Serum Antioxidants and Myocardial Infarction

Are Low Levels of Carotenoids and α-Tocopherol Risk Factors for Myocardial Infarction?

Debra A. Street, PhD; George W. Comstock, MD, DrPH; Richard M. Salkeld, MD; Willy Schüep, PhD; Michael J. Klag, MD, MPH

Background In vitro, animal and epidemiological studies suggest that lipoprotein oxidation may play an important role in atherosclerosis. Antioxidants may protect against lipoprotein oxidation and in that way inhibit atherosclerosis and its clinical sequelae. To investigate this possibility, we examined the association between levels of several antioxidants and myocardial infarction using serum specimens collected 7 to 14 years before the onset of myocardial infarction.

Methods and Results A nested case-control design was used. Cases and control subjects were selected from the 25 802 persons who had donated 15 mL of blood in 1974 for a serum bank. Cases comprised 123 persons with a subsequent first diagnosis of myocardial infarction who ranged from 23 through 58 years of age in 1974 and who had had their first diagnosis of myocardial infarction during 1981 to 1988. Two groups of control subjects matched to the cases for sex and age were selected from donors to the serum bank, one from those with hospital admissions during the same period and the other from the total group of donors. Sera were assayed for four carotenoids (β-carotene, lycopene, lutein, and zeaxanthin), α-tocopherol, and cholesterol. Because associations with these serum nutrients showed similar trends whether based on hospital or community controls, the two control groups were combined. There was a significantly increasing risk for subsequent myocardial infarction with decreasing levels of β-carotene in 1974 (*P* value for trend, .02) and a suggestive trend with decreasing levels of lutein ($P=.09$). When the results were stratified by smoking status, the excess risk of myocardial infarction associated with low serum levels of carotenoids was limited to smokers. A protective association with higher levels of α-tocopherol was suggested only among persons with high levels of serum cholesterol.

Conclusions Low serum levels of carotenoids were associated with an increased risk of subsequent myocardial infarction among smokers. (***Circulation*. 1994;90:1154-1161.**)

Key Words • antioxidants • myocardial infarction • atherosclerosis

Antioxidants are presently of interest for their potential role in inhibiting atherosclerosis, thus preventing some major clinical complications of atherosclerosis such as myocardial infarction. α-Tocopherol and several carotenoids have been shown to act as antioxidants.[1-4] Specifically, α-tocopherol and β-carotene can trap free radicals by breaking the chain reaction of lipid peroxidation, α-tocopherol doing this at high oxygen concentrations and β-carotene at low oxygen concentrations (partial pressures of oxygen <150 mm Hg).[4] β-Carotene and other carotenoids found in serum, namely, lycopene, lutein, and zeaxanthin, can also quench singlet oxygen, a potential initiator of lipid peroxidation.[2,5]

In vitro experiments have demonstrated that lipid peroxidation may modify low-density lipoprotein (LDL)[6] and that modified LDL may play a role in atherogenesis.[6-9] The oxidatively modified LDL appears to be (1) capable of signaling monocytes to enter the arterial intima, where they undergo transformation into macrophages, (2) capable of immobilizing macrophages in tissues, possibly leading to their retention in the subendothelial space, (3) recognized by a class of macrophage scavenger receptors that rapidly endocytose modified LDL, and (4) potentially cytotoxic, injuring endothelial cells and smooth muscle cells and perhaps perpetuating infiltration of LDL into arterial intima.[7-9] As a result of these processes, foam cells may be generated with potential progression of atherosclerotic lesions.

Few epidemiological studies have examined the association between serum antioxidants and cardiovascular disease or death.[10-15] Only two have examined the association between β-carotene and these outcomes prospectively.[10,15] To replicate their findings in a different population and to include the major carotenoids found in human serum, we studied prediagnostic serum levels of these carotenoids and serum α-tocopherol among 123 cases of myocardial infarction and 246 matched control subjects in Washington County, Md.

Methods

Participants for this case-control study were selected in 1988 from the roster of 25 802 persons who had donated 15 mL of blood for a serum bank in the autumn of 1974. At that time, participants also completed a brief questionnaire, which included questions on birth date, sex, occupation, medication use, and smoking; they also had their blood pressure mea-

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From the Department of Epidemiology (D.A.S., G.W.C., M.J.K.) and the Department of Health Policy and Management (M.J.K.), the Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md; the Department of Medicine (M.J.K.), the Johns Hopkins University School of Medicine, Baltimore; and the Department of Vitamin and Nutrition Research (R.M.S., W.S.), F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Reprint requests to Dr George W. Comstock, The Johns Hopkins Training Center, PO Box 2067, Hagerstown, MD 21742-2067.

sured. Blood pressure determinations were made in the sitting position after a few minutes' rest. The lowest of three readings was recorded. Cases and control subjects also participated in a private census of Washington County, Md, during the summer of 1975, nearly 1 year later. An estimated 90% of the population was enumerated (n=94 881 persons) and furnished additional information on demographic, socioeconomic, and smoking status.

