Associations of plasma carotenoids with risk factors and biomarkers related to cardiovascular disease in middle-aged and older women1–3

Lu Wang, J Michael Gaziano, Edward P Norkus, Julie E Buring, and Howard D Sesso

ABSTRACT
Background: Cardiovascular disease (CVD) risk factors may potentially influence plasma concentrations of carotenoids. However, data on the association of plasma carotenoids with CVD-related biomarkers are only limited.

Objective: We examined the cross-sectional association of plasma carotenoids with blood lipids, glycated hemoglobin (Hb A1c), and C-reactive protein (CRP) in middle-aged and older women initially free of CVD and cancer.

Design: Participants from 3 nested case-control studies in the Women's Health Study were pooled. Baseline plasma carotenoids, including \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, and lutein-zeaxanthin, blood lipids, Hb A1c, and CRP were available for 2895 women.

Results: Women who were current smokers or obese had lower plasma concentrations of most carotenoids expect for lycopene. After adjusting for age, race, lifestyle factors, clinical factors, plasma total cholesterol, and dietary carotenoids, an increase of 30 mg/dL in LDL cholesterol was associated with a 17% increase in \( \alpha \)-carotene, a 16% increase in \( \beta \)-carotene, and an 8.5% increase in lycopene; an increase of 10 mg/dL in HDL cholesterol was associated with a 5.3% decrease in lycopene; an increase of 0.3% in Hb A1c was associated with a 1.4% increase in lycopene; and an increase of 2 mg/L in CRP was associated with a 1.3% decrease in \( \beta \)-carotene (all \( P < 0.01 \)).

Conclusions: In middle-aged and older women free of CVD and cancer, plasma carotenoids were associated with smoking, obesity, LDL cholesterol, HDL cholesterol, Hb A1c, and CRP. The associations differ among individual carotenoids, possibly reflecting metabolic effects of lifestyle and physiologic factors on plasma carotenoids, and may partially explain the inverse association of plasma carotenoids with CVD outcomes observed in epidemiologic studies.

INTRODUCTION

Fruit and vegetables are rich in carotenoids, a group of plant-derived, fat-soluble pigments. With abundant conjugated double bonds, carotenoids have shown potent antioxidant properties (1, 2). High plasma or adipose carotenoid concentrations have been potentially associated with a reduced risk of cardiovascular disease (CVD) in population-based observational studies (3–5). However, large-scale clinical trials on \( \beta \)-carotene supplementation either showed no benefit or increased the risk of CVD outcomes (6–9). Inherent confounding in epidemiologic studies may partially explain the discrepancy. In addition to dietary intake, other lifestyle and physiologic factors that are involved in CVD development could have influenced the concentrations of carotenoids in plasma and body tissues.

Smoking and obesity, both established CVD risk factors, were associated with lower serum \( \beta \)-carotene concentrations in a number of studies (10–17). Relatively fewer studies have examined the association of plasma carotenoids with biomarkers related to CVD risk, such as blood lipid subtype, glycated hemoglobin (Hb A1c), and C-reactive protein (CRP). Most previous investigations on plasma carotenoids and CVD-related biomarkers were either relatively small or focused on only selected carotenoids, predominantly \( \beta \)-carotene. We therefore conducted a cross-sectional study to examine the association of 5 major plasma carotenoids, including \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, and lutein-zeaxanthin, with risk factors and biomarkers related to CVD in a subsample of middle-aged and older women free of CVD and cancer.

SUBJECTS AND METHODS

Study population

The Women’s Health Study (WHS) is a recently completed, randomized, double-blind, placebo-controlled clinical trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer (18–20). The \( \beta \)-carotene component was terminated in 1996 after median treatment duration of 2.1 y (9). In 1992, female US health professionals (\( n = 39 876 \)), aged \( \geq 45 \) y and free from self-reported CVD and cancer (except non-melanoma skin cancer), were randomly assigned into the WHS. Baseline blood samples were collected from 28 345 (71%) participants in chilled package by overnight courier, centrifuged immediately, divided into aliquots, and stored in liquid nitrogen freezers at

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−140 °C for 10 y until analyzed. The study protocol was approved by the Brigham and Women’s Hospital institutional review board. Written informed consent was obtained from all participants.

