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Serum α-Carotene Concentrations and Risk of Death Among US Adults

The Third National Health and Nutrition Examination Survey Follow-up Study

Chaoyang Li, MD, PhD; Earl S. Ford, MD, MPH; Guixiang Zhao, MD, PhD; Lina S. Balluz, MPH, ScD; Wayne H. Giles, MD, MS; Simin Liu, MD, ScD

Background: Much research has been conducted relating total carotenoids—and β-carotene in particular—to risk of cancer and cardiovascular disease (CVD). Limited data are emerging to implicate the important role of α-carotene in the development of CVD or cancer.

Methods: We assessed the direct relationship between α-carotene concentrations and risk of death among 15,318 US adults 20 years and older who participated in the Third National Health and Nutrition Examination Survey Follow-up Study. We used Cox proportional hazard regression analyses to estimate the relative risk for death from all causes and selected causes associated with serum α-carotene concentrations.

Results: Compared with participants with serum α-carotene concentrations of 0 to 1 µg/dL (to convert to micromoles per liter, multiply by 0.01863), those with higher serum levels had a lower risk of death from all causes (P < .001 for linear trend): the relative risk for death was 0.77 (95% confidence interval, 0.68-0.87) among those with α-carotene concentrations of 0 to 1 µg/dL, 0.66 (0.55-0.79) among those with concentrations of 6 to 8 µg/dL, and 0.61 (0.51-0.73) among those with concentrations of 9 µg/dL or higher after adjustment for potential confounding variables. We also found significant associations between serum α-carotene concentrations and risk of death from all causes was significant in most subgroups stratified by demographic characteristics, lifestyle habits, and health risk factors.

Conclusions: Serum α-carotene concentrations were inversely associated with risk of death from all causes, CVD, cancer, and all other causes. These findings support increasing fruit and vegetable consumption as a means of preventing premature death.

Therefore, carotenoids other than β-carotene may contribute to the reduction in disease risk, and their effects on risk of disease merit investigation.15

Relatively few studies have directly examined the association between α-carotene concentrations and risk of cancer or CVD. Findings from limited number of studies on the association between serum or plasma α-carotene concentrations and risk of death have been inconsistent. In a recent study of Japanese adults aged 39 to 80 years who were followed up for as many as 12 years, those with higher levels of serum α-carotene were found to be at lower risk of death from CVD and cancer than those with lower levels.16,17 In contrast, no significant association between serum α-carotene levels and risk of death from CVD was found in the Physicians’ Health Study II, in which a study population of primarily white US men and women was followed up for 2 years,18,19 or in a study of Dutch adults aged 65 to 85 years who were followed up for approximately 7 years.20 This inconsistency in study findings may have been the result of demographic differences in study cohorts, small sample sizes, or short follow-up periods. To assess the association between serum α-carotene concentrations and risk of death from all causes, CVD, cancer, and all other causes in a larger, more diverse study cohort over a longer study period, we analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) of US adults 20 years and older and linked mortality follow-up data on NHANES III participants.

# METHODS

## STUDY POPULATION

The NHANES III (1988-1994) recruited study participants using a multistage, stratified sampling process designed to produce a survey sample that was representative of the civilian noninstitutionalized US population 20 years and older.21 The NHANES III underwent institutional review board approval, and written informed consent was received from all participants. After being interviewed in their homes, participants were invited to a mobile examination center, where they had a clinical examination and provided a blood sample. Response rates were 80% for the household interviews and 78% for the medical examinations.22 Of 16 573 survey adults who attended a medical examination center, 15 631 (94.3%) provided serum samples for α-carotene measurements. After the exclusion of participants who were ineligible for mortality follow-up because of insufficient identifying data (social security number, full name, sex, race, date of birth, state of birth, state of residence) for matching or length of follow-up of 1 month or less (n=19), as well as those who had missing data on covariates (n=294), our study cohort consisted of 15 318 NHANES III participants (92.4% of all participants who appeared at a medical examination center).

