The Effects of Serum Beta-Carotene Concentration and Burden of Inflammation on All-Cause Mortality Risk in High-Functioning Older Persons: MacArthur Studies of Successful Aging

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Background. It remains unclear to what extent the associations between low serum beta-carotene concentration and increased risk for cardiovascular disease and cancers are attributable to inflammation. The objective of this study was to evaluate simultaneously the effects of serum beta-carotene concentration and inflammation on the subsequent all-cause mortality risk in high-functioning older persons.

Methods. The authors conducted a prospective cohort study using information from 672 participants from the MacArthur Studies of Successful Aging. Baseline information was obtained for serum concentrations of beta-carotene, C-reactive protein, interleukin-6, cholesterol, and albumin; body mass index; waist:hip ratio; prevalent medical conditions; health behaviors; and medications. Sex-specific univariate and multivariate logistic regression analyses were used to study the effects of low beta-carotene, high inflammation burden, or both on 7-year all-cause mortality rates while adjusting for other confounders.

Results. The serum beta-carotene concentration was inversely associated with C-reactive protein and interleukin-6 levels. After adjustment for inflammation markers and other covariates, the relative risks for low beta-carotene for the 7-year all-cause mortality risk were 2.30 (95% confidence interval [CI], 1.23 to 4.31) in men and 0.85 (95% CI, 0.42 to 1.75) in women. Compared with men with high beta-carotene levels and low inflammation, the multiply adjusted relative risk for low beta-carotene and high inflammation burden was 3.78 (95% CI, 1.69 to 8.47) in men.

Conclusions. Low levels of serum beta-carotene are independently associated with an increased all-cause mortality risk in older men, even after adjustment for the effects of inflammation and other risk factors. In men, but not women, a synergistic effect occurs between low beta-carotene concentration and high inflammation burden in predicting higher mortality rates.

BETA-CAROTENE, an antioxidant, has other well-established biologic effects, such as control of cell differentiation and modulation of immune function (1). Consistent epidemiologic evidence has suggested that high intake of carotenoid-rich vegetables and fruits and high blood concentrations of beta-carotene are associated with decreased risks for cardiovascular disease and some cancers (2–4). However, three large-scale randomized intervention trials have failed to show benefits of beta-carotene supplementation (5–7). In fact, two clinical trials reported higher incidence and mortality rates from lung cancer among heavy smokers who received beta-carotene (5,6), whereas the third trial, which had the longest follow-up period, showed no increased risk for cancer in the group that received beta-carotene supplementation (7).

The contradictory findings between epidemiologic data and the results of randomized controlled trials might be explained by, for example, differences in the amount and bioavailability of beta-carotene and the possible synergy of beta-carotene with other antioxidants in fruits and vegetables in preventing cardiovascular disease and cancer (1). Another possible explanation for the discrepancy is the presence of unmeasured or uncontrolled confounders (e.g., inflammation) in the observational studies, which may be responsible for the observed inverse association between beta-carotene concentration and cardiovascular and cancer risks.

Serum markers of inflammation have been identified as independent prognostic indicators for an increased incidence for cardiovascular events and death (8,9). Several cross-sectional studies have shown that inflammation markers are inversely associated with serum beta-carotene concentrations (10–12). Using data from the Third National Health and Nutrition Survey, Erlinger and colleagues (10) demonstrated a strong and inverse association between serum beta-carotene and C-reactive protein (CRP) concentrations in 14,470 current smokers, ex-smokers, and never-smokers aged 18 years and older. The association persisted after adjustment for multiple common cardiovascular risk factors. Accordingly, it has been postulated that the relation between serum beta-carotene concentration and disease risk observed in epidemiologic studies might be due to confounding by inflammation (10).
The benefit of using more than one marker to better predict health outcomes has been demonstrated in research into other diseases. For example, human immunodeficiency viral load and CD4+ cell counts are often inversely related, and each is a known risk factor for the development of the acquired immunodeficiency syndrome. Combining these two markers provides more accurate prediction of the progression of human immunodeficiency virus disease (13). It is biologically plausible that inflammation may serve as a marker of stress that promotes the pathogenesis of diseases, whereas high concentrations of antioxidants provide a “cushion” against the damage. A better understanding of the interaction between the two processes will not only improve the ability to predict adverse health outcomes but also help to identify subgroups of persons who may benefit the most from clinical interventions.