Case Definition

Cases were individuals who were admitted to Washington County Hospital from 1981 through 1988 for their first diagnosis of myocardial infarction, who had participated in the 1974 serum collection project and the 1975 private census, and who ranged in age from 35 through 65 years at the time of myocardial infarction. Washington County Hospital, the only general hospital in the county, admitted 94% of hospitalized cases of myocardial infarction among Washington County residents during 1988 (unpublished data, Training Center for Public Health Research, Hagerstown, Md).

All cases of first myocardial infarction had to have a hospital discharge diagnosis coded as 410.0-410.9 according to the Ninth Revision of the International Classification of Disease (ICD-9). They also had to meet additional criteria, which have been applied in other epidemiological studies[16,17] and have been shown to be highly sensitive and specific.[18] Although insistence on additional criteria undoubtedly caused a small decrease in sample size due to exclusion of some potentially true cases, it minimized dilution of the actual association between myocardial infarction and serum micronutrients that would have occurred if some noncases had been misclassified as cases.[19] To be included as a case, one of the following must have been recorded in the hospital record: (1) cardiac pain and significant elevation of serum enzymes, (2) diagnostic ECG findings at the time of the event, or (3) cardiac pain and equivocal ECG findings and equivocal enzymes.[16-18]

Cardiac pain was defined as pain without a noncardiac cause occurring anywhere in the anterior chest, left arm, or jaw. Elevation of enzymes qualified as a criterion for myocardial infarction only if after careful search of the hospital record, no nonischemic cause for their elevation could be found. A significant elevation of serum enzymes included one or more of the following. (1) CPK-MB was present (if laboratory did not report a numerical value) or CPK-MB was ≥10% of the total CPK value at any one and the same time as total CPK in the serial assay of enzymes, and total CPK greater than the upper limit of normal (>250 IU/L) on the date and time when CPK-MB met the definition. (2) Both total CPK and LDH were at least twice the upper limits of normal (upper limits: CPK, 250 IU/L; LDH, 210 IU/L) when CPK-MB was missing, not done, or done more than 36 hours after onset of symptoms. (3) The ratio of LDH_1 to LDH_2 was ≥1.0.

An equivocal elevation of serum enzymes was met by one of three criteria. (1) CPK-MB was 5% to 9% of total CPK, which in turn was greater than the upper limit of normal (>250 IU/L), or CPK-MB was "positive" (if no numerical laboratory value was reported) or was 10% of total CPK, which was less than or equal to the upper limit of normal for that enzyme (≤250 IU/L). (2) Either total CPK or total LDH was at least twice the upper limit of normal (upper limits: CPK, 250 IU/L; LDH, 210 IU/L). (3) Both total CPK and total LDH were between the upper limits of normal and twice the upper limits of normal.

If a patient did not qualify by cardiac pain and significant elevation of serum enzymes (additional criterion No. 1), the ECGs were photocopied and submitted for Minnesota coding to an internist (M.K.) trained in cardiovascular epidemiology and experienced in using the Minnesota codes. The internist was blinded to other study findings. Diagnostic and equivocal ECG findings were defined by Minnesota codes as follows.[16] (1) Diagnostic was defined as unequivocal Q or QS pattern (code 1-1) or Q or QS pattern (codes 1-2-1 to 1-2-7), plus any T wave item (codes 5-1 to 5-3). (2) Equivocal was defined as Q or QS pattern (codes 1-2-1 to 1-2-7), ST junction and segment depression (codes 4-1 to 4-3), T wave item (codes 5-1 to 5-2), or left bundle branch block (code 7-1). These review readings were used in the application of additional criteria No. 2 and No. 3.

The hospital record was also reviewed to determine if other atherosclerotic events had occurred before myocardial infarction: cerebral vascular accident, transient ischemic attack diagnosed on computed tomography scan or arteriogram, carotid endarterectomy for carotid artery stenosis, or femoral artery bypass surgery. These events excluded individuals from the study, as did a history of cancer on the hospital record or cancer register (in order to retain serum from individuals with cancer for other studies and because of the potential protective association of antioxidants for some cancers) or a report of diabetic or coronary heart disease medication use on the 1974 questionnaire. The reason for excluding persons who, before myocardial infarction, had clinical evidence of atherosclerosis-related events other than coronary artery disease, was to select, insofar as possible, cases with similar disease status, ie, cases of acute myocardial infarction only.