## LABORATORY PROCEDURES USED TO ASSESS BASELINE BIOMARKERS OF NHANES III PARTICIPANTS

A detailed description of laboratory procedures has been published elsewhere.23 In brief, serum samples were aliquoted, stored at −70°C, and shipped on dry ice to the NHANES laboratory at the Centers for Disease Control and Prevention, Atlanta, Georgia. Serum concentrations of α-carotene and β-carotene were quantified by isocratic high-performance liquid chromatography with detection at 3 different wavelengths (Waters HPLC system, Waters Chromatography Division, Milford, Massachusetts).

Total serum cholesterol concentrations were measured enzymatically in a series of coupled reactions that hydrolyze cholesterol esters and oxidize the 3-OH cholesterol group. High-density lipoprotein cholesterol (HDL-C) concentrations were measured after the precipitation of the other lipoproteins with a polyanion/divalent cation mixture. Total cholesterol and HDL-C analyses were performed with an automated chemistry analyzer (Hitachi 704 Analyzer; Boehringer Mannheim Diagnostics, Indianapolis, Indiana). Non–HDL-C concentrations were calculated by subtracting HDL-C concentrations from total cholesterol concentrations.

## DEMOGRAPHIC CHARACTERISTICS AND PHYSICAL EXAMINATION MEASURES AT BASELINE

Participants’ age (in years), race/ethnicity (white, non-Hispanic black, Mexican American, or other), education level (less than high school, high school, or beyond high school) smoking status (current smoker, former smoker, or never smoker), and alcohol consumption (times in the previous month) were based on self-reports of participants. Physical activity level was determined by participants’ self-reported frequency of engaging in specific types of leisure-time exercise or activities during the previous month multiplied by the rate of energy expenditure (intensity rating) for those activities according to a standardized coding method.24 Participants’ body mass index was calculated from their measured weight and height. The mean blood pressure was calculated as the average of the second and third readings for those who had 3 measurements, as the second reading for those who had 2 measurements, and as the only reading for those who had 1 measurement.25

## LINKED MORTALITY DATA THROUGH DECEMBER 31, 2006

The NHANES III participants with sufficient identifying data were matched to the National Death Index (NDI) to determine their survival status through December 31, 2006.26 The National Center for Health Statistics performed linkage with probabilistic matching between NHANES III and NDI data and reviewed death certificates to ensure that deaths reported in the NDI were matched to the correct NHANES III participants.

We used a standardized list of 113 causes based on the International Classification of Diseases, Ninth Revision, and the International Statistical Classification of Diseases, 10th Revision, codes to determine decedents’ underlying cause of death27 and divided the causes of death into 3 major categories: CVD, cancer, and all other causes. We divided CVD into the subcategories of ischemic heart disease, stroke, and other CVD. We divided cancer into cancer of the aerodigestive system (ie, cancers of the lip, oral cavity, pharynx, esophagus, stomach, colon, rectum, anus, liver, intrahepatic bile ducts, pancreas, and larynx); cancer of the lung, trachea, or bronchus; and all other cancers according to their frequencies and their possible relations to α-carotene concentrations. Also, we divided all other causes into the subcategories of diabetes mellitus, chronic lower respiratory disease, and all other diseases.

## STATISTICAL ANALYSIS

We first estimated the population distribution of serum α-carotene concentrations by sex and age. Because the distribution of serum α-carotene concentrations was skewed to...
the right, we used square root transformation to improve the distribution. We also divided the continuum of serum α-carotene concentrations into 5 categories (ie, 0-1, 2-3, 4-5, 6-8, and ≥9 µg/dL) to convert to micromoles per liter, multiply by 0.01863) according to its approximate quintile cutoff values and compared baseline characteristics of participants according to these 5 levels using 2-sample t tests. A total of 12 variables, including participants’ demographic characteristics, lifestyle and behavioral risk factors, and biomarkers, were used to control for individual characteristics that might confound or modify the association between α-carotene concentrations and risk of death. We conducted Cox proportional hazard regression analyses to estimate hazard ratios (relative risks) for death from all causes and major specific causes in which we treated serum α-carotene concentrations as either a categorical variable (5 levels) or a continuous variable. We used age in months as the time scale in survival analyses with left truncation (ie, participants by sex, race/ethnicity, education level, and smoking status (all P < .001) (Table 2). We found significant differences by serum α-carotene concentration in the following characteristics: mean age, alcohol consumption, leisure-time physical activity, body mass index, serum HDL-C concentration, and serum non–HDL-C concentration, as well as in the distribution of study participants by sex, race/ethnicity, education level, and smoking status (all P < .001) (Table 2).

