Prospective Study of Plasma Carotenoids and Tocopherols in Relation to Risk of Ischemic Stroke

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Background and Purpose—Intake of fruits and vegetables has been related to lower risk of ischemic stroke, but nutrients responsible for this apparent benefit remain ill-defined. Tocopherols (vitamin E) have also been proposed to be protective.

Methods—We conducted a prospective, nested case-control analysis among male physicians without diagnosed cardiovascular disease followed-up for up to 13 years in the Physicians’ Health Study. Samples from 297 physicians with ischemic stroke were analyzed with paired controls, matched for age and smoking, for 5 major carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene), retinol, and α- and γ-tocopherol.

Results—Baseline plasma levels of α-carotene and β-carotene and lycopene tended to be inversely related to risk of ischemic stroke with an apparent threshold effect. As compared with men whose plasma levels were in the lowest quintile, the multivariate adjusted odds ratios (ORs) of ischemic stroke among men with levels in the second through fifth quintiles were 0.59 (95% CI, 0.36 to 0.98) for α-carotene, 0.62 (95% CI, 0.38 to 1.01) for β-carotene, and 0.61 (95% CI, 0.37 to 1.00) for lycopene. A tendency toward an inverse association was found for β-cryptoxanthin, but the result was not statistically significant. No association was found for lutein, retinol, and tocopherols.

Conclusion—Our data suggest that higher plasma levels of carotenoids, as markers of fruit and vegetable intake, are inversely related to risk of ischemic stroke and provide support for recommendations to consume fruits and vegetables regularly. (Stroke. 2004;35:1584-1588.)

Key Words: antioxidants ■ carotenoids ■ tocopherols ■ ischemic stroke

Higher consumption of fruits and vegetables has been related to lower risk of ischemic stroke.1,2 Nutrients responsible for this benefit remain ill-defined. Antioxidants, such as carotenoids and vitamin E, may protect against atherosclerosis by blocking oxidation of low-density lipoprotein cholesterol3 and by favorably influencing plaque stability, vasomotor function, and tendency for thrombosis.4 Results of studies examining the association between intake of carotenoids and ischemic stroke are inconsistent.5–7 Difficulties in assessing carotenoid intake8 or lack of accurate food composition data, which became available only recently,9 may contribute to this inconsistency. In one prospective study, individuals with low plasma levels of both β-carotene and vitamin C had lower stroke mortality rates.10 In another prospective study, higher levels of lycopene were inversely related to stroke risk.11 No data are available on the association between blood levels of tocopherols, antioxidants high in plant oils (vitamin E), and risk of ischemic stroke.

We prospectively examined the associations between plasma levels of 5 major carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene), retinol, and α- and γ-tocopherol with ischemic stroke in male physicians.

Materials and Methods

Study Population, Dietary Intake, and Collection of Blood Samples

The Physicians’ Health Study has been described in detail elsewhere.12 Briefly, 22 071 male physicians, aged 40 to 84 in 1982, without history of cardiovascular disease were assigned at random in a factorial design to receive aspirin, β-carotene, or placebo. Informed consent was obtained. The research protocol was approved by the institutional review board at Brigham and Women’s Hospital in Boston.

At baseline, the physicians provided information on health status and risk factors for cardiovascular disease. Dietary intakes of selected fruits and vegetables were ascertained by 2 abbreviated, semiquantitative food frequency questionnaires.13 Seven response categories ranged from rarely/never to 2 or more per day. Reproducibility and validity of the questionnaire items on fruit and vegetable intake were found to be reasonable in a study of 127 male health professionals aged 45 to 70.8

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Before randomization, 14,916 (68%) participants provided blood samples in EDTA tubes; plasma was stored at −80°C. During storage, no specimen thawed or warmed substantially. Degradation of carotenoids, retinol, and tocopherols was nondetectable in plasma stored at −70°C for up to 51.5 months.14 and β-carotene, retinol, and α-tocopherol are stable for at least 15 years at temperatures < −70°C.15 Intraperson correlation coefficients for repeated blood samples taken from 22 control subjects at baseline and after 5 years ranged from 0.52 to 0.85 for carotenoids and tocopherols (P< 0.01).

