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## Original Contribution

# Serum Antioxidants, Inflammation, and Total Mortality in Older Women

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The inflammatory cytokine interleukin-6 (IL-6) has been linked to poor health outcomes in older adults. Oxidative stress triggers the production of IL-6, and antioxidant micronutrients play a critical role in decreasing this inflammatory response. The authors sought to identify the relations between serum levels of antioxidant nutrients and IL-6 and mortality in older women. Levels of α- and β-carotene, lycopene, lutein/zeaxanthin, α-cryptoxanthin, total carotenoids, retinol, α-tocopherol, zinc, and selenium were measured at baseline in 619 participants in Women's Health and Aging Study I (Baltimore, Maryland, 1992–1998). IL-6 was measured at baseline and at follow-up 1 and 2 years later, and all-cause mortality was determined over a 5-year period. Participants with the highest serum levels of α-carotene, total carotenoids, and selenium were significantly less likely to be in the highest tertile of serum IL-6 at baseline ($p < 0.0001$). Those with the lowest levels of α- and β-carotene, lutein/zeaxanthin, and total carotenoids were significantly more likely to have increasing IL-6 levels over a period of 2 years. Those with the lowest selenium levels had a significantly higher risk of total mortality over a period of 5 years (hazard ratio = 1.54, 95% confidence interval: 1.03, 2.32). These findings suggest that specific antioxidant nutrients may play an important role in suppressing IL-6 levels in disabled older women.

aging; antioxidants; carotenoids; inflammation; interleukin-6; mortality; selenium

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation; WHAS I, Women's Health and Aging Study I.

Older adults with the highest serum levels of interleukin-6 are more likely to develop disability and worsening chronic disease and are more likely to be frail and to die earlier than those with the lowest levels (1–5). These poor health outcomes are probably directly mediated by elevated interleukin-6 concentrations, which can induce muscle and bone loss, anemia, immune dysfunction, and altered production and function of multiple hormones (6–9). The etiology of chronic interleukin-6 elevations in older adults is multifactorial, with declines in sex steroid hormones, increased prevalence of inflammatory disease, increased fat mass, and increased generation of free radicals of oxygen all being known to enhance proinflammatory nuclear factor-κB signal transduction pathways and hence interleukin-6 production (6, 10).

Prior studies have suggested that several categories of dietary antioxidants, including the carotenoids, retinol, α-tocopherol, zinc, and selenium, may be effective in suppressing activation of these proinflammatory pathways through the quenching of free radical molecules (11, 12). Few studies have investigated the cross-sectional and longitudinal relations between specific antioxidants and inflammation, as measured by serum interleukin-6 level, and ultimately mortality in older adults. Therefore, we studied the relations of

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dietary carotenoids, retinol, α-tocopherol, zinc, and selenium with interleukin-6 and mortality in a cohort of older women.

## MATERIALS AND METHODS

### Human subjects

Women's Health and Aging Study I (WHAS I) is a longitudinal cohort study of the one third most disabled community-dwelling older women in Baltimore, Maryland. Subjects were women aged ≥65 years residing in 12 contiguous zip code areas in Baltimore who were recruited from a random sample of the Health Care Financing Administration's Medicare enrollment file ($N = 32{,}538$ women). An age-stratified (65–74, 75–84, and ≥85 years) sample of these women was randomly selected. Of those, 5,316 were eligible for screening, 4,135 were screened for disability, 1,409 met the study criteria, 1,002 agreed to participate in the study, and 783 agreed to have blood drawn starting in 1992 (13, 14). Of these 783 persons, 619 had stored serum samples and data on all reported variables recorded in the database. This left 383 WHAS I participants who were not included in these analyses.

Study participants received an extensive interview and examination in their homes at baseline and every 6 months for 3 years, for a total of seven examinations. Physical activity was measured by participant report, with persons who walked more than eight blocks per week being deemed active and those who walked less than eight blocks deemed inactive, as validated in prior WHAS I studies (15). Smoking status was analyzed by dividing pack-years into four categories: none, mild (1–30 pack-years), moderate (31–56 pack-years), and heavy (>56 pack-years) (16). Blood was drawn at baseline and at 1-year intervals. Information on physician diagnosis of 16 major chronic diseases was obtained at each examination, and the presence/absence of each disease was adjudicated by trained physicians using abstracted medical records and following standardized state-of-the-art algorithms (13). Information on vital status was obtained through follow-up interviews with proxies, obituaries, and matching with the National Death Index over a 5-year period. The Johns Hopkins University Institutional Review Board approved the study, and all participants gave informed consent.

