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Association of dietary and plasma carotenoids with urinary F_2-isoprostanes

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Abstract

Purpose: Carotenoids may protect against chronic diseases including cancer and cardiometabolic disease by mitigating oxidative stress and/or inflammation. We cross-sectionally evaluated associations between carotenoids and biomarkers of oxidative stress or inflammation.

Methods: From 2003-2009 the Sister Study enrolled 50,884 breast cancer-free US women aged 35 to 74. Post-menopausal participants (n=512) were randomly sampled to measure carotenoids and biomarkers of oxidative stress. Dietary carotenoid consumption was assessed using a validated 110-item Block 1998 food frequency questionnaire; use of β-carotene-containing supplements was also assessed. Plasma carotenoids were quantified, adjusting for batch. Urinary markers of lipid peroxidation, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) and its metabolite (8-iso-$PGF_{2\alpha}$-M) were also measured. Because the biomarker 8-iso-$PGF_{2\alpha}$ can reflect both oxidative stress and inflammation, we used a modeled 8-iso-$PGF_{2\alpha}$ to prostaglandin $F_{2\alpha}$ ratio approach to distinguish

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effects reflecting oxidative stress versus inflammation. Multivariable linear regression was used to assess the associations of dietary and plasma carotenoids with the estimated biomarker concentrations.

Results: Total plasma carotenoids were inversely associated with 8-iso-$PGF_{2\alpha}$-M concentrations (P for trend across quartile= 0.009). These inverse associations were also seen for α-carotene and β-carotene. In contrast, lutein/zeaxanthin showed associations with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations. The inverse association for total carotenoids appeared to be specific for oxidative stress (chemical 8-iso-$PGF_{2\alpha}$; $P_{\text{highest vs. lowest quartile}}$=0.04 and P for trend across quartiles=0.02). The pattern was similar for α-carotene. However, lutein/zeaxanthin tended to have a stronger association with enzymatic 8-iso-$PGF_{2\alpha}$, suggesting an additional anti-inflammatory effect. Supplemental β-carotene was inversely associated with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations, as well as with both chemical and enzymatic 8-iso-$PGF_{2\alpha}$. However, dietary carotenoids were not associated with either biomarker.

Conclusion: Plasma carotenoids and supplemental β-carotene were associated with lower concentrations of 8-iso-$PGF_{2\alpha}$ metabolite. Plasma carotenoids may be primarily associated with antioxidant effects.

Keywords

carotenoids; F_2-isoprostanes; oxidative stress; inflammation

INTRODUCTION

Carotenoids including α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene are a family of pigmented compounds that are synthesized by plants and microorganisms but not by animals. Fruits and vegetables comprise the major sources of carotenoids in human diet. [1] [2] Carotenoids may have direct or indirect beneficial health effects through inhibition of oxidative stress and/or inflammation pathways. [3] Oxidative stress is a state of imbalance between antioxidants and oxidative damage. Under oxidative conditions, prooxidants are dominant over antioxidants, potentially leading to damage to lipids, proteins, or DNA by free radical chemical mechanisms. [4] Inflammation is a host response to tissue injury, involving a multifactorial signaling network. Normal inflammation is self-limiting, but a failure of mechanisms for resolving the inflammatory response leads to chronic inflammation which may contribute to development or progression of cancer and cardiovascular disease. [5] [6]

Measurement of oxidative stress *in vivo* is difficult and many approaches have been used for measuring oxidative damage to lipids, proteins, DNA or levels of antioxidants. [7] However, there are some limitations in commonly used biomarkers of oxidative stress. For instance, malondialdehyde, a biomarker of lipid oxidation, has limited validity and storage stability and urinary 8-Hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of DNA oxidation, may be affected by metabolic rate and the degree of DNA excision repair. [8] [9] In contrast, F_2-isoprostanes, produced during non-enzymatic oxidation of arachidonic acid by different types of free radicals, are considered the best available biomarker of oxidative stress. It is preferable to measure urinary F_2-isoprostanes and metabolites rather than plasma F_2-

isoprostanes as these molecules can be generated *ex vivo* from plasma stores of arachidonic acid. [10] They are reliable and sensitive biomarkers that are stable in stored samples. [9] Intra-person variation across time in urinary F_2-isoprostanes measures is relatively low. [11] Urinary F_2-isoprostane metabolites may be a better marker of systemic oxidative stress than urinary F_2-isoprostanes. [12] Few studies have investigated the association between circulating carotenoids and urinary F_2-isoprostane metabolites. One primary F_2-isoprostane is 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), but this compound can be produced by non-enzymatic, free radical oxidation (chemical pathway) or can be generated from the interaction of arachidonic acid with cyclooxygenase enzymes (inflammatory pathway). The modeled ratio of 8-iso-$PGF_{2\alpha}$ to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been suggested as a measure to distinguish production between these two mechanisms [13,14].

We examined the associations between both plasma carotenoids measures and self-reported intake of carotenoids (dietary intake and supplement use) and urinary F_2-isoprostanes, including urinary 8-iso-$PGF_{2\alpha}$ and its major metabolite (2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$-M, 8-iso-$PGF_{2\alpha}$-M), and $PGF_{2\alpha}$, among postmenopausal women in the nationwide Sister Study cohort. We additionally used the ratio of 8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ to evaluate the relative contribution of beneficial anti-oxidant versus anti-inflammatory properties of carotenoids.