This cross-sectional analysis was conducted with the use of preexisting baseline measurements for both cases and controls in 3 independent nested case-control studies within the WHS. The case-control study of diabetes (n = 940) included 470 cases of incident type 2 diabetes and an equal number of controls that were individually matched with cases on age (±1 y) and follow-up time (∓6 mo). The case-control study of breast cancer (n = 1016) included 508 cases of incident breast cancer and an equal number of controls, individually matched on age, smoking status (never, former, and current smoker), and follow-up time. The case-control study of CVD (n = 966) included 483 cases of incident CVD and an equal number of controls, individually matched on age, smoking status, and follow-up time. For the current analysis, participants who reported a pre-randomization history of CVD or cancer after randomization (n = 5) and duplicate participants who were included in >1 case-control study (n = 22) were excluded. A total of 2895 women free of CVD and cancer remained for analysis, among whom the prevalence of self-reported diabetes, hypercholesterolemia, and hypertension was 3.2%, 35.1%, and 34.6%, respectively.

Blood assays

In each case-control study, blood samples were thawed and assayed for carotenoids at Our Lady of Mercy Medical Center, Bronx, NY. Plasma carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein-zeaxanthin, were quantified by reversed-phase HPLC after extraction and concentration with the use of standard methods (21). All investigators and laboratory personnel were blinded to the subjects’ case-control status. All blood samples were handled identically throughout the processes of blood collection, long-term storage, sample retrieval, and assays. Internal standards (echinone) were used to correct for recoveries of all samples analyzed. The laboratory prepared and assayed internal and external blinded quality control specimens in every run. From these control specimens, the accuracy for each measured carotenoid was within 7%, and the day-to-day and within-day precision (CV) for these analytes was 5%. The laboratory has participated in the US Quality Assurance Program to ensure methods consistent with other laboratories.

Plasma lipids, Hb A1c, and CRP were measured previously as part of a larger study in the WHS. Plasma lipids and CRP were assayed at a core laboratory facility and were available for 2878 (99.4%) of the 2895 participants. Total cholesterol and HDL cholesterol were measured enzymatically with the use of a Hitachi 911 autoanalyzer (Roche Diagnostics, Basel, Switzerland), and LDL cholesterol was directly measured (Genzyme, Cambridge, MA). High-sensitivity CRP was measured with the use of a validated immunoturbidimetric method (Denka Seiken, Tokyo, Japan), and the values were similar to expected values in a population of healthy middle-aged women (22). The CVs varied from 2.2% to 6.1% for CRP concentrations ranging from 0.15 to 1.90 mg/L. Hb A1c was measured by the Tina-Quant turbidimetric inhibition immunoassay (Hitachi 911 Analyzer; Roche Diagnostics, Indianapolis, IN) with the use of packed red blood cells and was available for 2869 (99.1%) of the 2895 participants. The CV for quality control samples of Hb A1c was 7.2%.

Other baseline covariates

Women provided baseline self-reports of age (in y), weight, and height [represented as body mass index (BMI) in kg/m²], smoking status (never, former, current < 15 cigarettes/d, current ≥ 15 cigarettes/d), alcohol use (rarely or never, 1–3 drinks/mo, 1–6 drinks/wk, ≥ 1 drink/d), vigorous exercise (rarely or never, <1, 1–3, ≥4 times/wk), and menopausal status (yes, no, uncertain). Information on multivitamin supplement use (never, former, current), postmenopausal hormone use (never, former, current), and diagnosis of diabetes, hypertension, and hypercholesterolemia (no, yes) were also collected in the baseline questionnaire. Hypertension was defined as having a physician diagnosis, self-reported systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or antihypertensive treatment. Hypercholesterolemia was defined as having a physician diagnosis, self-reported cholesterol concentration ≥ 240 mg/dL (6.22 mmol/L), or antihypercholesterolemia treatment.