### Laboratory analyses

Blood samples were obtained by venipuncture, and serum was separated by centrifugation and stored at –70°C until analysis. Levels of serum α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin (not separated with this procedure), and α-tocopherol were determined by high performance liquid chromatography (17). The internal standards used were tocol (Hoffmann-LaRoche, Inc., Nutley, New Jersey) at 320 nm and all-*trans*-ethyl-β-apo-8′-carotenoate (purified sample, courtesy of Dr. Fred Khachik, University of Maryland) at 450 nm. Within-run and between-run coefficients of variation for pooled standards were 10.7 percent and 23.9 percent for α-carotene, 7.0 percent and 19.1 percent for β-carotene, 4.7 percent and 8.5 percent for β-cryptoxanthin, 4.1 percent and 4.6 percent for lutein/zeaxanthin, and 4.1 percent and 9.7 percent for α-tocopherol, respectively. Total cholesterol was measured using an automated enzymatic method, and the values were used to compute the α-tocopherol:cholesterol ratio (18). Serum selenium and zinc levels were measured by graphite furnace atomic absorption spectrometry using a Perkin Elmer Analyst 600 with Zeeman background correction (Perkin Elmer Corporation, Norwalk, Connecticut). Within-run and between-run coefficients of variation were 5.8 percent and 4.8 percent for selenium and 2.8 percent and 3.9 percent for zinc, respectively. Plasma interleukin-6 was measured using an enzyme-linked immunosorbent assay (Quantikine human interleukin-6; R&D Systems, Inc., Minneapolis, Minnesota). Quality control was assessed by repeated analysis of standard reference material (SRMb; National Institute of Standards and Technology, Gaithersburg, Maryland) and pooled reference standards. All samples were analyzed in a masked fashion.

### Statistical analysis

Baseline demographic and health-related characteristics for the 619 WHAS I women with complete outcome and covariate information were compared by tertile of interleukin-6 values, using the $\chi^2$ test for categorical variables and analysis of variance for continuous variables. We calculated summary statistics for micronutrients, including means, medians, standard deviations (SDs), and ranges, and compared the log-transformed micronutrient values by tertile of interleukin-6 using analysis of variance. Sequential logistic regressions for being in the highest interleukin-6 tertile compared with the lowest two tertiles were fitted against each of the micronutrients, with adjustment for age, Black race, years of education, pack-years of smoking, body mass index (weight (kg)/height $(m)^2$), and physical activity. In addition, adjustments were made in the final model for four prevalent chronic diseases known to be associated with inflammation: chronic obstructive pulmonary disease, peripheral arterial disease, angina, and diabetes mellitus. Micronutrient values were logarithmically transformed to approximate normality in all regression models. To validly assess the relative strength of associations between interleukin-6 and micronutrients, we calculated odds ratios and 95 percent confidence intervals associated with a 1-SD increase in log micronutrient values. A random-effects model was used to examine both population-averaged and individual changes in log interleukin-6 levels over time while accounting for between-person heterogeneity in baseline interleukin-6 levels and individual rate of change over time.

To determine whether low levels of specific micronutrients at baseline predicted a significant increase in interleukin-6 over time, we divided subjects with nutrient measurements into tertiles, and the percentages of those who had a 0.5-SD increase in interleukin-6 level over 1-year and 2-year spans were determined. We selected the 0.5-SD increment to achieve a balance between sample size limitation

**TABLE 1. Demographic and health-related characteristics of 619* study participants with measurements of interleukin-6 and antioxidant nutrient levels, by tertile of interleukin-6 at baseline, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1993**

