Association between beta-carotene and acute myocardial infarction depends on polyunsaturated fatty acid status. The EURAMIC Study. European Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast

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Abstract: Because antioxidants may play a role in the prevention of coronary heart disease by inhibiting the peroxidation of polyunsaturated fatty acids (PUFAs), the combined association of diet-derived antioxidants and PUFAs with acute myocardial infarction (MI) was investigated. This multicenter case-control study included 674 patients and 725 control subjects in eight European countries and Israel. Fatty acid composition and alpha-tocopherol and beta-carotene levels were determined in adipose tissue; selenium level was determined in toenails. For alpha-tocopherol no association with MI was observed at any PUFa level. The overall multivariate odds ratio (OR) for low (10th percentile) versus high (90th percentile) beta-carotene was 1.98 (95% confidence interval [CI], 1.39 to 2.82). The strength of this inverse association with MI was dependent on PUFa levels: for low PUFa, the OR for low versus high beta-carotene was 1.79 (95% CI, 0.98 to 3.25), for medium PUFa the OR was 1.76 (95% CI, 1.00 to 3.11), and for high PUFa 3.47 (95% CI, 1.93 to 6.24). For selenium increased risk was observed only at the lowest PUFa tertile (OR, 2.49; 95% CI, 1.22 to 5.09). This interaction between selenium and PUFAs was not significant and may at least partly be explained by a higher proportion of smokers at the low PUFa level. These findings support the hypothesis that beta-carotene plays a role in the protection of PUFAs against oxidation and subsequently in the protection against MI. No evidence was found that alpha-tocopherol or selenium may protect against MI at any level of PUFa intake.

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Association Between β-Carotene and Acute Myocardial Infarction Depends on Polyunsaturated Fatty Acid Status

The EURAMIC Study

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Abstract

Because antioxidants may play a role in the prevention of coronary heart disease by inhibiting the peroxidation of polyunsaturated fatty acids (PUFAs), the combined association of diet-derived antioxidants and PUFAs with acute myocardial infarction (MI) was investigated. This multicenter case–control study included 674 patients and 725 control subjects in eight European countries and Israel. Fatty acid composition and α-tocopherol and β-carotene levels were determined in adipose tissue; selenium level was determined in toenails. For α-tocopherol no association with MI was observed at any PUFA level. The overall multivariate odds ratio (OR) for low (10th percentile) versus high (90th percentile) β-carotene was 1.98 (95% confidence interval [CI], 1.39 to 2.82). The strength of this inverse association with MI was dependent on PUFA levels (in tertiles): for low PUFA, the OR for low versus high β-carotene was 1.79 (95% CI, 0.98 to 3.25), for medium PUFA the OR was 1.76 (95% CI, 1.00 to 3.11), and for high PUFA 3.47 (95% CI, 1.93 to 6.24). For selenium increased risk was observed only at the lowest PUFA tertile (OR, 2.49; 95% CI, 1.22 to 5.09). This interaction between selenium and PUFAs was not significant and may at least partly be explained by a higher proportion of smokers at the low PUFA level. These findings support the hypothesis that β-carotene plays a role in the protection of PUFAs against oxidation and subsequently in the protection against MI. No evidence was found that α-tocopherol or selenium may protect against MI at any level of PUFA intake.

Key Words:
- antioxidants
- β-carotene
- fatty acids
- myocardial infarction
- α-tocopherol

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Experimental as well as epidemiological studies have accumulated evidence to suggest that antioxidants may play a part in the prevention of cardiovascular disease. Micronutrients such as vitamin E, β-carotene, and selenium may protect the LDL cholesterol particle against oxidation in the vascular subendothelium and thereby prevent the enhanced uptake of cholesterol by macrophages, leading to the formation of foam cells. In vitro studies have shown that the resistance of LDL against oxidation is dependent not only on antioxidant levels but also on its fatty acid composition. Polyunsaturated fatty acids (PUFAs) are most susceptible to oxidation. Increasing the ratio of oleic to linoleic acid in LDL by dietary intervention reduces the uptake of the LDL by macrophages in vitro. Several studies have shown an inverse association of dietary antioxidant intake or plasma or serum concentrations with risk of cardiovascular disease, but others have not. This lack of association may be attributed to relatively high levels of antioxidants in these populations or to deterioration of vitamin E during prolonged storage. It is also possible that the balance between antioxidants and polyunsaturates is the more important factor. Kok et al have reported lower...
selenium-to-PUFA ratios in patients with severe versus mild atherosclerosis.