Hospital Control Definition

Hospital control subjects were selected from individuals who were admitted to Washington County Hospital in the same year as the case to which they were matched, were the same sex as the case, were within 2 years of the case's age, and had participated in the 1974 serum collection project and the 1975 private census. Their diagnoses on discharge could not include myocardial infarction, atherosclerotic vascular disease, cerebrovascular disease, or cancer. Other exclusion criteria on hospital chart review and cancer registry were the same as for cases with the additional exclusion of persons with a history of myocardial infarction at any time preceding the time of the case's myocardial infarction. Medications for diabetes or coronary heart disease reported at the time of serum collection were also exclusion criteria.

Community Control Definition

Community control subjects were selected from the roster of participants in the 1974 serum collection project and the 1975 private census who were still residents of Washington County at the time of the matched case's admission to the hospital for myocardial infarction and were the same sex as the case and within 2 years of the case's age. Individuals were excluded as community control subjects if they had been admitted to Washington County Hospital for myocardial infarction, had been registered as having cancer, or had told the interviewer at the time of serum collection that they took medication for coronary heart disease or diabetes.

Laboratory Assays

Blood collected in 1974 was allowed to clot at room temperature and was then refrigerated at 4° to 5°C until centrifugation. The serum then was promptly frozen and stored at −70°C from 1974 until processing for this study in July 1990, 16 years later. Tubes of frozen sera from study participants were grouped in sets, each set consisting of sera from a case and its matched hospital and community control subjects. For each group of seven sets, an additional two tubes of reference serum were included to serve as quality control specimens for laboratory assays. Reference serum comprised blood collected from personnel at the Johns Hopkins Training Center. These specimens were pooled, allowed to clot at room temperature, and centrifuged; the serum was aliquotted to 36 tubes. The tubes were stored at −70°C for several days before removing all tubes to create sets. All tubes of sera from study participants and pooled blood were assigned identification numbers and shipped together on dry ice in one insulated container to

the Hoffmann-La Roche Laboratory in Basel, Switzerland, where they were stored at −70°C until analyzed. The laboratory personnel did not know which tubes contained serum from cases, control subjects, or reference specimens.

During a 2-week period in July 1990, levels of cholesterol, carotenoids, and α-tocopherol were assayed. The samples were thawed for the cholesterol estimation and immediately refrozen, then thawed for the carotenoid and α-tocopherol assays and refrozen, and thawed once more for repeat assays. Up to four freeze-thaw cycles have been shown not to affect levels of carotenoids and α-tocopherol.[20] Micronutrient levels were determined by high-performance liquid chromatography on reversed phase as described in detail in a recent publication.[21] Each set of sera was assayed on the same day with the same reagents. The samples were protected from light the entire time.

The replicate assays of the reference sera indicated acceptable low coefficients of interassay variation (<5%) for β-carotene, lycopene, lutein, and α-tocopherol but 23% for zeaxanthin, probably because of its low level in human serum.

Statistical Analysis

Analyses were performed with both untransformed values and transformed values (using either a natural log transformation or square root transformation of the serum antioxidant levels). Differences in mean serum levels of carotenoids or α-tocopherol were assessed by *t* tests. To estimate a trend in mean serum levels of micronutrients with age, number of cigarettes smoked per day, or cholesterol level, simple linear regression was used.[22] Exposure scores were assigned for each category of these variables using the midcategory value, and both weighted (the weight for each data point was defined as the inverse variance of the values in that category)[22] and unweighted linear regressions were performed. Odds ratios and the χ^2 test (Mantel-Haenszel extension) for linear trend of the odds ratios were computed for quintiles of each micronutrient to test the strength of the dose-response association between micronutrients and subsequent myocardial infarction.[23] For this analysis, the serum values of the two control groups were pooled to determine the quintile values for each micronutrient. The α-tocopherol to cholesterol ratio was calculated by dividing a participant's α-tocopherol level in milligrams per liter by the cholesterol level in milligrams per deciliter and multiplying by 100. Because of the matched design, conditional logistic regression was used to assess the association of serum micronutrients and myocardial infarction while controlling for potentially confounding variables and taking into account the effect of interaction between micronutrients and covariates.[23]

Results

All of the 123 persons selected as cases had a discharge diagnosis of myocardial infarction according to ICD-9 codes 410.0-410.9. Of the additional criteria used to further confirm the diagnosis, 98 (80%) of the cases had cardiac pain and significant elevation of enzymes. Among the remaining cases who did not meet both of these criteria, 15 (12%) had diagnostic ECG findings and 10 (8%) met the definitions for cardiac pain, equivocal enzymes, and equivocal ECG findings.

