Major metabolite of F_2-isoprostane in urine may be a more sensitive biomarker of oxidative stress than isoprostane itself[1–4]

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ABSTRACT

Background: There is limited literature on the contributors to isoprostane metabolite 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (15-F_{2t}-IsoP-M) compared with F_2-isoprostanes (F_2-IsoPs) as an oxidative stress biomarker.

Objective: The objective of this study was to investigate whether plasma concentrations of antioxidants, urinary excretion rates of polyphenols, and antioxidants in food and dietary supplements are attributable to both urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations.

Design: Dietary intake information and blood and urine samples were obtained from 845 healthy middle-aged and elderly female participants of the Shanghai Women's Health Study. Urinary isoprostanes (F_2-IsoPs and 15-F_{2t}-IsoP-M) were measured and adjusted for creatinine concentrations.

Results: Urinary 15-F_{2t}-IsoP-M and F_2-IsoP concentrations were lower in subjects who used a multivitamin. Lower F_2-IsoP concentrations were observed in ginseng users, whereas lower concentrations of 15-F_{2t}-IsoP-M were shown in subjects who used a vitamin E supplement. Plasma concentrations of several antioxidants (ie, β-carotenes, both *trans* and *cis* β-carotenes, lycopene other than *trans*, 5-*cis* and 7-*cis* isomers, *cis* anhydrolutein, and *cis* β-cryptoxanthin) were inversely associated with 15-F_{2t}-IsoP-M but not with F_2-IsoPs, whereas β-, γ-, and δ-tocopherols were positively associated with 15-F_{2t}-IsoP-M but not with F_2-IsoPs. Urinary polyphenol quercetin was positively associated with both F_2-IsoPs and 15-F_{2t}-IsoP-M.

Conclusion: The results suggest that the F_2-IsoP major metabolite 15-F_{2t}-IsoP-M may be a more sensitive marker of endogenous oxidative stress status than are F_2-IsoPs in the assessment of effects of antioxidants on age-related diseases. *Am J Clin Nutr* 2012;96:405–14.

INTRODUCTION

Evidence from experimental studies indicated that basal concentrations of endogenous reactive oxygen species or free radicals (1, 2), which act as secondary messengers, play an essential role in the regulation of various normal physiologies, including energy supply, signal transduction, cell proliferation, and homeostasis, as well as in the induction of apoptosis and senescence, which are 2 key mechanisms for cancer prevention (1–3). The overproduction of reactive oxygen species that leads to oxidative stress has been linked to the pathogenesis of many diseases and conditions, such as cancer, neurodegenerative and cardiovascular diseases, obesity, and aging (4–6).

Since their first discovery in 1990 (7), F_2-isoprostanes (F_2-IsoPs)[5], which are a series of free radical-catalyzed lipid peroxidation products of arachidonic acid, have been widely used in animal or human studies to measure in vivo lipid peroxidation and are generally accepted as the most reliable biomarker of oxidative stress (8, 9). However, unmetabolized F_2-IsoPs may be artificially produced in vitro in plasma by autoxidation, and their excretion in human urine may be affected by local renal isoprostane production, which is age dependent (10). After β-oxidation, 15-F_{2t}-isoprostane, which is one of the most common F_2-IsoPs, converts to 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (15-F_{2t}-IsoP-M), which is a metabolite that is not subject to autoxidation and renal production (10, 11). A method with both high sensitivity and accuracy has been developed to measure 15-F_{2t}-IsoP-M by using gas chromatography/negative-ion chemical ionization mass spectrometry (GC/NICI MS) (10, 12). 15-F_{2t}-IsoP-M has seldom been examined in epidemiologic studies. Recently, we showed that a high concentration of urinary 15-F_{2t}-IsoP-M was associated with increased risk of breast cancer in overweight and obese women (4). We also showed that the concentration of 15-F_{2t}-IsoP-M increased with age and BMI, whereas F_2-IsoPs decreased with age and were not related to BMI (13). These findings suggested that 15-F_{2t}-IsoP-M is an-

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[5] Abbreviations used: EGC, epigallocatechin; F_2-IsoP, F_2-isoprostane; GC/NICI MS, gas chromatography/negative-ion chemical ionization mass spectrometry; 15-F_{2t}-IsoP-M, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

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other important oxidative stress biomarker in age- and obesity-related diseases.

Antioxidant constituents, such as tocopherols, vitamin C, and polyphenols have been hypothesized to inhibit oxidative stress (3, 14, 15). The supplementation of α-tocopherol did not reduce urinary F_2-IsoP concentrations in healthy subjects; however, α-tocopherol supplementation reduced F_2-IsoP concentrations when the supplementation dose was very high (16), in individuals with high basal F_2-IsoP concentrations (17), or in overweight subjects (18). To the best of our knowledge, no study has compared the major antioxidant contributors to both urinary isoprostanes (F_2-IsoPs) and their major metabolite (15-F_{2t}-IsoP-M) excretions. In this study, we comprehensively evaluated the associations of intakes of fruit, vegetables, and antioxidant vitamins; dietary supplement use; plasma lipophilic antioxidants; and urinary tea polyphenols with both urinary F_2-IsoPs and 15-F_{2t}-IsoP-M.

