Progression of Carotid Intima-Media Thickness and Plasma Antioxidants: The Los Angeles Atherosclerosis Study

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Objective—Recent epidemiologic and animal model data suggest that oxygenated carotenoids are protective against early atherosclerosis. We assessed the association between atherosclerotic progression, measured by carotid intima-media thickness (IMT), and plasma levels of oxygenated and hydrocarbon carotenoids, tocopherols, retinol, and ascorbic acid.

Methods and Results—Participants were from an occupational cohort of 573 middle-aged women and men who were free of symptomatic cardiovascular disease at baseline. Ultrasound examination of the common carotid arteries, lipid level determination, and risk factor assessment were performed at baseline and 18-month follow-up. Plasma levels of antioxidants were determined at baseline only. Change in IMT was related to baseline plasma antioxidant levels in regression models controlling for covariates. In models adjusted for age, sex, and smoking status, 18-month change in IMT was significantly inversely related to the 3 measured oxygenated carotenoids (lutein, β-cryptoxanthin, zeaxanthin; $P<0.02$ for all) and one hydrocarbon carotenoid, α-carotene ($P=0.003$). After adjusting for additional cardiac risk factors and potential confounders, including high-sensitivity C-reactive protein, these associations remained significant ($P<0.05$).

Conclusions—These findings suggest that higher levels of plasma oxygenated carotenoids (lutein, zeaxanthin, β-cryptoxanthin) and α-carotene may be protective against early atherosclerosis. (***Arterioscler Thromb Vasc Biol.*** **2004;24:313-319.**)

Key Words: atherosclerosis ■ antioxidants ■ carotid arteries

The oxidative-modification hypothesis proposes that atherogenesis is initiated by oxidative damage to low-density lipoproteins (LDL) in the artery wall. The presence of oxidized LDL in the subendothelium of arteries stimulates monocyte recruitment and differentiation to macrophage, resulting in the formation of foam cells and increased thickness of arterial walls.[1] Antioxidants have been hypothesized to inhibit lipid peroxidation and play a protective role against chronic diseases such as cardiovascular disease.[2] In vitro studies suggest that vitamins C and E and carotenoids inhibit the damaging activities of oxidized LDL cholesterol.[3–5] Evidence from population studies, including descriptive, case-control, and cohort studies, has shown that dietary,[6–11] plasma, or serum level of vitamin E,[12–14] ascorbic acid,[15–17] and carotenoids[18–22] were inversely associated with cardiovascular mortality rate or early atherosclerosis. However, other epidemiological studies have reported no association between cardiovascular events with plasma[23,24] or serum antioxidants,[25–27] and dominantly negative results have been reported from intervention trials of β-carotene or vitamin E.[28–32]

We previously reported that dietary supplementation with lutein reduced atherosclerosis in two strains of susceptible mice.[22] We also found that plasma lutein was inversely associated with progression of atherosclerosis, as measured by carotid intima-media thickness (IMT), in a cohort of middle-aged women and men.[22] In this report from the same cohort, we present relations between IMT progression and baseline plasma levels of several possible antioxidants, including ascorbic acid, α- and γ-tocopherol, lutein, and other carotenoids. According to their chemical structure, carotenoids were categorized into two profiles for the analysis: non-polar hydrocarbon carotenoids without an oxygen atom (α-carotene, β-carotene, lycopene) and polar oxygenated carotenoids with 1 to 6 oxygen atoms (lutein, zeaxanthin, β-cryptoxanthin).[33]

Methods

Study Population

The Los Angeles Atherosclerosis Study (LAAS) is a prospective investigation of relationships between potential etiologic factors and

pre-clinical atherosclerosis and has been described previously.[22,34] Briefly, all participants were randomly sampled from a large utility company and were free of symptomatic cardiovascular disease. The participation rate was 85%, resulting in a baseline sample size of 573 women and men aged 40 to 60 years. The baseline examination was conducted between 1995 and 1996, and follow-up occurred approximately 18 months later (mean ±SD: 18.1±2.4 months). All participants signed an informed consent approved by the Institutional Review Board of the Keck School of Medicine at the University of Southern California before study participation.

Measurements

At each examination, subjects completed a questionnaire regarding information on demographic status, medication use, and health behaviors. Blood pressure, body weight, and height were also measured. Carotid IMT, an indicator of intimal thickening, was measured by high-resolution B-mode ultrasound with an ATL scanner.[35] Procedures for image acquisition and processing have been reported previously.[36] Briefly, bilateral carotid IMT was measured in two examinations. During the baseline examination, participants were scanned in two body positions (supine and lateral). In the 18-month examination, the ultrasound image was scanned in the supine position only. A mean IMT score, averaged over two sides, was used in the analysis. A reproducibility study conducted in association with the baseline examination found a between-sonographer coefficient of variation of 2.8% for IMT.[36] All measurements were conducted during a single examination in a mobile van that was driven to the work site.