Women also completed a 131-item validated semiquantitative food-frequency questionnaire (SFFQ). The average daily intakes of individual food items were calculated by multiplying the intake frequency by the specified portion size of each item. Nutrient intakes were computed by multiplying the intake frequency of each unit of food by the nutrient content of the specified portion size according to food composition tables from the Harvard Food Composition Database (23). All individual nutrients were adjusted for total energy intake with the use of the residual method (24). The SFFQ used in the WHS has shown reasonable validity as a measure of long-term average dietary intakes in populations of health professionals (25).

Statistical analysis

Statistical analyses were performed with the use of SAS software (version 9.1; SAS Institute, Cary, NC). Spearman’s correlations of plasma carotenoids with dietary carotenoids were calculated. Because the distributions of plasma carotenoids were highly skewed, we applied logarithmic transformation to these variables. Plasma carotenoids were then compared across categories of demographic factors (age, race), lifestyle factors (smoking, alcohol use, vigorous exercise, menopausal status, postmenopausal hormone use, and multivitamin use), and clinical factors (BMI, history of diabetes, hypertension, and hypercholesterolemia) with the use of analysis of variance. The association of plasma carotenoids with CVD-related biomarkers were evaluated by comparing plasma carotenoids across categories of each biomarker with the use of analysis of covariance, first adjusting for age, race, and total cholesterol, then further adjusting for other lifestyle factors, clinical factors, and dietary intake of respective carotenoids. After the same adjustment strategy, linear regression models were used to determine the difference in plasma carotenoids associated with unit change in biomarkers. Analyses on biomarkers were further stratified by lifestyle factors, including smoking status (current compared with not current), BMI (<25 compared with ≥25), and dietary fat intake (above median compared with below median). Interactions were tested with the use of Wald’s chi-square test. We used a lower value of P < 0.01 as the threshold to indicate significance because of the inherent multiple number of comparisons in our study. In sensitivity analyses, we excluded subjects who had
| TABLE 1 |
| Platelet concentrations of carotenoids across categories of baseline characteristics among 2895 women in the Women’s Health Study |

