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Higher Serum Concentrations of Dietary Antioxidants are Associated with Lower Levels of Inflammatory Biomarkers during the Year after Hip Fracture

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Abstract

Background & Aims—Chronic inflammation impairs recovery among the 1.6 million people who suffer from hip fracture annually. Vitamin E and the carotenoids are two classes of dietary antioxidants with profound anti-inflammatory effects, and the goal of this study was to assess whether higher post-fracture concentrations of these antioxidants were associated with lower levels of interleukin 6 (IL-6) and the soluble receptor for tumor necrosis factor-alpha (sTNF-αR1), two common markers of inflammation.

Methods—Serum concentrations of the dietary antioxidants and inflammatory markers were assessed at baseline and 2, 6, and 12 month follow-up visits among 148 hip fracture patients from The Baltimore Hip Studies. Generalized estimating equations modeled the relationship between baseline and time-varying antioxidant concentrations and inflammatory markers.

Results—Higher post-fracture concentrations of vitamin E and the carotenoids were associated with lower levels of inflammatory markers. Associations were strongest at baseline, particularly between the α-tocopherol form of vitamin E and sTNF-αR1 (p=0.05) and total carotenoids and both sTNF-αR1(p=0.01) and IL-6 (p=0.05). Higher baseline and time-varying α-carotene and time-varying lutein concentrations were also associated with lower sTNF-αR1 at all post-fracture visits ($p \leq 0.05$).

Conclusions—These findings suggest that a clinical trial increasing post-fracture intake of vitamin E and the carotenoids may be warranted.

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Keywords

antioxidants; inflammation; vitamin E; carotenoids; micronutrients; hip fracture

INTRODUCTION

Chronic inflammation is thought to play a role in an increasing number of adverse health outcomes[1, 2]. Approximately 1.6 million people suffer from hip fracture annually[3], and older adults recovering from hip fracture have particularly high levels of inflammatory markers that persist an entire year after the injury[4]. While the mechanism is currently unclear and the cause likely multi-factorial, this inflammation may be a result of the excessive oxidative stress shown to occur after bone fractures[5] and bone-corrective surgical procedures[6]. The typical hip fracture patient is functionally compromised for greater than a year after the injury[7] and those with higher peak and persistent levels of inflammation experience worse recovery[4]. Thus, while acute inflammation is a necessary part of the healing process, persistent inflammation appears to hamper post-fracture functional recovery and reducing this chronic inflammatory response might be an effective strategy to expedite and improve recovery from hip fracture.

Antioxidants may help lessen this persistent inflammatory response by reducing the high levels of oxidative stress after hip fracture. Oxidative stress is a result of oxygen metabolism and therefore a certain degree is necessary for life. However, excessive oxidative stress incurs substantial cellular damage and can elicit an inflammatory response[8]. Antioxidants are substances that reduce oxidative stress and are either produced endogenously or obtained through the diet. Vitamin E and the carotenoids, two of the most biologically active classes of dietary antioxidants, have been associated with reduced risk of chronic disease[9] and have demonstrated potent anti-inflammatory effects[10, 11]. Recent research among hip fracture patients specifically has shown that higher concentrations of vitamin E were associated with better post-fracture physical function[12]. The mechanism for this protective relationship is currently unknown, but antioxidant-mediated reduction of the inflammatory cytokines that have been strongly associated with loss of the muscle tissue[13] critical to physical function is one plausible explanation.

The goal of this study was to determine whether higher serum concentrations of vitamin E and the carotenoids at baseline and throughout the year after hip fracture were associated with lower levels of interleukin 6 (IL-6) and the soluble receptor for tumor necrosis factor-alpha (sTNF-αR1), two common markers of inflammation. IL-6 and TNF-α appear to be of clinical importance in hip fracture recovery as concentrations of these inflammatory markers have been associated with poor recovery and are persistently high after fracture[4]. We hypothesized that higher concentrations of vitamin E and the carotenoids both at baseline and throughout the year after hip fracture would be associated with lower levels of these important inflammatory biomarkers.