SUBJECTS AND METHODS

This cross-sectional study was conducted in 845 relatively healthy women from the Shanghai Women's Health Study, which is a population-based, ongoing cohort study. Details of the study were described previously (19). In brief, at baseline during March 1997 and May 2000, 74,942 Chinese women between 40 and 70 y of age were recruited in Shanghai (participation rate: 92.7%) and have been followed through multiple in-person follow-up surveys and record linkages for the occurrence of chronic diseases and mortality. Information on demographic characteristics, medical histories, and reproductive and lifestyle factors (eg, smoking, alcohol consumption, and physical activity) were collected by using in-person interviews that were conducted by trained midlevel health professionals. During the interview, anthropometric measurements, including height, weight, waist circumference, and hip circumference, were measured twice. The study was approved by all relevant institutional review boards in the People's Republic of China and United States. All participants provided informed written consent.

Sample collection, storage, and processing

Approximately 88% of cohort members donated a urine sample at baseline (1997–2000) or during the first follow-up ($\sim$2 y later). A spot urine sample was collected into a sterilized 100-mL cup that contained 125 mg ascorbic acid. Almost 76% of cohort participants donated a blood sample at baseline. A 10-mL blood sample was drawn into ethylene diamine tetraacetic acid evacuated tubes (Becton Dickinson and Company). Blood and urine samples were kept in a portable, insulated bag with ice packs (at $\sim$0–4°C), processed in $\leq$6 h of collection, and immediately stored at -80°C until laboratory analyses that were performed in 2009.

Assays for measurement of urinary F_2-IsoPs and 15-F_{2t}-IsoP-M

Assays were performed for 845 urine samples at 4 different time points or in 4 batches. Urinary concentrations of F_2-IsoPs (primarily 15-F_{2t}-isoprostane or 8-iso-prostaglandin-$F_{2\alpha}$) and their major metabolite 15-F_{2t}-IsoP-M (also termed 2,3-dinor-5,6-dihydro-8-iso-prostaglandin-$F_{2\alpha}$) were measured by using GC/NICI MS at Vanderbilt Eicosanoid Core Laboratory. The method has been reported in detail previously (10, 12), and urinary F_2-isoprostanes are not subject to autoxidation (10, 11). The precision of the assay was $\pm$4%, and accuracy was 97%. The lower limit of sensitivity was $\sim$20 pg (10). Creatinine concentrations were determined by using a test kit (kit 555l; Sigma Co) on the basis of Jaffe's reaction.

Dietary and supplemental intakes of antioxidants

Dietary intake data were collected during in-person interviews by using a validated food-frequency questionnaire that included 77 food items and groups commonly consumed in Shanghai (20). For each individual food item or group of foods, participants were first asked how frequently they consumed the food or food group (daily, weekly, monthly, annually, or never) over the 12 mo before the interview. Subjects were then asked about the amount of the food item they typically consumed per unit of time in liang (1 liang = 50 g). The dietary intake of antioxidants [vitamins A, C, and E (total, α, β-, γ-, and δ-tocopherols), retinol, and carotene] were calculated by multiplying the amount of food consumed by the nutrient content per gram of food according to Chinese food-composition tables (21). In addition, participants were asked whether they had ever taken any vitamin supplements (ie, multivitamins or vitamins A, B, C, and/or E) $\geq$3 times/wk for $>$2 mo continuously during the 12 mo before the interview. The use of ginseng was defined as taking ginseng and ginseng products (eg, extract, powder, tablet, or capsule) $\geq$5 times/y in the 3 y before enrollment. In addition, each participant was asked whether she ever drank tea $\geq$3 times/wk for $\geq$6 mo in her lifetime. If the participant answered yes, she was considered a regular tea drinker.

Assays for measurement of plasma retinol, carotenoids, and tocopherols

During thawing and apportioning into aliquots, the processing of plasma samples was performed in a darkroom equipped with a red light because lipophilic antioxidants, particularly carotenoids in plasma, are light sensitive and thus may be degraded after exposure to the light. Assays were performed for a total of 20 types of plasma lipophilic antioxidants including tocopherols (α-, β-, γ-, and δ-tocopherols), retinol, and carotenoids (carotene, lycopene, lutein and zeaxanthin, anhydrolutein, and cryptoxanthin) from 717 samples in 2 batches. The method of the use of isocratic reverse-phase HPLC with photodiode array detection has been described in detail in our previous study (22).