**Stroke Definition and Selection of Controls**
Six months after randomization and annually thereafter, participants completed mailed questionnaires inquiring about the occurrence of end points of interest, including stroke. Nonresponders were telephoned. Deaths among physicians usually were reported by family members or postal authorities. Morbidity and mortality follow-up exceeded 99%. The occurrence of stroke was confirmed after medical records were reviewed by a neurologist. Nonfatal stroke was defined as a typical focal neurological deficit sudden or rapid in onset with symptoms and signs persisting for > 24 hours, which was attributable to a cerebrovascular event. Fatal strokes were confirmed when clear evidence was found from all available records (death certificate, hospital records, eye-witness accounts) of a cerebrovascular event. When information was adequate, the neurologist subdivided strokes as ischemic or hemorrhagic.16 We restricted our current analyses to ischemic stroke cases only (interobserver agreement κ = 0.82).16 Each case was matched to 1 control free from diagnosed cardiovascular disease at the time of diagnosis of the matched case. Controls were randomly selected from participants who met the matching criteria of age (within 1 year), smoking (past, current, never), and time since randomization in 6-month intervals.

**Laboratory Analyses**
Plasma levels of carotenoids, retinol, and tocopherols were measured as described previously.17 Briefly, case and control samples were assayed in the same batch blinded for case-control status. Carote-
noids, retinol, and tocopherols were assayed by high-performance liquid chromatography. Mean intra-assay coefficients of variation based on blinded quality-control samples ranged from 7.6% for α-carotene to 11.9% for α-tocopherol. Total and high-density lipoprotein cholesterol levels and triglyceride concentrations were assayed as described.18

**Statistical Analysis**

For baseline cardiovascular risk factors, means or proportions were calculated for cases and controls. We assessed statistical significance using χ² tests for categorical variables and paired t tests for continuous variables.

For all analyses, we used weighted batch-specific values of plasma carotenoids, retinol, or tocopherols to account for differences in assay batches. Because each case and its matched control sample were assayed in the same batch, this procedure preserves the validity of our results.

The partial Spearman-rank correlation coefficient was used to test the multivariate-adjusted association between the baseline consumption of a specific fruit or vegetable known to be most predictive of the plasma carotenoid level and the measured plasma levels in controls. Because carotenoids are carried in lipoproteins, we adjusted the correlation coefficients for total and high-density lipoprotein cholesterol. When computing lipoprotein-adjusted plasma levels of α-tocopherol and γ-tocopherol according to baseline multivitamin use, we additionally adjusted for triglycerides, because of their strong association with α- and γ-tocopherol.

To estimate the relative risk of ischemic stroke according to baseline plasma levels of antioxidants, we first categorized men according to batch-specific quintiles determined by the distribution of plasma levels of carotenoids, retinol, or tocopherols in the controls. We then performed conditional logistic regression analyses, based on the case-control matching. Adjusted estimates of risk were obtained with multivariate models that also controlled for potential confounding factors. Because multivitamin use was the major determinant of tocopherols, we did not adjust for multivitamin use in models regarding tocopherols. A priori data suggest a nonlinear relation such that risk of ischemic stroke is reduced among those eating more than minimal amounts of fruits and vegetables, without a further dose effect.19 We therefore computed relative risks of ischemic stroke among men with plasma levels of antioxidants in quintile 2 to 5 as compared with men whose plasma levels of antioxidants were in the lowest quintile.

To assess whether results observed for plasma β-carotene were affected by subsequent assignment to either β-carotene or placebo, we fit unconditional logistic regression models within the active supplement and placebo groups, and fit an unconditional logistic regression model with a multiplicative interaction term involving β-carotene assignment and baseline plasma β-carotene level.

All P values are 2-tailed, and 95% CIs were computed.

**Results**

During follow-up, 297 men experienced a first ischemic stroke. The mean time from study enrollment to the occurrence of ischemic stroke was 7.3 years (SD ± 3.6 years), with a maximum follow-up of 13.0 years. Table 1 shows the baseline characteristics of the 297 men who had an ischemic stroke and their matched controls. As expected, compared with controls, ischemic stroke cases had a more adverse risk profile.