Here we present the results of a case–control study on the combined association of antioxidants and PUFAs with the risk of acute myocardial infarction (MI) in nine different countries. Levels of antioxidants and fatty acids in plasma or lipoproteins may be affected by recent dietary changes and by the acute event of an MI. Therefore, concentrations of α-tocopherol, β-carotene, and fatty acids in subcutaneous adipose tissue and selenium in toenails were compared between 674 case subjects with acute MI and 725 control subjects without a history of infarction.

Methods

Design and Subjects

Eligible subjects in this study, conducted during 1991 and 1992, were men under 70 years of age from 10 study centers in nine countries. They were native residents of their respective countries with stable dietary and weight patterns during the previous year, ie, no changes in the use of dietary supplements containing α-tocopherol, β-carotene, or selenium, no new or altered dietary prescription or advice for health reasons, and no weight loss over 5 kg. Cases were subjects diagnosed with a first acute MI (ICD code 410) that had been confirmed by specific abnormalities on an electrocardiogram and by elevated enzyme levels. These subjects had been admitted within 24 hours of manifesting symptoms and were recruited from the coronary care unit of participating hospitals. Control subjects had no history of MI and were frequency matched for age according to 5-year intervals. Control subjects were recruited from the population in the catchment area, from population registers, or from other appropriate sources. In some centers, control subjects were selected from among hospital patients with diseases that were not known to be associated with antioxidant status (renal colic, noninfectious prostatism, acute appendicitis, noninfectious otic pathology, hernia, volvulus, or rectal/anal pathology [except cancer, hemorrhoids, or chronic infections]). If low response rates from population–based samples might affect the internal validity, control subjects were selected from the catchment area via a random sample by the patient’s general practitioner (Netherlands) or by inviting friends and relatives of the case (Norway). Excluded from both groups were subjects with a history of treatment for alcohol or drug abuse, those diagnosed with major psychiatric disorders that would interfere with their ability to give informed consent, and institutionalized subjects. Informed consent was obtained from all subjects, and the study protocol was approved by the appropriate institutional committees on human experimentation. The study design has been reported in detail.\textsuperscript{17}

Biochemical Analyses

Subcutaneous adipose tissue was taken from the buttock by needle aspiration.\textsuperscript{18} In case subjects, the adipose sample was taken within 7 days of hospital admission. Samples were stored at −70°C; handling of the samples has been described.\textsuperscript{17} Samples were analyzed in a central laboratory. Concentrations of α-tocopherol and β-carotene in adipose tissue were determined by reverse-phase high–performance liquid chromatography\textsuperscript{19} and spectrophotometric detection. The coefficient of variation for the analysis of β-carotene and α-tocopherol was 7% (at mean values of 2.1 and 84 µg/g in the quality control samples, respectively). Detection limits were 0.02 µg/g for β-carotene and 2 µg/g for α-tocopherol at a mean sample weight of 29 mg. Vitamin concentration was expressed in micrograms per gram of total fatty acids.

Fatty acids were assayed centrally at the National Public Health Institute, Helsinki, Finland. The saponified sample was acidified with HCl, and the free fatty acids were extracted with hexane and methylated with acidic methanol. Fatty acid composition was determined by a gas chromatograph (HNU Nordion Oy, HRGC 412) with a 60–m–long SP-2380 column, an internal diameter of 0.32 mm, a phase layer of 0.2µm with a split injector, and helium as carrier gas. Fatty acid peaks from C12:0 (fatty acid with 12 carbon atoms/0 double bonds) to C22:6 were identified by an SC workstation (Sunicom Oy) in a temperature–programmed run. PUFAs include linoleic acid (C18:2), alpha-linolenic acid (C18:3), and arachidonic acid (C20:4); monounsaturated fatty acids (MUFAs) are C14:1, palmitoleic acid (C16:1), oleic acid (C18:1), and eicosaenoic acid (C20:1); saturated fatty acids (SFAs) include lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). All fatty acids are expressed as a proportion of total fatty acids. Because minor fatty acids are not included in these aggregated categories, proportions of PUFAs, MUFAs, and SFAs do not add up to 100%.