MATERIALS AND METHODS

Subjects

The study participants were drawn from the fourth cohort of The Baltimore Hip Studies (BHS4), a randomized clinical trial designed to assess the effects of an exercise intervention in reducing bone and muscle loss after hip fracture[14]. The study enrolled 180 community-dwelling women ≥ 65 years of age who were admitted to one of three hospitals in Baltimore with surgical repair of a non-pathological hip fracture. Due to the physical and cognitive demands involved in an exercise program, participants were also required to have been able

to walk without human assistance before the fracture and have scored ≥ 20 on the Folstein Mini-Mental State Examination[15] (MMSE). Participants were excluded on the basis of medical conditions that are contraindicated with exercise including; cardiovascular disease, respiratory problems, neuromuscular conditions, bone diseases, metastatic cancer, cirrhosis, end-stage renal disease, and other conditions that increase the risk of falls during exercise (seizures or substance abuse/dependency) or increase the risk of injury after a fall (Coumadin use or gastrointestinal bleeding). Only 19% of screened participants were deemed eligible for the study based upon these stringent selection criteria, resulting in a study population of hip fracture patients with a relatively high degree of health and function. Assessments consisting of an interview, functional measurements, dual energy X-ray absorptiometry (DXA), and a blood draw were conducted by research nurses at baseline (within 15 days after the hip fracture injury [mean 10.9 days after fracture] and following corrective surgery in all participants) and 2, 6 and 12 months post-fracture.

The final sample for this analysis consisted of the 148 unique BHS4 participants from whom a blood sample was successfully drawn at ≥1 study visit. Out of the 148 unique participants in this study, 40 had serum antioxidant data at all 4 visits, 59 had data at 3 visits, 36 had data at 2 visits, and 13 had data for 1 visit. The study was approved by the Institutional Review Boards of the University of Maryland and each study hospital.

Serum Antioxidant and Inflammatory Marker Assessment

Blood samples were obtained by venipuncture and processed following a standardized protocol. Serum samples were stored continuously at −70°C until the time of analysis for two of the major forms of vitamin E (α-tocopherol and γ-tocopherol), six of the major carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene), and the two inflammatory markers (IL-6 and sTNF-αR1) under study, all of which are stable under these long-term storage conditions[16]. Serum antioxidant concentrations were measured by reverse-phase high-pressure liquid chromatography (HPLC) and serum inflammatory marker concentrations were analyzed using commercially available kits (R&D Systems, Minneapolis, MN). All serum antioxidant and inflammatory marker assays were conducted at The Johns Hopkins University. The inter-assay and intra-assay coefficients of variation, respectively, were as follows; vitamin E – 11.4% and 7.8% for α-tocopherol and 11.5% and 6.2% for γ-tocopherol; the carotenoids – 10.31% and 7.56% for α-carotene, 3.21% and 3.64% for β-carotene, 2.62% and 2.24% for β-cryptoxanthin, 4.52% and 7.41% for lutein, 5.88% and 4.74% for zeaxanthin, and 3.97% and 1.55% for lycopene; and the inflammatory markers – 3.44% and 4.37% for IL-6 and 2.98% and 5.47% for sTNF-αR1.

Statistical Analysis

Descriptive analyses were conducted to characterize the study population and describe the distributions of the antioxidant and inflammatory biomarker concentrations during the year after hip fracture.

Generalized estimating equations (GEE)[17] were used to determine the associations between baseline antioxidant concentrations and inflammatory biomarkers at baseline and throughout the year after hip fracture (2, 6, and 12 month post-fracture visits) while adjusting for covariates and accounting for within-patient correlation. These analyses only used data from participants who had serum antioxidant data at baseline (n=96).

In addition to the baseline analysis, a time-varying analysis assessing the relationship between antioxidant concentrations at each of the four post-fracture study visits and the inflammatory markers throughout the year after fracture was also conducted. Marginal structural models (MSM)[18] were constructed in the time-varying analyses to account for

potential time-dependent confounding of the relationship between the dietary antioxidant concentrations and inflammatory markers by body-fat percentage and physical activity level. Each MSM was fit using a weighted GEE, where the weight was the estimated inverse probability density of the antioxidant concentration. Weights were estimated by linearly regressing antioxidant concentrations on the covariates described below. The probability densities of the observed antioxidants were then calculated assuming the antioxidants follow a normal probability density with the fitted antioxidant values and mean squared error from the linear regression as the mean and variance, respectively. The weights were then applied to the GEE models, which contained the antioxidant concentrations, time since fracture, and time-by-antioxidant interaction terms. These analyses used data from participants who had antioxidants measured during at least one study visit (n=148).

The relationships between the eight individual dietary antioxidants under study and the inflammatory biomarkers were assessed in separate regression models. Regression models were also used to determine the relationship between "Total Carotenoids", consisting of the sum of the six forms of carotenoids that are most abundant in the body and commonly analyzed together[19], and the inflammatory markers.