Distributions of cases and control subjects are shown in Table 1. Slightly more than 70% were men, and 30% were in the oldest age group (60 to 67 years) at the time of diagnosis of myocardial infarction. With respect to other known risk factors for myocardial infarction, a higher proportion of cases than control subjects smoked cigarettes at the time of serum collection (1974), had elevated blood pressure at that time, and had high levels of total serum cholesterol.

TABLE 1. Percentage Distribution of Cases and Control Subjects by Matching Factors and Other Risk Factors for Myocardial Infarction

	Cases (n=123)	Hospital Control Subjects (n=123)	Community Control Subjects (n=123)
Matching factors			
Sex			
Male	72.4%	72.4%	72.4%
Female	27.6%	27.6%	27.6%
Age at diagnosis of myocardial infarction			
35-49 y	27.6%	26.0%	26.8%
50-59 y	43.1%	43.1%	42.3%
60-67 y	29.3%	30.9%	30.9%
Risk factors in 1974			
Current smoker	54.5%	31.7%	30.1%
Systolic blood pressure (≥140 mm Hg)	47.1%	41.5%	39.0%
Diastolic blood pressure (≤90 mm Hg)	48.8%	33.3%	36.6%
Total serum cholesterol* (≥240 mg/dL)	55.3%	35.8%	31.7%

*One person, a case, is missing a cholesterol level.

The correlation between pairs of serum micronutrients (β-carotene, lutein, lycopene, zeaxanthin, and α-tocopherol) was assessed using data from all study participants, as were the correlations between each micronutrient and total cholesterol. Correlations >.50 were found between α-tocopherol and total cholesterol (r=.54, P=.0001) and between lutein and zeaxanthin (r=.64, P=.0001). All other correlations were weak (eg, the correlation between β-carotene and cholesterol, r=.16).

Because the associations with serum nutrients were similar whether based on hospital or community control subjects, the two control groups were combined. Similarity of the two control groups was based on the following observations: mean levels of micronutrients in hospital and community control subjects were similar; case-control differences in micronutrient levels were in the same direction for both control groups; and dose-response trends between serum micronutrients and myocardial infarction using hospital control subjects were similar to those obtained by using community control subjects.

Mean levels of serum antioxidants in the control subjects, stratified by level of risk factors for myocardial infarction, are shown in Table 2. These differences were examined among control subjects for the purpose of looking at relations apart from an association with myocardial infarction, that is, to determine whether cigarette smoking, cholesterol, or blood pressure might be potential confounders of the relation between the serum micronutrients and myocardial infarction. The significance levels were set lower than usual for individual *t* tests (P<.01) because multiple comparisons were performed. The significance of the trend tests was

TABLE 2. Mean Levels of Serum Antioxidants Among Control Subjects by Sex, Age, Smoking Status, Cholesterol Level, and Systolic Blood Pressure

Characteristics	Number of Control Subjects	Mean Serum Antioxidants Level (Standard Deviation)				
		β-Carotene, μg/dL	Lycopene, μg/dL	Lutein, μg/dL	Zeaxanthin, μg/dL	α-Tocopherol, mg/L
Sex						
Male	178	18.9 (13.3)	39.5 (18.8)	14.4 (6.2)	3.8 (1.9)	11.4 (3.7)
Female	68	25.0 (16.3)*	42.1 (18.8)	12.4 (5.7)	3.3 (1.9)	11.3 (4.0)
Age at serum collection						
22-39 y	64	18.1 (12.0)	41.7 (16.6)	11.7 (5.0)	3.3 (1.8)	9.5 (2.0)
40-49 y	103	21.3 (14.5)	42.2 (19.9)	14.7 (6.8)*	3.7 (2.0)	11.6 (3.8)*
50-60 y	79	21.7 (16.0)	36.5 (18.7)	14.6 (5.6)*	3.8 (1.8)	12.6 (4.2)*
Test for trend		*P*=.13	*P*=.11	*P*=.005	*P*=.10	*P*=.0001
Smoking status						
Nonsmoker	170	21.3 (14.4)	41.3 (19.1)	14.1 (6.1)	3.7 (1.9)	11.5 (3.9)
1-14 cigarettes/d	15	24.5 (23.0)	40.7 (20.6)	13.9 (7.2)	3.5 (2.1)	11.0 (2.5)
15-24 cigarettes/d	40	18.9 (10.7)	42.1 (17.9)	12.6 (4.7)	3.3 (1.7)	11.2 (3.9)
≥25 cigarettes/d	21	14.8 (11.9)	28.2 (12.3)*†	14.4 (7.5)	3.4 (2.1)	11.1 (3.3)
Test for trend		*P*=.05	*P*=.02	*P*=.60	*P*=.21	*P*=.81
Cholesterol						
<200 mg/dL	80	18.6 (13.8)	32.9 (16.3)	12.2 (4.9)	3.1 (1.8)	9.1 (2.0)
200-239 mg/dL	83	22.4 (14.4)	41.4 (15.6)	14.3 (5.8)	3.9 (1.9)	11.1 (3.0)*
≥240 mg/dL	83	20.6 (14.9)	46.2 (21.6)*	15.2 (7.1)*	3.8 (1.9)	13.8 (4.3)*†
Test for trend		*P*=.42	*P*=.0001	*P*=.0002	*P*=.02	*P*=.0001
Systolic blood pressure						
<140 mm Hg	147	21.8 (15.4)	41.0 (19.3)	13.3 (6.3)	3.5 (2.0)	10.9 (3.9)
≥140 mm Hg	99	18.8 (12.6)*	39.2 (18.0)	14.8 (5.7)*	3.7 (1.8)	12.0 (3.5)*