Plasma Assays

Fasting blood samples were collected by venipuncture at baseline. Specimens were centrifuged, and plasma was separated, treated with nitrogen, aliquoted, stored at −20°C for up to 2 days, and then frozen at −70°C. Storage time before analysis averaged (mean±SD) 1.2±0.3 years. Plasma ascorbic acid was analyzed by high-pressure liquid chromatography (HPLC) according to the method of Kutnink et al.[37] To prevent oxidation during sample storage, an equal volume (500 μL) of 5% or 10% meta-phosphoric acid (MPA) was added to plasma before the sample was stored. Statistical analyses were adjusted for concentration of MPA added to the plasma to adjust for the effects of MPA concentration on measured ascorbic acid levels. Isoascorbic acid was used as an internal standard to compensate for losses of ascorbic acid during sample processing.

Plasma levels of α-tocopherol, γ-tocopherol, carotenoids (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene), and retinol were determined with an HPLC-based assay derived from the method described by Epler et al.[38] All measurements were performed at the Heber Laboratory (UCLA, California), which participates in the National Cancer Institute/National Institute of Standards and Technology Quality Assurance Program.[39]

Serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured within 2 months of collection by an enzymatic method using an automated clinical chemistry analyzer at the University of Southern California. Non-HDL-C was calculated as TC minus HDL-C.

Data Analysis

Rank order correlations and linear regression models were used to investigate the association between plasma antioxidants and progression of IMT at 18 months. Because distributions of plasma antioxidant concentrations were skewed, each plasma antioxidant was divided into quintiles for regression analysis. Trend tests across quintiles were computed by regressing change in IMT on the median level of each antioxidant within each quintile. Model 1 was adjusted for age, sex, and smoking status (current, former, and never smoker). The multivariate model (model 2) further adjusted for BMI (kg/m²), serum total cholesterol, HDL-C, systolic blood pressure (SBP), current treatment for hypertension and elevated cholesterol, history of diabetes, ethnicity (non-Hispanic white, black, Hispanic, Asian/Pacific Islander, and other), alcohol intake (g/d), height, natural log

of high-sensitivity C-reactive protein (hsCRP), and number of days between baseline and follow-up visit. SBP is the average of 2 seated blood pressure measurements taken at baseline. For analyses of ascorbic acid, the models were additionally adjusted for MPA levels.

Results

Of the 573 participants at baseline, IMT at 18-month follow-up was available for 480 (84%) participants. There were no significant differences at baseline between participants with and without follow-up, but those lost to follow-up were 1 year older (*P*=0.06). An additional 3 subjects did not have baseline plasma data and were excluded. Therefore, all analyses for this study are based on a sample size of 477. Characteristics of the cohort are presented in Table 1.

The correlations between plasma antioxidant levels and atherosclerotic risk factors are presented in Table 2. Two of the three oxygenated carotenoids, both tocopherols, and retinol were significantly and positively related to serum total cholesterol level, whereas the hydrocarbon carotenoids were all significantly inversely related to total cholesterol. This suggests that the polarity of oxygenated carotenoids, tocopherols, and retinol may allow these antioxidants to rely on lipids, like cholesterol, for transportation through the blood stream to various parts of the body. The water-soluble antioxidant, ascorbic acid, had a significant inverse association with total cholesterol (*P*=0.006). The marker of chronic inflammation (hsCRP) was significantly inversely related to plasma levels of ascorbic acid and all carotenoids, except zeaxanthin, and was positively related to γ-tocopherol.

Progression of IMT averaged 15 μm/18 months (SD=40) or 10 μm/12 months. Tests of linear trend for IMT progression rates over antioxidant quintiles are presented in Table 3. After adjusting for age, sex, interaction between antioxidant and sex, and smoking status (model 1), higher plasma levels of lutein (*P*=0.017), zeaxanthin (*P*=0.0004), β-cryptoxanthin (*P*=0.015), and α-carotene (*P*=0.003) were associated with reduced progression of carotid IMT. On average, for every 1 μmol/L increase of plasma lutein, zeaxanthin, β-cryptoxanthin, or α-carotene, IMT progression was reduced by 3.2, 4.7, 3.4, and 4.2 μm/18 months, respectively. These inverse relations are depicted across quintiles of antioxidants in Figure 1. Associations with IMT progression were not significant for plasma levels of lycopene and α- and γ-tocopherols (Figure 2).