A number of potential confounders of the relationship between antioxidants and inflammatory biomarkers were assessed. Covariates included in the *baseline* regression analyses were baseline age, comorbidity (Charlson Comorbidity Index[20]), cognitive function (MMSE), urinary tract infection, Instrumental Activities of Daily Living[21] (IADLS), and study group allocation (exercise group vs. control group). Covariates included in the *time-varying* regression analyses were the baseline covariates as well as body-fat percentage (using DXA), urinary tract infection, physical activity (using total activity time in the Yale Physical Activity Scale[22]), and IADLS throughout the year after hip fracture. Each of the time-varying covariates were assessed at the 2, 6, and 12 month post-fracture follow up visits. A number of acute medical complications are known to occur during the year after hip fracture[23] that could potentially confound the relationship between antioxidant micronutrients and inflammatory biomarkers. Therefore, a wide variety of complications that were assessed in BHS4 including congestive heart failure, cardiac arrest, renal failure, bone fracture, pressure ulcers, pneumonia, urinary tract infections, and wound infections were considered as potential covariates in regression models. However, urinary tract infection was the only complication that arose in at least one participant at each study visit in this relatively healthy cohort of hip fracture patients. Consequently, incidence of urinary tract infection was the only one of these complications that was included as a covariate in regression models. Associations were considered statistically significant when $p < 0.05$. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Table 1 characterizes the study population at each visit. The study sample was composed of 148 unique female hip fracture patients with a mean age of 82.0 (standard deviation [SD]=7.0) years, Charlson Comorbidity Index of 1.1 (SD=1.3) points, and body-fat percentage of 33.5 (SD=9.2) at baseline which increased slightly throughout the year after fracture. Post-fracture physical activity increased from 2 months to 6 months, but decreased slightly from 6 months to 12 months. Disability, as expressed by Instrumental Activities of Daily Living (IADLs), increased after hip fracture and decreased at each subsequent visit during the year after hip fracture. There were very few complications in this robust cohort of hip fracture patients with the exception of urinary tract infections, which decreased throughout the year after fracture (from 4.0% at baseline to 0.7% at 12 months). Antioxidant concentrations were lowest and inflammatory biomarkers were highest at baseline. Though there were differences between the individual forms, the greatest increases in vitamin E and

carotenoids concentrations generally occurred between baseline and 2 months. The greatest decreases in IL-6 and sTNF-αR1 also occurred between baseline and 2 months, though these inflammatory biomarkers continued to decrease throughout the year after fracture.

Tables 2–5 summarize the results of the covariate-adjusted regression models assessing the relationships between the antioxidants and inflammatory markers after hip fracture.

Table 2 reveals that higher baseline α-tocopherol was associated with lower sTNF-αR1 at baseline (beta= −19.6, p=0.05) but at no other post-fracture visits. Time-varying α-tocopherol was also associated with lower sTNF-αR1 at baseline (beta= −18.7, p=0.05) but at no other post-fracture visits. The γ-tocopherol form of vitamin E was not significantly associated with sTNF-αR1 at any point during the year after fracture.

Table 3 shows that neither form of vitamin E was significantly associated with IL-6 at any point during the year after hip fracture.

Table 4 reveals that higher baseline total carotenoids were associated with lower sTNF-αR1 at baseline (beta= −513, p=0.01) but at no other post-fracture visits. All of the individual baseline carotenoids were significantly associated with lower baseline sTNF-αR1 ($p < 0.04$), with the exception of baseline lycopene (p=0.10). Baseline α-carotene was also associated with lower sTNF-αR1 at all study visits ($p \leq 0.02$) and baseline β-cryptoxanthin (p=0.05) and β-carotene (p=0.05) were also associated with lower sTNF-αR1 at 2 and 6 months post-fracture, respectively, but most of the individual baseline carotenoids were not associated with sTNF-αR1 at the 2, 6, or 12 month post-fracture visits. Time-varying total carotenoids were also associated with lower sTNF-αR1 at baseline (beta= −454, p=0.008) but at no other post-fracture visits. All of the individual time-varying carotenoids were significantly associated with lower baseline sTNF-αR1 ($p < 0.05$) except for baseline lycopene (p=0.01). Unlike the baseline individual carotenoids, most of the time-varying individual carotenoids were significantly associated with lower sTNF-αR1 at 2, 6, or 12 month post-fracture visits. In particular, higher concentrations of time-varying α-carotene and lutein were significantly associated with lower sTNF-αR1 at all four post-fracture study visits ($p<0.05$). Higher concentrations of time-varying zeaxanthin were also significantly associated with lower sTNF-αR1 at baseline and 2 and 6 month post-fracture visits ($p<0.04$).