| | Total | Tertile of interleukin-6 | | | *p* value† |
|---|---|---|---|---|---|
| | | ≤2.80 pg/ml ($n = 208$) | >2.80–≤4.81 pg/ml ($n = 209$) | >4.81 pg/ml ($n = 202$) | |
| Mean age (years) | 77.3 (7.8)‡ | 76.7 (7.8) | 77.5 (7.9) | 77.6 (7.6) | 0.45 |
| Black race (%) | 27.3 | 23.1 | 26.3 | 32.7 | 0.09 |
| Mean years of education | 9.9 (4.9) | 10.5 (6.7) | 9.9 (3.5) | 9.3 (3.4) | 0.08 |
| Level of smoking and pack-years (%) | | | | | <0.01 |
| None: 0 | 52.3 | 64.3 | 48.8 | 43.5 | |
| Mild: 1–30 | 27.0 | 24.2 | 30.4 | 26.5 | |
| Moderate: 31–56 | 11.1 | 7.2 | 12.1 | 14.0 | |
| Heavy: >56 | 9.6 | 4.3 | 8.7 | 16.0 | |
| Body mass index§ | 28.8 (6.8) | 27.3 (5.3) | 29.2 (7.2) | 29.8 (7.6) | <0.01 |
| Ability to walk ≥8 blocks (%) | 31.1 | 40.5 | 32.0 | 20.4 | <0.01 |
| Prevalent chronic diseases (%) | | | | | |
| Cardiovascular disease | 23.6 | 22.7 | 34.5 | 37.6 | 0.003 |
| Peripheral arterial disease | 20.2 | 17.3 | 15.8 | 28.7 | <0.01 |
| Chronic obstructive pulmonary disease | 15.0 | 12.0 | 17.2 | 15.8 | 0.30 |
| Diabetes mellitus | 16.2 | 9.1 | 15.8 | 23.8 | <0.01 |

* All participants with baseline data on serum carotenoid levels, corrected interleukin-6 level, age, race, education, pack-years of smoking, body mass index, chronic obstructive pulmonary disease, peripheral arterial disease, cardiovascular disease, and diabetes.

† *p* value for comparison between the three interleukin-6 tertiles.

‡ Numbers in parentheses, standard deviation.

§ Weight (kg)/height $(m)^2$.

and clinical significance. Crude incidence rates for having a greater than 0.5-SD increase in interleukin-6 were plotted by micronutrient tertile; logistic regression analyses were used to calculate the adjusted odds ratios, with the highest tertiles of micronutrients being used as reference groups. We also applied random-effects models by pooling all available longitudinal interleukin-6 measurements together and modeling log interleukin-6 as a continuous outcome in order to explicitly model the effect of baseline micronutrient levels in tertiles on individual rate of change in log interleukin-6 levels over time. Cox proportional hazards regression models were used to determine the relations between

**TABLE 2. Summary data on levels of micronutrients and interleukin-6 among 619 participants at baseline, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1993***

| Micronutrient | Mean | Standard deviation | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| α-Carotene (μmol/liter) | 0.09 | 0.09 | 0.07 | 0.00 | 0.93 |
| β-Carotene (μmol/liter) | 0.44 | 0.38 | 0.31 | 0.03 | 3.34 |
| Lycopene (μmol/liter) | 0.56 | 0.31 | 0.51 | 0.02 | 2.00 |
| Lutein/zeaxanthin (μmol/liter) | 0.38 | 0.20 | 0.35 | 0.04 | 1.72 |
| β-Cryptoxanthin (μmol/liter) | 0.14 | 0.15 | 0.1 | 0.01 | 1.41 |
| Total carotenoids (μmol/liter) | 1.60 | 0.73 | 1.5 | 0.13 | 4.49 |
| Retinol (μmol/liter) | 2.60 | 0.93 | 2.4 | 0.67 | 7.16 |
| α-Tocopherol (μmol/liter) | 21.83 | 8.90 | 19.7 | 5.05 | 66.53 |
| α-Tocopherol:cholesterol ratio (mg/g) | 4.23 | 1.65 | 3.8 | 0.90 | 12.39 |
| Zinc (μg/liter) | 889.7 | 229.8 | 854.4 | 188.2 | 2,661.5 |
| Selenium (μg/liter) | 118.2 | 19.2 | 116.4 | 58.2 | 245.8 |
| Interleukin-6 (pg/ml) | 5.51 | 12.69 | 3.70 | 0.66 | 289.72 |

* $n = 619$ for all analyses except α-tocopherol:cholesterol ratio ($n = 605$), zinc ($n = 615$), and selenium ($n = 591$).