MATERIALS AND METHODS

Study population

Data for this analysis came from the Sister Study, a nationwide prospective cohort study that identifies risk factors for breast cancer. [15] A total of 50,884 breast cancer-free women (ages 35-74 at enrollment) who were the sisters or half-sisters of women diagnosed with breast cancer were recruited from the US including Puerto Rico between 2003 and 2009. At enrollment, study participants had anthropometric measurements taken and provided biological samples in a home exam and completed telephone interviews and written questionnaires on demographic, medical, lifestyle, and reproductive history. Details of the study design and data collection are described elsewhere. [15,16] All participants provided written informed consent.

Participants included in this analysis were randomly sampled from the Sister Study cohort, irrespective of future breast cancer status. It is part of a larger case-cohort sample selected to investigate the association between carotenoids, oxidative stress and breast cancer risk in postmenopausal women. Our analysis was limited to postmenopausal women because estrogen and other hormones may influence carotenoid concentrations. [17] Women were considered postmenopausal if they had not menstruated for over one year, had had both ovaries removed, or had had a hysterectomy with ovarian preservation but were over 55 years old. A total of 512 postmenopausal women (including 35 women who developed breast cancer during follow-up) who reported plausible energy intakes (≥500 and ≤5000 kcals/d) and who had plasma samples analyzed for carotenoids and urine samples analyzed for 8-iso-$PGF_{2\alpha}$ were eligible for this analysis.

Dietary assessment

Dietary carotenoids were ascertained at baseline using a modified 1998 Block 110-item food frequency questionnaire (FFQ). [18] This FFQ has been validated in women [19]. Participants reported their mean dietary intake in the previous 12 months of each listed food and beverage item, including 9 possible frequencies ranging from “never” to “every day” with 3 or 4 quantity (portion size) choices per food item or group of similar food items. Based on FFQ responses, nutrient consumption was calculated using the United States Department of Agriculture Food and Nutrient Database for Dietary Studies for US women. [20] Intake of β-carotene supplements was calculated from both use of multivitamins (including regular Once-A-Day, Centrum, or Thera type; Stress-tabs or B-Complex type, Once-A-Day; and antioxidant combination type) and use of a separate β-carotene supplement, incorporating both dose and frequency of use.

Measurement of plasma carotenoids

Baseline fasting plasma samples were stored in 0.5 mL straws at −80°C before being shipped to Craft Technologies, Inc. (Wilson, NC) where α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were assayed. Specimens were randomly placed across 64 batches. Each batch included 1 post-menopausal pooled plasma quality control sample and 17 participant samples. All the carotenoids were assessed using high-performance liquid chromatography (HPLC) described by Craft [21]. This method was also calibrated with standards within the physiological range which are assigned concentrations using absorption coefficients ($E^{1\%}_{cm}$) and corrected for HPLC purity. [22] Inter-batch coefficients of variation (CV) for pooled quality control samples were 6.1% for α-carotene, 5.7% for β-carotene, 7.1% for β-cryptoxanthin, 5.3% for lycopene, and 5.5% for lutein/zeaxanthin, respectively. Total carotenoid concentration was the sum of the individual concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin.

Oxidative stress measurement

Participants provided first morning urine samples during their home exam at enrollment. Samples were stored at −80°C before being shipped to the Eicosanoid Core Laboratory at Vanderbilt University Medical Center for measurement of oxidative stress biomarkers. Urinary 8-iso-$PGF_{2\alpha}$ and its metabolite (8-iso-$PGF_{2\alpha}$-M) and $PGF_{2\alpha}$ concentrations were measured by liquid chromatography/negative ion chemical ionization mass spectrometry (LC/MS). Detailed protocols for these methods have been published. [23] [24] A total of 64 batches were run and the batching was the same as the one used for measuring plasma carotenoids. Each batch contained 17 samples from study subjects and 1 post-menopausal pooled urine quality control sample. The CV for QC were 9.9% for 8-iso-$PGF_{2\alpha}$, 17.0% for 8-iso-$PGF_{2\alpha}$-M, and 15.5% for $PGF_{2\alpha}$. All concentration of 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M were adjusted for urine creatinine concentrations to account for urine diluteness using covariate-adjusted standardization. [25]

As mentioned above, the biomarker 8-iso-$PGF_{2\alpha}$ can reflect both oxidative stress and inflammation. [13] Using an 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ modeled ratio approach as described by van ‘t Erve et al, [13] the 8-iso-$PGF_{2\alpha}$ biomarker was apportioned and quantified into a more specific oxidative stress component as distinct from effects of inflammation.

The oxidative stress component (chemical 8-iso-$PGF_{2\alpha}$: the chemical fraction of the 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ modeled ratio) reflects concentrations generated from lipid peroxidation, whereas the inflammatory component (enzymatic 8-iso-$PGF_{2\alpha}$: the enzymatic fraction of the 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ modeled ratio) indicates concentrations produced from prostaglandin-endoperoxide synthases. [26] The calculation was made by a custom interface for the R package Constrained Linear Mixed Effects. [14] No reference values are assigned for the fractions because the proportions are dependent on the situation-specific production of 8-iso-PGF2α. [27]

Statistical Analysis

Individual carotenoids concentrations were adjusted for batch effects. The effect of batch on carotenoids was modeled using a random effects model, then standardized across batches by subtracting the batch-specific random effect estimate from each concentration in that batch. The batch-adjusted plasma carotenoids concentrations and dietary carotenoids consumption were categorized in quartiles. Intake of β-carotene supplements was categorized into four groups (none or missing, <1200, 1200-2999, and ≥3000 mcg/day).