Serum total cholesterol levels were determined in Helsinki by using enzymatic methods (kits by Boehringer–Mannheim GmbH), HDL cholesterol was determined after precipitation with dextran sulfate and magnesium chloride, and LDL cholesterol was calculated by the Friedewald formula. In case subjects, cholesterol concentrations were inversely related to time from onset of symptoms (Pearson
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$r = -0.18, P < .001$), which may be due to the effect of acute MI on serum cholesterol.

Toenail clippings were collected within 8 weeks of a subject’s inclusion in the study and were stored in small plastic bags at room temperature. Nails were cleaned before clipping. The selenium content of the toenails was assessed by instrumental neutron-activation analysis of the metastable selenium-77 isotope (Interfaculty Reactor Institute, Delft University, Netherlands).\(^{20}\) Samples were irradiated for 17 seconds in a thermal flux of $1.2 \times 10^{13}$ neutrons $\cdot s^{-1} \cdot cm^{-2}$. After a decay time of 20 seconds, gamma radiation of \(^{77m}Se\) was measured for 60 seconds. Mean level of selenium ($n=87$) in certified bovine liver reference material (NBS-1577A) was $0.76 \pm 0.04$ ppm against a certified value of $0.80 \pm 0.04$ ppm. Reproducibility of measurement was evaluated by repeated analysis of 19 samples; the coefficient of variation was 5%.

Data Analysis

Questionnaire data were available for 1499 eligible subjects. Vitamin results were unavailable (no adipose tissue in adaptor) for 34 subjects (30 case and 4 control subjects). Extreme values caused by measurement error due to very small sample size were excluded (38 case and 28 control subjects), leaving 674 case and 725 control subjects for data analysis. Fifty-five case and 24 control subjects lacked toenail samples; 4 case and 5 control subjects had selenium values below the detection level and were handled as missing values as well. Of the remaining subjects, 28 case subjects and 4 control subjects had provided no biopsy, leaving 655 case and 724 control subjects for the data analysis of selenium in combination with fatty acid composition.

Crude means for major risk factors and potential confounders were computed; the difference between case and control groups was tested by using Student’s $t$ test and $\chi^2$ analysis. Mean center-adjusted levels of antioxidants and fatty acids (as a proportion of total fatty acids) were calculated for both groups. As the distribution of PUFAs and MUFAs was skewed, the $\log_e$-transformed values were used. Potential confounders of the association between antioxidants and MI were identified by using stratified analysis. Partial (center-adjusted) correlations between antioxidants and fatty acids were calculated. The odds ratio (OR) of MI was estimated for the 10th percentile level of the antioxidants relative to the 90th percentile level based on the distribution among control subjects by multiple logistic regression, with maximum-likelihood estimation of the regression coefficients. This continuous OR was preferred to calculating ORs in quintiles of the antioxidant distribution to avoid irrelevant fluctuations of ORs that may occur due to small numbers when examining interactions. The fitted model included age, center, smoking, and body mass index (BMI) for the relation with α-tocopherol and β-carotene; the model for selenium and MI included only age, center, and smoking. Smoking categories included never smokers, ex-smokers, pipe/cigar smokers, and current cigarette smokers, the last category further divided in subjects smoking fewer than 5, 6 to 10, 11 to 20, and more than 20 cigarettes per day. The significance of the interaction of antioxidants and fatty acid composition was tested (with the log-likelihood ratio test) by including an interaction term of the continuous, $\log_e$-transformed variables in the logistic regression model. Subsequently, the risk of MI of the 10th compared with the 90th percentile level of the antioxidants was estimated at tertiles of PUFAs, MUFAs, and SFAs. Nine separate models were required so that each combination of the three antioxidants and the three types of fatty acids was considered.

Results

The prevalence of risk factors of coronary heart disease (CHD) among case and control subjects is summarized in Table 1. Age, serum total and HDL cholesterol, history of hypertension, smoking, angina pectoris, diabetes mellitus, family history for CHD, socioeconomic status, and BMI differed significantly between the two groups. The reduced concentrations of serum cholesterol in case subjects may reflect the cholesterol-lowering effect of the acute event.