After controlling for additional atherosclerotic risk factors and covariates potentially confounding the association between IMT progression and plasma antioxidant levels (model 2), inverse linear trends remained significant for the 3 oxygenated carotenoids and α-carotene. Including these 4 predictors in one regression model adjusted for age, sex, interaction with sex, and smoking status, plasma zeaxanthin and α-carotene remained statistically significant predictors of IMT progression (β±SE: −4.51±1.30; *P*=0.0006 and −4.02±1.38; *P*=0.0038, respectively). After controlling for additional atherosclerotic risk factors, these associations remained significant (β±SE: −4.38±1.34, *P*=0.001 for zeaxanthin and −3.41±1.46, *P*=0.02 for α-carotene).

TABLE 1.   Characteristics of the Study Cohort at Baseline: The Los Angeles Atherosclerosis Study, 1995–1998.

Characteristics	Women	Men	Total
N (%)	(n=220)	(n=257)	(n=477)
Ethnicity (% White)	121 (55)	140 (54)	261 (55)
Education (% ≤ high school)	38 (17)	21 (8)*	59 (12)
Current smokers (%)	42 (19)	73(28)†	115 (24)
Former smokers (%)	54 (25)	73 (28)	127 (27)
Diabetes mellitus (%)	7 (3)	7 (3)	14 (3)
Medication for hypertension (%)	44 (20)	36 (14)	80 (17)
Cholesterol-lowering medication (%)	7 (3)	22(9)†	29 (6)
Mean±SD			
Age at baseline	51.4±4.3	48.6±4.6*	49.9±4.7
Body Mass Index (kg/m²)	27.2±6.1	28.6±4.8†	28.0±5.5
Systolic blood pressure (mmHg)	127.0±16.2	129.5±12.7	128.3±14.5
Diastolic blood pressure (mmHg)	88.0±9.5	91.6±8.8*	89.9±9.3
Alcohol intake (g/day)	3.8±7.7	8.9±12.9*	6.5±11.1
Common Carotid IMT (μm)			
Baseline	648.8±85.0	679.8±106.5*	665.0±98.4
18 month progression	18.0±39.4	12.8±43.2	15.2±41.5
Serum Lipid Levels (mmol/L)	(n=217)	(n=252)	(n=469)
Total Cholesterol	5.49±0.91	5.65±0.98	5.58±0.95
High Density Lipoprotein fraction	1.67±0.37	1.31±0.24*	1.47±0.36
Non High Density Lipoprotein fraction	3.82±0.97	4.34±1.00*	4.10±1.02
Plasma Levels			
High Sensitivity C-Reactive Protein (mg/L)	3.52±3.27	2.03±2.01	2.72±2.76
Antioxidants (μmol/L)	(n=206)	(n=250)	(n=456)
Ascorbic acid	31.9±21.6	28.2±17.1†	29.9±19.3
Oxygenated carotenoids	(n=220)	(n=257)	(n=477)
Lutein	0.28±0.12	0.27±0.12	0.28±0.12
Zeaxanthin	0.06±0.04	0.06±0.03	0.06±0.04
β-cryptoxanthin	0.09±0.07	0.09±0.06	0.09±0.06
Hydrocarbon carotenoids			
α-carotene	0.22±0.19	0.16±0.15*	0.19±0.17
β-carotene	0.86±0.83	0.64±0.75*	0.74±0.79
Lycopene	0.76±0.73	0.76±0.71	0.76±0.72
Tocopherols			
α-tocopherol	30.06±13.37	30.38±12.46	30.23±12.88
γ-tocopherol	5.16±3.60	5.68±3.18	5.44±3.39
Retinol	2.08±0.62	2.35±0.67*	2.23±0.66

*$P<0.001$ for difference between gender groups.
†$P<0.05$ for difference between gender groups

A statistically significant interaction was observed between zeaxanthin and sex ($P=0.02$), suggesting that zeaxanthin has a greater effect in reducing 18-month IMT progression in women than in men. This interaction was not observed with the other 9 plasma antioxidants examined, suggesting that in general the effects of plasma levels of antioxidants on IMT progression are similar in women and men.

We also tested for interactions between the relation of plasma antioxidant to IMT progression with current smoking and former smoking status (relative to never-smokers). Only 2 significant interactions were observed for current smoking,

lycopene ($P=0.03$) and retinol ($P=0.001$). Lycopene was protective against IMT progression among smokers whereas retinol was a risk factor for IMT progression only among current smokers. There were no significant interactions with former smoking status (all $P>0.05$).