Adjustment for batch effects of oxidative stress biomarkers was done as described above. Log transformation of 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$ was then performed to approximate a normal distribution. Generalized linear models were used to calculate the geometric means of 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$ in each quartile of dietary and plasma carotenoids. In multivariable models, geometric means were adjusted for age at blood draw, self-reported race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), highest educational attainment (high school or less, some college, or 4-year degree or higher), body mass index (BMI; continuous) as measured at home visit, smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015 as a proxy indicator of diet quality, and multivitamin use (none, < 1–3 days/week, < 4–6 days/week, every day, missing). We also adjusted for percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, and self-reported high cholesterol defined by diagnosed hypercholesterolemia or current use of cholesterol-lowering medication. [1] [28] We further adjusted for urinary creatinine concentration to control residual confounding by diluteness of urine. [25] Models of the association between supplemental β-carotene and oxidative stress markers adjusted for dietary β-carotene. Linear regression models, with log-transformed 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, or $PGF_{2\alpha}$ as the dependent variable, were used to evaluate linear trends.

Potential effect measure modification was evaluated with likelihood ratio tests for smoking, obesity (body mass index and waist-to-hip ratio), and ever or never having had any chronic disease including type 2 diabetes, hypertension, hypercholesterolemia, cardiovascular disease, or cancer. We also performed a sensitivity analysis with an additional adjustment for fruit and vegetable consumption (separately in quintiles) to explore associations after

adjusting for the effects linked to oxidative stress and inflammation other than through carotenoids.

The P values provided are two-sided, with the level of significance at 0.05. All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Participant characteristic associations with dietary vs. plasma carotenoids:

Most participants were non-Hispanic white (88.2 %) and the a mean age was 60.3 years. Baseline characteristics are presented in Table 1 stratified by quartile of total plasma carotenoids. Women with higher total plasma carotenoids were more physically active, had a lower BMI and waist to hip ratio, and reported higher consumption of fruit and vegetable and higher diet quality. They were more likely to be non-Hispanic White and were more likely to have more education (Table 1). In contrast, consumption of total dietary carotenoids was not associated with BMI or waist to hip ratio, and women with higher total dietary carotenoids had a higher mean energy intake (Supplemental Table 1). Multivitamins were the major source of supplemental β-carotene and most multivitamin users reported taking them (Supplemental Table 2).

Urinary Isoprostanes associations with dietary carotenoids:

The geometric mean concentrations of 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$ and their associations with quartiles of dietary carotenoids in multivariable linear regression models are shown in Table 2. Linear models were carried out on the natural log transformed concentrations, with the estimated means within quartiles exponentiated to estimate adjusted geometric means. Overall, there was little association between total dietary carotenoids and 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$. Among individual carotenoids, increasing quartiles of cryptoxanthin were positively associated with $PGF_{2\alpha}$ (P for trend =0.03). β-carotene supplementation was inversely associated with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations (P trend across quartiles=0.004 and 0.03). Combined intake of dietary carotenoids and supplemental β-carotene was not associated with 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$ (Supplemental Table 3).

Urinary Isoprostanes associations with Plasma Carotenoids:

The geometric mean concentrations of 8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$-M, and $PGF_{2\alpha}$ and their associations with quartiles of plasma carotenoids in multivariable linear regression models are shown in Table 3. Compared with the lowest quartile, the highest quartile of total plasma carotenoids was inversely associated with 8-iso-$PGF_{2\alpha}$-M concentrations (P trend across quartiles= 0.009). An inverse association with 8-iso-$PGF_{2\alpha}$-M concentrations was specifically apparent for α-carotene and β-carotene (P trend across quartiles=0.02 and 0.003, respectively). In contrast, increasing concentration of lutein/zeaxanthin was inversely associated with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations (both P trend across quartiles=0.02).

Associations with modeled enzymatic and chemical fractions of isoprostanes:

The geometric mean concentrations of enzymatic 8-iso-$PGF_{2\alpha}$ (the enzymatic fraction of the 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ modeled ratio) and chemical 8-iso-$PGF_{2\alpha}$ (the chemical fraction of the 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ modeled ratio) and their associations with quartiles of plasma carotenoids in multivariable linear regression models are shown in Table 4. Compared with the lowest quartile, the highest quartile of total plasma carotenoids was inversely associated with the chemical 8-iso-$PGF_{2\alpha}$ (P trend across quartiles=0.02), representing generation by the oxidative stress pathway. More specifically, α-carotene was inversely associated with the chemical 8-iso-$PGF_{2\alpha}$ ($P_{\text{highest vs. lowest quartile}}$=0.02; P trends across quartiles=0.02). Interestingly, the inverse association of lutein/zeaxanthin was stronger with the enzymatic 8-iso-$PGF_{2\alpha}$ representing generation by the enzymatic inflammation pathway ($P_{\text{highest vs. lowest quartile}}$=0.02; P trend across quartiles=0.04). β-carotene supplementation was inversely associated with both chemical and enzymatic 8-iso-$PGF_{2\alpha}$ (both P trend across quartiles=0.02).

In analysis of the association between plasma carotenoids, 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations stratified by having any chronic disease (ever vs. never) including type 2 diabetes, hypertension, hypercholesterolemia, cardiovascular disease, and cancer, the inverse association between α-carotene and 8-iso-$PGF_{2\alpha}$-M was observed only in women with chronic diseases ($P_{\text{interaction}}$=0.005). There was also evidence for differential associations by chronic diseases status in the association of α-carotene and lutein +zeaxanthin with 8-iso-$PGF_{2\alpha}$, and in the association of total carotenoids, β-carotene, β-cryptoxanthin, and lutein +zeaxanthin with 8-iso-$PGF_{2\alpha}$-M although the interaction p values were not low (Supplemental Table 4). Associations did not notably differ across strata defined by obesity (body mass index and waist-to-hip ratio) or smoking status (data not shown). Sensitivity analysis with additional adjustment for fruit and vegetable consumption did not materially alter the overall results (data not shown).