**TABLE 3. Odds ratios (cross-sectional association) for being in the highest tertile of interleukin-6 level as compared with the two lowest tertiles, according to micronutrient intake at baseline, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1993***

| Micronutrient | Odds ratio† | 95% confidence interval | *p* value |
|---|---|---|---|
| α-Carotene (μmol/liter) | 0.65 | 0.53, 0.80 | <0.0001 |
| β-Carotene (μmol/liter) | 0.72 | 0.59, 0.87 | 0.001 |
| Lycopene (μmol/liter) | 0.75 | 0.63, 0.91 | 0.003 |
| Lutein/zeaxanthin (μmol/liter) | 0.72 | 0.59, 0.89 | 0.004 |
| β-Cryptoxanthin (μmol/liter) | 0.77 | 0.63, 0.94 | 0.016 |
| Retinol (μmol/liter) | 0.87 | 0.72, 1.05 | 0.038 |
| α-Tocopherol (μmol/liter) | 0.91 | 0.74, 1.11 | 0.5 |
| α-Tocopherol:cholesterol ratio (mg/g) | 1.01 | 0.82, 1.24 | 0.777 |
| Total carotenoids (μmol/liter) | 0.65 | 0.53, 0.79 | <0.0001 |
| Selenium (μg/liter) | 0.65 | 0.52, 0.80 | <0.0001 |
| Zinc (μg/liter) | 0.99 | 0.82, 1.20 | 0.948 |

* $n = 619$ for all analyses except α-tocopherol:cholesterol ratio ($n = 605$), zinc ($n = 615$), and selenium ($n = 591$).

† Calculated for a one-standard-deviation increase in log-transformed micronutrient level in a logistic regression model with adjustment for age, race, years of education, smoking status, body mass index, chronic obstructive pulmonary disease, peripheral arterial disease, angina, diabetes, physical activity, and incident cardiovascular disease.

the micronutrients with the strongest inverse association with interleukin-6 and mortality.

## RESULTS

Demographic and health-related characteristics of the 619 WHAS I participants with complete blood measurements are displayed in table 1 by interleukin-6 tertile (≤2.80 pg/ml, >2.80–≤4.81 pg/ml, and >4.81 pg/ml). Mean and median nutrient and interleukin-6 values for these 619 participants are displayed in table 2. Persons in the highest tertile of interleukin-6 were more likely to be smokers, to have a greater body mass index, to have peripheral arterial disease, diabetes, or cardiovascular disease, and to be inactive (table 1).

Given that there were 383 WHAS I participants with missing data, we compared demographic characteristics in persons with blood values and those without blood values. We found that, compared with the 383 persons who did not have blood information available for analysis, persons with data on all blood variables were younger (77.3 years (SD, 7.8) vs. 80.0 years (SD, 8.3); $p < 0.01$), had a higher body mass index (28.8 (SD, 6.8) vs. 27.5 (SD, 6.6); $p < 0.01$), and had higher levels of physical activity (31.1 percent vs. 19.3 percent; $p < 0.01$), as measured by the percentage in each group who had walked more than eight blocks in the past week. To explore the impact of these differences on our inference, we stratified the results shown in table 3 by age, body mass index, and physical activity and calculated

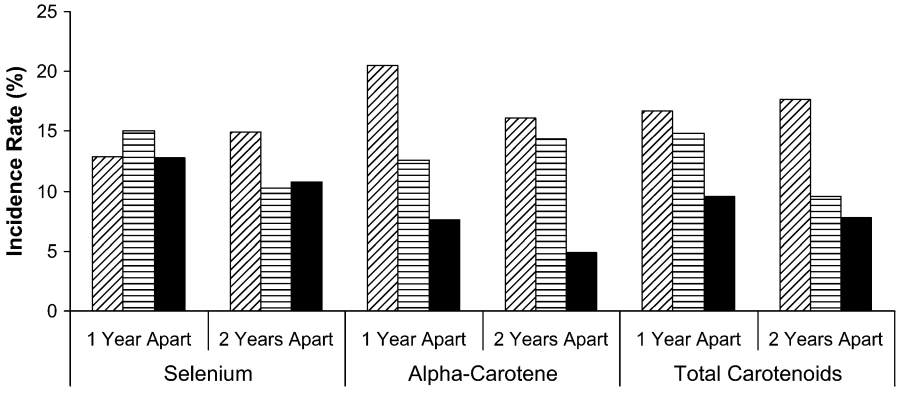


**FIGURE 1.** Crude incidence rate for an increase of more than 0.5 standard deviation (3.21 pg/ml) in interleukin-6 level, by micronutrient tertile and duration of follow-up, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1998. The lowest, middle, and highest tertiles are distinguished by bars with diagonal lines, bars with horizontal lines, and black bars, respectively. The differences in 1-year incidence rates by α-carotene tertile were significant at the 0.01 level; the differences in 2-year incidence rates by α-carotene and total carotenoid tertiles were significant at the 0.05 level.

