Lycopene and Myocardial Infarction Risk in the EURAMIC Study

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A multicenter case-control study was conducted to evaluate the relations between antioxidant status assessed by biomarkers and acute myocardial infarction. Incidence cases and frequency matched controls were recruited from 10 European countries to maximize the variance in exposure within the study. Adipose tissue needle aspiration biopsies were taken shortly after the infarction and analyzed for levels of carotenoids and tocopherols. An examination of colinearity including all covariates and the three carotenoids, a-carotene, b-carotene, and lycopene, showed that the variables were sufficiently independent to model simultaneously. When examined singularly, each of the carotenoids appeared to be protective. Upon simultaneous analyses of the carotenoids, however, using conditional logistic regression models that controlled for age, body mass index, socioeconomic status, smoking, hypertension, and maternal and paternal history of disease, lycopene remained independently protective, with an odds ratio of 0.52 for the contrast of the 10th and 90th percentiles (95% confidence interval 0.33–0.82, p = 0.005). The associations for a- and b-carotene were largely eliminated. We conclude that lycopene, or some substance highly correlated which is in a common food source, may contribute to the protective effect of vegetable consumption on myocardial infarction risk. Am J Epidemiol 1997;146:618–26.

coronary heart disease; myocardial infarction

Coronary heart disease remains a major cause of mortality in developed countries and is increasingly recognized as an important cause of morbidity in the developing world as well. A number of important risk factors for coronary heart disease have been identified including hypertension, hypercholesterolemia, insulin resistance, and cigarette smoking. However, these factors can only partly account for the variations in the incidence of coronary heart disease either between or within populations (1–3). Studies of lipid metabolism have suggested that oxidative modifications of low density lipoprotein accelerate atherogenesis (4–6), and supplements of the antioxidant vitamin E reduce the incidence of nonfatal myocardial infarction (7).

Hypothesized methods of promotion of atherogenesis by oxidized low density lipoprotein include stimulation of monocyte and platelet adhesion to endothelium, inhibition of vasodilation, stimulation of synthesis of autoantibodies, and promotion of proliferation of smooth muscle cells leading to the promotion of foam cells and fatty streaks in the arterial intima (8–10). Natural antioxidants present in the diet may inhibit the oxidative modification of low density lipoprotein and slow the progression of atherosclerosis (11).

Observational epidemiologic studies that explored the antioxidant vitamin hypothesis using ecologic studies and cross-sectional studies (12), case-control studies (13–16), and cohort studies (17, 18) generally provide evidence supportive of the hypothesis that some antioxidant vitamins may reduce the risk of coronary heart disease. However, several large scale trials have not confirmed a protective effect of b-carotene (19–21) and are inconsistent for vitamin E (7,
A variety of nutrients have antioxidant activity (e.g., carotenoids, vitamin C, tocopherols). Carotenoids are fat-soluble pigments found in many fresh fruits and vegetables transported in the human body via lipoproteins. They have been shown to have antioxidant abilities in vitro, being most effective as quenchers of singlet oxygen. β-Carotene is by far the most widely studied carotenoid. Increased intake and tissue levels of β-carotene have been shown in epidemiologic studies to be associated with decreased risk of coronary heart disease (12, 15, 16). The EURAMIC Study suggested that dietary β-carotene plays a protective role for myocardial infarction, especially in heavy smokers (15). However, the Finnish trial of β-carotene and α-tocopherol failed to show a reduction in coronary heart disease in smokers with β-carotene supplements (19). The authors inferred from the unexpected findings that “β-carotene may not be the active . . . component of the fruits and vegetables identified as protective in observational studies” (19, p. 1034). Similarly, both the CARET and Physician's Health studies failed to show a chemoprotective effect of β-carotene on cardiovascular disease (18, 20, 21). Thus, the postulated protection of β-carotene as an antioxidant against either cancer or cardiovascular disease remains unproved.