Baseline plasma levels of carotenoids were modestly correlated with the reported consumption of specific fruits or vegetables known to be predictive of these levels (Table 2). Among controls, geometric mean baseline plasma levels of α-tocopherol were higher in multivitamin users (13 138 ng/mL) than nonusers (11 281, P = 0.001), whereas levels of γ-tocopherol were lower (1648 and 1841 ng/mL, respectively; P = 0.04).

Table 3 shows the relation of baseline plasma levels of antioxidants to risk of ischemic stroke. Men with levels of the carotenoids α-carotene, β-carotene, and lycopene in the second through fifth quintile tended to have lower risks of ischemic stroke, suggesting a threshold effect. For example, men with levels of β-carotene in the second up to fifth quintile had a 43% lower risk of ischemic stroke (OR, 0.57; 95% CI, 0.39 to 0.85), adjusted for age and smoking. Multivariate adjustment slightly affected the association (OR, 0.62; 95% CI, 0.38 to 1.01). Men with plasma levels of β-cryptoxanthin in the second up to fifth quintile tended to have lower risks of ischemic stroke, but results were not statistically significant. Levels of lutein, retinol, α-tocopherol, or γ-tocopherol were not associated with ischemic stroke.

In subgroup analyses, we observed no material differences between baseline plasma β-carotene levels and risk of ischemic stroke according to random assignment to either β-carotene or placebo (P interaction = 0.69).

**Discussion**

In this prospective, nested case-control study of generally well-nourished healthy male physicians without diagnosed cardiovascular disease at enrollment, baseline plasma levels of α-carotene, β-carotene, and lycopene tended to be inversely associated with risk of ischemic stroke. The association exhibited a threshold effect, with a consistent inverse association from the second quintile of plasma carotenoid level onwards. A tendency toward an inverse association was found for β-cryptoxanthin, but the result was not statistically significant. No inverse association with ischemic stroke risk was found with higher baseline levels of lutein, retinol, and tocopherols.

Our analyses are based on a single baseline measurement of carotenoids and tocopherols and therefore may not reflect levels over a longer period with complete accuracy. However, baseline measurements were reasonably correlated with levels measured 5 years later. In addition, we observed reasonable correlations of plasma carotenoids with intake of specific fruits and vegetables, and plasma tocopherol levels differed between multivitamin users and nonusers. These results are
TABLE 3. Relative Risk of Ischemic Stroke by Quintiles of Baseline Antioxidant Plasma Levels