**t* test, *P*<.01 for this category compared with the lowest category.
†*t* test, *P*<.01 for this category compared with the category below it.

essentially unchanged whether weighted or unweighted simple linear regression was performed; therefore, unweighted regression results are reported. The most notable findings were (1) significantly higher mean β-carotene levels among women than men, (2) a significant positive trend of increasing levels of lutein and α-tocopherol with increasing age, (3) lower mean levels of β-carotene with higher levels of cigarette smoking, although differences between means did not achieve statistical significance, and the lowest mean level of lycopene among the heaviest smokers (≥25 cigarettes per day), which was significantly different from mean levels of lycopene in other smoking categories, a suggestive trend of decreasing levels of these micronutrients with number of cigarettes smoked, (4) a significant positive trend of higher mean levels of micronutrients with higher levels of cholesterol for all micronutrients with the exception of β-carotene and zeaxanthin, and (5) significantly higher mean levels of α-tocopherol and lutein and lower mean levels of β-carotene with increased systolic blood pressure.

Table 3 presents a comparison of the mean levels of the micronutrients in cases and control subjects. Because the results from transformed data were not appreciably different from results using original data, the untransformed means are shown here. For all the carotenoids, the control subjects had higher mean serum levels than the cases, with the difference for β-carotene achieving a *P* value of .03. Mean serum α-tocopherol was higher in the cases than the control subjects, but the mean ratio of α-tocopherol to cholesterol was nearly the same for cases and control subjects. Mean levels of serum micronutrients were also com-

TABLE 3. Percent Difference in Mean Serum Nutrient Levels Between Case and Combined Control Groups

Serum Nutrient	Mean (SD)		% Difference	*P*
	Cases	Control Subjects		
β-Carotene, μg/dL	17.2 (12.2)	20.6 (14.4)	−16.5	.03
Lycopene, μg/dL	39.0 (18.6)	40.2 (18.8)	−3.0	.53
Lutein, μg/dL	12.8 (6.2)	13.9 (6.1)	−7.9	.12
Zeaxanthin, μg/dL	3.5 (1.9)	3.6 (1.9)	−2.8	.44
α-Tocopherol, mg/L	12.0 (3.9)	11.4 (3.8)	+5.3	.13
α-Tocopherol: cholesterol ratio ×100	4.9 (1.4)	5.1 (1.4)	−3.9	.20

*One person, a case, is missing a cholesterol value.

TABLE 4. Risk for Myocardial Infarction Associated With Quintiles of Serum Nutrients Compared With the Highest Quintile

	Odds Ratios* at Quintiles of Serum Nutrients					
Serum Nutrient	**Highest=5**	**4**	**3**	**2**	**Lowest=1**	***P* Value for Trend**
β-Carotene, μg/dL	1.00	1.18	1.12	1.67	2.23	.02
Lycopene, μg/dL	1.00	1.48	0.67	1.35	1.33	.54
Lutein, μg/dL	1.00	0.72	1.28	1.06	1.71	.09
Zeaxanthin, μg/dL	1.00	1.18	1.14	1.34	1.37	.34
α-Tocopherol, mg/L	1.00	0.61	0.67	0.71	0.42	.03
α-Tocopherol:cholesterol ratio ×100	1.00	0.62	0.48	0.64	1.41	*

*Test for trend not done.

pared across the three categories of additional criteria for myocardial infarction (not shown in table) and showed no significant differences.