**TABLE 4. Adjusted odds ratio for having an interleukin-6 level that increased longitudinally by more than 0.5 standard deviation (3.21 pg/ml) over a 2-year period, with the highest tertile of each micronutrient at baseline used as the reference group, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1995**

| Baseline nutrient tertile | Year 1 | | | Year 2 | | |
|---|---|---|---|---|---|---|
| | No. | OR†,‡ | 95% CI† | No. | OR‡ | 95% CI |
| α-Carotene (μmol/liter) | | | | | | |
| ≤0.039 | 146 | 2.48 | 1.05, 5.88* | 112 | 7.99 | 2.27, 28.21** |
| >0.039, ≤0.094 | 126 | 1.49 | 0.60, 3.72 | 111 | 7.12 | 2.08, 24.38** |
| >0.094 | 155 | 1 | | 119 | 1 | |
| β-Carotene (μmol/liter) | | | | | | |
| ≤0.23 | 138 | 1.68 | 0.74, 3.84 | 115 | 4.09 | 1.38, 12.11* |
| >0.23, ≤0.45 | 141 | 0.96 | 0.42, 2.21 | 108 | 3.52 | 1.19, 10.39 * |
| >0.45 | 148 | 1 | | 119 | 1 | |
| Lycopene (μmol/liter) | | | | | | |
| ≤0.38 | 134 | 1.01 | 0.44, 2.31 | 109 | 1.71 | 0.63, 4.62 |
| >0.38, ≤0.64 | 138 | 1.40 | 0.63, 3.09 | 118 | 2.14 | 0.83, 5.52 |
| >0.64 | 155 | 1 | | 115 | 1 | |
| Lutein/zeaxanthin (μmol/liter) | | | | | | |
| ≤0.27 | 132 | 1.12 | 0.46, 2.74 | 105 | 5.57 | 1.74, 17.80** |
| >0.27, ≤0.41 | 144 | 1.34 | 0.61, 2.94 | 114 | 3.18 | 1.08, 9.39* |
| >0.41 | 151 | 1 | | 123 | 1 | |
| β-Cryptoxanthin (μmol/liter) | | | | | | |
| ≤0.074 | 129 | 1.58 | 0.69, 3.62 | 102 | 2.00 | 0.75, 5.37 |
| >0.074, ≤0.14 | 147 | 1.40 | 0.63, 3.11 | 118 | 1.71 | 0.67, 4.39 |
| >0.14 | 151 | 1 | | 122 | 1 | |
| Retinol (μmol/liter) | | | | | | |
| ≤2.13 | 148 | 0.70 | 0.31, 1.56 | 111 | 0.48 | 0.19, 1.23 |
| >2.13, ≤2.90 | 143 | 0.70 | 0.31, 1.59 | 121 | 0.66 | 0.27, 1.61 |
| >2.90 | 136 | 1 | | 110 | 1 | |
| α-Tocopherol (μmol/liter) | | | | | | |
| ≤17.43 | 138 | 0.46 | 0.18, 1.13 | 116 | 1.00 | 0.37, 2.72 |
| >17.43, ≤23.08 | 141 | 1.00 | 0.46, 2.18 | 106 | 2.17 | 0.86, 5.47 |
| >23.08 | 148 | 1 | | 120 | 1 | |
| α-Tocopherol:cholesterol ratio (μmol/liter) | | | | | | |
| ≤3.31 | 141 | 0.84 | 0.35, 2.02 | 109 | 1.05 | 0.38, 2.94 |
| >3.31, ≤4.47 | 131 | 1.23 | 0.54, 2.81 | 105 | 1.70 | 0.64, 4.52 |
| >4.47 | 134 | 1 | | 112 | 1 | |
| Total carotenoids (μmol/liter) | | | | | | |
| ≤1.17 | 132 | 2.05 | 0.86, 4.91 | 113 | 3.98 | 1.51, 10.49** |
| >1.17, ≤1.80 | 142 | 1.94 | 0.83, 4.52 | 103 | 1.40 | 0.47, 4.14 |
| >1.80 | 153 | 1 | | 126 | 1 | |
| Selenium (μg/liter) | | | | | | |
| ≤110.00 | 131 | 0.53 | 0.23, 1.27 | 99 | 0.94 | 0.36, 2.45 |
| >110.00, ≤122.90 | 138 | 0.76 | 0.34, 1.68 | 115 | 0.81 | 0.32, 2.03 |
| >122.90 | 140 | 1 | | 122 | 1 | |
| Zinc (μg/liter) | | | | | | |
| ≤770.85 | 135 | 0.94 | 0.39, 2.26 | 103 | 1.05 | 0.38, 2.94 |
| >770.85, ≤938.68 | 147 | 1.76 | 0.81, 3.85 | 124 | 1.70 | 0.64, 4.52 |
| >938.68 | 145 | 1 | | 119 | 1 | |