Since supplementation has not been successful, whereas epidemiologic studies suggest a protective effect of high vegetable consumption, attention is focusing on other compounds in vegetables that could account for their protective effect. For example, other carotenoids that are present in human tissues in substantial concentrations are α-carotene and lycopene. α-Carotene is present in relatively high concentrations in pumpkins and carrots as is lycopene in tomatoes, guava, and watermelon. Intakes of these compounds are often closely correlated with β-carotene intakes. This fact might explain why in observational studies β-carotene appears to be protective, but in the largest reported randomized trial it was not (19). It is necessary therefore to assess if there are other micronutrients with antioxidant activity that may play a protective role against coronary heart disease, especially other carotenoids apart from β-carotene (e.g., α-carotene and lycopene).

The EURAMIC Study, a multicenter case-control study, reported on the relation between levels of α-tocopherol and β-carotene in adipose tissue and first acute myocardial infarction (15). This has been expanded to examine other carotenoids available in large quantities. We analyzed the levels of α-carotene, β-carotene, and lycopene in adipose tissue of cases and controls of the EURAMIC Study with the aim of assessing the role of other carotenoids in explaining the protective association seen between β-carotene and myocardial infarction.

MATERIALS AND METHODS

Subject recruitment

The EURAMIC Study design has been described in detail elsewhere (24). Briefly, centers in 10 countries recruited incidence cases of first acute myocardial infarction in men under 70 years of age from coronary care units of participating hospitals. Of the eligible cases, 81 percent participated in the study, as did 57 percent of the controls, who consisted of men recruited from the population in the catchment area of the hospitals providing the acute myocardial infarction cases (15). The sampling of controls was frequency matched for age (5-year intervals). The study excluded all persons reporting within the past year a physician-prescribed change in diet, alteration in dietary vitamin supplement use, or weight change exceeding 5 kg. A history of alcohol or drug abuse or of major psychiatric disorder also served as grounds for exclusion. Informed consent was obtained for all participants in accordance with the requirements of responsible committees on human experimentation. A standard questionnaire was used in all centers to maintain comparability. Anthropometric measures were taken directly from all subjects.

Fat aspirate

Subcutaneous adipose tissue was taken from the buttock by needle aspiration (25). To assist in acquiring the appropriate skills for sampling and to ensure standardized procedures, a videotape showing the technique was distributed to all participating centers. The adipose samples were taken from most cases within 3 days and often on the day of the infarction. Samples were collected directly into Vacutainer adapters (Becton, Dickinson & Company, East Rutherford, New Jersey) and, without further handling or exposure to light or air, immediately placed on dry ice or in liquid nitrogen. They were stored at —70°C and transported on dry ice. Quality control samples were included in the shipments. Carotenoids and tocopherol were analyzed using reverse-phase high-performance liquid chromatography and spectrophotometric detection.

Carotenoid concentrations were based on the amount of fat in the sample. This was achieved by analysis of fatty acids from a split sample from each individual. After saponification and acidification, the
free fatty acids were extracted with hexanol and methylated. Gas-liquid chromatography (HRGC 5300 Mega Series; Carlo Erba, Modena, Italy) with split injection was conducted in Zeist, Netherlands, using a 30-m-long DB-23 column, inner diameter = 0.253-mm phase layer, and helium as carrier gas, in a temperature-programmed run. Heptadecanoic acid was added as an internal standard to the sample prior to saponification. The addition of three reference samples taken from a single large specimen of tissue allowed assessment of within and between run analytic variation. Approximately equal numbers of samples from cases and controls were analyzed in each run. Coefficients of analytic variation for the individual fatty acids ranged from 13 to 38 percent. The coefficients of variation for α-carotene, β-carotene, and lycopene were 9.0 percent, 6.7 percent, and 8.5 percent, respectively.

