method, the limit of detection for lycopene was 0.01 to 0.02 μmol/L. In the statistical analysis, values below the limits of detection for the assay batch were marked as 0.00 μmol/L. The coefficients of variation were determined with a plasma pool analyzed in 14 separate batches. The coefficient of variation for lycopene was 10.1%.

Dietary intake of foods and nutrients was assessed at baseline by 4-day instructed food recording. Instructions were given, and completed food recordings were checked by a nutritionist. The intake of nutrients was calculated by use of NUTRICA version 2.5 software. The data bank of NUTRICA is compiled by using mainly Finnish values of nutrient composition of foods. All nutrients were adjusted for dietary energy intake by use of the residual method. Energy adjustment is based on the rationale that a larger, more physically active person requires a high 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 the present study population.

Serum cholesterol was determined from fresh samples with an enzymatic colorimetric method (Konelab). Serum LDL cholesterol was measured after precipitation with polyvinyl sulfate (Boehringer-Mannheim), and HDL cholesterol was measured from supernatant after magnesium chloride dextran sulfate precipitation. Serum triglycerides were measured colorimetrically (Boehringer-Mannheim). Plasma total homocysteine (tHcy) concentration was determined with HPLC. Blood pressure was measured manually with the subject in a sitting position after a rest of 10 minutes; there were 3 measurements at 3-minute intervals. The body mass index was computed as the ratio of weight to the square of height (in kilograms per square meter).

### Statistical Analysis

Data were analyzed by using either SPSS statistical software for the IBM RS/6000 workstation or SPSS 9.01 for Windows 98. Mean age, ratio of waist-to-hip circumference, systolic blood pressure, diastolic blood pressure, serum triglycerides, serum HDL cholesterol, LDL cholesterol, total cholesterol, and intake of nutrients are reported as mean±SD, and cigarette smoking is reported as a percentage. Subjects were classified into 2 categories according to their concentrations of plasma lycopene and β-carotene. We compared the higher-than-median level with the lower level. The statistical significance of differences between these 2 lycopene groups in the main characteristics of the subjects was studied by the Student t test.

The association between plasma lycopene and β-carotene and ultrasonographically assessed CCA-IMT was tested for statistical significance also by using ANCOVA, adjusting for age, smoking, serum triglycerides, serum HDL and LDL cholesterol, plasma concentration of tHcy, systolic blood pressure, ultrasound observer, and intake of 4 nutrients (saturated fatty acids, vitamin C, vitamin E, and fiber). All tests were 2-tailed, and a value of P<0.05 was considered significant.

### Results

The mean±SD concentration of plasma lycopene in the study population was 0.16±0.11 μmol/L. Mean plasma levels of lycopene were higher in women (0.17±0.11 μmol/L) than in men (0.14±0.12 μmol/L, P=0.007 for difference). The plasma concentration of lycopene ranged from 0 to 0.62 μmol/L in men and from 0 to 0.64 μmol/L in women.

The main characteristics of the subjects are presented in the Table. For men, age (P=0.001), plasma concentration of tHcy (P=0.004), and dietary vitamin C (P=0.010) had a statistically significant difference between the men who had plasma levels of lycopene lower than the median (<0.12 μmol/L) and those who had values higher than the median (≥0.12 μmol/L). The women with lower levels of plasma lycopene (<0.15 μmol/L) differed significantly with regard to age (P<0.001), HDL cholesterol (P<0.001), systolic blood pressure (P=0.016), plasma concentration of tHcy (P=0.010), and dietary vitamin C intake (P=0.016) from women with higher levels of plasma lycopene. Mean IMT of...
Main Characteristics of Subjects