* $p \leq 0.05$; ** $p \leq 0.01$.

† OR, odds ratio; CI, confidence interval.

‡ Adjusted for age, Black race, years of education, smoking status, body mass index, baseline cardiovascular disease, chronic obstructive pulmonary disease, diabetes, peripheral vascular disease, physical activity, incident cardiovascular disease, and baseline interleukin-6 level.

**TABLE 5. Mortality hazard over a 5-year period in relation to baseline levels of three micronutrients in a Cox proportional hazards model, Women's Health and Aging Study I, Baltimore, Maryland, 1992–1998**

| Micronutrient | No. of deaths over 5 years | Unadjusted HR† | 95% CI† | Age- and race-adjusted HR | 95% CI | Fully adjusted HR‡ | 95% CI |
|---|---|---|---|---|---|---|---|
| α-Carotene (μmol/liter) | | | | | | | |
| ≤0.040 | 71 | 1.19 | 0.85, 1.68 | 1.44* | 1.02, 2.04 | 1.06 | 0.70, 1.59 |
| >0.040, ≤0.094 | 73 | 1.21 | 0.86, 1.69 | 1.30 | 0.92, 1.82 | 1.19 | 0.81, 1.74 |
| >0.094 | 62 | 1 | | 1 | | 1 | |
| Total carotenoids (μmol/liter) | | | | | | | |
| ≤1.167 | 75 | 1.23 | 0.88, 1.72 | 1.32 | 0.95, 1.85 | 1.07 | 0.72, 1.58 |
| >1.167, ≤1.806 | 67 | 1.03 | 0.73, 1.45 | 1.09 | 0.78, 1.54 | 1.02 | 0.69, 1.50 |
| >1.806 | 64 | 1 | | 1 | | 1 | |
| Selenium (μg/liter) | | | | | | | |
| ≤109.9 | 78 | 1.66* | 1.17, 2.37 | 1.48* | 1.03, 2.13 | 1.54* | 1.03, 2.32 |
| >109.9, ≤122.8 | 68 | 1.40 | 0.98, 2.02 | 1.24 | 0.86, 1.79 | 1.30 | 0.86, 1.96 |
| >122.8 | 51 | 1 | | 1 | | 1 | |

* $p \leq 0.05$.

† HR, hazard ratio; CI, confidence interval.

‡ Adjusted for age (years), Black race, years of education, current smoking, body mass index, chronic obstructive pulmonary disease, peripheral arterial disease, angina, diabetes, and physical activity at baseline.

coefficients for the relations between micronutrients and log interleukin-6 levels. We found that associations and correlations were generally higher for older women, women with a higher body mass index, and women with lower physical activity than for women without these risk factors (data not shown).

In the cross-sectional analysis adjusting for multiple confounders, we identified highly significant inverse relations between serum interleukin-6 levels and α-carotene (odds ratio (OR) = 0.65, 95 percent confidence interval (CI): 0.53, 0.80), total carotenoids (OR = 0.65, 95 percent CI: 0.53, 0.79), and selenium (OR = 0.65, 95 percent CI: 0.52, 0.80) ($p < 0.0001$ for each)—a 35 percent reduction in risk of being in the highest interleukin-6 tertile for every 1-SD increase in log nutrient value (table 3). Persons with the highest levels of β-carotene, lycopene, lutein/zeaxanthin, β-cryptoxanthin, and retinol were also significantly less likely to be in the highest interleukin-6 tertile (table 3). There was no identifiable relation between serum levels of zinc, α-tocopherol, or α-tocopherol:cholesterol ratio and serum interleukin-6 in these analyses (table 3).