Data analyses

Analyses use the pooled data set MI002 from the EURAMIC Study. An algorithm identified unreliable assays by comparing fat mass estimated from assay values with actual tissue sample weight. In addition, complete chromatographic results were examined for samples showing extreme or inconsistent carotenoid or fatty acid values, and these were verified against the original chromatograms. Subjects with assays deemed unreliable or with evident chromatographic problems were excluded from all subsequent analyses. Two chromatograms showed no peaks for the carotenoids and were excluded. In another 91 individuals, the total fat content of the adipose samples, as estimated using a C17 standard in the chromatograms, was >1 standard deviation from the total biopsy weight. This could be due to unreliable biopsy weight measurements or flawed chromatography. Their samples were also excluded from the final analyses.

A total polyunsaturated fatty acid variable was created and includes the three omega-3 fatty acids and five omega-6 fatty acids measured in the adipose tissue samples.

Initial analyses used simple descriptive statistics to compare carotenoid levels among cases and controls in the different centers. The primary analyses were based on conditional logistic regression modeling after colinearity diagnostics to ascertain the independence of the covariates. Models are conditioned on age and recruitment center unless otherwise noted. All statistical analyses used SAS version 6.10 software.

Factors included in the logistic regression models in this process included the known risk factors for cardiovascular disease: age, body mass index, current and past smoking, and family history of myocardial infarc-

tion. Other potential confounders from the initial array of demographic and health history variables were selected for inclusion based upon their statistical significance and effect on improving the model fit in this data set. Models were then run with and without inclusion of other carotenoids simultaneously in the equation. All variables meeting a statistical significance criterion of $p = 0.10$ were selected for inclusion. Potential interactions were investigated by stratification. To ensure that the model estimates are not unstable because of one covariate's being approximately linearly associated with a combination of the others, we conducted colinearity diagnostics on each set of variables entered into a model. The procedure Proc Reg, using the option “collin” in SAS, was used to estimate the variance inflation, conditional indices, and the eigen values (26). If either of the former were high, or the latter low, the set of variables causing the colinearity was not used simultaneously in one equation. This was used, among other things, to test the modeling of carotenoids simultaneously (27).

Odds ratio measures of association were derived from the conditional logistic regression models. Odds ratios were also calculated separately for each center, conditioned on age. With the exception of quintile comparisons, all odds ratios represent estimates of the difference in risk between the 10th and 90th percentiles for each carotenoid, with the percentiles based on the respective carotenoid's distribution in the control population.

RESULTS

The distribution of risk factors for myocardial infarction in cases and controls is presented in table 1. The average age of the subjects was 54 years. Men with acute myocardial infarction were more overweight than controls, and a greater proportion were current smokers who also smoked more heavily, were more likely to report a maternal and paternal history, and had a greater prevalence of self-reported hypertension.

The median adipose tissue concentrations of the three carotenoids for cases and controls by center are shown in table 2. There was a sixfold difference in the concentrations of α-carotene among the controls between centers, a threefold difference in β-carotene, and less than a twofold difference in lycopene concentrations. Higher α- and β-carotene concentrations were found among controls in the northwest countries. Malaga had the lowest median levels of α-carotene (0.03 mg/g of fatty acid) and β-carotene (0.18 mg/g of fatty acid in controls). The geographic distribution of lycopene was less consistent, with Helsinki and Malaga having the lowest mean lycopene concentra-

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cases (n = 662)</th>
<th>Controls (n = 717)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Mean SD†</td>
<td>54.0 9.1</td>
<td>54.7* 9.0</td>
</tr>
<tr>
<td>Body mass index Mean SD</td>
<td>26.3 3.7</td>
<td>26.5* 3.9</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>43 9.7 14.5 35 12 22 21</td>
<td></td>
</tr>
<tr>
<td>Exsmokers (%)</td>
<td>55* 13.9* 16.6 32* 17* 26* 26*</td>
<td></td>
</tr>
<tr>
<td>Maternal history of MI (%)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Paternal history of MI (%)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>High blood pressure (%)</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from controls at \( p < 0.05 \).
† MI, myocardial infarction; SD, standard deviation.