<table>
<thead>
<tr>
<th>Plasma Lycopene, µmol/L</th>
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<tbody>
<tr>
<td><strong>Men (n=256)</strong></td>
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<tr>
<td>(&lt;0.12)</td>
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<tr>
<td>n</td>
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<tr>
<td><strong>Age, y</strong></td>
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<tr>
<td>61.6±5.0</td>
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<tr>
<td><strong>Body mass index, kg/m²</strong></td>
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<td><strong>Waist-to-hip ratio</strong></td>
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<td><strong>Systolic blood pressure, mm Hg</strong></td>
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<td><strong>Diastolic blood pressure, mm Hg</strong></td>
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<td><strong>Serum total cholesterol, mmol/L</strong></td>
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<td><strong>Serum HDL cholesterol, mmol/L</strong></td>
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<td><strong>Serum LDL cholesterol, mmol/L</strong></td>
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<td><strong>Serum triglycerides, mmol/L</strong></td>
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<tr>
<td><strong>Serum homocysteine, µmol/L</strong></td>
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<td><strong>Dietary saturated fatty acids, E %</strong></td>
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<td><strong>Dietary fiber, g/d</strong></td>
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<td><strong>Dietary vitamin C, mg/d</strong></td>
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<td><strong>Dietary vitamin E, mg/d</strong></td>
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<tr>
<td><strong>Smoking, %†</strong></td>
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<td><strong>IMT, mm</strong></td>
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</table>

Values are mean±SD. E% indicates percentage of energy.

*Adjusted for energy intake.
†Proportion of each group of plasma levels of lycopene.

In ANCOVA, adjusting for other cardiovascular risk factors (age, serum triglycerides, serum HDL and LDL cholesterol, plasma tHcy, and systolic blood pressure), ultrasound observer, and intake of 4 nutrients (proportion of saturated fatty acids of total daily energy, vitamin C, vitamin E, and fiber), low plasma lycopene levels were associated with 17.8% increased IMT in men compared with plasma levels of lycopene higher than median \((P=0.003\) for difference). In women, the difference did not remain significant after the adjustments.

We conducted analyses for β-carotene similar to those presented above for lycopene. In ANCOVA, adjusting for risk factors, there was no statistically significant association between plasma concentration of β-carotene and IMT.

We also repeated analysis in smokers and nonsmokers. The association between plasma levels of lycopene and IMT seems to be stronger among nonsmokers than among smokers. Adjusted IMT was significantly higher \((P=0.006)\) in nonsmokers and nonsignificantly higher \((P=0.070)\) in smokers with lower plasma levels of lycopene compared those with higher plasma lycopene levels. The association between the plasma level of β-carotene and the IMT did not differ in smokers and nonsmokers.

We also divided subjects into 2 categories according to IMT (≤1.00 mm and >1.00 mm). The mean level of plasma lycopene was 0.16 µmol/L in men and 0.17 µmol/L in women with lower IMT and 0.12 µmol/L in men \((P=0.003\) for difference) and 0.16 in women \((P=0.225\) for difference) with higher IMT.

**Discussion**

Our main finding is that in middle-aged men but not in middle-aged women from eastern Finland, a low concentra-
tion of plasma lycopene is associated with early atherosclerosis, as manifested by increased thickness of the CCA wall.

Two previous studies have dealt with the concentration of blood lycopene and the thickness of the artery wall. In the Atherosclerosis Risk in Communities (ARIC) study,16 there were 231 age-matched, sex-matched, race-matched, and field center–matched case-control pairs selected from the larger study population. Cases exceeded the 90th percentile of IMT in the cohort, and the control subjects were below the 75th percentile of IMT for all arterial segments. The cases had nonsignificantly lower levels of serum lycopene and α- and β-carotene. The odds ratio of being above the 90 percentile of IMT, related to low serum levels of lycopene, after adjusting for risk factors was 0.81 (95% CI 0.60 to 1.08). In the ARIC study,17 high dietary intake of provitamin A carotenoids was associated with lower prevalence of carotid plaques, but this association was not statistically significant. The dietary lycopene intake was not assessed. In the prospective Rotterdam Study18 on serum carotenoids and atherosclerosis, serum lycopene was the only carotenoid associated with decreased risk of aortic atherosclerosis (odds ratio 0.55). However, the association was nonsignificant. In the Etude sur le Vieillissement Artériel (EVA) Study,19 in the highest quarter of plasma total carotenoids was associated with lower CCA-IMT in women ($P=0.014$ for a linear trend between quarters) and men ($P=0.02$ for a linear trend between quarters), but after adjustment this association was nonsignificant. Plasma levels of lycopene were not measured.