<table>
<thead>
<tr>
<th>Category</th>
<th>Subjects</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lycopene</th>
<th>Lutein-zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>n</td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>μmol/L</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Overall</td>
<td>1300</td>
<td>0.105</td>
<td>0.167–3.84</td>
<td>0.157 (0.151, 0.164)</td>
<td>0.261 (0.253, 0.270)</td>
<td>0.288 (0.280, 0.295)</td>
</tr>
<tr>
<td>Age 3</td>
<td>607</td>
<td>0.101 (0.094, 0.109)</td>
<td>0.354 (0.333, 0.376)</td>
<td>0.153 (0.144, 0.162)</td>
<td>0.258 (0.246, 0.270)</td>
<td>0.294 (0.283, 0.305)</td>
</tr>
<tr>
<td>Race 5</td>
<td>490</td>
<td>0.101 (0.093, 0.110)</td>
<td>0.348 (0.326, 0.373)</td>
<td>0.157 (0.147, 0.167)</td>
<td>0.252 (0.239, 0.266)</td>
<td>0.285 (0.273, 0.297)</td>
</tr>
<tr>
<td>BMI 6</td>
<td>498</td>
<td>0.112 (0.103, 0.122)</td>
<td>0.388 (0.363, 0.414)</td>
<td>0.168 (0.157, 0.179)</td>
<td>0.244 (0.232, 0.257)</td>
<td>0.288 (0.277, 0.301)</td>
</tr>
<tr>
<td>Multivitamin use 7</td>
<td>2730</td>
<td>0.105 (0.102, 0.109)</td>
<td>0.360 (0.350, 0.370)</td>
<td>0.157 (0.153, 0.162)</td>
<td>0.256 (0.250, 0.261)</td>
<td>0.288 (0.283, 0.293)</td>
</tr>
<tr>
<td>History of postmenopausal exercise 8</td>
<td>135</td>
<td>0.100 (0.085, 0.117)</td>
<td>0.363 (0.319, 0.412)</td>
<td>0.170 (0.150, 0.193)</td>
<td>0.277 (0.251, 0.307)</td>
<td>0.293 (0.271, 0.318)</td>
</tr>
<tr>
<td>Smoking 9</td>
<td>1420</td>
<td>0.100 (0.105, 0.115)</td>
<td>0.373 (0.359, 0.388)</td>
<td>0.167 (0.160, 0.173)</td>
<td>0.258 (0.250, 0.266)</td>
<td>0.291 (0.284, 0.298)</td>
</tr>
<tr>
<td>Alcohol use 10</td>
<td>1386</td>
<td>0.102 (0.097, 0.107)</td>
<td>0.356 (0.342, 0.370)</td>
<td>0.154 (0.148, 0.160)</td>
<td>0.256 (0.248, 0.264)</td>
<td>0.282 (0.275, 0.299)</td>
</tr>
<tr>
<td>Exercise 11</td>
<td>1211</td>
<td>0.101 (0.096, 0.106)</td>
<td>0.346 (0.331, 0.361)</td>
<td>0.152 (0.146, 0.159)</td>
<td>0.260 (0.251, 0.269)</td>
<td>0.279 (0.271, 0.286)</td>
</tr>
<tr>
<td>History of diabetes 12</td>
<td>1267</td>
<td>0.100 (0.100, 0.112)</td>
<td>0.349 (0.335, 0.364)</td>
<td>0.158 (0.152, 0.165)</td>
<td>0.254 (0.246, 0.263)</td>
<td>0.292 (0.285, 0.300)</td>
</tr>
<tr>
<td>History of hypercholesterolemia 13</td>
<td>1398</td>
<td>0.102 (0.097, 0.107)</td>
<td>0.356 (0.342, 0.370)</td>
<td>0.154 (0.148, 0.160)</td>
<td>0.256 (0.248, 0.264)</td>
<td>0.282 (0.275, 0.299)</td>
</tr>
<tr>
<td>History of hypertension 14</td>
<td>1055</td>
<td>0.109 (0.103, 0.116)</td>
<td>0.364 (0.347, 0.381)</td>
<td>0.159 (0.152, 0.167)</td>
<td>0.256 (0.247, 0.266)</td>
<td>0.296 (0.288, 0.305)</td>
</tr>
<tr>
<td>Reference 15</td>
<td>155</td>
<td>0.089 (0.077, 0.103)</td>
<td>0.327 (0.290, 0.368)</td>
<td>0.141 (0.125, 0.159)</td>
<td>0.235 (0.214, 0.258)</td>
<td>0.274 (0.254, 0.295)</td>
</tr>
<tr>
<td>1 drink/d 16</td>
<td>279</td>
<td>0.078 (0.069, 0.087)</td>
<td>0.311 (0.284, 0.340)</td>
<td>0.123 (0.113, 0.134)</td>
<td>0.257 (0.240, 0.276)</td>
<td>0.258 (0.244, 0.272)</td>
</tr>
<tr>
<td>Rarely or never (ref) 17</td>
<td>1386</td>
<td>0.102 (0.097, 0.107)</td>
<td>0.356 (0.342, 0.370)</td>
<td>0.154 (0.148, 0.160)</td>
<td>0.256 (0.248, 0.264)</td>
<td>0.282 (0.275, 0.299)</td>
</tr>
<tr>
<td>1–3 drinks/wk 18</td>
<td>390</td>
<td>0.103 (0.094, 0.114)</td>
<td>0.362 (0.336, 0.390)</td>
<td>0.158 (0.147, 0.171)</td>
<td>0.253 (0.239, 0.269)</td>
<td>0.286 (0.273, 0.300)</td>
</tr>
<tr>
<td>1–6 drinks/wk 19</td>
<td>854</td>
<td>0.112 (0.105, 0.119)</td>
<td>0.372 (0.353, 0.391)</td>
<td>0.163 (0.155, 0.172)</td>
<td>0.261 (0.251, 0.272)</td>
<td>0.300 (0.291, 0.310)</td>
</tr>
<tr>
<td>≥1 drink/d 20</td>
<td>264</td>
<td>0.099 (0.088, 0.111)</td>
<td>0.346 (0.316, 0.379)</td>
<td>0.160 (0.146, 0.175)</td>
<td>0.242 (0.225, 0.260)</td>
<td>0.291 (0.275, 0.308)</td>
</tr>
<tr>
<td>Rarely or never (ref) 21</td>
<td>1211</td>
<td>0.101 (0.096, 0.106)</td>
<td>0.346 (0.331, 0.361)</td>
<td>0.152 (0.146, 0.159)</td>
<td>0.260 (0.251, 0.269)</td>
<td>0.279 (0.271, 0.286)</td>
</tr>
<tr>
<td>&lt;1 time/wk 22</td>
<td>573</td>
<td>0.104 (0.096, 0.112)</td>
<td>0.353 (0.332, 0.376)</td>
<td>0.153 (0.144, 0.163)</td>
<td>0.254 (0.242, 0.267)</td>
<td>0.284 (0.274, 0.296)</td>
</tr>
<tr>
<td>1–3 times/wk 23</td>
<td>834</td>
<td>0.110 (0.103, 0.117)</td>
<td>0.379 (0.360, 0.399)</td>
<td>0.168 (0.159, 0.176)</td>
<td>0.253 (0.243, 0.264)</td>
<td>0.300 (0.290, 0.309)</td>
</tr>
<tr>
<td>≥4 times/wk 24</td>
<td>276</td>
<td>0.109 (0.098, 0.122)</td>
<td>0.387 (0.354, 0.424)</td>
<td>0.166 (0.152, 0.181)</td>
<td>0.251 (0.234, 0.269)</td>
<td>0.310 (0.293, 0.328)</td>
</tr>
</tbody>
</table>