The unmatched odds ratios and tests for trend for myocardial infarction by quintiles of serum carotenoids and α-tocopherol are shown in Table 4. Odds ratios in this table are estimates of the relative risks of having a first myocardial infarction compared with the risk of persons whose serum levels were in the highest quintile in 1974. For example, persons whose serum β-carotene was in the fourth highest quintile had a risk of having a myocardial infarction that was 1.18 times the risk of that experienced by persons whose β-carotene levels were in the highest quintile. As can be seen in Table 4, levels of carotenoids, except lycopene, were inversely associated with myocardial infarction. The trend of increasing risk for myocardial infarction with decreasing levels of carotenoids was strongest for β-carotene ($P=.02$) and suggestive for lutein ($P=.09$). Persons with β-carotene levels in the lowest quintile were more than twice as likely to have a myocardial infarction than persons with β-carotene levels in the highest quintile (odds ratio, 2.23; 95% confidence interval, 1.11 to 4.48).

In contrast, a trend of decreasing risk for myocardial infarction with decreasing levels of α-tocopherol was observed ($P=.03$), but not for the α-tocopherol to cholesterol ratio. To further take into account the correlation of serum α-tocopherol with cholesterol, the relation between α-tocopherol and myocardial infarction was also assessed for three strata of cholesterol levels using conditional logistic regression. As shown in Table 5, there was an apparent interaction between cholesterol and α-tocopherol levels with respect to subsequent myocardial infarction. At low levels of α-tocopherol, there was a definite trend of increasing risk with increasing concentrations of serum cholesterol, a trend that was not observed if serum levels of α-tocopherol were above the median. Only at cholesterol levels ≥240 mg/dL did low serum levels of α-tocopherol appear to be associated with the risk of myocardial infarction. Adjustment for smoking, blood pressure, or interactions between α-tocopherol and age or sex had little effect on these findings.

Because carotenoid levels were lower in smokers than nonsmokers (Table 2), the possibility was examined that low serum carotenoid levels might merely reflect the intensity of smoking. This did not appear to be the case. For each quintile of carotenoid levels, smokers reported smoking a median of close to 20 cigarettes per day. Smoking status was entered as a covariate in conditional logistic regression models that assessed the relation between myocardial infarction and serum carotenoids. Table 6 demonstrates that smoking increased the risk of myocardial infarction regardless of carotenoid level. Among nonsmokers, there was no demonstrable association of low levels of any of the four carotenoids with myocardial infarction. In contrast, among smokers in 1974, low levels of each of the carotenoids were associated with an increased risk of subsequent myocardial infarction. Again, intensity of smoking did not appear to be an explanation for this finding. The association between carotenoid levels and myocardial infarction did

TABLE 5. Odds Ratios and 95% Confidence Intervals for Association of Myocardial Infarction and α-Tocopherol by Level of Cholesterol

	Odds Ratios* (95% CI)		Adjusted Odds Ratios† (95% CI)	
	Serum α-Tocopherol		Serum α-Tocopherol	
Cholesterol Level	**Above Median‡**	**At or Below Median**	**Above Median**	**At or Below Median**
<200 mg/dL	1.00	0.36 (0.11-1.16)	1.00	0.39 (0.11-1.35)
200-239 mg/dL	0.89 (0.29-2.74)	0.79 (0.25-2.48)	0.72 (0.22-2.38)	0.63 (0.18-2.15)
≥240 mg/dL	1.39 (0.49-3.89)	3.01 (0.84-10.80)	1.03 (0.35-3.08)	2.02 (0.52-7.89)

*Matched analysis (age and sex).
†Matched analysis, adjusted for blood pressure and smoking.
‡Median value for α-tocopherol among control subjects: 10.5 mg/L (after 16 years of storage).

TABLE 6. Odds Ratios and 95% Confidence Intervals for Association of Myocardial Infarction and Serum Carotenoids by Smoking Status

	Odds Ratios* (95% CI)		Adjusted Odds Ratios† (95% CI)	
	Serum Carotenoids		Serum Carotenoids	
Smoking in 1974	Above Median‡	At or Below Median	Above Median	At or Below Median
		β-Carotene		
No	1.00	1.06 (0.56-2.01)	1.00	1.12 (0.57-2.21)
Yes	1.75 (0.84-3.64)	3.71 (1.97-6.98)	1.91 (0.89-4.13)	3.60 (1.87-6.93)
		Lycopene		
No	1.00	0.81 (0.44-1.51)	1.00	1.11 (0.57-2.16)
Yes	1.81 (0.92-3.53)	3.14 (1.67-5.87)	2.03 (1.00-4.12)	3.65 (1.88-7.10)
		Lutein		
No	1.00	1.10 (0.60-2.01)	1.00	1.10 (0.58-2.10)
Yes	2.00 (0.99-4.07)	3.66 (1.93-6.91)	1.98 (0.93-4.20)	3.67 (1.88-7.16)
		Zeaxanthin		
No	1.00	0.73 (0.39-1.38)	1.00	0.73 (0.38-1.42)
Yes	1.91 (0.97-3.74)	2.78 (1.51-5.10)	1.90 (0.98-3.92)	2.71 (1.44-5.10)