DISCUSSION

In this study of postmenopausal women, we found that dietary consumption of carotenoids was not associated with decreased concentrations of 8-iso-$PGF_{2\alpha}$ or 8-iso-$PGF_{2\alpha}$-M; however supplemental β-carotene was associated with lower concentrations of both measures. Total plasma carotenoids were associated with lower concentrations of 8-iso-$PGF_{2\alpha}$-M, after adjusting for potential confounders. This pattern was most apparent for α-carotene and β-carotene. In contrast, lutein/zeaxanthin was inversely associated with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M. The association between plasma carotenoids and 8-iso-$PGF_{2\alpha}$-M concentrations tended to be stronger in women with any chronic diseases. Our findings suggest that plasma α-carotene possesses antioxidant effects, and that lutein/zeaxanthin may have additional anti-inflammatory effects, offering a potential explanation for their beneficial health effects.

Numerous studies have investigated the relationship between circulating carotenoids and various oxidative stress biomarkers related to lipid peroxidation, reactive oxygen species, and non-enzymatic antioxidant activity in observational study settings, and inverse associations have typically been reported. [8] However, only a few studies have evaluated

the association between circulating carotenoids and F_2-isoprostanes. [8] In a study of 192 postmenopausal breast cancer survivors in the US, increasing total plasma carotenoid and β-carotene concentrations were inversely associated with 8-iso-$PGF_{2\alpha}$ (P trend across quartiles=0.051 and 0.066, respectively) after adjusting for age, BMI, plasma or dietary cholesterol, and physical activity. [29] In a study of 298 healthy U.S. adults, plasma β-carotene was inversely associated with plasma F_2-isoprostanes, after adjusting for age, sex, race, smoking status, and BMI. [30] In the Coronary Artery Risk Development in Young Adults study (n=2,395), total concentrations of serum α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin were inversely associated with plasma F_2-isoprostanes concentration. [31]

However, none of these studies evaluated the association between circulating carotenoids and F_2-isoprostane metabolites. In a study of 717 relatively healthy Chinese women, plasma concentrations of β-carotenes, lycopene other than trans, 5-cis and 7-cis isomers, and cis β-cryptoxanthin were inversely associated with urinary F2-isoprostane metabolites but not with F2-isoprostanes after adjusting for potential confounders including age, smoking, BMI, multivitamin use and consumption of fruit and vegetables. [12] In our study, β-carotene was also inversely associated with 8-iso-$PGF_{2\alpha}$-M concentrations only. It has been suggested that non-natural production of F_2-isoprostanes may occur *in vitro* in plasma by auto-oxidation, and their urinary excretion may be influenced by local production of F_2-isoprostanes in the kidney. [32] However, F_2-isoprostanes metabolites may not be affected by autooxidation and renal production, [33] and they have been shown to be more sensitive than F_2-isoprostanes as a biomarker for assessing the effect of antioxidants. [12,32]

Our analysis distinguished between the enzymatic and chemical 8-iso-$PGF_{2\alpha}$, representing production from the inflammation pathway and generation from the oxidative stress pathway, respectively. We observed that total plasma carotenoids were inversely associated with the chemical 8-iso-$PGF_{2\alpha}$, suggesting that carotenoids may predominantly regulate chemical oxidative stress pathways, which could explain their beneficial health effects. The inverse association with the chemical 8-iso-$PGF_{2\alpha}$ was more apparent for plasma α-carotene. Interestingly, an inverse association with the enzymatic 8-iso-$PGF_{2\alpha}$ was seen for plasma lutein/zeaxanthin. A relatively stronger anti-inflammatory effect of plasma lutein/zeaxanthin than other individual carotenoids has also been shown in another study. In a clinical study of patients with coronary artery disease, plasma lutein/zeaxanthin was the only type of carotenoid showing an inverse correlation with IL-6 and anti-inflammatory effects of lutein were confirmed through *ex vivo* experiments. [34] Lutein and its stereoisomer zeaxanthin are classified as xanthopylls, which are oxidative products of carotenes and structurally different from other carotenoids. [1] Lutein may have anti-inflammatory properties due to its antioxidant activity and tendency to alter oxidant-sensitive inflammatory signaling pathways. Lutein is also known to decrease the level of reactive oxygen species (ROS) such as hydrogen peroxide by inhibiting NF-κB activation and NF-κB-dependent inflammatory gene expression in lipopolysaccharide-stimulated macrophages. [35,36] A study using gastric epithelial cells showed that the inhibitory effect of lutein on ROS levels, NF-κB activation, and hydrogen peroxide-induced IL-8 expression was stronger than that of other carotenoids such as β-carotene. [37]

In our stratified analysis, the association between plasma carotenoids, 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations was stronger in women with any chronic diseases including type 2 diabetes, hypertension, hypercholesterolemia, cardiovascular disease, and cancer. Previous studies reported inverse correlations between circulating carotenoids and F_2-isoprostanes in individuals with chronic disease such as patients with congestive heart failure [38] and patients with hepatitis C virus, who are at high risk of liver cancer [39]. In contrast, circulating carotenoids were reported to have no association [40] or even positive association with other oxidative stress markers such as glutathione and malon-dialdehyde in healthy individuals. [41] This finding might suggest that lower concentrations of carotenoids may indicate reduced protection for oxidative stress in individuals with chronic diseases, whereas in healthy individuals who don't have chronic diseases, homeostasis of oxidative stress may be well balanced with pre-existing antioxidant defense mechanisms in the body. [8]