<table>
<thead>
<tr>
<th>Quintile of Antioxidant*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>2–5†</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Carotene</td>
<td>30.4</td>
<td>45.8</td>
<td>55.6</td>
<td>71.3</td>
<td>96.8</td>
<td></td>
</tr>
<tr>
<td>(n=277/277)</td>
<td>1 (ref)</td>
<td>0.63 (0.38–1.05)</td>
<td>0.45 (0.25–0.78)</td>
<td>0.64 (0.38–1.09)</td>
<td>0.66 (0.39–1.12)</td>
<td>0.59 (0.39–0.89)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>113.8</td>
<td>170.0</td>
<td>235.5</td>
<td>320.2</td>
<td>435.3</td>
<td></td>
</tr>
<tr>
<td>(n=296/296)</td>
<td>1 (ref)</td>
<td>0.63 (0.39–1.04)</td>
<td>0.58 (0.35–0.97)</td>
<td>0.37 (0.21–0.66)</td>
<td>0.69 (0.41–1.16)</td>
<td>0.57 (0.39–0.85)</td>
</tr>
<tr>
<td>Lutein†</td>
<td>25.7</td>
<td>51.4</td>
<td>84.1</td>
<td>122.0</td>
<td>181.9</td>
<td></td>
</tr>
<tr>
<td>(n=152/152)</td>
<td>1 (ref)</td>
<td>1.07 (0.53–2.15)</td>
<td>0.64 (0.39–1.34)</td>
<td>0.77 (0.38–1.53)</td>
<td>0.72 (0.35–1.48)</td>
<td>0.79 (0.45–1.37)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>216.4</td>
<td>325.9</td>
<td>398.7</td>
<td>495.2</td>
<td>606.8</td>
<td></td>
</tr>
<tr>
<td>(n=295/295)</td>
<td>1 (ref)</td>
<td>0.69 (0.42–1.16)</td>
<td>0.66 (0.39–1.09)</td>
<td>0.78 (0.47–1.32)</td>
<td>0.80 (0.47–1.36)</td>
<td>0.72 (0.48–1.08)</td>
</tr>
<tr>
<td>Retinol</td>
<td>294.2</td>
<td>356.5</td>
<td>398.4</td>
<td>464.9</td>
<td>523.1</td>
<td></td>
</tr>
<tr>
<td>(n=265/265)</td>
<td>1 (ref)</td>
<td>0.66 (0.36–1.23)</td>
<td>0.54 (0.29–1.00)</td>
<td>0.55 (0.29–1.05)</td>
<td>0.72 (0.38–1.37)</td>
<td>0.61 (0.37–1.00)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>8279.5</td>
<td>10 216.0</td>
<td>11 842.4</td>
<td>13 523.4</td>
<td>16 986.9</td>
<td></td>
</tr>
<tr>
<td>(n=245/245)</td>
<td>1 (ref)</td>
<td>0.82 (0.48–1.40)</td>
<td>0.88 (0.50–1.53)</td>
<td>0.58 (0.33–1.04)</td>
<td>1.27 (0.74–2.18)</td>
<td>0.86 (0.55–1.34)</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>1170.5</td>
<td>1547.9</td>
<td>1817.7</td>
<td>2172.8</td>
<td>2866.1</td>
<td></td>
</tr>
<tr>
<td>(n=293/293)</td>
<td>1 (ref)</td>
<td>0.88 (0.52–1.47)</td>
<td>0.91 (0.54–1.52)</td>
<td>1.06 (0.63–1.77)</td>
<td>1.09 (0.66–1.80)</td>
<td>0.98 (0.66–1.46)</td>
</tr>
<tr>
<td>(n=245/245)</td>
<td>1 (ref)</td>
<td>1.01 (0.50–2.01)</td>
<td>0.87 (0.45–1.68)</td>
<td>1.07 (0.53–2.15)</td>
<td>0.85 (0.41–1.74)</td>
<td>0.93 (0.55–1.57)</td>
</tr>
</tbody>
</table>

*Shown values are OR and 95% CI in parentheses; ref indicates reference category (lowest quintile of baseline antioxidant level).
†Shown values are median values (ng/mL) for each quintile, based on weighted batch-specific medians of controls.
‡N of cases and controls in analyses adjusted for matching variables age and smoking (current, former, never).
§N of cases and controls in analyses additionally adjusted for body mass index; total and high-density lipoprotein (HDL) cholesterol; history of hypertension, diabetes mellitus, and parental history of MI before the age of 60 years; frequency of vigorous exercise (6 categories); alcohol consumption (7 categories); multivitamin use (never, past, current); and assignment to aspirin or β-carotene treatment or placebo.
¶Data on β-cryptoxanthin and lutein for 152 and 170 cases and matched controls, respectively.
intake of α-carotene, β-carotene, and lycopene, as measured by a food frequency questionnaire and risk of ischemic stroke.6 Difficulties in collecting information on carotenoid intake may have contributed to this inconsistency.8 Our results tend to support the previously reported finding suggesting an inverse association between lycopene and ischemic stroke.11 Higher levels of β-cryptoxanthin tended to be associated with lower risk of ischemic stroke. Our results were not statistically significant but are consistent with data showing an inverse association between consumption of citrus fruit and ischemic stroke.2 Lutein has been associated with reduced atherosclerosis.26 We, however, did not find a reduced risk of ischemic stroke with higher levels of lutein. Our null finding for retinol is not surprising, because retinol levels are highly regulated and vary little with intake.27 Although an inverse association between vitamin E intake may have contributed to this inconsistency.8 Our tocopherol levels are highly regulated and vary little with intake.27

In conclusion, our data suggest that higher plasma levels of carotenoids, as markers of fruit and vegetable intake, are inversely related with risk of ischemic stroke and provide further support for the recommendation to consume fruits and vegetables regularly.

Acknowledgments

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