<table>
<thead>
<tr>
<th>Center</th>
<th>α-Carotene (mg/g of fatty acid)</th>
<th>β-Carotene (mg/g of fatty acid)</th>
<th>Lycopene (mg/g of fatty acid)</th>
<th>α-Tocopherol (mg/g of fatty acid)</th>
<th>Body mass index (kg/m²)</th>
<th>Smoker (%)</th>
<th>Poly-unsaturated fatty acid (% of fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 1,387)</td>
<td>0.09</td>
<td>0.45</td>
<td>0.27</td>
<td>198</td>
<td>25.6</td>
<td>32</td>
<td>14.2</td>
</tr>
<tr>
<td>Helsinki (n = 120)</td>
<td>0.10</td>
<td>0.54</td>
<td>0.23</td>
<td>161</td>
<td>26.6</td>
<td>25</td>
<td>12.2</td>
</tr>
<tr>
<td>Jerusalem (n = 97)</td>
<td>0.09</td>
<td>0.27</td>
<td>0.28</td>
<td>124</td>
<td>25.4</td>
<td>36</td>
<td>26.2</td>
</tr>
<tr>
<td>Berlin (n = 162)</td>
<td>0.08</td>
<td>0.46</td>
<td>0.26</td>
<td>213</td>
<td>25.5</td>
<td>38</td>
<td>12.3</td>
</tr>
<tr>
<td>Zeist (n = 107)</td>
<td>0.10</td>
<td>0.54</td>
<td>0.27</td>
<td>206</td>
<td>25.4</td>
<td>40</td>
<td>16.2</td>
</tr>
<tr>
<td>Granada (n = 106)</td>
<td>0.04</td>
<td>0.30</td>
<td>0.28</td>
<td>205</td>
<td>26.1</td>
<td>52</td>
<td>14.3</td>
</tr>
<tr>
<td>Edinburgh (n = 100)</td>
<td>0.18</td>
<td>0.61</td>
<td>0.35</td>
<td>180</td>
<td>25.8</td>
<td>24</td>
<td>12.9</td>
</tr>
<tr>
<td>Sarpsborg (n = 191)</td>
<td>0.11</td>
<td>0.59</td>
<td>0.30</td>
<td>194</td>
<td>25.1</td>
<td>23</td>
<td>14.8</td>
</tr>
<tr>
<td>Moscow (n = 186)</td>
<td>0.11</td>
<td>0.50</td>
<td>0.36</td>
<td>243</td>
<td>25.1</td>
<td>38</td>
<td>15.5</td>
</tr>
<tr>
<td>Zurich (n = 123)</td>
<td>0.13</td>
<td>0.54</td>
<td>0.31</td>
<td>317</td>
<td>26.0</td>
<td>14</td>
<td>13.1</td>
</tr>
<tr>
<td>Malaga (n = 195)</td>
<td>0.03</td>
<td>0.16</td>
<td>0.21</td>
<td>192</td>
<td>26.9</td>
<td>33</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Pearson's correlation coefficients were calculated to assess the relation between the carotenoids and myocardial infarction-associated risk factors. All three carotenoids were significantly \( (p < 0.05) \) negatively correlated with body mass index \((r = -0.22 \text{ for } \alpha\text{-carotene, } r = -0.23 \text{ for } \beta\text{-carotene, and } r = -0.24 \text{ for lycopene})\). \( \alpha\text{-Carotene and } \beta\text{-carotene were also significantly negatively correlated with the number of cigarettes smoked per day (} r = -0.14 \text{ for } \alpha\text{-carotene, } r = -0.12 \text{ for } \beta\text{-carotene). The carotenoids were strongly correlated with each other. For } \alpha\text{- and } \beta\text{-carotene, } r = 0.78 \text{ (} p < 0.0001 \text{), for } \alpha\text{-carotene and lycopene, } r = 0.60 \text{ (} p < 0.0001 \text{), and for } \beta\text{-carotene and lycopene, } r = 0.65 \text{ (} p < 0.0001 \text{). Despite these correlations, the median carotenoid concentrations by center for controls are presented in figures 1–3.
strong correlations between the carotenoids, colinearity diagnostics revealed that, in the complete models, individual carotenoids were adequately separated.