We found that serum α-carotene concentration was inversely associated with unadjusted risk of death from all causes, CVD, cancer, and all other causes (all P < .001 for linear trend) (Table 3). After adjusting for the demographic characteristics, lifestyle habits, and health risk factors presented in Table 2, we found that serum α-carotene concentration was inversely associated with adjusted risk of death from all causes (P < .001 for linear trend), CVD (P = .007 for linear trend), cancer (P = .02 for linear trend), and all other causes (P < .001 for linear trend) (Table 3). In the various subcategories for cause of death, we found a significant inverse association between serum α-carotene concentra-
Serum α-carotene values were obtained from values were obtained from 


<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Cohort</th>
<th>0-1</th>
<th>2-3</th>
<th>4-5</th>
<th>6-8</th>
<th>≥9</th>
<th>P Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unweighted sample size</td>
<td>15,518</td>
<td>3615</td>
<td>3814</td>
<td>3950</td>
<td>2349</td>
<td>1590</td>
<td></td>
</tr>
<tr>
<td>Weighted, %</td>
<td>100</td>
<td>21.93 (0.75)</td>
<td>23.66 (0.63)</td>
<td>25.84 (0.56)</td>
<td>16.62 (0.47)</td>
<td>11.95 (0.63)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum α-carotene concentration, µg/dL</td>
<td>4.79 (0.09)</td>
<td>0.93 (0.01)</td>
<td>2.59 (0.02)</td>
<td>4.43 (0.01)</td>
<td>6.79 (0.03)</td>
<td>14.18 (0.25)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Demographic variables

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Male sex, %</th>
<th>Race/ethnicity, %</th>
<th>Smoker status, %</th>
<th>Education, %</th>
<th>Alcohol consumption (times in previous month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.82 (0.46)</td>
<td>47.88 (0.43)</td>
<td>62.52 (0.67)</td>
<td>28.14 (0.79)</td>
<td>41.60 (1.26)</td>
<td>*Carotene Concentration at Baseline (1988-1994)$^a$</td>
</tr>
<tr>
<td>23.66 (1.34)</td>
<td>74.78 (1.27)</td>
<td>74.78 (1.27)</td>
<td>28.14 (0.79)</td>
<td>41.60 (1.26)</td>
<td>*Carotene Concentration at Baseline (1988-1994)$^a$</td>
</tr>
<tr>
<td>12.01 (0.88)</td>
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</tr>
<tr>
<td>0.62 (1.41)</td>
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<td>*Carotene Concentration at Baseline (1988-1994)$^a$</td>
</tr>
<tr>
<td>0.43 (0.43)</td>
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<td>0.43 (0.43)</td>
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<td>*Carotene Concentration at Baseline (1988-1994)$^a$</td>
</tr>
</tbody>
</table>

**Abbreviations:** BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; METs, metabolic equivalents.

$^a$All data are weighted mean (SE) for continuous variables and weighted percentage (SE) for categorical variables.

In this prospective study of a nationally representative sample of US adults over a mean follow-up period of 13.9 years, we found that serum α-carotene concentrations were inversely associated with risk of death from all causes, CVD, cancer, and all causes other than CVD and cancer. The negative association between serum α-carotene concentrations and overall risk of death was also significant in most subgroups stratified by demographic characteristics, lifestyle habits, and health risk factors. The strengths of our study include its large sample size and relatively long follow-up period. Our results showed inverse associations of serum α-carotene concentrations with the risk of death from all causes, CVD, cancer, and all other causes. Similar results were reported in a study of 3061 Japanese adults, among whom serum concentrations of α-carotene were inversely associated with decreased risk of death from cancer at its lower concentrations (Figure, C); however, the association appeared to be attenuated at serum α-carotene concentrations of 16 µg/dL and above.