Mean center-adjusted values for the antioxidants and fatty acids are shown in Table 2. β-Carotene and selenium concentrations were significantly lower in case subjects than in control subjects. Mean proportions of the SFAs myristic acid (C14:0) and stearic acid (C18:0) were 6% and 7% lower in case subjects, respectively. MUFAs, in particular palmitoleic acid (C16:1) and C18:1(n-7), were higher in case subjects (both 6%), whereas PUFAs were similar for both groups.
Fatty acid composition among subjects from different centers clearly reflected variation in dietary intake: proportion of PUFAs varied between 11.2% in Finland and 25.4% in Israel. The lowest proportion of MUFAs was observed in Israel (43.5%), the highest in Spain (59.1%); Scotland had the highest SFA content (35.4%).

Table 2.
Center-Adjusted Means of Antioxidants and Fatty Acids in Adipose Tissue by Disease Status

We examined the relation between antioxidant concentrations and CHD risk factors in the control group. For β-carotene, negative associations were observed for the number of cigarettes smoked per day (\(r = -0.17, P < .05\)) and BMI (\(r = -0.36, P < .001\)). Subjects with a positive family history of CHD and those with higher socioeconomic status had significantly higher β-carotene levels (\(P < .001\)). α-Tocopherol concentration was also negatively associated with BMI (\(r = -0.12, P < .001\)) and number of cigarettes smoked per day (\(r = -0.13, P = .06\)) and positively with socioeconomic status (\(P < .05\)). There were no significant correlations with other risk factors. Subjects who currently smoked cigarettes or had a negative family history of CHD had significantly higher β-carotene levels (\(P < .001\)). Other classic risk factors were not associated with selenium concentration. The association between antioxidants and proportion of PUFAs varied among countries. The center–adjusted partial correlation was .22 (\(P < .01\)) for α-tocopherol, .09 (\(P < .05\)) for β-carotene, and .18 (\(P < .01\)) for selenium.

To evaluate whether an association between antioxidants and MI depends on the fatty acid composition of the adipose tissue, we tested the significance of the interaction for each of the fatty acid categories (PUFAs, MUFAs, and SFAs) with each antioxidant (β-carotene, α-tocopherol, and selenium). These variables were included in a logistic regression model as continuous variables, with adjustment for age, center, smoking, and BMI (ie, nine separate models for all possible interactions). A significant interaction was observed for α-tocopherol and SFAs (\(\chi^2 = 6.68, P < .01\)). The interactions for β-carotene and PUFAs (\(\chi^2 = 3.20, P = .07\)) and for selenium and SFAs (\(\chi^2 = 3.51, P = .06\)) were borderline significant. Subsequently, the multivariate risk of MI at low (10th percentile value) versus high (90th percentile value) antioxidant concentrations was calculated, both overall and for tertiles of PUFAs, MUFAs, and SFAs (Table 3). For β-carotene the association with MI appeared to be dependent on fatty acid composition. At all levels of PUFAs an increased risk for low β-carotene compared with high β-carotene was observed. The highest ORs were seen at the highest PUFAs level (OR for low versus high β-carotene, 3.47; 95% confidence interval [CI], 1.93 to 6.24) and at the lowest MUFAs and SFAs levels. Since PUFAs, MUFAs, and SFAs are expressed as proportions of total fatty acids, this interrelation is to be expected. Similar calculations were done for tertiles of the ratio between MUFAs and SFAs (m/p ratio). The OR for MI at low versus high β-carotene at low, medium, and high m/p ratio was 2.86 (95% CI, 1.61 to 5.11), 2.76, and 1.38, respectively. For α-tocopherol, a significant positive association with MI was observed at the low SFA level, and a (nonsignificant) negative association at the high SFA level. This relation was not reflected in the associations at different PUFAs or MUFAs levels. For selenium, an inverse association with MI was seen at low PUFA (OR, 2.49; 95% CI, 1.22 to 5.09) and high SFA (OR, 1.75; 95% CI, 0.89 to 3.42) levels.