Results of conditional logistic regression (conditioning on age and center) after adjustment for body mass index, socioeconomic status, smoking, family history of disease, and history of high blood pressure are presented in table 3. The odds ratios for myocardial infarction with the continuous carotenoid variables per unit of change as modeled both separately and simultaneously revealed lycopene to be the only carotenoid with a significant independent association with lower risk of myocardial infarction. The odds ratio for lycopene, when modeled separately, was 0.50 (95 percent confidence interval 0.34–0.73) for the contrast between the 10th and 90th percentiles in this population. When modeled simultaneously with \( \alpha \)- and \( \beta \)-carotene, the odds ratio was almost identical, 0.52 (95 percent confidence interval 0.33–0.82). \( \beta \)-Carotene, on the other hand, was not significantly inversely associated with protection against myocardial infarction (odds ratio = 0.73, 95 percent confidence interval 0.55–0.96) prior to simultaneous modeling, but after adjustment for other carotenoids, the point estimate of 1.01 (95 percent confidence interval 0.66–1.55) remained insignificantly associated with myocardial infarctions. Similarly, a protective association of \( \alpha \)-carotene on its own was virtually eliminated when modeled together with the other carotenoids.

Table 4 reports the odds ratios for myocardial infarction by quintiles of adipose tissue carotenoid concentrations, based on the distributions in the control population, with the bottom fifth serving as the referent category. Trend analyses showed all three carotenoids to be protective when modeled separately. In simultaneous analyses, accounting for all three carotenoids at the same time, no trend toward lower risk with higher \( \alpha \)-carotene levels was seen \( (p = 0.74) \). The trend for \( \beta \)-carotene remained statistically significant after inclusion of the other carotenoids in the
model. However, the association within the upper four quintiles was not consistent, and only for the fifth quintile of the \( \beta \)-carotene distribution was the odds ratio substantially protective in the simultaneous model at 0.47 (95 percent confidence interval 0.26–0.99). Lycopene showed the greatest protective effect with a tendency toward increasing inverse odds ratios with increased concentrations, evident in both the separate and simultaneous models. The probability of the inverse association’s arising by chance is 0.008 (from the test of trend).

### Polyunsaturated fats and carotenoid effects

An examination of the carotenoid interactions with polyunsaturated fats and acute myocardial infarction using interaction terms and categorizing polyunsaturated fat levels in adipose tissues by tertiles revealed a significant interaction (table 5). The protective effect of lycopene increased at each increasing level of polyunsaturated fat and was significant in the individuals whose adipose tissue contained more than 16.1 percent of the fat in the form of polyunsaturates. \( \beta \)-Carotene and \( \alpha \)-carotene were never significantly associated with polyunsaturated fat levels but tended also to show a trend toward protection only in the presence of the highest concentrations of polyunsaturated fats.

### Smoking status and carotenoid effects

Stratification by smoking status indicated that the effect of lycopene among nonsmokers (table 6) was strongest. \( \beta \)-Carotene was not protective in any group.
**TABLE 5.** Conditional odds ratios for myocardial infarction and carotenoids by tertile of polyunsaturated fatty acid (PUFA), EURAMIC Study, 1991–1992*

<table>
<thead>
<tr>
<th>Adipose tissue sample concentration of</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low, &lt;12.8†</td>
<td>1.31 (0.67–2.54)‡</td>
<td>1.21 (0.73–1.99)</td>
<td>0.71 (0.36–1.40)</td>
</tr>
<tr>
<td>Medium, 12.8–16.0§</td>
<td>0.94 (0.57–1.54)</td>
<td>1.14 (0.64–2.05)</td>
<td>0.70 (0.38–1.27)</td>
</tr>
<tr>
<td>High, 16.1–36.2¶</td>
<td>0.67 (0.36–1.25)</td>
<td>0.65 (0.36–1.18)</td>
<td>0.38 (0.21–0.71)</td>
</tr>
<tr>
<td>p for trend#</td>
<td>0.02</td>
<td>0.02</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Conditional on age and center; covariates are body mass index, socioeconomic status, smoking, maternal and paternal history of disease, and history of high blood pressure. The odds ratios are from controls of the 10th and 90th percentiles of the carotenoid in the population at large.
† Median PUFA for the low tertile is 11.3% of fat.
‡ Numbers in parentheses, 95% confidence interval.
§ Median PUFA for the medium tertile is 14.3% of fat.
¶ Median PUFA for the high tertile is 19.3% of fat.
# Trend test weighted by tertile medians.