Discussion

The primary findings in the current study were that higher plasma levels of the oxygenated carotenoids (lutein, zeaxanthin, and β-cryptoxanthin) and α-carotene at baseline were associated with reduced IMT progression over 18 months.

TABLE 2.   Regression Coefficients from Rank Order Correlation [Spearman] Among Plasma Antioxidant Levels and Atherosclerosis Risk Factors at Baseline After Adjustment for Age and Sex. The Los Angeles Atherosclerosis Study, 1995–1998

| Risk Factor | Vit C | Carotenoids | | | | | | Tocopherols | | |
| | Ascorbic Acid* | Oxygenated Carotenoids | | | Hydrocarbon Carotenoids | | | | | |
		Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Lycopene	γ-Tocopherol	α-Tocopherol	Retinol
Cigarettes Smoked (packs/day)	−0.03	−0.14†	0.05	−0.24†	−0.18†	−0.22†	−0.08	0.04	0.06	−0.05
Alcohol intake (g/day)	−0.01	0.04	0.10†	−0.11†	0.00	0.02	0.10†	0.01	0.10†	0.10†
BMI (kg/m^2)	−0.26†	−0.14†	0.00	−0.18†	−0.27†	−0.29†	−0.16†	0.26†	0.01	−0.03
SBP (mm Hg)	−0.11†	−0.04	0.01	−0.08	−0.21†	−0.20†	−0.09†	0.12†	0.08	0.12†
DBP (mm Hg)	−0.12†	−0.04	0.04	−0.11†	−0.27†	−0.21†	−0.13†	0.15†	0.06	0.08
Plasma hs-CRP (mg/L)	−0.16†	−0.19†	−0.02	−0.23†	−0.30†	−0.31†	−0.24†	0.19†	0.03	−0.01
Serum lipid levels (mmol/L)										
Total cholesterol	−0.13†	0.15†	0.19†	0.08	−0.09†	−0.10†	−0.10†	0.35†	0.38†	0.23†
HDL-C	0.25†	0.11†	0.07	0.06	0.21†	0.14†	0.11†	−0.19†	−0.03	0.02
Non-HDL-C	−0.20†	0.11†	0.16†	0.06	−0.16†	−0.15†	−0.12†	0.40†	0.37†	0.21†

N=477 for all antioxidants except ascorbic acid (n=456). N=469 for all cholesterol levels except those for ascorbic acid (n=450)

*Ascorbic acid is adjusted for MPA concentration added to the blood specimen during processing in addition to age and sex.

†$P<0.05$.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high sensitivity C-Reactive Protein; HDL-C, high density lipoprotein fraction of total cholesterol; Non-HDL-C, total cholesterol–HDL-C.

Plasma levels of 2 hydrocarbon carotenes (β-carotene and lycopene), retinol, ascorbic acid, and tocopherols were not significantly associated with IMT progression.

Carotid IMT was used to assess atherosclerosis in early stages of disease and has been shown to predict cardiac events including myocardial infarction and stroke.[40] Furthermore, it was recently reported that rate of common carotid IMT progression was increased 3-fold in persons with angiographically confirmed coronary artery disease relative to normal controls.[41]

Some previous studies found that carotenoids were more protective against cardiovascular outcomes among smokers than non-smokers.[19,42] We tested for interactions between plasma antioxidant levels and current or former smoking status (relative to non-smokers) as they relate to IMT progression. Because there were significant interactions with current smoking for only 2 of the 10 plasma measures examined, it is plausible that these results were caused by chance. However, the interaction between smoking and retinol was highly significant ($P=0.001$), suggesting an atherogenic effect of elevated plasma retinol or dietary retinol, and should be examined further in future studies.

One of the criticisms of previous plasma antioxidant studies, and a potential explanation for the conflicting results observed, is that study designs did not account for the impact of inflammation on plasma antioxidant levels and atherosclerosis. For example, serum antioxidant levels are lower during acute illness as measured by inflammatory markers such as CRP.[43,44] Our data are consistent with these findings in that higher plasma levels of hsCRP were correlated with lower plasma levels of ascorbic acid and each carotenoid (Table 2). For this reason, regression model 2 included adjustment for (logarithm) hsCRP. The important finding was that adjustment for hsCRP did not alter the inverse associations between

IMT progression and plasma oxygenated carotenoids or α-carotene, suggesting that observed inverse associations were not caused by confounding by the effects of chronic inflammation.