We found an increasing but not statistically significant time trend in the population mean interleukin-6 level using the random-effects model. There was substantial between-person heterogeneity in the rate of change (i.e., time slope) in interleukin-6 levels over time ($p < 0.01$; data not shown), suggesting that any population-average approach in this case could underestimate critical changes in interleukin-6 on an individual level. Therefore, we examined the longitudinal effect of baseline micronutrient levels on individual-level changes in serum interleukin-6. The percentage increase of more than 0.5 SD in interleukin-6 values (3.21 pg/ml) over 1-year and 2-year periods significantly increased as the α-carotene level decreased (figure 1). A similar finding was observed among the total carotenoid groups over a period of 2 years (figure 1). The associations between increasing interleukin-6 and α-carotene remained significant in the fully adjusted model for year 1 (OR = 2.48, $p < 0.05$). For year 2, the associations remained significant for α-carotene (OR = 7.99, $p < 0.01$), β-carotene (OR = 4.09, $p < 0.05$), lutein/zeaxanthin (OR = 5.57, $p < 0.01$), and total carotenoids (OR = 3.98, $p < 0.01$) (table 4). Selenium levels were unrelated to interleukin-6 increase in both models (table 4), probably partly because of the large number of subjects in the lowest selenium tertile who did not return for follow-up visits in this longitudinal study. Further investigation of the potential reasons why many persons in the lowest selenium tertile at baseline were missing from subsequent analyses showed that those participants had a significantly greater risk of all-cause mortality over a 5-year period than participants in the other tertiles (hazard ratio = 1.54, 95 percent CI: 1.03, 2.32; $p < 0.05$), even in unadjusted and fully adjusted models (table 5). We identified no significant increase in 5-year cardiovascular disease mortality hazard in the two lower tertiles of selenium in relation to the highest tertile (data not shown). We also identified an increased risk of mortality for persons with the lowest levels of α-carotene in the model adjusted for age and race, but we identified no increased mortality risk in persons with the lowest baseline levels of α-carotene or total carotenoids over 5 years in the fully adjusted model (table 5). No other cause-of-death grouping was large enough for analysis of differences between groups.

## DISCUSSION

These findings demonstrate robust inverse cross-sectional relations between the potent inflammatory cytokine interleukin-6 and several antioxidant carotenoids and selenium.

No relations were demonstrated between α-tocopherol or zinc levels and serum interleukin-6. Importantly, longitudinal analyses demonstrated that baseline levels of α-carotene, β-carotene, lutein/zeaxanthin, and total carotenoids were significantly associated with increasing levels of interleukin-6 over a period of 2 years and that low levels of selenium were associated with increased risk of all-cause mortality over a period of 5 years. The identification of differences in relations between individual antioxidant micronutrients and interleukin-6 may provide specific clues as to which oxidative pathways most contribute to the inflammatory characteristics observed in frail and disabled older adults (1, 3, 19). Additional longitudinal nutritional data and analyses may help in determining which serum antioxidant measurements might be useful guides in the suppression of oxidative stress-induced inflammation.

Dietary carotenoids are powerful antioxidants that are embedded within lipid bi-layers (the two lipid layers that make up cell membranes) and function to quench free radicals generated by intracellular oxidative processes (20). Although all of the individual carotenoids analyzed for this study showed significant relations with interleukin-6, β-carotene and (especially) α-carotene demonstrated the most robust cross-sectional and longitudinal relations. Lower levels of α-carotene have been linked to atherosclerotic processes in older adults, and low levels of α-carotene and β-carotene correlate with a higher risk of coronary artery disease in adult women (21, 22). This may be partly because α- and β-carotene constitute a major benign sink for oxidation in lipid particles; hence, lower levels of these carotenoids could lead to more oxidized lipids and increased activation of inflammatory pathways (23–25). Although deficiencies in lutein/zeaxanthin have most often been linked to macular degeneration, studies also show a strong relation between low levels of these nutrients and cardiovascular disease (26). Thus, our longitudinal findings suggest that low levels of α- and β-carotene, lutein/zeaxanthin, and total carotenoids may drive interleukin-6 increases, perhaps through decreased availability of these antioxidants for quenching free radicals in the cardiovascular system.