While there was an inverse association between plasma carotenoids and 8-iso-$PGF_{2\alpha}$, and 8-iso-$PGF_{2\alpha}$-M, no association was observed for dietary consumption of carotenoids. This differential association was also found in a previous study. [29] Considering measurement error in carotenoid consumption from self-administered dietary questionnaires, the bioavailability of carotenoids from different foods, and individual differences in absorption and metabolism, biomarker measurements of circulating carotenoids may need to be accounted for in assessing the underlying carotenoid exposure. [42] However, there was an inverse association between consumption of total carotenoids, β-carotene, and lutein/zeaxanthin with both F_2-isoprostanes and its metabolites in the premenopausal women from the Sister Study. [43] It is unclear why the association would differ by menopausal status, but others have reported that a positive association between diet and plasma carotenoids was observed only in younger people (24-45 years), not in older people (≥ 65 years). [44]

In contrast, there was an inverse association between β-carotene supplementation and both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations as well as both chemical and enzymatic 8-iso-$PGF_{2\alpha}$. Since most of the women taking ≥1200 mcg/day of β-carotene supplements reported daily use, supplement use may well support the effect of plasma carotenoids on oxidative stress markers. Interestingly, the association between supplemental β-carotene and oxidative stress markers seemed to be clearer than the association between plasma β-carotene and oxidative stress markers. Concurrent intake of the other nutrients included in multivitamins may also play a role in the association between supplemental β-carotene and oxidative stress markers. A possible alternative explanation might be that women with high intake of β-carotene supplementation or high plasma carotenoids tended to follow a healthy lifestyle such as being more physically active or consuming higher-quality diets, both of which are also known to be associated with reduced oxidative stress. [43] [45] Our findings showed that there was an independent inverse association of high intake of β-carotene supplementation and plasma carotenoids with urinary isoprostanes after adjusting for potential confounders including sociodemographic and behavioral/lifestyle characteristics, but that adjustment could be incomplete.

Strengths of the present study include highly valid markers of oxidative stress. In addition to measuring 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M, we assessed $PGF_{2\alpha}$, allowing us to evaluate specific chemical oxidative stress pathways in relation to plasma carotenoids. We were also

able to address batch effects as well as potential confounding using the comprehensive information at baseline. Our study has limitations. Urinary F_2-isoprostanes and plasma carotenoids were both measured in samples collected at the same time, which does not allow assessment of a temporal relationship. In addition, our sample included only postmenopausal women, meaning that the findings may not be generalizable to men or premenopausal women.

In summary, we observed an inverse association between carotenoid concentrations and several oxidative stress measures. Supplemental β-carotene was inversely associated with both 8-iso-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$-M concentrations, as well as with both chemical and enzymatic 8-iso-$PGF_{2\alpha}$. Plasma carotenoids were inversely associated with urinary 8-iso-$PGF_{2\alpha}$-M concentrations, especially in plasma α-carotene, β-carotene, and lutein/zeaxanthin. While health effects of plasma carotenoids may be primarily due to their antioxidant effects, plasma lutein/zeaxanthin might have additional anti-inflammatory effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

General characteristics at baseline by quartiles of total plasma carotenoids: The Sister Study 2003–2009

	Total plasma carotenoids (mcg/mL)							
	Quartile 1 <1.01		Quartile 2 1.01-1.33		Quartile 3 1.33-1.74		Quartile 4 ≥ 1.74	
N	128		128		128		128	
Mean (SD)								
Age at enrollment, year	60	(7)	59	(7)	61	(7)	61	(6)
Body mass index, kg/m^2	31.9	(7)	28.3	(6)	27.0	(6)	24.4	(4)
Waist to hip ratio	0.85	(0.08)	0.82	(0.10)	0.80	(0.07)	0.79	(0.07)
Total MET-hours of leisure time physical activity/week	9.4	(13)	12.1	(17)	16.4	(19)	21.0	(19)
Healthy Eating Index-2015	69	(10)	70	(9)	74	(9)	76	(8)
Fruit consumption, cup eq.	1.3	(1.0)	1.2	(0.9)	1.4	(0.9)	1.8	(1.1)
Vegetable consumption, cup eq.	1.4	(1.0)	1.6	(1.1)	2.0	(1.2)	2.3	(1.5)
Total energy intake (kcal/d)	1639	(675)	1569	(634)	1560	(466)	1643	(594)
Percentage of total energy intake from fat	37.4	(7)	37.2	(7)	38.3	(8)	37.0	(8)
Polyunsaturated fatty acids consumption as a percentage of total fat	24.2	(4)	23.9	(4)	25.0	(4)	24.7	(5)
Number (%)								
Race/ethnicity								
Non-Hispanic White	106	(83)	110	(86)	117	(92)	119	(93)
Non-Hispanic Black	15	(12)	9	(7)	3	(2)	4	(3)
Other than White or Black	7	(5)	9	(7)	8	(6)	5	(4)
Educational attainment								
High school degree or less	26	(20)	24	(19)	24	(19)	14	(11)
Some college	53	(42)	57	(44)	30	(23)	47	(37)
College degree or higher	49	(38)	47	(37)	74	(58)	67	(52)
Alcohol consumption								
Never	8	(6)	9	(7)	3	(2)	5	(4)
Former	28	(22)	22	(17)	20	(16)	14	(11)
Current drinker, < 1 drink/day	83	(65)	79	(62)	85	(66)	87	(68)
Current drinker, ≥ 1 and <2 drink/day	5	(4)	8	(6)	15	(12)	18	(14)