Table 5 shows that higher baseline total carotenoids were associated with lower IL-6 at baseline (beta= −8.9 pg/mL, p=0.05), but not at the other post-fracture visits. While all of the individual baseline carotenoids approached statistically significant relationships with lower baseline sTNF-αR1 ($p \leq 0.1$), only lutein attained statistical significance (beta = −41.1, p=0.05). Higher concentrations of time-varying total carotenoids were also associated with lower IL-6 at baseline (beta= −8.6 pg/mL, p=0.05), but at no other post-fracture visits. Again, all of the individual time-varying carotenoids approached statistically significant associations with lower baseline IL-6 ($p \leq 0.1$), but only lutein was statistically significant (beta = −47.8, p=0.04). Higher concentrations of time varying β-cryptoxanthin at 6 months (beta= −11.8, p=0.05) were associated with lower IL-6, but most of the individual time-varying carotenoids were not associated with IL-6 at the 2, 6, or 12 month post-fracture visits.

DISCUSSION

Higher serum concentrations of vitamin E and the carotenoids were associated with lower concentrations of inflammatory markers in this cohort of hip fracture patients. However, the strength of association varied substantially by both the class of antioxidant and the inflammatory marker. While serum concentrations of both classes of these dietary antioxidants were associated with less inflammation, the carotenoids generally demonstrated

stronger relationships than vitamin E with both sTNF-αR1 and IL-6. The reason for this discrepancy is unknown, but the fact that the half-lives of the serum carotenoids (between 26 to 76 days[24]) are much longer than those of vitamin E (13 hours for γ-tocopherol and 57 hours for α-tocopherol[25]) suggests that carotenoids might potentially provide more sustained anti-inflammatory activity than vitamin E due to their extended period of systemic circulation.

In addition, the strength of associations with inflammatory markers varied substantially across the individual micronutrients within each class of dietary antioxidants. For instance, the α-tocopherol form of vitamin E was much more strongly associated with sTNF-αR1 than the γ-tocopherol form. While the evidence in humans is fairly limited at present, this finding was somewhat surprising as γ-tocopherol has been shown to possess stronger anti-inflammatory effects than α-tocopherol in animal models[10]. Among the individual carotenoids, serum α-carotene, lutein, and zeaxanthin were the most strongly and consistently associated with lower concentrations of inflammatory markers. In particular, higher concentrations of both baseline and time-varying α- carotene were strongly associated with lower concentrations of sTNF-αR1 at every study visit. These findings suggest that α-carotene, an important micronutrient that has received relatively little attention compared to the other dietary carotenoids, may be warrant future study among hip fracture patients. Lutein and zeaxanthin often accompany one another in leafy green vegetables and other dietary sources, but the reason that these particular carotenoids and α-carotene were more strongly related to lower inflammatory markers than the other dietary antioxidants under study is unclear and worthy of further study.

The findings of this study may have important implications to the growing number of older adults suffering from hip fracture, in whom persistent inflammation has been associated with poor recovery[4]. There are several plausible explanations for the associations between these common dietary antioxidants and inflammatory markers. While the relationships between the particular antioxidant micronutrients and inflammatory markers in this analysis have not previously been studied after hip fracture or any traumatic injury in humans, clinical studies have demonstrated that a number of other antioxidants are associated with reduced levels of inflammatory markers[26, 27]. It is postulated that the mechanism through which antioxidants elicit anti-inflammatory effects may be the buffering of highly reactive oxygen species that can no longer activate nuclear factor-kappa beta (NF-κB), a transcription factor that enacts the expression of inflammatory cytokines. In situations of excessive oxidative stress, reactive oxygen species can activate Toll-like receptor 4, which in turn stimulates NF-κB activity[28]. Such a situation is likely to occur after hip fracture as both bone fractures[29] and long-bone fixative surgery[6] generate high levels oxidative stress. Thus, the antioxidant activity of vitamin E and the carotenoids may reduce these high levels of oxidative stress following hip fracture, resulting in decreased NF-κB activity and inflammatory cytokine expression.

While higher concentrations of these antioxidants were associated with less inflammation throughout the year after hip fracture, both the carotenoids and vitamin E were more strongly associated with sTNF-αR1 than IL-6. The explanation for this difference is unclear, though it has been shown that NF-κB plays an especially critical role in the expression of the inflammatory cytokine TNF-α. Therefore, antioxidant suppression of NF-κB activity could explain the particularly strong associations noted between sTNF-αR1, vitamin E, and the carotenoids. The pathways for expression of IL-6 are considerably more complicated than TNF-α and it is possible that antioxidants may not have as much of a direct effect on IL-6. Future studies will be needed to verify this hypothesis.