Therefore, we analyzed the data from the MacArthur Studies of Successful Aging to evaluate the potential interaction between serum beta-carotene concentration and inflammation burden on the subsequent 7-year all-cause mortality rate in high-functioning community-dwelling older persons. Specifically, we hypothesized that an inverse relationship exists between serum beta-carotene concentration and inflammation in high-functioning older persons. We also hypothesized that low beta-carotene concentration and high inflammation burden would each predict mortality risk and that there would be a synergistic effect between the two in their association with higher subsequent overall mortality rate.

Methods

Study Participants

The participants in this study were part of the MacArthur Research Network Study of Successful Aging, a subset of the Established Populations for Epidemiologic Studies of the Elderly. The details of this 7-year cohort study have been described elsewhere (14). Briefly, the Established Populations for Epidemiologic Studies of the Elderly was a community-based cohort study of persons aged 65 years and older residing in Durham, North Carolina; East Boston, Massachusetts; and New Haven, Connecticut. The participants were eligible for the MacArthur study if they were 70 to 79 years old at inception in 1988 and met the criteria designed to identify those functioning in the top one third of the age group.

Selection criteria for cognitive performance included scores of 6 or more correct on the 9-item Short Portable Mental Status questionnaire (15) and ability to remember 3 or more of 6 elements on a delayed recall of a short story. Selection criteria for physical function included no reported disability on a 7-item scale of activities of daily living, no more than 1 disability on 8 items tapping gross mobility and range of motion, ability to hold a semitandem balance for at least 10 seconds, and ability to stand from a seated position 5 times within 20 seconds without using their arms (14).

Of 1313 volunteers in the Established Populations for Epidemiologic Studies of the Elderly who met the criteria, 1189 (91%) agreed to participate at inception. Nineteen hundred seventy-one participants agreed to provide blood samples. Forty-seven (4.8%) refused follow-up visits. Two hundred fifty-one were excluded from analyses because of incomplete information on blood chemistry, serum antioxidant concentrations, or markers of inflammation. Compared with the 672 older persons who had complete information on biomarkers and 7-year mortality risk, persons who were excluded were more likely to be part of a racial group that was not white. However, the two groups did not differ in the distributions of age, sex, other common cardiovascular risk factors, and cancer.

Measures

Serum beta-carotene concentration was determined using an isocratic liquid chromatography method at the Lipids Laboratory, University of Southern California, Los Angeles (16). An enzyme-linked immunosorbent assay (ELISA) test was used to measure serum CRP (ELISA CRP kit, University of Vermont, Burlington, VT) and interleukin-6 (IL-6) levels (High Sensitivity Quantikine kit, R&D Systems, Minneapolis, MN). Serum levels of cholesterol and albumin were measured at Nichols Laboratories, San Juan Capistrano, California, using an automated sequential multiple analyz

Deaths among cohort members were identified through contact with next of kin at the time of follow-up for the cohort, on-going local monitoring of obituary notices, and National Death Index searches.

At baseline, study participants completed a standardized self-reported assessment of demographic characteristics; medical history, including chronic conditions such as coronary artery disease, hypertension, diabetes, stroke, or cancer; cigarette smoking and alcohol consumption; and use of prescription and over-the-counter medications. Patients were classified as receiving vitamin A or beta-carotene supplementation if they reported use of vitamin A, beta-carotene, fish oil, or any multivitamins containing vitamin A or beta-carotene. Body mass index (weight in kilograms divided by height in meters squared) was calculated based on self-reported height and weight at baseline. The waist:hip ratio was calculated based on waist circumference (measured at its narrowest point between the ribs and iliac crest) and hip circumference (measured at the maximal buttocks).

Data Analyses

The 7-year overall mortality risks by the quartiles of serum concentrations of beta-carotene, CRP, and IL-6 were calculated to determine possible threshold effects of these variables on mortality risk. Because mortality risk was much greater (and similar) in the bottom two quartiles of beta-carotene level (26.2% and 25%, respectively) compared with the top two quartiles (18.5% and 20.2%, respectively), high or low concentrations of these three biomarkers were classified using medians of the distributions in the cohort. The associations between beta-carotene concentration and other variables were first evaluated in bivariate analyses. For continuous variables, the means and standard deviations were calculated for participants with high or low serum beta-carotene concentrations. Because the distributions of some of the variables, such as CRP and IL-6, were right skewed, the Wilcoxon rank-sum test was used to determine the significance of the differences. For categorical variables,
such as sex, the percentage of participants with that characteristic was calculated for each category of beta-carotene. Statistical significance was determined using the chi-square test. The difference was considered significant if the two-sided probability value was less than .05.