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**COMMENT**

In this prospective study of a nationally representative sample of US adults over a mean follow-up period of 13.9 years, we found that serum α-carotene concentrations were inversely associated with risk of death from all causes, CVD, cancer, and all causes other than CVD and cancer. The negative association between serum α-carotene concentrations and overall risk of death was also significant in most subgroups stratified by demographic characteristics, lifestyle habits, and health risk factors. The strengths of our study include its large sample size and relatively long follow-up period. Our results showed inverse associations of serum α-carotene concentrations with the risk of death from all causes, CVD, cancer, and all other causes. Similar results were reported in a study of 3061 Japanese adults, among whom serum concentrations of α-carotene were inversely associated with decreased risk of death from cancer at its lower concentrations (Figure, C); however, the association appeared to be attenuated at serum α-carotene concentrations of 16 µg/dL and above.
with the risk of CVD,17 and in a recent study of 559 elderly Dutch men, among whom dietary intake of \(\alpha\)-carotene was inversely associated with risk of death from CVD.20 However, no inverse association was found between \(\alpha\)-carotene levels and overall risk for death in a study of 638 elderly Dutch persons20 or between \(\alpha\)-carotene levels and risk for CVD events in a case-control study of 499 American men who experienced CVD events and 499 who did not experience CVD events during a mean follow-up period of 2.1 years.21 These studies, however, had relatively small sample sizes and short follow-up periods and thus less statistical power to detect associations. In contrast, our analyses were based on data from a much larger, population-based cohort, which was followed up for nearly 4 times as long; therefore, our findings should be more reliable and generalizable to the general population than those of previous studies of the relationship between \(\alpha\)-carotene levels and risk of various adverse health outcomes.

Consistent with findings from previous studies22-25 our results showed an especially strong association between serum \(\alpha\)-carotene concentrations and risk for death from some specific causes, including cancers of the aerodigestive tract, diabetes, and chronic lower respiratory disease. Specifically, our results were consistent with those from a 1997 nested case-control study among Japanese American men in Hawaii,26 which showed that (1) low serum \(\alpha\)-carotene concentrations were associated with increased risk of cancers of the upper aerodigestive tract, including esophageal cancer, laryngeal cancer, and oral-pharyngeal cancer; (2) consumption of fruits and vegetables and serum \(\alpha\)-carotene concentrations were inversely associated with the incidence of type 2 diabetes mellitus,27,31,33 and (3) serum \(\alpha\)-carotene concentrations were directly associated with lung function,22 which in turn may protect against chronic lower respiratory disease and its progression in persons who already have the disease.

In the past several decades, \(\beta\)-carotene has received attention for its possible role in the prevention of several chronic diseases, including cancer and CVD. However, randomized clinical trials have failed to link \(\beta\)-carotene supplements to reduced incidence and mortality of cancer or CVD or to reduced incidence of adverse effects among smokers and workers exposed to asbestos.11-13 Although \(\alpha\)-carotene is recognized as a major component of carotenoids, its protective effects against cancer and CVD have been investigated less than those of \(\beta\)-carotene.
Although α-carotene is chemically similar to β-carotene, in vivo study results suggest that α-carotene is about 10 times more effective than β-carotene in inhibiting the proliferation of human neuroblastoma cells; that α-carotene, but not β-carotene, has a potent inhibitory effect against liver carcinogenesis; and that α-carotene is more effective than β-carotene in inhibiting the tumor-promoting action of glycerol in lung carcinogenesis and skin tumor promotion. Moreover, results from a population-based case-control study of the association between the consumption of fruits and vegetables and risk of lung cancer suggest that consumption of yellow-orange (carrots, sweet potatoes or pumpkin, and winter squash) and dark-green (broccoli, green peas, spinach, turnip greens, collards, and leaf lettuce) vegetables, which have a high α-carotene content, was more strongly associated with a decreased...
that members of our study cohort obtained than 75% of their dietary health.4-10

Also, the association between serum \( \alpha \)-carotene concentrations and lung cancer appeared to be confounded and/or modified by smoking status. Future research is warranted to elucidate the mechanisms underlying the differential effects of \( \alpha \)-carotene and \( \beta \)-carotene against cancer and CVD.