**DISCUSSION**

Lycopene is present in the blood in concentrations roughly equal to those of β-carotene. For unknown reasons, certain organs (28) selectively take up or concentrate lycopene so that tissue levels greatly exceed those of β-carotene. Although little is known about the uptake and turnover of carotenoids in adipose tissue, the adipose tissue levels of lycopene, as with other carotenoids, are derived largely from the diet and serve as a better indicator of long-term exposure than do serum or recent dietary records (29). Even if tissues such as adrenal glands or prostate concentrate lycopene, the vast majority of the carotenoids will be found in the adipose tissue. It is important to note that lycopene was not available as a supplement to the individuals in this study prior to their recruitment. Major food sources appear to be tomatoes and tomato products, watermelon, grapefruit, and seafood such as lobster and crab. Foods rich in lycopene have recently been suggested to be inversely associated with prostate cancer (30). There are indications in the literature that lycopene is a more potent antioxidant than β-carotene. DiMascio et al. (31) showed the disappearance in vitro of lycopene in cells under singlet oxygen stress to be greater than that of α-carotene, β-carotene, and α-tocopherol. A study of the sequential order of antioxidant consumption in Cu²⁺-mediated oxidation of low density lipoprotein found that lycopene was consumed first, with cryptoxanthin and lutein/zeaxanthin next, and β-carotene following in last place (32). Furthermore, it has recently been discovered that skin exposure to ultraviolet radiation under controlled experimental conditions results in a strong (31–46 percent) and rapid reduction in skin lycopene levels but in no change in β-carotene levels in the skin (33). Thus, lycopene may be the underlying carotenoid providing antioxidant protection.

Since individuals consuming diets high in vegetables tend to have higher levels of all carotenoids, the strong association between lycopene and β-carotene...
can conceivably explain the β-carotene-myocardial infarction association evident in some observational studies. β-Carotene may be a good marker of lycopene intake, and its effect disappears when both are considered simultaneously. In the same line of argument, lycopene could be a marker of other active phytochemicals in tomatoes and possibly in other major food sources of this antioxidant. The protective potential of lycopene was greatest among individuals with the highest polyunsaturated fat stores, consistent with an antioxidant effect. The interaction was not, however, strongest among smokers.

Adipose tissue provides quantitative measures of prior exposure unbiased by problems of dietary assessment by questionnaire or imprecise food composition values. It is subject to individual differences in metabolism and absorption. Despite these weaknesses, for components widely distributed in the diet with high day-to-day variation in intakes—as is the case with carotenoids—it remains a useful biomarker of exposure. In a case-control study, there is the potential for the disease condition to affect the biomarker. To prevent this, adipose tissue was used, and aspirations were done during hospitalization soon after the infarction. Since adipose tissue is a stable depository of fat-soluble substances whose turnover rate is quite slow (29, 34), and since it was collected shortly after the infarctions, it is unlikely to be significantly affected by the occurrence of myocardial infarction. In this case-control design, only patients who survived until admission to the hospital and until adipose tissue aspiration were included. This may be a potential source of bias if the association among the deaths differs from that in the surviving patients.

Our results are consistent with a protective effect of adipose tissue levels of lycopene, but not of β-carotene or α-carotene, on myocardial infarction risk. Alternately, lycopene may be a marker of another protective substance in a common food source. The protective association with lycopene was not seen in smokers. It did, however, interact with another major source of oxidative stress, stores of polyunsaturated fats, which are markers of consumption of high polyunsaturated fat diets. This suggests that lycopene may be operating under a tissue-specific antioxidant mechanism.

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