The observed associations between oxygenated and hydrocarbon carotenoid levels and 18-month IMT progression may result, in part, from the unique geometric structure of the carotenoid molecules.[45] Oxygenated carotenoids have polar ring structures at the end of conjugated double-bond chains, whereas the hydrocarbon carotenes lack polar chains and ring structures, thus making them non-polar.[45,46] The polarity afforded to the oxygenated carotenoids allows them to be incorporated into lipid micelles in larger concentrations than the nonpolar hydrocarbon carotenes.[46] The micelles are then transported to the intestinal mucosal cells, where the carotenoids are absorbed through passive diffusion into the small intestine.[45,46] Thus, the polarity of oxygenated carotenoids may, in general, improve bioavailability and have more impact on atherosclerosis than the hydrocarbon carotenoids. The mechanisms by which such effects may operate are suggested by the finding that lutein inhibits the inflammatory response to damaged LDL in a model of the artery wall.[22]

Results of the present study are similar to those from a Dutch cohort that related baseline serum carotenoids and α-tocopherol to 7.2-year all-cause mortality in 638 elderly participants. De Waart et al found the strongest protective associations with mortality for β-cryptoxanthin, lutein, and zeaxanthin.[21] In contrast, as found in the current study, α- and β-carotene and α-tocopherol were not significantly associated with the outcome.

A nested case-control study from the ARIC cohort used cross-sectional differences in carotid IMT as a measure of asymptomatic atherosclerosis. Cases (n=231) were defined

TABLE 3.   Change in IMT (μm) Over 18 Months Regressed on Plasma Antioxidant Levels (μmol/L). The Los Angeles Atherosclerosis Study, 1995–1998

Independent Variable		Regression Coefficient	SE	P for Trend
Ascorbic acid*	Model 1	−1.13	1.51	0.45
	Model 2	0.26	1.65	0.87
Oxygenated carotenoids				
Lutein	Model 1	−3.23	1.35	0.017
	Model 2	−2.88	1.43	0.045
Zeaxanthin	Model 1	−4.67	1.31	0.0004
	Model 2	−4.62	1.37	0.0008
β-Cryptoxanthin	Model 1	−3.42	1.39	0.015
	Model 2	−3.36	1.53	0.028
Hydrocarbon carotenoids				
α-Carotene	Model 1	−4.20	1.41	0.003
	Model 2	−4.21	1.51	0.005
β-Carotene	Model 1	−2.24	1.40	0.11
	Model 2	−1.40	1.53	0.36
Lycopene	Model 1	−0.45	1.36	0.74
	Model 2	0.19	1.42	0.89
Tocopherols				
α-Tocopherol	Model 1	−0.53	1.37	0.70
	Model 2	−0.42	1.49	0.78
γ-Tocopherol	Model 1	−0.60	1.36	0.66
	Model 2	−1.44	1.51	0.34
Retinol	Model 1	0.34	1.40	0.81
	Model 2	0.07	1.50	0.96

Model 1: Adjusted for age, sex, antioxidant and sex interaction, and smoking status (N=477). Model 2: Adjusted for age, sex, antioxidant and sex interaction, smoking status, BMI, serum total cholesterol, HDL-C, systolic blood pressure, treatment for hypertension and high cholesterol, diabetes, ethnicity, alcohol intake, height, natural log of high sensitivity C-Reactive Protein, and the number of days between baseline and follow-up visit (N=469). Units are (μm IMT/yr)/(0.1 μmol/L plasma antioxidant).

*Ascorbic acid models are adjusted for levels of meta-phosphoric acid (added during blood specimen processing) in addition to all other covariates for each respective model.

as the subjects categorized as exceeding the 90th percentile of IMT, and control subjects (n=231) were categorized as below the 75th percentile. They reported that carotid IMT was inversely and significantly related with serum β-cryptoxanthin and lutein plus zeaxanthin. Carotid IMT was not significantly related to α-carotene, β-carotene, lycopene, retinol, or α-tocopherol.[20]