1. Ref. reference.  
2. Geometric mean; CI in parentheses (all such values).  
3. Different from the reference, 0.01 < P < 0.05 (t test).  
4. Different from the reference, 0.001 > P < 0.01 (t test).  
5. Different from the reference, P < 0.001 (t test).
prevalent diabetes at baseline as well as those who developed incident diabetes or incident CVD during the cohort follow-up; the study results were similar and therefore not presented.

RESULTS

Plasma concentration and dietary intake were significantly correlated with one another for each carotenoid except lycopene, although the magnitude of correlations was only moderate (Spearman’s r, ranging from 0.034 for lycopene to 0.13 for β-cryptoxanthin). For all plasma carotenoids, the geometric means were similar across categories of age, race, menopausal status, postmenopausal hormone use, and multivitamin use (Table 1). Women who were current smokers and obese had lower concentrations of all carotenoids except lycopene. Women who vigorously exercised ≥1 time/wk tended to have higher plasma β-carotene, β-cryptoxanthin, and lutein-zeaxanthin, and women who consumed alcohol 1–6 drinks/wk had higher plasma lutein-zeaxanthin only. Women with hypercholesterolemia had higher plasma lycopene, and women with hypertension had lower plasma β-carotene.

For all plasma carotenoids and in both simple adjusted and multivariate adjusted models, the geometric means were similar across concentrations of total cholesterol (Table 2). After adjusting for age, race, and total cholesterol, the geometric means of plasma α-carotene, β-carotene, and lycopene tended to increase with increasing concentrations of LDL cholesterol; the geometric means of all plasma carotenoids except lycopene tended to increase with increasing concentrations of HDL cholesterol and to decrease with increasing Hb A1c and CRP. In contrast to other plasma carotenoids, plasma lycopene was inversely associated with HDL cholesterol and positively associated with Hb A1c and CRP in the model adjusting for age, race, and total cholesterol. Some, but not all, of these associations remained significant after further adjusting for lifestyle factors, clinical factors, and dietary intake of respective carotenoids (Table 2). In the multivariate-adjusted model, women who had higher LDL cholesterol had a significantly higher plasma concentration of lycopene, and women who had higher CRP had lower concentrations of α-carotene and β-carotene. β-cryptoxanthin and lutein-zeaxanthin were not significantly associated with any CVD-related biomarkers in multivariate models.