	Total plasma carotenoids (mcg/mL)							
	Quartile 1 <1.01		Quartile 2 1.01-1.33		Quartile 3 1.33-1.74		Quartile 4 ≥ 1.74	
Current drinker, ≥ 2 drink/day	4	(3)	10	(8)	5	(4)	4	(3)
Smoking								
Never smoker	68	(53)	65	(51)	63	(49)	67	(52)
Past smoker	47	(37)	51	(40)	59	(46)	56	(44)
Current smoker	13	(10)	12	(9)	6	(5)	6	(4)
Use of hormone therapy								
None	50	(39)	47	(37)	54	(42)	44	(35)
Estrogen only	43	(34)	42	(33)	31	(24)	35	(27)
Progesterone or combination therapy	35	(27)	38	(30)	43	(34)	49	(38)
Multivitamin use								
None	14	(11)	10	(8)	17	(13)	15	(12)
< 1-3 days/wk	14	(11)	10	(8)	9	(7)	12	(9)
< 4-6 days/wk	14	(11)	17	(13)	10	(8)	10	(8)
Every day	58	(45)	68	(53)	74	(58)	80	(62)
Missing	28	(22)	23	(18)	18	(14)	11	(9)
Chronic disease *								
Yes	101	(79)	85	(66)	91	(71)	70	(55)
High cholesterol **								
Yes	68	(53)	58	(45)	58	(45)	45	(35)

Data are presented as mean (standard deviation), or number (percentage).

Abbreviation: MET, metabolic equivalent.

* Having type 2 diabetes, hypertension, high cholesterol, cardiovascular disease, or cancer

** Diagnosed with hypercholesterolemia or current use of cholesterol-lowering medication

Table 2.

Adjusted geometric means and 95% confidence intervals of urinary oxidative stress biomarkers (ng/mL) by quartiles of dietary carotenoids and use of β-carotene supplements (mcg)

		8-iso-$PGF_{2\alpha}$				8-iso-$PGF_{2\alpha}$ metabolite				$PGF_{2\alpha}$			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**	**aGM**	**95% CI**		**P**
Total dietary carotenoids	<8248	0.65	0.58	0.73	.	3.83	3.35	4.37	.	1.96	1.74	2.21	.
	8248-12630	0.69	0.62	0.77	0.48	4.11	3.64	4.65	0.42	2.10	1.88	2.34	0.39
	12631-19764	0.75	0.67	0.83	0.09	4.40	3.90	4.97	0.14	2.14	1.92	2.38	0.31
	≥ 19765	0.69	0.62	0.78	0.50	3.87	3.39	4.42	0.91	2.03	1.81	2.28	0.70
	P trend				0.36				0.74				0.68
α-carotene	<268	0.62	0.56	0.70	.	4.17	3.67	4.74	.	1.98	1.77	2.22	.
	268-436	0.75	0.68	0.84	0.02	4.07	3.59	4.61	0.79	2.10	1.88	2.34	0.47
	437-800	0.73	0.65	0.81	0.05	3.94	3.49	4.45	0.55	2.13	1.91	2.37	0.38
	≥ 801	0.69	0.61	0.76	0.25	4.03	3.55	4.57	0.72	2.02	1.81	2.26	0.82
	P trend				0.35				0.66				0.80
β-carotene	<2548	0.67	0.60	0.76	.	3.88	3.39	4.44	.	2.11	1.87	2.38	.
	2548-4213	0.65	0.58	0.72	0.62	4.12	3.65	4.66	0.51	1.94	1.74	2.16	0.29
	4214-7053	0.75	0.68	0.84	0.20	4.16	3.68	4.70	0.48	2.16	1.93	2.41	0.80
	≥ 7054	0.71	0.63	0.79	0.62	4.05	3.55	4.62	0.67	2.03	1.81	2.28	0.69
	P trend				0.33				0.70				0.99
Cryptoxanthin	<66	0.64	0.57	0.72	.	3.88	3.39	4.43	.	1.85	1.65	2.09	.
	66-128	0.67	0.61	0.75	0.49	4.06	3.60	4.59	0.60	2.05	1.85	2.29	0.20
	129-239	0.76	0.68	0.85	0.04	4.06	3.58	4.60	0.64	2.05	1.83	2.29	0.25
	≥ 240	0.71	0.64	0.79	0.22	4.21	3.70	4.80	0.41	2.29	2.04	2.57	0.02
	P trend				0.15				0.44				**0.03**
Lutein+zeaxanthin	<1833	0.66	0.59	0.74	.	3.72	3.25	4.26	.	2.02	1.79	2.28	.
	1833-3167	0.71	0.64	0.79	0.34	4.37	3.87	4.93	0.08	2.11	1.89	2.35	0.62
	3168-5619	0.69	0.62	0.77	0.62	4.06	3.59	4.59	0.38	2.04	1.83	2.28	0.89
	≥ 5620	0.71	0.64	0.80	0.40	4.09	3.59	4.67	0.36	2.05	1.83	2.30	0.86

		8-iso-$PGF_{2\alpha}$				8-iso-$PGF_{2\alpha}$ metabolite				$PGF_{2\alpha}$			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**	**aGM**	**95% CI**		**P**
	P trend				0.52				0.56				0.97
Lycopene	<2399	0.75	0.67	0.84	.	4.24	3.72	4.83	.	2.13	1.90	2.39	.
	2399-3767	0.65	0.58	0.72	0.07	3.91	3.45	4.42	0.35	2.05	1.84	2.28	0.62
	3768-5652	0.72	0.64	0.79	0.57	4.12	3.65	4.65	0.75	2.17	1.95	2.42	0.81
	≥ 5653	0.67	0.60	0.75	0.21	3.95	3.45	4.52	0.49	1.88	1.67	2.12	0.18
	P trend				0.40				0.62				0.31
β-carotene supplements *	None (n=151)	0.77	0.70	0.85	.	4.40	3.93	4.93	.	2.21	2.00	2.44	.
	<1200 (n=71)	0.74	0.64	0.85	0.60	4.33	3.69	5.08	0.86	2.18	1.88	2.51	0.86
	1200-2999 (n=232)	0.66	0.61	0.71	0.01	3.90	3.56	4.27	0.11	2.05	1.89	2.22	0.26
	≥ 3000 (n=58)	0.62	0.53	0.72	0.02	3.55	2.97	4.24	0.05	1.61	1.37	1.88	0.001
	P trend				**0.004**				**0.03**				**0.006**