Common carotid plaques and increased IMT have been shown to predict coronary events.20,21 The association between blood or tissue concentration of lycopene and risk of a CHD has been studied in a cross-sectional study. In a multicenter European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer (EU-ROMIC) study,3 participants who had suffered a myocardial infarction (MI) had lower concentrations of lycopene in adipose tissue than did the control subjects. In a nested case-control study22 that compared subjects who developed MI with healthy control subjects, the cases had lower serum concentration of lycopene than did the control subjects, but the difference was not significant. Only in smokers was a low serum level of lycopene associated with a significantly increased risk of subsequent MI.

In the present study, the association between the plasma level of lycopene and IMT appeared to be stronger among nonsmokers than among smokers. We found that an association between the plasma level of lycopene and IMT exists in nonsmokers and is weak in smokers. There are 2 explanations for this difference. First, mean daily intake of carotenoids could be lower in smokers than in nonsmokers. An alternative explanation is that because smoking is such a strong risk factor itself, smokers do not benefit from lycopene intake as much as nonsmokers, or they have a greater need of antioxidants.

The effect of lycopene on IMT was different in women than in men. There are some possible causes of this difference. Women have a better diet than do men. This is the most likely explanation for the lack of association with IMT. Another explanation would be the more effective endogenous antioxidative system of women.

The oxidative modification of LDL particles may play a role in the formation of foam cells, atherosclerotic lesions, and CHD.9,23 Antioxidants can inhibit the oxidative modification of LDL, may retard atherosclerotic progression, and, consequently, prevent clinical complications of atherosclerosis, such as MI.24,25 Lycopene and other carotenoids have been shown to act as antioxidants.1,11,26,27 Carotenoids found in plasma can quench singlet oxygen, a potential initiator of lipid peroxidation.26 Lycopene, the open-chain isomer of β-carotene, exhibits the highest physical quenching rate constant of all carotenoids with singlet oxygen.7

The mean CCA-IMT in the present subjects was somewhat higher than that reported in most other studies. In the ARIC study,16 the mean IMT of the carotid artery was 1.2 mm for the cases and 0.6 mm for the controls. In the Perk Carotid Ultrasound Disease Assessment Study (CUDAS),28 the average mean of carotid artery IMT in men was 0.73 mm. In the EVA study,19 the mean IMT was 0.70 in men and 0.65 in women. In the Cardiovascular Health Study,21 the mean CCA-IMT was 1.03 mm in subjects aged ≥65 years; thus, the subjects were >10 years older than in the present study. In the population in eastern Finland, CCA-IMT seems to be rather high. This is consistent with the high occurrence of clinical CHD in eastern Finland. Moreover, the serum LDL cholesterol in the present study was higher than that in other studies, and half of the present study subjects smoked regularly. The mean plasma concentration of lycopene in the present study was lower than that in other population-based studies.16,22,28 in only 1 earlier study28 was the mean level of plasma lycopene similar to that in the present study. Evidently, the consumption of tomatoes or tomato products in Finland may be lower than that in most other European countries.

It is possible that the plasma level of lycopene could be an indicator for other favorable dietary factors. However, the effect of lycopene was significant after an adjustment for 3 other dietary factors, the intakes of saturated fatty acids, fiber, and vitamin C. Also, smokers had lower plasma levels of lycopene than did nonsmokers. This could be due to either dietary differences or a consequence of smoking itself. However, we adjusted for smoking in our statistical analysis to show that the increased CCA-IMT in subjects with low plasma levels of lycopene was not simply a consequence of a higher proportion of smokers.

In conclusion, the present study shows that low levels of plasma lycopene are associated with increased CCA-IMT in middle-aged men, but not in women, from eastern Finland. Because increased IMT has been shown to predict coronary events,20,21 this finding suggests that the plasma level of lycopene, particularly a biomarker of tomato-rich food intake, may play a role in the early stages of atherogenesis. Consequently, our results support the previous evidence that plant-dominated diet contributes to cardiovascular health.

Acknowledgments
This study was partly funded by the Finnish Academy and the Juho Vainio Foundation. We are grateful to our staff of almost 30 people and Oy Jurilab Ltd for helping with data collection.

References
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Arterioscler Thromb Vasc Biol. 2000;20:2677-2681
doi: 10.1161/01.ATV.20.12.2677
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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