*Matched analysis (age and sex).
†Matched analysis, adjusted for serum cholesterol and blood pressure.
‡Median values for carotenoids among control subjects: β-carotene, 16.7 μg/dL; lycopene, 38.5 μg/dL; lutein, 13.0 μg/dL; zeaxanthin, 3.3 μg/dL (after 16 years of storage).

not differ significantly among persons smoking 1 to 24 cigarettes per day and those smoking ≥25 cigarettes per day at baseline. When cholesterol and blood pressure levels were included in the logistic regression analysis, there were only minimal changes in the odds ratios shown in Table 6. Additionally, accounting for the possible interactions between age and carotenoids or between sex and carotenoids caused essentially no change in the odds ratios.

Discussion

The major findings of this study were a significant trend of increasing risk for subsequent myocardial infarction associated with low levels of serum β-carotene and a suggestive trend with low levels of serum lutein. The increased risk for myocardial infarction with low levels of carotenoids appeared to be limited to smokers. Results from other studies that examined the association between atherosclerosis-related disease or mortality and serum carotenoids without stratification by smoking status were consistent with these findings. In an ecologic study, Gey et al[10] analyzed the association of serum carotene (β-carotene and α-carotene) with ischemic heart disease mortality in 16 European populations. Groups of about 100 men, ages 40 to 49 years, were randomly sampled from each of these regions and blood samples taken. When the three Finnish populations were excluded, based on the postulate that part of the high risk for ischemic heart disease in these three populations was due to genetic differences in apolipoproteins, a significant inverse correlation between incident rate of ischemic heart disease death and the median level of plasma carotene in the groups of men from these populations was observed ($r=-.71$). In a case-control study of men responding to the World Health Organization chest pain questionnaire in Scotland, angina pectoris was significantly associated with the lowest quintile of plasma carotene (predominately β-carotene; odds ratio, 2.64; 95% confidence interval, 1.32 to 5.29), but the strength of this association was reduced to nonsignificance after adjustment for smoking, blood pressure, cholesterol, and several other risk factors (adjusted odds ratio, 1.41; 95% confidence interval, 0.63 to 3.13).[11]

Gey et al[10] also analyzed the effect of low serum carotene in the Basel Prospective Study during a 12-year follow-up period and demonstrated that after adjusting for age, smoking, blood pressure, and cholesterol, persons with the lowest quartile of serum carotene had 1.53 times higher risk of dying from ischemic heart disease than did persons with the upper three quartiles of serum carotene (relative risk, 1.53; 95% confidence interval, 1.07 to 2.20). As in the Basel study, the present nested case-control study measured micronutrients in serum obtained before the diagnosis of myocardial infarction, thereby removing the possibility that postdiagnostic dietary changes might have affected the serum levels.

Another prospective study was based on findings from the Lipid Research Clinics Coronary Primary Prevention Trial.[15] The level of total serum carotenoids had a protective association with coronary heart disease incidence. This was statistically significant among the placebo group but not among the group treated with cholestyramine, a medication that interferes with the absorption of carotenoids. All study participants had type IIA hyperlipoproteinemia.

Perhaps the most striking finding of the present study was that the association between low levels of serum carotenoids and subsequent myocardial infarction was observed only among smokers. This cannot be directly observed in the studies in Scotland and Basel because

they both adjusted for the effects of smoking and other cardiovascular risk factors. Only the Scottish study reported both unadjusted and adjusted values. After adjustment, there was essentially no association of carotene serum levels with angina pectoris, a finding consistent with the possibility that the association could have been limited to smokers. A large prospective study, the Health Professionals Follow-up Study,[24] did investigate the dietary intake of carotenoids with subsequent coronary heart disease among persons who never smoked, former smokers, and current smokers at baseline. An increasing protective association for coronary heart disease with increasing levels of carotene intake was found to be significant only among former and current smokers. On the other hand, the protective association observed in the Lipid Research Clinics Coronary Primary Prevention Trial was strongest among nonsmokers in the placebo group.[15]

Another study, the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, enrolled only smokers, 29 133 men aged 50 to 69 years from southwestern Finland, in a randomized, double-blind, placebo-controlled trial to assess the effect of supplementation with α-tocopherol, β-carotene, both micronutrients, or placebo on the incidence of lung cancer and other cancers.[25] As a secondary finding of this trial, investigators observed a nonsignificant increase in ischemic heart disease death in persons supplemented with β-carotene compared with those in the control group.[25,26]