Adjusted for age at sample collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), education (high school or less, some college, or 4-year degree or higher), examiner-measured BMI (continuous), smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015, multivitamin use (none, < 1–3 days/week, < 4–6 days/week, every day, missing), percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, high cholesterol, and urinary creatinine concentration

*Adjusted for age at sample collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), education (high school or less, some college, or 4-year degree or higher), examiner-measured BMI (continuous), smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015, consumption of dietary β-carotene (quintile), percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, high cholesterol, and urinary creatinine concentration

Geometric mean and 95% CI were calculated on the basis of natural logarithm of 8-iso-PGF2α and 8-iso-PGF2α-M.

Beta, 95% CI, and P value for each category with respect to the reference category were calculated using generalized linear regression.

Abbreviation: 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; aGMD, adjusted geometric mean

Table 3.

Adjusted geometric means and 95% confidence intervals of urinary oxidative stress biomarkers (ng/mL) by quartiles of plasma carotenoids (mcg/mL)

		8-iso-$PGF_{2\alpha}$				**8-iso-$PGF_{2\alpha}$ metabolite**				**$PGF_{2\alpha}$**			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**	**aGM**	**95% CI**		**P**
Total plasma carotenoids	<1.01	0.77	0.69	0.87	.	4.68	4.11	5.33	.	2.14	1.91	2.41	.
	1.01-1.33	0.69	0.62	0.77	0.13	4.21	3.72	4.76	0.22	2.08	1.86	2.32	0.69
	1.33-1.74	0.65	0.58	0.72	0.04	3.72	3.29	4.21	0.02	1.98	1.78	2.21	0.36
	≥ 1.74	0.67	0.60	0.76	0.12	3.66	3.21	4.17	0.02	2.03	1.81	2.28	0.55
	P trend				0.11				**0.009**				0.49
α-carotene	<0.05	0.78	0.70	0.87	.	4.78	4.20	5.44	.	2.19	1.94	2.46	.
	0.05-0.08	0.65	0.59	0.73	0.03	3.81	3.37	4.30	0.012	1.92	1.72	2.14	0.11
	0.08-0.12	0.70	0.63	0.77	0.16	4.03	3.57	4.55	0.07	2.11	1.89	2.35	0.67
	≥ 0.12	0.66	0.59	0.73	0.04	3.66	3.22	4.16	0.007	2.02	1.81	2.27	0.39
	P trend				0.11				**0.02**				0.68
β-carotene	<0.24	0.74	0.66	0.83	.	4.82	4.23	5.50	.	2.13	1.89	2.41	.
	0.24-0.37	0.65	0.59	0.73	0.11	3.96	3.52	4.47	0.03	1.89	1.69	2.11	0.14
	0.37-0.55	0.73	0.65	0.81	0.78	4.08	3.61	4.61	0.08	2.12	1.90	2.36	0.93
	≥ 0.55	0.66	0.59	0.74	0.19	3.41	2.98	3.90	0.001	2.09	1.86	2.35	0.83
	P trend				0.39				**0.003**				0.83
β-cryptoxanthin	<0.07	0.71	0.63	0.80	.	4.62	4.05	5.27	.	1.99	1.77	2.23	.
	0.07-0.11	0.65	0.59	0.73	0.30	3.88	3.43	4.39	0.05	2.03	1.82	2.26	0.81
	0.11-0.17	0.71	0.63	0.78	0.94	3.97	3.52	4.48	0.11	2.05	1.84	2.29	0.72
	≥ 0.17	0.71	0.63	0.79	0.99	3.77	3.30	4.31	0.05	2.16	1.93	2.43	0.34
	P trend				0.80				0.07				0.35
Lutein+zeaxanthin	<0.20	0.83	0.74	0.93	.	4.75	4.17	5.40	.	2.47	2.20	2.77	.
	0.20-0.27	0.65	0.58	0.72	0.002	4.05	3.58	4.57	0.08	1.78	1.60	1.98	<.0001
	0.27-0.38	0.65	0.58	0.72	0.002	3.70	3.29	4.17	0.007	1.96	1.77	2.18	0.006
	≥ 0.38	0.67	0.60	0.75	0.01	3.78	3.31	4.31	0.02	2.07	1.85	2.32	0.05
	P trend				**0.02**				**0.02**				0.18

		8-iso-PGF$_{2\alpha}$				8-iso-PGF$_{2\alpha}$ metabolite				PGF$_{2\alpha}$			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**	**aGM**	**95% CI**		**P**
Lycopene	<0.33	0.73	0.66	0.82	.	4.51	3.98	5.11	.	2.12	1.89	2.37	.
	0.33-0.42	0.71	0.64	0.79	0.67	3.82	3.39	4.31	0.06	1.94	1.74	2.15	0.26
	0.42-0.57	0.68	0.61	0.76	0.37	4.03	3.57	4.54	0.21	2.12	1.91	2.36	0.96
	≥ 0.57	0.66	0.59	0.73	0.19	3.89	3.44	4.40	0.11	2.06	1.84	2.29	0.72
	P trend				0.17				0.19				0.97

Adjusted for age at sample collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), education (high school or less, some college, or 4-year degree or higher), examiner-measured BMI (continuous), smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015, multivitamin use (none, < 1–3 days/week, < 4–6 days/week, every day, missing), percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, high cholesterol, and urinary creatinine concentration

Geometric mean and 95% CI were calculated on the basis of natural logarithm of 8-iso-PGF2α and 8-iso-PGF2α-M.