This study is the first to assess the relationship between common dietary antioxidants and inflammatory markers in a cohort of hip fracture patients, a population commonly burdened with persistent inflammation. Moreover, one of the strengths of this analysis was the longitudinal assessment of both antioxidant and inflammatory biomarker concentrations at various points throughout the year after hip fracture. This enabled us to track how these important dietary antioxidants changed throughout the year after hip fracture. Additionally, we were able to compare the magnitude and direction of the associations between the antioxidants and inflammatory markers at various stages of hip fracture recovery. In doing so, we found that the associations between vitamin E, the carotenoids, and inflammatory markers were generally strongest at baseline. On a potentially related note, our analyses also revealed that antioxidant concentrations were lowest and inflammatory marker concentrations were highest at the baseline visit. This particular finding might explain why the relationship between the dietary antioxidants and inflammatory markers was strongest at baseline. It has been suggested that serum antioxidants are utilized, and thus depleted, around the time of traumatic injury to help ameliorate the damage incurred in part by inflammatory cytokines[30].

A related strength of this study was our serum assessment of the carotenoids and vitamin E. Serum concentrations are considered the "gold standard" of dietary intake of these micronutrients as the potential for recall bias associated with most questionnaire-based measurement devices is minimized[31], thereby strengthening the dietary inferences of this study. When taken in consideration with previous evidence of near complete depletion of antioxidants after surgery[32], our findings suggest that the trauma of the hip fracture injury and subsequent surgery might also deplete serum stores of dietary antioxidants shortly after the injury. Pre- and post-injury assessments would be necessary to confirm this hypothesis. Nevertheless, consumption of nuts, leafy green vegetables, fruits and other foods rich in vitamin E and carotenoids shortly after hip fracture might help normalize concentrations of these important antioxidants shown to be associated with lower levels of inflammatory markers. Though clinical trials are necessary to corroborate these findings, consumption of nutrient-dense foods might offer some protection against the potentially adverse effects of inflammation following hip fracture.

There were also a number of notable limitations of this work. Dietary intake data were not collected in BHS4, and we were consequently unable to directly correlate serum vitamin E and carotenoid concentrations to dietary intake of these micronutrients in this cohort. Thus, despite the fact that prior analyses have established that serum vitamin E and carotenoid concentrations are useful indicators of dietary intake of these micronutrients[31], future studies of hip fracture patients should include both serum measurements and dietary intake assessment to further strengthen dietary conclusions. Our findings may also not be generalizable to all hip fracture patients as the BHS4 cohort was exclusively female, relatively healthy, and highly physically and cognitively functioning. This highly functioning cohort was the result of a stringent set of inclusion criteria in which only 19% of screened hip fracture patients were deemed eligible to participate in the study. Such a highly functioning cohort was required due to the rigors of participation in an exercise intervention. These rigorous inclusion criteria contributed to the very low incidence of acute complications (aside from urinary tract infections) during the year after hip fracture. The lack of complications and statistical adjustment for urinary tract infections in the regression models minimized this potentially important source of confounding of the relationship between micronutrients and inflammatory markers. While the high functional status may limit the generalizability to more robust hip fracture patients, it also increases applicability to the general population of older adults with similarly high functional levels.

A related limitation is that BHS4 was an exercise intervention study and physical activity has been shown to modulate markers of inflammation[33]. We implemented several analytical measures to account for the potential confounding of the relationship between antioxidants and inflammation introduced by physical activity. First, we adjusted our regression models for study group allocation (exercise vs. control) since the BHS4 exercise group performed more physical activity than the control group. Our analyses were further protected from confounding by physical activity as additional adjustment was made in regression models for the total time spent in physical activity as measured by the Yale Physical Activity Scale. Neither allocation to the exercise group nor total physical activity was meaningfully associated with the inflammatory markers under study at any time point ($p \geq 0.3$). While these findings were surprising, it was hypothesized in previous work that the BHS4 exercise intervention was not of sufficient intensity to elicit reductions in bone and muscle loss after hip fracture[34]. The relatively low level of exercise intensity may also explain the lack of relationship between participation in the exercise program and inflammatory markers that was expected in this study.

Most post-fracture nutritional interventions to date have focused almost exclusively upon increasing protein and total caloric intakes through nutritional supplement shakes. These interventions have demonstrated mixed results in improving hip fracture outcomes[35, 36], and more comprehensive nutritional interventions may be necessary to optimize recovery from hip fracture. The associations between higher concentrations of serum antioxidants and lower concentrations of inflammatory markers uncovered in this study suggest that a clinical trial increasing the intake of nuts, vegetables, fruits and other foods rich in vitamin E and carotenoids may be warranted. Alternatively, a trial evaluating the typical post-fracture nutritional supplement approach with the addition of the six major carotenoids and vitamin E may be a viable and practical strategy for functionally-compromised hip fracture patients with impaired ability to purchase, prepare, and consume healthy meals. In light of the moderate success of post-fracture protein shake supplementation, adding reasonable levels of the six major dietary carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene) and mixed tocopherols (including naturally sourced dl-alpha tocopherol, which offers superior absorption[37] to the synthetic d-alpha tocopherol used in most clinical trials to date) to such a shake may be worthy of evaluation. Such a dietary intervention geared towards attenuating persistently elevated levels of inflammation could reveal a safe and cost-effective route towards potentially expedited and more complete recovery from hip fracture.