Because of the significant sex difference in the distribution of beta-carotene concentrations, we performed sex-specific analyses to determine the relations among beta-carotene, inflammation burden, and mortality risk. Logistic regression models were used to evaluate the associations between low beta-carotene concentration and 7-year mortality risk in men and women separately and to determine how these associations varied after adjustment for other common cardiovascular risk factors. Based on previous knowledge and significant associations between serum beta-carotene and covariates in bivariate analyses, the final sex-specific multivariate models were adjusted for age; race; serum CRP and IL-6 levels; total and high-density lipoprotein cholesterol levels; body mass index; waist-hip ratio; history of coronary artery disease, hypertension, diabetes, stroke, and cancer; smoking (pack-years) and alcohol consumption; and vitamin A and beta-carotene supplementation. The values of CRP and IL-6 were log-transformed in the multivariate models because the distributions of these variables were right skewed.

Participants were further classified as having high inflammation burden if they had both high CRP and high IL-6 levels. To assess the interaction between serum beta-carotene concentration and inflammation, participants with high beta-carotene and low inflammation burden were used as the reference group. The remaining participants were separated again into 3 exposure subgroups: high burden of inflammation only, low beta-carotene-only, and both low beta-carotene and high inflammation burden. Logistic regression models were used to assess the associations between the three exposure categories and 7-year mortality risk in men and women while controlling for the confounding effects of the covariates. The Hosmer-Lemeshow test statistic was used to assess the goodness of fit of the logistic regression models.

To further investigate the possible contributing factors for the observed sex difference in the effect of beta-carotene, variables related to oxidative stress, such as pack-year smoking history and waist:hip ratios, were compared in men and women. The associations between serum beta-carotene concentration and mortality risk were evaluated in men, while stratifying for oxidative stress as indicated by levels of smoking and waist:hip ratio. All analyses were performed using SAS software, Windows version 8.1 (SAS Institute, Cary, NC) (17).

RESULTS
The average age for the entire cohort was 74.2 years. Approximately 46% were men and 84% were white. The mean serum beta-carotene concentration in men and women was 0.20 μmol/L (standard deviation, 0.20 μmol/L) and 0.30 μmol/L (standard deviation, 0.37 μmol/L), respectively (p < .001). Table 1 shows a comparison of baseline characteristics of the study population by high versus low serum concentrations of beta-carotene. The age distribution was similar in different serum concentrations of beta-carotene.

The participants with low serum beta-carotene concentrations were more likely to be white and had significantly higher levels of CRP, IL-6, body mass index, and waist:hip ratio but lower serum high-density lipoprotein cholesterol. Low concentrations of beta-carotene were also positively associated with more pack-years of smoking, current alcohol use, and diabetes, but they were inversely associated with vitamin A or beta-carotene supplementation. Serum beta-carotene concentration was not associated with history of coronary heart disease, hypertension, stroke, or cancer or with serum total cholesterol and albumin levels. Using different cutoff points to define high beta-carotene concentration, such as top tertile, resulted in minimal changes in the relations between beta-carotene and other common cardiovascular risk factors.

Ninety-eight men (31.9%) and 54 women (14.8%) in the cohort died during the 7-year follow-up period (p < .001). In women, the unadjusted relative risk for the effect of low beta-carotene on the overall mortality rate was 0.90 (95% CI, 0.50 to 1.60; Table 2). Even after adjustment for multiple common cardiovascular risk factors, the relationship was...
not significant. In men, however, low beta-carotene concentration was significantly associated with increased 7-year mortality risk, both unadjusted (1.67; 95% CI, 1.02 to 2.76) and after simultaneous adjustment for markers of inflammation and other covariates (2.30; 95% CI, 1.23 to 4.31).

Table 3 summarizes the interaction of beta-carotene and inflammation burden with respect to the 7-year overall mortality risk. Women who had both low beta-carotene and high inflammation burden did not have an increased mortality risk compared with the reference group. In men, the multiply adjusted relative risk for low beta-carotene and high inflammation burden was 3.78 (95% CI, 1.69 to 8.47). Men with low beta-carotene or high inflammation levels alone also had greater mortality risks, but the differences were not statistically significant. Using an alternative definition for increased inflammation burden based on high CRP or high IL-6 alone did not change our findings. Logistic regression modeling was used to assess the relations between the exposures and mortality risk in the entire cohort including both men and women. The probability value for the interaction term for sex and low beta-carotene and high inflammation was 1.91 (95% CI, 0.90 to 4.06). The Hosmer-Lemeshow test did not suggest lack of fit for any of the multivariate models.