Linear regression performed on log-transformed plasma carotenoids obtained generally consistent results (Table 3). After
TABLE 2 (Continued)

<table>
<thead>
<tr>
<th>β-Cryptoxanthin</th>
<th>Lycopene</th>
<th>Lutein-zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Simple adjusted</strong></td>
<td><strong>Multiadjusted</strong></td>
<td><strong>Simple adjusted</strong></td>
</tr>
<tr>
<td>μmol/L</td>
<td>μmol/L</td>
<td>μmol/L</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>0.158 (0.144, 0.175)</td>
<td>0.158 (0.142, 0.175)</td>
<td>0.265 (0.245, 0.286)</td>
</tr>
<tr>
<td>0.161 (0.153, 0.169)</td>
<td>0.160 (0.152, 0.168)</td>
<td>0.249 (0.239, 0.259)</td>
</tr>
<tr>
<td>0.154 (0.148, 0.161)</td>
<td>0.155 (0.148, 0.163)</td>
<td>0.253 (0.244, 0.262)</td>
</tr>
<tr>
<td>0.161 (0.152, 0.171)</td>
<td>0.164 (0.154, 0.175)</td>
<td>0.270 (0.258, 0.283)</td>
</tr>
</tbody>
</table>

1. Hb A1c, glycated hemoglobin, ref. reference.
2. Adjusted for age and race for total cholesterol, additionally adjusted for total cholesterol for the other biomarkers.
3. Adjusted additionally for smoking (never, former, current < 15 cigarettes/d, current ≥ 15 cigarettes/d), alcohol use (rarely/never, 1–3 drinks/mo, 1–6 drinks/wk, ≥ 1 drink/d), vigorous exercise (rarely or never, <1, 1–3, ≥4 times/wk), menopausal status (yes, no, uncertain), postmenopausal hormone use (never, former, current), multivitamin use (never, former, current), history of hypercholesterolemia (yes, no), hypertension (yes, no), diabetes (yes, no), BMI (in kg/m²; continuous), and dietary intake of respective carotenoid (continuous).
4. Geometric %; 95% CI in parentheses (all such values).
5. Linear trend was tested by using the median values of each category as ordinal variable.
6. Different from the reference, 0.01 < P < 0.05 (t test).
7. Different from the reference, 0.001 < P < 0.01 (t test).
8. Different from the reference, P < 0.001 (t test).

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adjusting for age, race, total cholesterol, lifestyle factors, clinical factors, and dietary intake of respective carotenoids, an increase of 30 mg/dL in LDL cholesterol was associated with a 17% (calculated as \( \frac{0.16}{10} = 1.17 \)) increase in plasma α-carotene, a 16% increase in plasma β-carotene, and an 8.5% increase in plasma lycopene; an increase of 10 mg/dL in HDL cholesterol was associated with a 5.3% decrease in plasma lycopene; an increase of 0.3% in Hb A1c was associated with a 1.4% increase in plasma lycopene; and an increase of 2 mg/L in CRP was associated with a 1.3% decrease in plasma β-carotene. A marginally significant inverse association was observed between CRP and plasma α-carotene (\( P = 0.06 \)). When these linear regression analyses were stratified by smoking status, BMI, and dietary fat intake, the regression coefficients were somewhat different across strata, but the interactions were not statistically significant (all \( P \) for interaction > 0.01).

DISCUSSION

In this cross-sectional sample of 2895 middle-aged and older women free of CVD and cancer, we confirmed findings from other studies that smokers and obese women had lower plasma concentrations of carotenoids. We also found that plasma α-carotene, β-carotene, and lycopene were positively associated with LDL cholesterol, plasma lycopene was positively associated with Hb A1c, and inversely associated with HDL cholesterol, and plasma β-carotene was inversely associated with CRP.
These associations were independent of various lifestyle factors, clinical factors, and dietary intake of respective carotenoids. Lower plasma concentrations of carotenoids among smokers (10, 12–16) and obese persons (16, 17) were previously reported in a number of studies. These associations may reflect different intakes across subgroups of study subjects and metabolic effect of lifestyle and anthropometry factors on plasma carotenoids. Smoking is known to increase the production of oxygen-derived free radicals. Carotenoids, as potent antioxidants, retard the proliferation of free radicals and protect against free radical-mediated tissue damage (26, 27). The interaction with free radicals results in the fragmentation and loss of carotenoid molecules (28). Excessive oxidative stress and depletion of antioxidants are also present among obese persons. An alternative explanation of the inverse association between plasma carotenoids and BMI is that compared with normal weight persons, overweight or obese persons with greater body fat storage may have lower circulating carotenoids in plasma because of a high proportion of carotenoids, as lipid-soluble compounds, being stored in adipose tissue (10).