Table 3.
Risk of Myocardial Infarction at Low (10th Percentile) Compared With High (90th Percentile) Levels of Antioxidants at Different Fatty Acid Levels

The association between β-carotene and MI at different levels of PUFAs was recomputed after excluding persons with angina pectoris; the same trend was observed (ORs were 1.46, 2.03, and 3.20 for low, medium, and high PUFA tertiles, respectively). To evaluate the contribution of centers with very high PUFA (Israel) or MUFAs (Spain) levels to the observed interaction between PUFA and β-carotene, ORs were also computed that excluded these centers; this exclusion did not considerably change the estimates (results not shown).

The association of proportion of PUFAs with risk of MI, without accounting for antioxidant levels, was examined in a logistic regression model including tertiles of PUFAs with adjustment for age, center, smoking, and BMI. A positive association was observed, with an OR of 1.26 (95% CI, 0.92 to 1.71) in the middle tertile.
Association Between β-Carotene and Acute Myocardial Infarction De...

In this multicenter case-control study, the strongest association of low adipose tissue β-carotene with increased risk of MI was observed when the proportion of PUFAs in adipose tissue was high. Because a higher proportion of one type of fatty acid implicates a lower proportion of other types, opposite results were seen for levels of MUFAs and SFAs. Since adipose tissue levels of PUFAs best reflect dietary intake, levels of MUFAs and SFAs may be considered derived values that are not directly related to intake. For α-tocopherol no association with MI was observed at any PUFA level. An inverse association of selenium with MI was observed at low PUFA and high SFA levels; this may be attributed to the larger proportion of smokers in the low PUFA category.

To avoid changes in antioxidant status due to previous disease, the case subjects in this study were those patients with acute, first-time MI. Hospital control subjects or friends and relatives, in some of the participating centers, might not have represented the distribution of the antioxidant status in the population from which the case subjects had originated, although due care was taken to avoid such a bias. If, however, a control subject’s disease was related to antioxidant status, it is most likely to have reduced the antioxidant status and thus diminish the risk estimates for MI. The use of friends or relatives as control subjects may lead to overmatching on lifestyle (including diet) exposures. This would also result in a decreased estimate of association between antioxidants and MI, thus leading to a conservative bias. Because smoking habits and BMI were found to be confounders of the relation between β-carotene and MI and smoking habits alone for selenium and MI, we adjusted for these factors in the multivariate analysis. At low PUFA levels we observed an inverse association of selenium and MI. As smoking is also associated with PUFA status (lower PUFA levels in smokers compared with nonsmokers), the interaction between selenium and PUFAs may also be explained by an interaction between selenium and smoking, although we cannot say to what extent. For β-carotene, the differential risk of MI at different proportions of PUFAs could not be explained by an interaction with smoking. Even if there were such an interaction, it would affect the ORs in the opposite way, as observed for selenium; better adjustment for smoking could only enhance our findings for β-carotene and PUFAs. The interaction between α-tocopherol and SFAs cannot easily be interpreted, since it is not reflected in any interaction with PUFAs. Adipose tissue SFA levels by themselves have a poor correlation with dietary fat intake.

Experimental studies indicate that the balance between antioxidant status, oxidative stress, and PUFAs as the main substrate for oxidation may determine the amount of damage to cells and tissues and eventually the occurrence of disease. The oxidation of LDL is thought to be a major factor in atherogenesis, mainly by causing an increased uptake of lipids in the macrophages in the arterial wall and by its cytotoxic effects on endothelial cells. Oxidation of LDL results in the formation of hydroxylated derivatives of both oleic and linoleic acid. However, a more modest increase in amounts of hydroxy derivatives is seen for oleic acid compared with linoleic acid. Moreover, diets rich in oleic acid relative to PUFAs reduce the uptake of LDL by macrophages. Increasing the amount of vitamin E in the LDL particle by oral supplementation increases the resistance to in vitro oxidation. However, the initial concentration of vitamin E in LDL is not related to oxidation resistance; this has been attributed not only to other components of LDL, such as other antioxidants, but also to the fatty acid composition. Long-term supplementation with β-carotene results in increased β-carotene levels in LDL, but these enriched LDL particles are not more resistant to in vitro oxidation. As Reaven et al. have remarked, from the fact that β-carotene does not confer direct

Discussion

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compared with the lowest and 1.76 (95% CI, 1.24 to 2.50) for the highest tertile.