The average numbers of pack-years of smoking in men and women were 34.4 (standard deviation, 39.6) and 13.5 (standard deviation, 24.8), respectively (p < .001). Men also had higher mean waist:hip ratios (0.94 versus 0.84; p < .001) compared with women. When medians of the distributions in men were used to define heavy smoking and high waist:hip ratio, the multiply adjusted relative risk of low beta-carotene for all-cause death was 10.39 (95% CI, 1.67 to 64.72) in men with both high waist:hip ratios and heavy smoking. The relative risk of low beta-carotene in men with 1 risk factor only was 2.90 (95% CI, 1.09 to 7.72). In men with neither factor, the relative risk was 1.25 (95% CI, 0.35 to 4.46).

**DISCUSSION**

The findings from this population of high-functioning community-dwelling older persons showed that men had lower serum beta-carotene concentrations than did women. Serum beta-carotene was inversely associated with markers of inflammation. When we studied the relationship between serum beta-carotene and 7-year all-cause mortality risk, we found a striking sex difference. A low serum beta-carotene concentration was independently associated with an increased all-cause mortality risk in men, even after adjustment for markers of inflammation and other risk factors. Furthermore, we observed a significant synergistic effect between low beta-carotene and high inflammation burden in predicting higher mortality risk in men. In contrast, we found no relation between serum beta-carotene and 7-year mortality risk in women.

Table 2. Bivariate and Multivariate Logistic Regression Analyses of the Association Between Low Serum Beta-Carotene Concentration and 7-Year All-Cause Mortality, Stratified by Sex

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Men Odds Ratios (95% CI)</th>
<th>Women Odds Ratios (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (Low CRP/IL6 and high β-carotene)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Unadjusted RR (High CRP/IL6 only)</td>
<td>1.50 (0.64, 3.54)</td>
<td>1.45 (0.63, 3.33)</td>
</tr>
<tr>
<td>Adjusted RR (High CRP/IL6 only)</td>
<td>1.22 (0.50, 3.00)</td>
<td>1.51 (0.65, 3.47)</td>
</tr>
<tr>
<td>Adjusted RR (Low CRP/IL6 only)</td>
<td>1.17 (0.43, 3.20)</td>
<td>2.04 (0.78, 5.36)</td>
</tr>
<tr>
<td>Unadjusted RR (Low β-carotene only)</td>
<td>1.34 (0.71, 2.52)</td>
<td>1.09 (0.53, 2.23)</td>
</tr>
<tr>
<td>Adjusted RR (Low β-carotene only)</td>
<td>1.65 (0.83, 3.26)</td>
<td>1.08 (0.53, 2.23)</td>
</tr>
<tr>
<td>Adjusted RR (High CRP/IL6 &amp; Low β-carotene)</td>
<td>1.83 (0.86, 3.92)</td>
<td>1.30 (0.56, 3.00)</td>
</tr>
<tr>
<td>Unadjusted RR (High CRP/IL6 &amp; Low β-carotene)</td>
<td>3.01 (1.54, 5.86)</td>
<td>0.86 (0.36, 2.05)</td>
</tr>
<tr>
<td>Adjusted RR (High CRP/IL6 &amp; Low β-carotene)</td>
<td>3.93 (1.92, 8.08)</td>
<td>0.85 (0.36, 2.05)</td>
</tr>
<tr>
<td>Adjusted RR (High CRP/IL6 &amp; Low β-carotene)</td>
<td>3.78 (1.69, 8.47)</td>
<td>0.76 (0.24, 2.40)</td>
</tr>
</tbody>
</table>

Notes: *Models adjusted for age and race.
1Model 2 adjusted for age, race, C-reactive protein, and interleukin-6.
2Model 3 adjusted for age, race, C-reactive protein, interleukin-6, total and high-density lipoprotein cholesterol, body mass index, waist-hip ratio, coronary artery disease, hypertension, diabetes, stroke, cancer, smoking, alcohol consumption, and vitamin A or beta-carotene supplementation.

CI = confidence interval; CRP = C-reactive protein; IL6 = interleukin-6; RR = relative risk.
Our finding of an inverse association between serum beta-carotene concentration and markers of inflammation is consistent with results reported in earlier studies. A strong and inverse association of serum beta-carotene with CRP was reported among participants of the Third National Health and Nutrition Examination Survey. The association was present in non-smokers, ex-smokers, and current smokers (10). Although data are more limited for older persons, CRP levels were shown to be inversely correlated with alpha-carotene, beta-carotene, and total carotenoids in a cross-sectional study of 85 elderly nuns whose mean age was 86 years (18). Our study is unique because it has not only shown an inverse association between beta-carotene and inflammation but also used longitudinal data to evaluate simultaneously the effects of these two factors on subsequent mortality risk. The results show an association between low serum beta-carotene and increased mortality risk in men, independent of inflammation burden and other potential confounders. Furthermore, our study extends previous findings by suggesting a synergistic effect between serum beta-carotene and inflammation in predicting the subsequent all-cause mortality risk. The combined effect of the 2 risk factors is significantly stronger than either process alone, suggesting that persons with both low beta-carotene levels and high inflammation burden may potentially benefit the most from clinical interventions that reduce inflammation, such as aspirin and statins (8,19). It remains unclear whether these men might reduce their mortality risk by high intake of carotenoid-rich fruits and vegetables or beta-carotene supplementation at a dose lower than what had been used in previous clinical trials.