The positive association between plasma carotenoids and serum cholesterol concentrations observed in previous studies (10, 12–17) reflect that the lipophilic carotenoids are absorbed with dietary fats (29) and transported in lipoproteins (30). In our study, although plasma carotenoids were unassociated with total cholesterol, α-carotene, β-carotene, and lycopene were strongly and positively associated with LDL cholesterol, and all carotenoids except lycopene were positively associated with HDL cholesterol after adjusting for total cholesterol. The positive association of plasma carotenoids with LDL cholesterol remained significant after multivariate adjustment. Although hypercholesterolemia is complicated with greater free radical production (31), it appeared that the potentially increased use of carotenoids in hypercholesterolemia is less biologically important in determining plasma carotenoids than is the role of cholesterol as a non-specific carrier.

An inverse association between plasma carotenoids and blood glucose was reported in previous cross-sectional studies (32–34), suggesting a relation between impaired glucose metabolism, increased free radical activities, and reduced antioxidant concentrations. Fewer data are available about the association with elevated Hb A1c, an indicator of chronic hyperglycemia. Our study found that higher Hb A1c values were associated with low plasma -carotene, -carotene, and -cryptoxanthin, which is consistent with an earlier report (35), but the inverse association in our study was attenuated and no longer significant after multivariate adjustment. Our finding of a positive association of plasma lycopene with Hb A1c contrasted with some earlier studies (35, 36).

An inverse association between plasma carotenoids and inflammatory markers was reported previously among elderly nuns (37) and patients with lung cancer (38). In adults with acute inflammatory conditions such as tuberculosis (39) and pancreatitis (40), there were transient decreases in serum carotenoids and an increase in CRP concentration, which normalize with resolution of the illness. In the Third National Health and Nutrition Examination Survey, serum β-carotene was strongly and inversely associated with CRP concentrations and white blood cell count after adjusting for carotene intake and other possible confounders (41). Our study results agree with that earlier study in a representative population sample by showing that high CRP was associated with low plasma α- and β-carotene after multivariate adjustment. In many inflammatory disorders, the inflammatory responses induce the release of chemical mediators and activate peripheral blood mononuclear cells, which in turn

<table>
<thead>
<tr>
<th>Biomedical markers</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lycopene</th>
<th>Lutein-zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (40 mg/dL)</td>
<td>-0.012 (0.49)</td>
<td>0.0071 (0.61)</td>
<td>0.0036 (0.79)</td>
<td>0.015 (0.17)</td>
<td>0.0063 (0.47)</td>
</tr>
<tr>
<td>LDL cholesterol (30 mg/dL)</td>
<td>-0.012 (0.54)</td>
<td>0.0050 (0.75)</td>
<td>0.012 (0.44)</td>
<td>0.0040 (0.75)</td>
<td>0.0065 (0.51)</td>
</tr>
<tr>
<td>HDL cholesterol (10 mg/dL)</td>
<td>0.12 (0.0002)</td>
<td>0.13 (&lt;0.0001)</td>
<td>0.027 (0.30)</td>
<td>0.076 (0.0003)</td>
<td>-0.011 (0.49)</td>
</tr>
<tr>
<td>Hb A1c (0.3%)</td>
<td>0.032 (0.008)</td>
<td>0.017 (0.09)</td>
<td>0.034 (0.0004)</td>
<td>-0.051 (&lt;0.0001)</td>
<td>0.026 (&lt;0.0001)</td>
</tr>
<tr>
<td>C-reactive protein (2 mg/L)</td>
<td>0.0057 (0.70)</td>
<td>-0.0049 (0.68)</td>
<td>0.014 (0.24)</td>
<td>-0.054 (&lt;0.0001)</td>
<td>0.011 (0.12)</td>
</tr>
</tbody>
</table>