Because current antioxidant supplements or food additives contain little if any \( \alpha \)-carotene,37,38 we assumed that members of our study cohort obtained \( \alpha \)-carotene primarily from consumption of fruits and vegetables. Studies have shown that plasma \( \alpha \)-carotene is highly correlated with total consumption of fruits and vegetables, especially carrots and other root vegetables,39,40 and that \( \alpha \)-carotene coexists with \( \beta \)-carotene in fruits and vegetables, particularly in carrots.39 Results from the Framingham Heart Study showed that participants obtained more than 75% of their dietary \( \alpha \)-carotene from carrots.42 Taken together, the results of these studies indicate that serum \( \alpha \)-carotene concentrations can serve as a reliable and useful biomarker for fruit and vegetable consumption. The inverse relationship that we found between serum \( \alpha \)-carotene concentrations and risk of death from various causes adds further support to previous findings that fruit and vegetable consumption is beneficial to people’s health.4-10

We also found that although high serum concentrations of \( \alpha \)-carotene may be needed to protect against death from CVD, a very high concentration of \( \alpha \)-carotene may be less effective than lower concentrations against death from cancer. Previous study findings have similarly suggested that \( \beta \)-carotene seems to lose its antioxidant effects at very high levels.43 This possible loss of protective effect at extremely high serum concentrations indicates a need for studies to determine the threshold serum concentration at which \( \alpha \)-carotene produces positive health effects in the general population and the fruit and vegetable consumption necessary to reach this threshold concentration. Currently, the US Department of Agriculture’s food pyramid recommends the consumption of 2 to 4 servings of fruits and 3 to 5 servings of vegetables daily.43

Our results are subject to 3 potential limitations. First, because only a single measurement of serum \( \alpha \)-carotene concentration at baseline was available, bias from regression toward the mean may have led us to underestimate the strength for the association between serum \( \alpha \)-carotene concentrations and risk of death. Second, possible misclassifications in the underlying cause of death of deceased study participants, particularly the misclassification of diabetes,44 may also have biased our estimates for the association between \( \alpha \)-carotene concentrations and risk of death toward null. Finally, our results may be subject to residual confounding owing to unmeasured biomarkers or health behaviors. It is possible that serum \( \alpha \)-carotene concentrations may act as an in-

![Graph showing relative risk of death vs. \( \alpha \)-carotene concentration](http://web.archive.org/web/20170619061700/http://archinte.jamanetwork.com/pdfaccess.ashx?url=/data/journals/intemed/5804/)
ticator of multiple interactive forces that are more proximal to the mortality outcomes of interest in our study, providing insights linking intake of vegetables and fruits to lower mortality risk.

In conclusion, our findings, based on data from a large representative sample of US adults, showed that serum α-carotene concentrations were inversely associated with the risk of death from all causes and death from CVD, cancer, and all causes other than CVD and cancer. They also showed that the inverse association was independent of demographic characteristics, lifestyle habits, and traditional health risk factors. Our results, if replicated in other studies and populations, suggest a need for clinical research into the health benefits of serum α-carotene.

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Author Contributions: Drs Li and Ford had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Li, Ford, and Liu. Acquisition of data: Li, Ford, Zhao, Balluz, Giles, and Liu. Drafting of the manuscript: Li. Critical revision of the manuscript for important intellectual content: Li, Ford, Zhao, Balluz, Giles, and Liu. Statistical analysis: Li, Ford, and Liu. Administrative, technical, and material support: Li, Ford, Zhao, Balluz, and Giles. Study supervision: Ford, Balluz, and Giles.

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REFERENCES