Relationship Between Systemic Markers of Inflammation and Serum β-Carotene Levels

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Background: Low serum levels of β-carotene have been associated with increased risk of cancer and cardiovascular disease. However, in clinical trials, supplementation of the diet with β-carotene either had no benefit or caused harm. This pattern of findings raises the possibility that confounding by other factors might explain the association between serum β-carotene level and disease risk.

Methods: We used data from 14,470 current smokers, ex-smokers, and never smokers aged 18 years or older who participated in the Third National Health and Nutrition Examination Survey to assess the relationship between serum β-carotene and markers of inflammation (C-reactive protein and white blood cell count).

Results: After adjustment for β-carotene intake and other factors, geometric mean levels of serum β-carotene for individuals with undetectable (<0.22 mg/dL), mildly elevated (0.22-0.99 mg/dL), and clinically elevated (≥1.0 mg/dL) C-reactive protein levels were 18.0, 16.1, and 13.6 µg/dL, respectively, in never smokers; 18.1, 15.7, and 13.9 µg/dL in ex-smokers; and 11.3, 10.2, and 9.4 µg/dL in current smokers (P<.001 for all). In corresponding analyses, white blood cell count was also inversely related to serum β-carotene concentration (P<.05 for all).

Conclusions: The strong and inverse association of serum β-carotene level with C-reactive protein level and white blood cell count suggests that the relationship between serum β-carotene concentration and disease risk might be confounded by inflammation. More broadly, for β-carotene and likely other nutrients, it seems unwise to interpret biomarker data as prima facie evidence of dietary intake without a more complete understanding of the physiologic processes that affect nutrient levels.

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In a landmark article, Peto et al hypothesized that low intake of β-carotene was a modifiable risk factor for cancer. This hypothesis was strongly supported by the consistent finding of an inverse association between serum β-carotene level and risk of cardiovascular disease and certain types of cancer, especially lung cancer. However, in large-scale trials, supplementation of the diet with β-carotene either had no benefit or caused harm. There are several possible explanations for the discrepancy between results of observational studies and clinical trials, including the possibility of confounding by other nutrients or lifestyle factors that might be associated with β-carotene intake. An alternative explanation is that serum β-carotene levels reflect not only β-carotene intake but also other physiologic processes related to disease occurrence. In that case, low serum β-carotene concentration might be an epiphénomènemon, and increased intake of β-carotene would not be expected to reduce the risk of disease. Although this is a well-known theoretical limitation of serum biomarkers, it is seldom considered in the interpretation of biomarker data.

Preliminary evidence suggests that β-carotene levels are associated with inflammation. For example, it is well known that smoking increases systemic markers of inflammation and that smokers have lower levels of serum β-carotene than nonsmokers independent of β-carotene intake. In elderly women and in persons with lung cancer, an inverse relationship between inflammatory markers and serum β-carotene concentration has been documented. In middle-aged adults, serum level of sialic acid, a systemic marker of inflammation, was inversely associated with serum β-carotene level. Inverse associations between serum β-carotene level and C-reactive protein (CRP) level, an acute-phase reactant, have also been found in persons who are critically ill or have other acute in-
PARTICIPANTS AND METHODS

STUDY POPULATION

The NHANES III is a national probability survey of Americans conducted between 1988 and 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention. This survey used a complex, multi-stage, stratified, cluster-sampling design to obtain a representative sample of the noninstitutionalized civilian US population. Of the 19618 NHANES III participants aged 18 years and older, we excluded 2989 with missing serum β-carotene data, 1943 with unrealistic total caloric intake (<800 or >4200 kcal/d in men and <600 or >3500 kcal/d in women), and 216 who were pregnant, leaving 14 470 individuals available for analysis.

MEASUREMENTS

A detailed description of survey methods and data collection procedures has been published elsewhere.26,29 In brief, questionnaire data included self-reported age, race or ethnicity, sex, and medical history. Nutrient intake was estimated from a single 24-hour dietary recall. Nonfasting blood samples were used for analysis of inflammatory markers, total cholesterol level, and β-carotene concentration.

Serum β-carotene level was measured using high-performance liquid chromatography. The interbatch coefficient of variation of pooled samples used for quality control varied between 5.7% and 10.0%. This assay could detect a serum β-carotene level of 0.67 µg/dL or greater. Only 5 participants had serum β-carotene levels below the limit of detection. The CRP level was measured using latex-enhanced nephelometry. Pooled controls had a coefficient of variation of 3.2% to 16.1% through the period of data collection. Because 74% of individuals had CRP levels below the detection limit for this assay (0.22 mg/dL), we treated CRP level as a categorical variable: undetectable (<0.22 mg/dL), mildly elevated (0.22-0.99 mg/dL), and clinically elevated (≥1.00 mg/dL). The WBC count was determined using a fully automated hematology analyzer (Counter Model S-PLUS JR; Coulter Electronics, Hialeah, Fla). Serum cholesterol level was measured enzymatically (Hitachi 704 Analyzer; Boehringer Mannheim Diagnostics, Indianapolis, Ind).