1. Hb A1c, glycated hemoglobin.
2. Adjusted for age and race for total cholesterol and additionally adjusted for total cholesterol for the other biomarkers.
3. Regression coefficients (β) were standardized to the units specified in parenthesis (P) for each biomarker. The percentage changes presented in text were calculated by taking the exponential of these regression coefficients (all such values).
4. Adjusted additionally for smoking (never, former, current <15 cigarettes/d, current ≥15 cigarettes/d), alcohol use (rarely or never, 1–3 drinks/mo, 1–6 drinks/wk, ≥1 drink/d), vigorous exercise (rarely or never, <1, 1–3, ≥4 times/wk), menopausal status (yes, no, uncertain), postmenopausal hormone use (never, former, current), multivitamin use (never, former, current), history of hypercholesterolemia (yes, no), hypertension (yes, no), diabetes (yes, no), BMI (in kg/m²; continuous), and dietary intake of respective carotenoid (continuous).
produce excessive reactive oxygen species or oxygen free radicals (42–45), and then increase the utilization of carotenoids.

In the current study, the observed associations with CVD risk factor and related biomarkers were not uniform for all carotenoids. The carotenoids differ in tissue localization and in antioxidant properties (46). Lutein-zeaxanthin and β-cryptoxanthin were less-reactive antioxidants than were other carotenoids in vitro and were not associated with any CVD-related biomarkers in our study. Lycopene has the most powerful antioxidant properties among major carotenoids detected in human tissues (47). However, our study, in consistency with others (10, 34), noted that many associations observed for plasma lycopene were in directions opposite to other carotenoids, for reasons yet to be fully understood. Dietary lycopene is derived predominantly from consumption of tomatoes and tomato products (48). It is possible that subjects who consume large amounts of lycopene-rich foods have some distinctive but unrecognized characteristics. Another possible explanation could be the different features of cis and trans lycopene, which were not determined in the present study.

Several potential limitations of our study deserve consideration. First, we cannot establish any cause-effect association from this cross-sectional study. Second, although we do not anticipate major errors in the biomarker measurement resulting from sample processing, storage, and assays, single measurement of both plasma carotenoids and CVD-related biomarkers could introduce nondifferential misclassification, which will bias the association toward the null. This may also explain the relatively low correlation between dietary carotenoids and plasma carotenoids in the current study compared with other studies (10, 32, 49). In addition to variability in blood measurements, the diversity of carotenoid food sources also serves to reduce individual magnitudes of dietary plasma carotenoid correlations. Of note, the Harvard Food Consumption database used USDA Agriculture Handbook no. 8 published in 1993 and had not included the most updated carotenoid values of foods. Third, although multiple factors were comprehensively adjusted for in our analyses, residual confounding from unknown or poorly measured determinants of plasma carotenoids cannot be completely ruled out. Finally, our study results apply to middle-aged and older predominantly white women from unknown or poorly measured determinants of carotenoids were comprehensively adjusted for in our analyses, residual confounding from unknown or poorly measured determinants of plasma carotenoids cannot be completely ruled out. Finally, our study results apply to middle-aged and older predominantly white women who were initially free of CVD and cancer. Studies in other populations are needed to confirm our findings.

In conclusion, we found in a cross-sectional sample of middle-aged and older women, that plasma carotenoids were associated with smoking, obesity, Hb A1c values, and LDL-cholesterol, HDL-cholesterol, and CRP concentrations. These associations were different between individual carotenoids and may partially explain the observed inverse association of plasma carotenoids with CVD outcomes in previous population studies. These findings emphasize the importance of adequately controlling for confounders of plasma carotenoids in epidemiologic studies of CVD.

We thank the 39 876 participants in the Women’s Health Study for their dedicated and conscientious collaboration and the entire staff of the Women’s Health Study for their continuous efforts.

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REFERENCES