Beta, 95% CI, and P value for each category with respect to the reference category were calculated using generalized linear regression.

Abbreviation: 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$; aGMD, adjusted geometric mean

Table 4.

Adjusted geometric means and 95% confidence intervals of the modeled enzymatic and chemical fractions of isoprostanes (ng/mL) by quartiles of plasma carotenoids (mcg/mL) and use of β-carotene supplements (mcg)

		8-iso-PGF$_{2\alpha}$, enzymatic				**8-iso-PGF$_{2\alpha}$, chemical**			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**
Total carotenoids	<1.01	0.34	0.30	0.38	.	0.40	0.35	0.46	.
	1.01-1.33	0.32	0.28	0.35	0.36	0.36	0.32	0.41	0.27
	1.33-1.74	0.30	0.27	0.33	0.13	0.32	0.28	0.37	0.03
	≥ 1.74	0.30	0.27	0.34	0.17	0.32	0.28	0.37	0.04
	P trend				0.15				**0.02**
α-carotene	<0.05	0.34	0.30	0.38	.	0.40	0.35	0.46	.
	0.05-0.08	0.29	0.26	0.33	0.07	0.35	0.30	0.39	0.10
	0.08-0.12	0.31	0.28	0.35	0.36	0.33	0.29	0.38	0.04
	≥ 0.12	0.31	0.28	0.35	0.29	0.32	0.28	0.36	0.02
	P trend				0.51				**0.02**
β-carotene	<0.24	0.33	0.30	0.38	.	0.38	0.33	0.43	.
	0.24-0.37	0.29	0.26	0.32	0.07	0.35	0.30	0.39	0.36
	0.37-0.55	0.33	0.29	0.37	0.84	0.37	0.33	0.42	0.90
	≥ 0.55	0.31	0.27	0.34	0.34	0.30	0.26	0.35	0.05
	P trend				0.68				0.09
β-cryptoxanthin	<0.07	0.31	0.27	0.34	.	0.39	0.34	0.44	.
	0.07-0.11	0.30	0.27	0.34	0.90	0.32	0.28	0.37	0.06
	0.11-0.17	0.32	0.29	0.36	0.56	0.35	0.31	0.39	0.29
	≥ 0.17	0.33	0.29	0.37	0.47	0.34	0.30	0.39	0.26
	P trend				0.38				0.40
Lutein+zeaxanthin	<0.20	0.37	0.33	0.42	.	0.40	0.35	0.46	.
	0.20-0.27	0.29	0.26	0.32	0.002	0.34	0.30	0.38	0.07
	0.27-0.38	0.30	0.27	0.33	0.011	0.33	0.29	0.37	0.03
	≥ 0.38	0.30	0.27	0.34	0.02	0.33	0.29	0.38	0.06

		8-iso-PGF$_{2\alpha}$, enzymatic				8-iso-PGF$_{2\alpha}$, chemical			
		aGM	**95% CI**		**P**	**aGM**	**95% CI**		**P**
	P trend				**0.04**				0.06
Lycopene	<0.33	0.32	0.29	0.36	.	0.38	0.33	0.43	.
	0.33-0.42	0.29	0.26	0.33	0.26	0.37	0.33	0.42	0.86
	0.42-0.57	0.32	0.29	0.36	0.95	0.33	0.29	0.37	0.11
	≥ 0.57	0.32	0.28	0.35	0.84	0.32	0.28	0.36	0.08
	P trend				0.89				0.05
β-carotene supplements*	None (n=151)	0.34	0.31	0.38	.	0.39	0.35	0.44	.
	<1200 (n=71)	0.32	0.28	0.37	0.48	0.36	0.30	0.43	0.38
	1200-2999 (n=232)	0.31	0.28	0.33	0.15	0.32	0.29	0.36	0.01
	≥ 3000 (n=58)	0.26	0.22	0.31	0.009	0.33	0.27	0.40	0.12
	P trend				**0.02**				**0.02**

Adjusted for age at sample collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), education (high school or less, some college, or 4-year degree or higher), examiner-measured BMI (continuous), smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015, multivitamin use (none, < 1–3 days/week, < 4–6 days/week, every day, missing), percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, high cholesterol, and urinary creatinine concentration

*Adjusted for age at sample collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, or other), attained education (high school or less, some college, or 4-year degree or higher), examiner-measured BMI (continuous), smoking status (never, current, or past), self-reported leisure-time physical activity (metabolic equivalent hours/week, quintile), alcohol consumption (never, former, current ≤1 drink per day, or current >1 drink per day), hormone therapy (none, estrogen only, both estrogen and progesterone), total energy intake (kcals/d), Healthy Eating Index-2015, consumption of dietary β-carotene (quintile), percentage of energy from fat intake, polyunsaturated fatty acids consumption as a percentage of total fat, high cholesterol, and urinary creatinine concentration

Geometric mean and 95% CI were calculated on the basis of natural logarithm of 8-iso-PGF2α and 8-iso-PGF2α-M.

Beta, 95% CI, and P value for each category with respect to the reference category were calculated using generalized linear regression.

Abbreviation: 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$; aGMD, adjusted geometric mean