The association of vitamin E with coronary heart disease also has been examined in other studies, but with contradictory results. In the ecologic study among European communities and the study of angina in Scotland, high levels of serum α-tocopherol were associated with a decreased risk of disease.[10,11] The Health Professionals Follow-up Study[24] and the Nurses Health Study[27] also found that use of vitamin E supplements was associated with a decreased risk of coronary disease, and the Alpha-Tocopherol Beta Carotene Cancer Prevention Study found a nonsignificant decrease in the incidence of ischemic heart disease death among persons taking vitamin E compared with the control group.[25] However, three studies that assessed prediagnostic levels of serum α-tocopherol failed to demonstrate that high levels of serum α-tocopherol at baseline were associated with subsequent protection against coronary disease.[10,13,14] In the Basel cohort, failure to find a significant association was attributed to the high levels of α-tocopherol among the participants, with too little variability to make it possible to study the effects of low α-tocopherol levels in the serum. All quartiles of lipid-standardized α-tocopherol were above the presumed critical threshold for adverse cardiovascular events, and it was judged that all (or virtually all) participants had levels that were protective.[10] In the Dutch study, persons who died from coronary heart disease had a mean serum α-tocopherol level at baseline that was only 5% lower than the mean level for control subjects.[14] In the Eastern Finnish study, the cases had levels that were 5% higher on average than the control subjects,[13] in agreement with the present study in which the mean prediagnostic serum level of α-tocopherol was also 5% higher among cases than among control subjects.

A variety of reasons for different outcomes among studies with respect to β-carotene and α-tocopherol have been considered. Most important is that observational studies of antioxidant nutrients and coronary disease, whether based on dietary intake histories or serum levels, can only detect associations, which may or may not be causal in nature. Specifically, persons who differ with respect to intake or serum level of a certain nutrient are likely to differ with respect to other potentially important exposures such as the intake of still other protective nutrients. The association of these nutrients with β-carotene or α-tocopherol might well vary from one population to another.

In the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, the primary hypothesis was related to lung cancer, with the result that other findings are not as solidly based.[25] However, the finding that serum levels of β-carotene on admission to the study were inversely related to lung cancer incidence while supplementation was not is consistent with other observational studies and the possibility that β-carotene merely accompanies a true protective factor.

Disagreement also exists among studies that assessed the effect of β-carotene supplementation on lipid peroxidation. One study using breath pentane as the indicator found that β-carotene inhibited lipid peroxidation among smokers.[28] Another study using other methods came to the opposite conclusion, namely, that β-carotene supplementation failed to show a meaningful effect.[29] Studies do agree, however, that β-carotene does not appear to inhibit lipid peroxidation among nonsmokers.[28,30] Studies that assessed the effect of α-tocopherol supplementation on lipid peroxidation have mostly shown an inhibitory effect.[29-31]

Another reason for different outcomes among case-control studies could be that most control subjects will have some coronary atherosclerosis and some may even have silent ischemic heart disease. This misclassification of control subjects would lessen the difference between cases and control subjects and thereby reduce the likelihood of finding an association.

Loss of micronutrients in frozen sera over time is sometimes suggested as a reason for not being able to demonstrate an association. A recent review of long-term storage effects, however, has shown that concentrations of α-tocopherol appear to be fairly stable for at least 15 years if stored at -70°C or colder.[32] The serum for the present study was stored at -70°C for 16 years before assay. Although some loss of β-carotene did occur, the levels of serum α-tocopherol and β-carotene were both sufficiently high to demonstrate important differences between cases and control subjects. Most important, sera from each case-control set were stored, processed, and assayed similarly, so that differences between cases and control subjects could not have resulted from differences in storage or technical factors.

In this study, the numbers of cases and control subjects are sufficiently large to make it somewhat unlikely that conclusions based on the total group resulted from chance. The situation is different with respect to the stratified analyses. Even though the associations with different levels of smoking and serum cholesterol were not selected from a myriad of subgroup analyses, the number of participants in some of the stratified cells are sufficiently small to make these findings only tentative.

Conclusions

The possibility that β-carotene and possibly other carotenoids may aid in preventing myocardial infarction seems worthy of further attempts at confirmation, especially as it may be related to smoking. The fact that this possibility has been raised in relatively young study populations suggests its potential usefulness in preventing premature deaths. Other ongoing trials of β-carotene supplementation, such as the Physicians Health Study[33] with a primary outcome of cardiovascular disease, will help to clarify whether protection is associated with β-carotene. A study currently under way in one of the centers in the Atherosclerosis Risk In Communities (ARIC) study will indicate whether or not serum levels of antioxidants are associated with carotid artery wall thickening and/or various clotting factors.[17] The results will yield further clues to the mode of action of these potentially protective antioxidant nutrients.

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D A Street, G W Comstock, R M Salkeld, W Schüep and M J Klag

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