Selenium is a critical constituent of glutathione peroxidase, the major reducing enzyme for both hydrogen peroxide and lipid peroxides (27). Zinc, like selenium, is an important component of a cytosolic antioxidant enzyme, copper-zinc superoxide dismutase (28). Although we found a strong relation between low selenium and high interleukin-6, we found no relation between zinc and interleukin-6. Superoxide molecules are converted to hydrogen peroxide by copper-zinc superoxide dismutase, which in turn activates inflammatory pathways (25). Elevation of copper-zinc superoxide dismutase to selenium-dependent glutathione peroxidase activity has been demonstrated to correlate with increased lipid peroxidation and nuclear factor-κB activation through increased hydrogen peroxide activity (29, 30). This evidence from prior studies, along with our findings of a strong relation between selenium and interleukin-6 and no relation between zinc and interleukin-6, suggests that oxidative processes driven by increased hydrogen peroxide and lipid peroxides rather than superoxide radicals may partly drive generation of interleukin-6 in older adults (31).

The increased 5-year mortality in persons with the lowest selenium levels was identified after we found that many persons in the lowest selenium tertile did not return for subsequent study visits. Selenium deficiency is associated with a host of inflammatory tissue responses and with disease progression, including myocarditis related to Coxsackievirus and human immunodeficiency virus, thyroid dysfunction, arthritis, cancer, depression, and cardiovascular disease (27, 32). This selenium deficiency-related acceleration of many disease processes may partly explain the association between mortality and lower selenium levels observed in this population. Further exploration of the specific etiology of mortality beyond cardiovascular disease may help add biologic and tissue specificity to this finding.

It is not clear why we did not identify a relation between the carotenoids and mortality. Although we cannot rule out residual confounding, other plausible explanations exist. First, the set of micronutrients included in this study comprises only a small part of a large family of antioxidants and nutrients. It may well be that other nutrients and biomediators that we did not measure (e.g., vitamin C) can modulate interleukin-6 as well, thereby independently contributing to mortality. Second, we had hypothesized that micronutrients represent more distal correlates relative to interleukin-6 in relation to mortality; therefore, the effects are more likely to be indirect. Third, WHAS I was designed to study the one third most disabled older women, which could have resulted in limited variability of micronutrient levels—that is, a flooring effect—in the study population. Finally, since we do not have longitudinal data on serum antioxidants because of the prohibitive cost, we are not able to confirm a causal relation without taking into account the changes in carotenoid levels over time.

A number of questions remained unanswered in this study. First, it is unclear whether low dietary intake, high oxidative stress, or both contributed to the observed lower antioxidant levels. Although inflammation has minimal impact on selenium levels, serum carotenoid levels are modestly decreased by inflammatory processes in younger and older adults (33, 34). Second, although we adjusted for diseases known to trigger inflammation, it was not possible to characterize all clinical and subclinical inflammation-inducing conditions and hence capture all potentially confounding variables in this population. Third, although there is some knowledge of specificity in function of antioxidant nutrients and enzymes, many studies have been performed in in-vitro systems, making any findings regarding specificity of function of individual nutrients less than conclusive. Fourth, we had 383 missing data points because of lack of or insufficient amounts of serum for measuring the relevant variables. Given that persons with insufficient data were older, less obese, and less physically active, we would hypothesize that our findings would have been even stronger if we had had those missing data. Finally, we did not have longitudinal measurements of antioxidant levels for this analysis, which, combined with longitudinal interleukin-6 measurements, might help determine directionality and the utility of specific antioxidant nutrient interventions in suppressing elevated interleukin-6 levels in at-risk older adults.

In summary, in this study, we identified robust inverse cross-sectional and longitudinal relations between several specific carotenoids and serum interleukin-6. We identified a robust cross-sectional inverse relation between selenium and interleukin-6 and increased mortality among persons with lower selenium levels. These findings suggest that specific antioxidant nutrients, which act mechanistically to decrease levels of hydrogen peroxide and lipid peroxides, may play an important role in suppressing expression of interleukin-6 in disabled older women.

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