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Non-standard abbreviations

IL-6	Interleukin 6
sTNF-αR1	Tumor necrosis factor-alpha soluble receptor 1

NF-κB	Nuclear factor-kappa beta
BHS4	Baltimore Hip Studies cohort 4
DXA	Dual energy X-ray absorptiometry
HPLC	Reverse-phase high-pressure liquid chromatography
GEE	Generalized estimating equations
MSM	Marginal structural models

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Table 1

Participant Characteristics

	Mean (SD) [n]			
Variable [units]	**Baseline**	**2 Month**	**6 Month**	**12 Month**
Age at Fracture [years]	82.0 (6.9) [148]	---	---	---
Charlson Comorbidity Index [number of comorbidities]	1.1 (1.3) [148]	---	---	---
Mini-Mental State Examination (MMSE) [points]	26.8 (2.6) [148]	---	---	---
Yale Physical Activity Survey: Total Activity Time (YPAS) [hours] *	25.0 (18.1) [147]	12.8 (12.9) [130]	18.5 (13.6) [134]	17.0 (13.6) [133]
Body-fat [%]	33.5 (9.2) [105]	34.4 (9.4) [108]	34.4 (9.7) [127]	36.2 (9.7) [120]
Instrumental Activities of Daily Living (IADLS) [# of limitations] *	1.1 (1.3) [143]	2.8 (1.4) [116]	2.0 (1.5) [126]	1.9 (1.6) [124]
Urinary Tract Infection [% Yes]	4.1% [148]	3.0% [132]	3.0% [134]	0.8% [133]
Antioxidant Concentrations				
Total Vitamin E [μmol/L]	52.3 (11.9) [96] **	59.5 (16.8) [95] **	58.6 (16.1) [108] **	58.2 (14.5) [92] **
α-tocopherol [μmol/L]	44.0 (11.9)	51.8 (16.6)	50.5 (15.9)	50.6 (15.1)
γ-tocopherol [μmol/L]	8.3 (4.2)	7.7 (3.6)	8.1 (4.1)	7.6 (3.9)
Total Carotenoids [μmol/L]	1.41 (0.63)	1.80 (0.98)	2.00 (1.32)	1.97 (1.4)
α-carotene [μmol/L]	0.11 (0.08)	0.10 (0.08)	0.12 (0.10)	0.11 (0.08)
β-carotene [μmol/L]	0.47 (0.31)	0.67 (0.77)	0.80 (1.11)	0.81 (1.19)
β-cryptoxanthin [μmol/L]	0.13 (0.08)	0.17 (0.10)	0.16 (0.10)	0.19 (0.14)
Lutein [μmol/L]	0.16 (0.09)	0.18 (0.10)	0.21 (0.11)	0.20 (0.11)
Zeaxanthin [μmol/L]	0.04 (0.02)	0.05 (0.02)	0.06 (0.02)	0.06 (0.03)
Lycopene [μmol/L]	0.51 (0.27)	0.62 (0.30)	0.66 (0.33)	0.59 (0.32)
Inflammatory Biomarker Concentrations				
IL-6 [pg/mL]	17.9 (25.4) [94]	7.2 (13.6) [94]	5.6 (11.1) [107]	4.6 (9.4) [92]
sTNF-αR1 [pg/mL]	2760 (1051) [95]	2219 (920) [96]	2063 (878) [108]	2002 (790) [92]

* Baseline values refer to 2 week period prior to hip fracture

** The sample size for each antioxidant concentration was the same at all time points

Table 2

Adjusted Associations between Vitamin E and sTNF-αR1

Vitamin E Concentration [μmol/L]	sTNF-αR1 [pg/mL]			
Baseline Vitamin E	**Baseline**	**2 months**	**6 months**	**12 months**
α-tocopherol	**−19.6 [−0.7, −39.9] (0.05)**	−17.9 [−39.8, 3.9] (0.1)	−8.4 [−27.1, 10.4] (0.4)	−2.3 [−21.3, 16.7] (0.8)
γ-tocopherol	−6.3 [−56.6, 44.0] (0.8)	−1.5 [−88.6, 85.7] (0.9)	−15.6 [−60.0, 28.7] (0.5)	−23.3 [−62.6, 15.9] (0.2)
Time-varying Vitamin E				
α-tocopherol	**−18.7 [−0.3, −37.9] (0.05)**	−0.1 [−10.7, 10.4] (0.9)	−0.2 [−8.1, 7.8] (0.9)	−1.2 [−10.8, 8.4] (0.8)
γ-tocopherol	−7.1 [−52.7, 38.5] (0.8)	−2.1 [−42.1, 37.8] (0.9)	−11.1 [−38.8, 16.7] (0.4)	−19.9 [−64.5, 24.7] (0.4)