In contrast to the protective effects of oxygenated carotenoids found in our study and the two studies described,[20,47] a recently reported nested case control study from the Physicians' Health Study found that plasma levels of five carotenoids (α- and β-carotene, β-cryptoxanthin, lycopene, and lutein), retinol, and α- and γ-tocopherol were not significantly related to risk of myocardial infarction in males.[42] The physicians' study also found a protective relation for β-carotene against myocardial infarction among current and
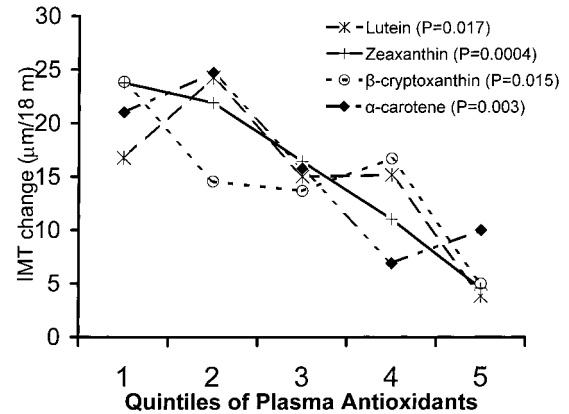

Figure 1. Plasma carotenoid quintiles by 18-month IMT change (adjusted for age, sex, antioxidant and sex interaction, and smoking status). The Los Angeles Atherosclerosis Study. Probability values are for trend. Antioxidant quintile ranges (μmol/L): lutein: 0.02 to 0.18, 0.18 to 0.23, 0.24 to 0.29, 0.29 to 0.36, 0.36 to 0.81; zeaxanthin: 0.01 to 0.04, 0.04 to 0.05, 0.05 to 0.06, 0.06 to 0.08, 0.09 to 0.28; β-cryptoxanthin: 0.01 to 0.04, 0.05 to 0.06, 0.06 to 0.08, 0.08 to 0.12, 0.12 to 0.60; α-carotene: 0.01 to 0.07, 0.07 to 0.11, 0.11 to 0.15, 0.16 to 0.27, 0.27,1.06; β-carotene: 0.02 to 0.24, 0.24 to 0.42, 0.42 to 0.64, 0.64 to 1.04, 1.05 to 8.00; and lycopene: 0.03 to 0.31, 0.31 to 0.47, 0.47 to 0.67, 0.67 to 1.02, 1.03 to 6.47.

former smokers, contrary to our results. The reasons for the apparent divergence of these findings from the other 3 studies of oxygenated carotenoids are unknown. Of the 3 positive studies, the current study and the ARIC findings[20] used IMT endpoints, whereas the third recorded total mortality.[21] The 3 studies also included women. In contrast, the physicians' study was limited to men and used myocardial infarction as an endpoint. Further epidemiologic research and clinical trials will be needed to determine the effects of dietary intake of oxygenated carotenoids on cardiovascular outcomes.

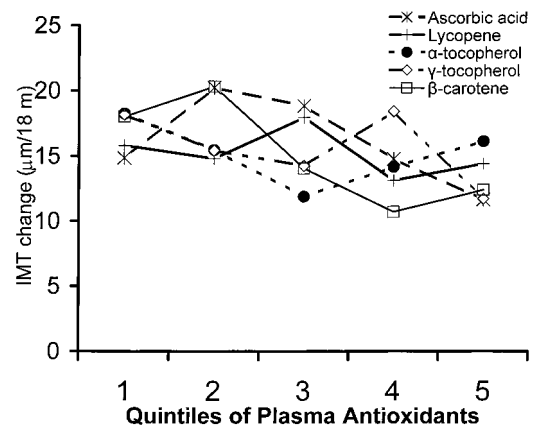We have previously reported the protective effect of lutein on the progression of carotid IMT at 18-month follow-up.[22]

Figure 2. Non-carotenoid antioxidant quintiles by 18-month IMT change (adjusted for age, sex, antioxidant and sex interaction, and smoking status). The Los Angeles Atherosclerosis Study. All probability values are for trend >0.05. Antioxidant quintile ranges (μmol/L): ascorbic acid: 0 to 14.09, 14.20 to 22.14, 22.23 to 31.23, 31.25 to 43.09, 43.15 to 203.27; α-tocopherol: 1.64 to 20.47, 20.53 to 24.82, 24.98 to 29.86, 30.02 to 38.77, 38.92 to 100.20; γ-tocopherol: 0.19 to 2.74, 2.77 to 4.23, 4.23 to 5.86, 5.87 to 7.42, 7.44 to 24.04; and retinol: 0.73 to 1.73, 1.73 to 1.99, 2.00 to 2.27, 2.27 to 2.67, 2.69 to 5.44

This finding motivated coculture and mouse model experiments.[22] Pretreatment of the coculture cells with lutein as low as 10 nmol/L inhibited the inflammatory response of monocytes to LDL trapped in the artery wall (reduced monocyte migration 8-fold). In mouse models, lutein supplementation reduced lesion size 43% in LDL receptor-null mice ($P=0.02$) and 44% in apoE-null mice ($P=0.009$).[22]