Both sex-stratified analyses and logistic models with interaction terms for the exposures and sex showed a sex difference in the effects of serum beta-carotene concentration and inflammation. Low beta-carotene, high inflammation burden, or both were associated with increased mortality risk in older men only. Similar patterns have been reported in other observational studies. In the Scottish Heart Health Study, higher intake of antioxidants had beneficial effects on mortality risk in men but not in women (20). Furthermore, in the Iowa Women’s Health Study, the intake of vitamin A was not associated with the risk for death from coronary heart disease in 34,486 postmenopausal women (21). Lung cancer risk was also unrelated to consumption of the 3 food groups defined as “high carotenoid” in this cohort (22).

The exact mechanisms for this possible sex difference are unknown. However, in our study, older men and women had different levels of smoking and different waist:hip ratios, which is a proxy variable for visceral fat. Among men with high levels of smoking and a high waist:hip ratio, the effect of low beta-carotene on mortality risk was particularly pronounced. Smoking and visceral fat have been shown to be related to oxidative stress (23,24). Therefore, it is possible that oxidative stress may be a potential effect modifier and contribute to the observed sex difference in the relation between serum beta-carotene and mortality risk.

These results should be interpreted in the context of the strengths and limitations of this cohort study, which evaluated the relationship between serum beta-carotene concentration and inflammation burden in predicting mortality risk in an elderly population. The study had a 7-year follow-up period. Study participants were high-functioning community-dwelling older persons, who might be expected to have fewer fluctuations in serum concentrations of antioxidants and acute-phase reactants. Therefore, the potential misclassification of biomarkers in the study participants was probably modest, although the serum markers were measured only once, at the study baseline.

Several possible limitations must be noted. There has been no consensus regarding the best measure of inflammation. We used both CRP and IL-6 to define inflammation burden, because CRP is a common measure of inflammation and IL-6 is a major stimulant of CRP. A previous study suggested that high CRP level without concurrent elevation of IL-6 may not confer the same risk (25). Using an alternative definition for increased inflammation burden based on high CRP or high IL-6 alone did not change our findings. However, other inflammation markers such as IL-1b and tumor necrosis factor, which might reflect certain components of inflammation, were not measured in our study.

The problem of selection bias also must be considered. Only 672 persons (56.5% of the baseline cohort) agreed to have blood drawn and had complete information on biomarkers. The true magnitude of the possible selection bias cannot be assessed directly using our data. However, when we compare the participants who had complete information with those who were excluded, the two groups were not significantly different in the distributions of common risk factors for death.

Finally, the power to detect the effect of beta-carotene in women was limited due to a lower 7-year all-cause mortality rate in women compared with men. Nevertheless, our study had a statistical power of 76% to identify a relative risk of 2.0 and 36% to identify a relative risk of 1.5 in women. Thus, we are relatively confident that we did not miss a large independent effect of beta-carotene on mortality risk.

Despite these limitations, our data suggest that a low serum beta-carotene level is independently associated with an increased all-cause mortality risk in men, even after adjustment for the effects of inflammation burden and other common risk factors. Furthermore, there is a significant synergistic effect between low beta-carotene and inflammation in predicting higher mortality risk. Further research is needed to explore the effects of the other antioxidants, inflammation, or both on mortality risk and functional outcomes in older persons and to provide guidance for new clinical intervention options that may improve the health and functional status of older persons.

ACKNOWLEDGMENTS

Work was supported by National Institute on Aging grants AG-17056 and AG-17265, the MacArthur Research Network on Successful Aging and the MacArthur Research Network on SES and Health through grants from the John D. and Catherine T. MacArthur Foundation, and the UCLA Claude Pepper Older American Independence Center (P06 AG10415-11).

The authors thank Dr. Russell Tracy and his colleagues at the University of Vermont who performed the assays for C-reactive protein and interleukin-6, and Dr. Alex Sevanian and his colleagues at the University of Southern California who performed the assays for beta-carotene.
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Received April 3, 2003
Accepted April 30, 2003