The most important associate of MI in all multivariate models was smoking. The ORs for the smoking categories in the model estimating coefficients for the interaction between β-carotene and PUFAs increased from 1.72 (95% CI, 1.20 to 2.45) in ex-smokers and 1.74 (95% CI, 0.86 to 3.51) in subjects smoking fewer than 5 cigarettes per day to 9.63 (95% CI, 6.03 to 15.4) in subjects smoking over 20 cigarettes daily compared with those who had never smoked. Smoking was also associated with PUFA level; the mean proportion of PUFAs was 14.4% in nonsmokers and 13.7% in smokers (P=.06). To test whether the interaction between selenium and PUFAs was perhaps the reflection of an interaction with smoking, we compared the fit of a no-interaction model with the fit of a model with the selenium–PUFA interaction (both as continuous variables) and with a model that included the selenium–smoking interaction (smoking in seven categories). The relative improvement with the smoking interaction was larger (χ²=12.30 [6 df], P=.06) than with the PUFA interaction (χ²=1.64 [1 df], P=.20). A similar approach was used for β-carotene: improvement of the model with the β-carotene–PUFA interaction was, as mentioned above, of borderline significance (χ²=3.20, P=.07); an interaction of β-carotene and smoking did not improve the model (χ²=2.30, P=.14).
protection to LDL in in vitro experiments it should not be concluded that it has no role as an antioxidant in the prevention of CHD. The oxidation of LDL by different types of cells in the artery wall may well be influenced by the β-carotene content of these cells.

We measured antioxidants in adipose tissue and toenails rather than in LDL or plasma mainly because of the effects of recent dietary changes and the acute event of the MI itself on plasma levels, which would severely bias the outcome of a case-control study. When we investigated the relation between α-tocopherol and β-carotene in adipose tissue and in plasma, we observed a correlation of .31 for α-tocopherol and r=.62 for β-carotene (r=.77 when measurement variability was taken into account). The concentration of these antioxidants in adipose tissue is also associated with dietary intake, although perhaps not very strongly. Inadequacies in dietary assessment methods may explain rather low correlations with levels in adipose tissue or plasma. An important recent suggestion is that plasma α-tocopherol levels are regulated and thus kept relatively constant. The resulting small variation in tissue levels would prevent finding an association with risk of MI, at least at normal levels of intake.

Selenium in toenails, like serum selenium, has a dose–dependent relation with selenium intake. The fatty acid composition of adipose tissue, especially of essential fatty acids, is a reliable indicator of dietary intake over a longer period of time.

There is a growing body of epidemiological evidence for an inverse relation between antioxidant nutrients and CHD risk. However, not all studies have found this association. For vitamin E, possibly only very high intake through supplementation is associated with decreased risk of CHD. The (mostly preliminary) findings for β-carotene indicate an inverse association at normal dietary intake levels. Results of the first large preventive trial with antioxidants do not support these findings and even suggest that large doses of β-carotene might be harmful. Population–based studies have scarcely addressed the combined effect of antioxidants and dietary fatty acids on CHD risk. Kok et al have observed lower selenium-to–PUFA ratios in cases with severe versus mild coronary atherosclerosis, but these results were not adjusted for smoking status. The ratio of α-tocopherol to PUFA did not differ between these groups. Riemersma et al report no significant interaction between adipose linoleic acid and plasma vitamin E in the relation with angina pectoris, which agrees with our findings for α-tocopherol. To our knowledge the interaction between β-carotene and PUFAs has not been addressed in other studies.

In conclusion, the association of low β-carotene levels in adipose tissue with increased risk of MI is modified by adipose tissue PUFAs levels. Since adipose tissue fatty acid composition and β-carotene concentration are reported to be indicators of dietary intake, this finding suggests that low β-carotene intake may increase the risk of MI in men, particularly when they consume large amounts of PUFAs. In the evaluation of PUFA relative to MUFA and SFA levels, both the susceptibility to oxidative stress and the cholesterol-lowering potential should be taken into account. It is too early to make firm recommendations in favor of MUFA over PUFAs on the basis of available evidence. It must be stressed that the observed association is no proof of a cause–and–effect relation: it may be possible that β-carotene is not the protective factor, but another constituent of the diet that is present in the same food products. In both cases, increased consumption of yellow fruits and green leafy vegetables may improve the CHD risk profile of middle-aged men.

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