*Beta coefficient represents mean difference in sTNF-αR1 with each additional unit of vitamin E, [95% CI], (p value)

**__Bold__ type represents associations significant at the p = 0.05 level

Table 3

Adjusted Associations between Vitamin E and IL-6

Vitamin E Concentration [μmol/L]	**IL-6 [pg/mL]**			
Baseline Vitamin E	**Baseline**	**2 months**	**6 months**	**12 months**
α-tocopherol	−0.17 [−0.85, 0.51] (0.6)	−0.12 [−0.46, 0.22] (0.5)	0.30 [−0.11, 0.70] (0.2)	0.02 [−0.11, 0.70] (0.9)
γ-tocopherol	−0.38 [−1.73, 0.69] (0.6)	−0.90 [−2.21, 0.42] (0.2)	−0.55 [−1.27, 0.18] (0.1)	−0.30 [−0.89, 0.29] (0.3)
Time-varying Vitamin E				
α-tocopherol	−0.18 [−0.87, 0.51] (0.6)	−0.05 [−0.16, 0.06] (0.4)	0.07 [−0.02, 0.17] (0.2)	0.04 [−0.03, 0.11] (0.3)
γ-tocopherol	−0.34 [−1.63, 0.94] (0.6)	0.06 [−0.29, 0.42] (0.7)	−0.36 [−0.88, 0.16] (0.2)	−0.32 [−0.89, 0.25] (0.3)

*Beta coefficient represents mean difference in IL-6 with each additional unit of vitamin E, [95% CI], (p value)

**__Bold__ type represents associations significant at the p = 0.05 level

Table 4

Adjusted Associations between Carotenoids and sTNF-αR1

Carotenoid Concentration [μmol/L]	sTNF-αR1 [pg/mL]			
Baseline Carotenoids>	**Baseline**	**2 months**	**6 months**	**12 months**
Total Carotenoids	**−513 [−906, −120] (0.01)**	−285 [−677, 106] (0.1)	−302 [−694, 88] (0.1)	−197 [−579, 184] (0.3)
α-carotene	**−2,955 [−5,224, −685] (0.01)**	**−2,454 [−4,476, −432] (0.02)**	**−2,194 [−3,408, −980] (0.0004)**	**−2,034 [−3,415, −671] (0.004)**
β-carotene	**−951 [−1,669, −234] (0.009)**	−477 [−1,269, 316] (0.2)	**−684 [−1,396, −28] (0.05)**	−264 [−1,030, 501] (0.5)
β-cryptoxanthin	**−4,099 [−7,333, −866] (0.01)**	**−2,855 [−5,698, −12] (0.05)**	−2,332 [−4,943, 279] (0.08)	−1,388 [−3,544, 769] (0.2)
Lutein	**−1,515 [−3,179, 148] (0.04)**	−44 [−1,775, 1,686] (0.9)	−298 [−1,978, 1,381] (0.7)	−851 [−2,667, 964] (0.4)
Zeaxanthin	**−13,968 [−25,840, −2,096] (0.02)**	−6,000 [−17,984, 5,983] (0.3)	−7,641 [−18,404, 3,122] (0.1)	−10,484 [−23,734, 2,765] (0.1)
Lycopene	−831 [−1,963, 301] (0.1)	−595 [−1,644, 455] (0.2)	−387 [−1,562, 787] (0.5)	−369 [−1,357, 620] (0.4)
Time-varying Carotenoids>				
Total Carotenoids	**−454 [−791, −116] (0.008)**	−101 [−335, 132] (0.4)	−72 [−233, 90] (0.4)	−51 [−185, 83] (0.5)
α-carotene	**−2,955 [−5,224, −685] (0.01)**	**−2,454 [−4,476, −432] (0.02)**	**−2,194 [−3,408, −980] (0.0004)**	**−2,043 [−3,415, −671] (0.004)**
β-carotene	**−777 [−1,393, −161] (0.01)**	1 [−194, 196] (0.9)	−18 [−146, 109] (0.8)	−21 [−146, 103] (0.7)
β-cryptoxanthin	**−3,899 [−6,815, −982] (0.009)**	−1,502 [−3,506, 502] (0.1)	−548 [−2,515, 1,418] (0.5)	−216 [−1,120, 687] (0.6)
Lutein	**−1,535 [−3,061, −89] (0.05)**	**−1,237 [−2,662, −188] (0.05)**	**−1,408 [−2,539, −277] (0.01)**	**−1,300 [−2,605 −6] (0.05)**
Zeaxanthin	**−12,583 [−23,320, −1,846] (0.02)**	**−5,758 [−12,639, −1,123] (0.04)**	**−5,523 [−11,448, −402] (0.03)**	−2,310 [−5,867,1,247] (0.2)
Lycopene	−855 [−1,897, 186] (0.1)	−584 [−1,255, 87] (0.09)	**−513 [−972, −55] (0.03)**	−317 [−706, 73] (0.1)