Participants were classified as never smokers, ex-smokers, and current smokers. Never smokers and ex-smokers were defined by self-report, whereas current smokers were defined by self-report or by a serum cotinine level greater than 20 ng/mL, as measured by high-performance liquid chromatography and atmospheric-pressure chemical ionization tandem mass spectroscopy. Diabetes mellitus was defined by self-report of a physician diagnosis, by the presence of a fasting plasma glucose level greater than 126 mg/dL (≥7.0 mmol/L), or by the presence of a 2-hour glucose tolerance test result greater than 200 mg/dL (≥11.1 mmol/L). Prevalent cardiovascular disease was defined by self-report of physician-diagnosed myocardial infarction or stroke or by angina as assessed by the Rose questionnaire. Information on current use of estrogen replacement therapy, use of vitamin or mineral supplements in the past month, and use of aspirin or other nonsteroidal anti-inflammatory drugs during the past month was based on self-report.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the level of the high point of the iliac crest and hip circumference at the level of maximum extension of the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Blood pressure measurement was the average of measurements obtained at the household interview and the mobile examination center (maximum of 3 measurements at each).

STATISTICAL METHODS

Because the distribution of serum β-carotene levels was right-skewed, we log-transformed this variable and then back-transformed the results for this study. The association between serum β-carotene concentration and participant characteristics was evaluated by quintiles of serum β-carotene level using multiple linear regression for continuous outcomes and logistic regression for dichotomous outcomes. Multiple linear regression was used to determine whether serum β-carotene level was independently associated with markers of inflammation. In addition to age, race, and sex, variables in this model included known determinants of serum β-carotene levels (total caloric intake, dietary fat and carotenoid intake, serum cholesterol level, BMI, WHR, and use of vitamin or mineral supplements) and factors or conditions associated with systemic markers of inflammation (estrogen replacement therapy, aspirin, or other nonsteroidal anti-inflammatory drug use; diabetes mellitus; and prevalent cardiovascular disease). These variables were selected a priori, before introducing systemic markers of inflammation into the models. Because of the well-known association of smoking with low levels of serum β-carotene and systemic markers of inflammation, all analyses were stratified by smoking status (never smokers, ex-smokers, and current smokers).11,25,30 Tests for trend were performed by adding a continuous variable with the median of each category into the regression models.

To account for the complex survey design and to obtain results generalizable to the US noninstitutionalized population, we used SUDAAN software31 and applied NHANES III weights in all analyses. P<.05 was considered statistically significant (2-sided).

flammatory conditions, such as pancreatitis and tuberculosis.18,19 However, interpretation of these study results is complicated by the lack of control of important confounders, including smoking status and dietary intake of β-carotene.

The importance of these findings is highlighted by the fact that systemic markers of inflammation are consistently associated with atherosclerotic cardiovascular disease (ASCVD) events and tumor recurrence.20,21 Numerous studies22-27 have now shown that CRP level and white blood cell (WBC) count independently predict ASCVD events. In this setting, we hypothesized that serum β-carotene level is inversely associated with systemic markers of inflammation in the general population and that this association is independent of smoking status, dietary intake of β-carotene, and other potential confounders. To test this
hypothesis, we used data from the nationally representative cohort of the Third National Health and Nutrition Examination Survey (NHANES III).

Table 1 displays characteristics of the 14 470 participants included in our analyses. On average, ex-smokers were older and were more likely to be white than never or current smokers. Ex-smokers also had higher blood pressure, cholesterol levels, BMI, and WHR, as well as a higher prevalence of diabetes mellitus and ASCVD. Current smokers had higher CRP levels and WBC counts and lower levels of serum β-carotene than ex-smokers or never smokers.

In the 3 smoking categories, individuals with higher levels of serum β-carotene tended to be older and were more likely to be female and white than those with lower serum β-carotene levels (Table 2). After adjusting for age, sex, and race, serum β-carotene level was positively associated with total serum cholesterol level, carotenoid intake, and use of vitamin or mineral supplements during the past month and inversely associated with BMI and WHR in the 3 smoking categories. Total caloric intake was positively associated with serum β-carotene level, but the trend reached statistical significance in never smokers only (P < .001). Fat intake was inversely associated with serum β-carotene level in never smokers and ex-smokers but positively associated in current smokers. Finally, an inverse association between alcohol intake and serum β-carotene level was present only in current smokers.