Limitations of the current study stem from its observational design. Unmeasured confounding factors may be associated with both plasma levels of oxygenated carotenoids and atherosclerosis. For example, there may be other components of foods containing these compounds that explain the protective effects observed in epidemiologic studies. There may also be other factors, such as inflammation, that are associated with atherosclerosis and impact blood levels of carotenoids.[47] However, the effects of lutein supplementation in mouse models,[22] and our finding that adjustment for hsCRP did not explain protective associations, argue against a confounding explanation of our findings. The consistency of our findings for oxygenated carotenoids and IMT progression with those from ARIC for cross-sectional IMT[20] also argue against explanation of findings in terms of artifacts of study design particular to either study.

In summary, this study provides evidence for an inverse association between plasma levels of oxygenated carotenoids and α-carotene with progression of carotid IMT. The consistency of these findings with two other epidemiologic studies, together with in vitro and animal model evidence for an anti-atherogenic effect of lutein,[22] suggest that oxygenated carotenoids are protective against the pathogenesis of atherosclerosis. The anti-inflammatory effects of lutein in vitro suggest a mechanism for anti-atherogenic effects.[22] However, randomized trials with oxygenated carotenoids and cardiovascular endpoints are needed to determine if the observed epidemiologic associations are causal in humans.

References

1. Lusis AJ. Atherosclerosis. *Nature.* 2000;407:233–241.
2. Diaz MN, Frei B, Vita JA, Keaney JF, Jr. Antioxidants and atherosclerotic heart disease. *N Engl J Med.* 1997;337:408–416.
3. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med.* 1992;13:341–390.
4. Devaraj S, Jialal I. Oxidized low-density lipoprotein and atherosclerosis. *Int J Clin Lab Res.* 1996;26:178–184.
5. Stahl W, Junghans A, de Boer B, Driomina ES, Briviba K, Sies H. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Lett.* 1998;427:305–308.
6. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med.* 1993;328:1450–1456.
7. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med.* 1993;328:1444–1449.
8. Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr.* 1996;64:190–196.
9. Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH, Boeing H, Hofman A, Grobbee DE, Witteman JC. Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. *Am J Clin Nutr.* 1999;69:261–266.
10. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witteman JC. Serum carotenoids and atherosclerosis. The Rotterdam Study. *Atherosclerosis.* 2000;148:49–56.
11. Klipstein-Grobusch K, den Breeijen JH, Grobbee DE, Boeing H, Hofman A, Witteman JC. Dietary Antioxidants and Peripheral Arterial Disease: The Rotterdam Study. *Am J Epidemiol.* 2001;154:145–149.
12. Gey KF, Puska P. Plasma vitamins E and A inversely correlated to mortality from ischemic heart disease in cross-cultural epidemiology. *Ann N Y Acad Sci.* 1989;570:268–282.
13. Riemersma RA. Dietary fatty acids and antioxidant vitamins and the risk of coronary heart disease: the Scottish experience. *Acta Cardiol.* 1989;44:482–483.
14. Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet.* 1991;337:1–5.
15. Ramirez J, Flowers NC. Leukocyte ascorbic acid and its relationship to coronary heart disease in man. *Am J Clin Nutrition.* 1980;33:2079–2087.
16. Sahyoun NR, Jacques PF, Russell RM. Carotenoids, vitamins C and E, and mortality in an elderly population. *Am J Epidemiol.* 1996;144:501–511.
17. Nyyssonen K, Parviainen MT, Salonen R, Tuomilehto J, Salonen JT. Vitamin C deficiency and risk of myocardial infarction: prospective study of men in eastern Finland. *BMJ.* 1997;314:634–638.
18. Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease: the Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. *J Am Med Assoc.* 1994;272:1439–1441.
19. Street DA, Comstock GW, Salkfeld RM, Schuep W, Klag MJ. Serum antioxidants and myocardial infarction: Are levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? *Circulation.* 1994;90:1154–1161.
20. Iribarren C, Folsom AR, Jacobs Jr DR, Gross MD, Belcher JD, Eckfeldt JH. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. *Arterioscler Thromb Vasc Biol.* 1997;17:1171–1177.
21. De Waart FG, Schouten EG, Stalenhoef AF, Kok FJ. Serum carotenoids, alpha-tocopherol and mortality risk in a prospective study among Dutch elderly. *Int J Epidemiol.* 2001;30:136–143.
22. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore AM, Hama S, Hough G, Wang X, Drake T, Bairey Merz CN, Fogelman AM. The oxygenated carotenoid *lutein* and progression of early atherosclerosis. The Los Angeles Atherosclerosis Study. *Circulation.* 2001;103:2922–2927.
23. de Waart FG, Smilde TJ, Wollersheim H, Stalenhoef AF, Kok FJ. Smoking characteristics, antioxidant vitamins, and carotid artery wall thickness among life-long smokers. *J Clin Epidemiol.* 2000;53:707–714.
24. Duthie GG, Beattie JA, Arthur JR, Franklin M, Morrice PC, James WP. Blood antioxidants and indices of lipid peroxidation in subjects with angina pectoris. *Nutrition.* 1994;10:313–316.
25. Hense HW, Stender M, Bors W, Keil U. Lack of association between vitamin E and myocardial infarction in a population with high vitamin E levels. *Atherosclerosis.* 1993;103:21–28.
26. Salonen JT, Salonen R, Penttila I, Herranen J, Jauhiainen M, Kantola M, Lappetelainen R, Maenpaa P, Alfthan G, Puska P. Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and risk of death from coronary artery disease. *Am J Cardiol.* 1985;56:226–231.
27. Evans RW, Shaten BJ, Day BW, Kuller LH. Prospective association between lipid soluble antioxidants and coronary heart disease in men. The Multiple Risk Factor Intervention Trial. *Am J Epidemiol.* 1998;147:180–186.
28. Alpha-tocopherol beta-carotene cancer prevention study group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med.* 1994;330:1029–1035.
29. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med.* 1996;334:1145–1149.
30. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med.* 1996;334:1150–1155.
31. Greenberg ER, Baron JA, Karagas MR, Stukel TA, Nierenberg DW, Stevens MM, Mandel JS, Haile RW. Mortality associated with low