*Beta coefficient represents mean difference in sTNF-αR1 with each additional unit of carotenoid, [95% CI], (p value)

**Bold type represents associations significant at the p = 0.05 level

Table 5

Adjusted Associations between Carotenoids and IL-6

Carotenoid Concentration [μmol/L]	IL-6 [pg/mL]			
Baseline Carotenoids	**Baseline**	**2 months**	**6 months**	**12 months**
Total Carotenoids	**−8.9 [−18.6, −0.7] (0.05)**	−5.1 [−13.9, 3.6] (0.3)	−2.4 [−6.9, 2.0] (0.3)	−3.7 [−9.8, 2.4] (0.3)
α-carotene	−48.4 [−99.5, 2.8] (0.06)	−23.7 [−53.2, 5.9] (0.1)	−8.8 [−24.1, 6.6] (0.3)	−13.8 [−30.9, 3.3] (0.1)
β-carotene	−13.9 [−33.0, 5.2] (0.1)	−7.9 [−24.4, 8.7] (0.3)	−1.4 [−7.9, 5.2] (0.6)	−6.7 [−18.7, 5.3] (0.2)
β-cryptoxanthin	−62.0 [−130.8, 6.9] (0.08)	−30.0 [−91.1, 31.8] (0.3)	−13.1 [−53.8, 27.6] (0.5)	−19.2 [−58.1, 19.6] (0.3)
Lutein	**−41.1 [−84.1, −2.0] (0.05)**	1.0 [−27.4, 29.5] (0.9)	9.3 [−16.4, 35.0] (0.5)	−7.6 [−33.7, 18.5] (0.5)
Zeaxanthin	−186.5 [−448.8, 75.6] (0.1)	−79.6 [−334.3, 175.2] (0.5)	−62.3 [−223.2, 97.7] (0.4)	−129.8 [−319.5, 59.9] (0.1)
Lycopene	−18.4 [−41.8, 5.1] (0.1)	−13.9 [−33.3, 5.5] (0.1)	−10.4 [−23.9, 3.1] (0.1)	−7.1 [−18.8, 4.6] (0.2)
Time-varying Carotenoids				
Total Carotenoids	**−8.6 [−17.1, −0.1] (0.05)**	−1.7 [−4.1, 0.8] (0.2)	−0.6 [−2.0, 0.7] (0.3)	−0.5 [−1.4, 0.3] (0.2)
α-carotene	−48.4 [−99.5, 2.8] (0.06)	−23.7 [−53.2, 5.9] (0.1)	−8.8 [−24.1, 6.6] (0.2)	−13.8 [−30.9, 3.3] (0.1)
β-carotene	−13.4 [−30.5, 3.6] (0.1)	−1.2 [−3.6, 1.1] (0.3)	−0.1 [−0.9, 0.9] (0.9)	−0.2 [−0.8, 0.4] (0.5)
β-cryptoxanthin	−60.9 [−126.9, 5.1] (0.07)	−15.5 [−35.2, 4.3] (0.1)	**−11.8 [−25.1, −0.3] (0.05)**	−3.4 [−10.6, 3.9] (0.3)
Lutein	**−47.8 [−92.4, −3.0] (0.04)**	2.0 [−12.5, 16.6] (0.8)	−3.1 [−13.3, 7.0] (0.5)	−9.8 [−23.9, 4.3] (0.2)
Zeaxanthin	−173 [−423.5, 78.0] (0.1)	52.2 [−41.1, 145.5] (0.3)	−39.3 [−101.5, 22.9] (0.1)	−17.6 [−45.0, 9.9] (0.2)
Lycopene	−18.0 [−39.6, 3.6] (0.1)	−6.7 [−14.5, 1.0] (0.09)	−6.7 [−14.5, 1.0] (0.09)	−4.4 [−9.0, 0.2] (0.06)

*Beta coefficient represents mean difference in IL-6 with each additional unit of carotenoid, [95% CI], (p value)

**Bold type represents associations significant at the p = 0.05 level