**ASSOCIATIONS BETWEEN β-CAROTENE AND CRP LEVELS**

The level of CRP was strongly and inversely related to serum level of β-carotene. After adjusting for age, sex, and race (Table 3, model 1), the geometric mean levels of serum β-carotene in never-smokers with undetectable, mildly elevated, and clinically elevated CRP levels were 18.9, 14.7, and 11.0 µg/dL, respectively (P < .001 for trend). After further adjustment for serum cholesterol level, BMI, WHR, total caloric intake, alcohol intake, use of vitamin or mineral supplements, systolic blood pressure, use of aspirin or nonsteroidal anti-inflammatory drugs, estrogen replacement therapy, diabetes mellitus, and prevalent cardiovascular disease (Table 3, model 2), the relationship between β-carotene and CRP levels persisted. The corresponding geometric means were 18.0, 16.1, and 13.6 µg/dL, respectively (P < .001 for trend).

The association between CRP and serum β-carotene levels in ex-smokers was similar to that of never smokers. In multivariate analysis (Table 3, model 2), geometric mean levels of serum β-carotene in ex-smokers with undetectable, mildly elevated, and clinically elevated CRP were 18.1, 15.7, and 13.9 µg/dL, respectively (P < .001 for trend). Current smokers had markedly lower levels of serum β-carotene, but an inverse association with CRP level was still evident. In multivariate analysis (Table 3, model 2), serum β-carotene levels in smokers with undetectable, mildly elevated, and clinically elevated CRP levels were 11.6, 11.7, and 8.3 µg/dL, respectively (P < .001 for trend).

**ASSOCIATION BETWEEN β-CAROTENE LEVEL AND WBC COUNT**

A strong inverse association was also present between WBC count and serum β-carotene level (Table 4). Af-
Table 2. Factors Associated With Serum β-Carotene Level According to Smoking Status*

<table>
<thead>
<tr>
<th>C-Retinol Equivalent Level</th>
<th>Never Smokers</th>
<th>Ex-smokers</th>
<th>Current Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest</td>
<td>Highest</td>
<td>P‡</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smokers</td>
<td>34.3±10.8</td>
<td>50.8±17.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>52±15.6</td>
<td>73±20.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current Smokers</td>
<td>64±18.2</td>
<td>75±22.5</td>
<td>.02</td>
</tr>
<tr>
<td>Serum cholesterol, mg/dL§</td>
<td>195.9±71.9</td>
<td>208.9±76.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²§</td>
<td>29.1±4.4</td>
<td>24.4±3.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist-hip ratio§</td>
<td>0.92±0.08</td>
<td>0.88±0.07</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total carotene intake, µg/dL§</td>
<td>2008.8±705.2</td>
<td>2151.3±750.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fat intake, % of total calorie intake§</td>
<td>32.7±7.9</td>
<td>30.6±7.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Carotenoid intake, RE/1000 kcal/d§</td>
<td>176.0±41.9</td>
<td>511.3±124.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Alcohol intake, % current drinkers§</td>
<td>18±20</td>
<td>17±19</td>
<td>.87</td>
</tr>
<tr>
<td>Vitamin or mineral use, %§</td>
<td>33±15</td>
<td>59±32</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Data are given as geometric means. ‡Adjusted for age, race, and sex. §Further adjusted for carotene intake and other possible confounders, persons with elevated systemic markers of inflammation or cardiovascular disease.

Table 3. Serum β-Carotene Level by Level of C-Reactive Protein*

<table>
<thead>
<tr>
<th>C-Retinol Equivalent Level</th>
<th>Neve Smokers</th>
<th>Ex-smokers</th>
<th>Current Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest</td>
<td>Highest</td>
<td>P†</td>
</tr>
<tr>
<td>Model 1‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable (&lt;0.22 mg/dL)</td>
<td>18.9±10.9</td>
<td>19.0±11.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Mildly elevated (0.22-0.99 mg/dL)</td>
<td>14.7±9.4</td>
<td>14.4±9.7</td>
<td>11.7</td>
</tr>
<tr>
<td>Clinically elevated (&gt;1.00 mg/dL)</td>
<td>11.0±8.5</td>
<td>11.7±8.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Model 2§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable (&lt;0.22 mg/dL)</td>
<td>18.0±11.3</td>
<td>18.1±11.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Mildly elevated (0.22-0.99 mg/dL)</td>
<td>16.1±10.7</td>
<td>15.7±10.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Clinically elevated (&gt;1.00 mg/dL)</td>
<td>13.6±9.4</td>
<td>13.9±9.4</td>
<td>9.4</td>
</tr>
</tbody>
</table>

*Data are given as geometric means. †Adjusted for age, race, and sex. §Further adjusted for carotene intake and other nonsteroidal anti-inflammatory drugs, estrogen replacement therapy in women, diabetes mellitus, and prevalent cardiovascular disease.