plasma concentration of beta carotene and the effect of oral supplementation. *JAMA.* 1996;275:699–703.

32. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20, 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet.* 2002;360:23–33.

33. Castenmiller JJM, West CE. Bioavailability and bioconversion of carotenoids. *Ann Rev Nutrition.* 1998;18:19–38.

34. Sun P, Dwyer KM, Bairey Merz CN, Sun W, Johnson CA, Shircore AM, Dwyer JH. Blood pressure, LDL cholesterol, and intima-media thickness: A test of the "Response to Injury" hypothesis of atherosclerosis. The Los Angeles Atherosclerosis Study. *Arterioscler Thromb Vasc Biol.* 2000;20:2005–2010.

35. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation.* 1986;74:1399–1406.

36. Dwyer JH, Sun P, Kwong-Fu H, Dwyer KM, Selzer RH. Automated intima-media thickness: The Los Angeles Atherosclerosis Study. *Ultrasound Med Biol.* 1998;24:981–987.

37. Kutnink M, Hawkes WC, Schauss EE, Omaye ST. An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components. *Anal Biochem.* 1987;166:424–430.

38. Epler KS, Ziegler RG, Craft NE. Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. *J Chrom.* 1993;619:37–48.

39. Margolis SA, Duewer DL. Measurement of ascorbic acid in human plasma and serum: stability, intralaboratory repeatability, and interlaboratory reproducibility. *Clin Chem.* 1996;42:1257–1262.

40. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med.* 1999;340:14–22.

41. Crouse JR, 3rd, Tang R, Espeland MA, Terry JG, Morgan T, Mercuri M. Associations of extracranial carotid atherosclerosis progression with coronary status and risk factors in patients with and without coronary artery disease. *Circulation.* 2002;106:2061–2066.

42. Hak AE, Stampfer MJ, Campos H, Sesso HD, Gaziano JM, Willett W, Ma J. Plasma Carotenoids and Tocopherols and Risk of Myocardial Infarction in a Low-Risk Population of US Male Physicians. *Circulation.* 2003;108:802–807.

43. Louw JA, Werbeck A, Louw ME, Kotze TJ, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. *Crit Care Med.* 1992;20:934–941.

44. Kritchevsky SB, Bush AJ, Pahor M, Gross MD. Serum carotenoids and markers of inflammation in nonsmokers. *Am J Epidemiol.* 2000;152:1065–1071.

45. Zaripheh S, Erdman JW, Jr. Factors that influence the bioavailability of xanthophylls. *J Nutr.* 2002;132:531S–534S.

46. Yeum KJ, Russell RM. Carotenoid bioavailability and bioconversion. *Annu Rev Nutr.* 2002;22:483–504.

47. Kritchevsky SB. beta-Carotene, carotenoids and the prevention of coronary heart disease. *J Nutr.* 1999;129:5–8.