COMMENT

In a nationally representative survey (NHANES III), we documented that serum β-carotene concentration is strongly and inversely associated with systemic markers of inflammation (CRP level and WBC count). After adjustment for carotene intake and other possible confounders, persons with elevated systemic markers of inflammation or cardiovascular disease. As displayed in the Figure, the results were essentially unchanged.

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flammation had significantly lower levels of serum β-carotene. This inverse association between serum β-carotene levels and systemic markers of inflammation was demonstrated in never smokers, ex-smokers, and current smokers and persisted after exclusion of persons with clinical conditions that might confound the association.

An inverse association between serum β-carotene level and systemic markers of inflammation in a healthy population is biologically plausible. In adults with an acute illness, there is a transient decrease in serum β-carotene level with a simultaneous increase in CRP level, both of which normalize with resolution of the illness. A similar acute-phase reaction has been shown for vitamin A and other serum vitamins and minerals. In children, serum retinol levels decrease during acute infection and return to normal, without vitamin A supplementation, once the acute process has passed. This pattern of findings could result from decreased production of retinol binding protein by the liver and/or increased urinary excretion of retinol during acute inflammation. Thus, although stores of vitamin A might be depleted during acute inflammation, the decreases seem to be primarily due to the timing and magnitude of the acute-phase response. With the recent identification of a binding protein for β-carotene, a similar relationship between the acute-phase response and serum β-carotene level could be hypothesized.

Although reduced serum β-carotene concentration is probably the result of systemic markers of inflammation, another interpretation of these findings is that β-carotene has anti-inflammatory properties. This conclusion is not supported by trials that show either no effect or a modest enhancement of immune system activity with supplemental β-carotene, but additional data from clinical trials are needed to determine whether supplemental β-carotene affects systemic markers of inflammation.

Among the strengths of our analyses are the large, nationally representative survey and the remarkable consistency of our results in each category of smoking status, which persisted after adjustment for multiple potential confounders. One potential limitation is the imprecision of the measurements of CRP, WBC, serum β-carotene, and dietary intake, all based on single determinations. Still, we found highly significant associations between inflammatory markers and serum levels of β-carotene.

Results from our analyses have several implications. These findings might partially explain the discrepancy between observational studies that associated low serum β-carotene levels with increased disease risk and clinical trials of β-carotene supplements. For instance, in the Alpha-Tocopherol, Beta-Carotene trial, low baseline serum levels of β-carotene were associated with an increased risk of lung cancer, whereas supplementation of the diet with β-carotene for 5 to 8 years actually increased incident lung cancer and cardiovascular disease events. One reason for these discordant results might be that low levels of serum β-carotene reflect systemic markers of inflammation, itself a risk factor for cardiovascular disease and perhaps cancer. To this end, prospective observational studies of serum β-carotene and subsequent disease risk, adjusted for inflammatory markers, would be informative, as would clinical trials that assess the effect of β-carotene supplementation on markers of inflammation.

More broadly, our findings document the potential limitations of using serum nutrient levels as a surrogate for dietary intake, particularly in observational studies that assess the relationship between nutrient intake and subsequent disease. Serum nutrient levels have appeal in epidemiologic studies in that they are more objective and might even be more precise than corresponding estimates from a single food frequency questionnaire or multiple 24-hour dietary recalls. However, as documented in this study, physiologic processes also affect serum levels and might reduce precision. Furthermore, serum nutrient levels are still subject to confounding with other nutrients and, in fact, are subject to additional confounding from physiologic determinants.

In summary, serum β-carotene level is strongly and inversely associated with systemic markers of inflammation, which themselves are markers of increased ASCVD risk and perhaps cancer. These findings have important implications for the interpretation of studies that show an increased risk of cancer and ASCVD in persons with reduced levels of serum β-carotene. More broadly, these results highlight the potential limitations of using serum nutrient levels as a surrogate for dietary intake in observational studies. For β-carotene and likely other nutrients, it seems unwise to interpret biomarker data as prima facie evidence of dietary intake without a more complete...
plete understanding of the physiologic processes that affect nutrient levels.

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