Assays for measurement of urinary tea polyphenols and flavonoids

The measurement of the urinary excretion of polyphenols and flavonols was carried out by using liquid chromatography electrospray mass spectrometry, which is a method that was recently established (23). Quercetin, kaempferol, epicatechin, and epigallocatechin (EGC) and their respective metabolites 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and 5-(3′,4′,-dihydroxyphenyl)-γ-valerolactone, as well as the methylated form of EGC, were measured by using HPLC with electrospray ionization (negative mode) high-resolution tandem mass

spectrometry (model TSQ; Thermo). The method has previously been reported in detail (24, 25).

Statistical analysis

Log-transformation was conducted to approach normal distribution for urinary F_2-IsoPs and 15-F_{2t}-IsoP-M. Geometric means and 95% CIs of F_2-IsoPs and 15-F_{2t}-IsoP-M were obtained on the basis of the least-square means estimated from general linear regression models or ANOVA according to the dichotomous categories or quartile or tertile distributions of exposure variables. We performed both unadjusted and adjusted analyses. Age (continuous), education and occupation (categorical), cigarette smoking (yes or no), BMI (continuous), multivitamin supplement use, fruit and vegetable intakes (continuous), plasma total tocopherols, retinol and total carotenoids (where appropriate), and batches for isoprostanes assays (categorical) were considered as potential confounding factors in the final multivariate analysis (**Tables 1–3**). Tests for trends were performed by entering the continuous variable in the model. All statistical tests were 2-sided and performed with SAS statistical software (version 9.2; SAS Institute). $P \leq 0.05$ was interpreted as statistically significant.

RESULTS

The average (±SD) age of the study population was 52.9 ± 8.9 y. The log-transformed mean concentrations of urinary F_2-IsoPs and 15-F_{2t}-IsoP-M were 1.62 ± 1.51 and 0.56 ± 0.57 ng/mg creatinine, respectively. The correlation coefficient was 0.34 between log-transformed F_2-IsoPs and 15-F_{2t}-IsoP-M ($P < 0.01$). Geometric means and 95% CIs for F_2-IsoPs and 15-F_{2t}-IsoP-M according to demographic and lifestyle variables are shown in **Table 4**. Urinary 15-F_{2t}-IsoP-M was significantly higher with age in a dose-response manner (P-trend < 0.01), whereas F_2-IsoPs were significantly lower with age (P-trend = 0.02). Likewise, 15-F_{2t}-IsoP-M concentrations were higher in postmenopausal women than in premenopausal women ($P < 0.01$). In general, after adjustment for age, women with a higher socioeconomic status, as indicated by an education more than high school, and professional occupation had lower concentrations of F_2-IsoPs and 15-F_{2t}-IsoP-M (P-trend < 0.05 for all). Both F_2-IsoPs and 15-F_{2t}-IsoP-M were higher in cigarette smokers than in nonsmokers; however, the association was significant only for F_2-IsoPs. Concentrations of 15-F_{2t}-IsoP-M were positively associated with BMI levels in a significant dose-response manner, but not for F_2-IsoPs. Regular alcohol drinking, physical activity, history of diabetes mellitus, and history of cardiovascular diseases (ie, stroke, coronary heart diseases, and hypertension) were not related to concentrations of isoprostanes. In this study, a few women regularly took vitamin supplements. Users of a multivitamin supplement had significantly lower concentrations of F_2-IsoPs ($P = 0.05$) and 15-F_{2t}-IsoP-M ($P = 0.01$) than did nonusers. Similarly, users of a vitamin E supplements had a lower concentration of 15-F_{2t}-IsoP-M than did nonusers. In addition, a large proportion (26%) of women regularly took a ginseng supplement. Concentrations of F_2-IsoPs were lower in women who regularly used ginseng ($P = 0.03$).

Although unadjusted concentrations of 15-F_{2t}-IsoP-M were significantly lower in women who consumed higher quantities of fruit, vegetables, carotene, vitamin A, α-tocopherol, and vitamin C than in women who consumed lower amounts (P-trend ≤ 0.05 for all), none of these associations were significant after adjustment for confounding factors (Table 1).

Associations between urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations and circulating concentrations of lipophilic antioxidants (tocopherols, retinol and carotenoids) are presented in Table 2. Circulating concentrations of a number of antioxidants were significantly associated with urinary excreting rates of F_2-IsoPs in unadjusted analyses. However, after adjustment for confounding factors, only high plasma concentrations of *cis* β-carotene were associated with lower concentrations of F_2-IsoPs. In contrast, after confounding factors were controlled for, higher concentrations of 15-F_{2t}-IsoP-M were associated with higher plasma concentrations of β-, γ-, and δ-tocopherols (P-trend < 0.01). Also, lower concentrations of 15-F_{2t}-IsoP-M were related to higher circulating concentrations of α-tocopherol (P-trend = 0.05), β-carotene (P-trend = 0.07), as well as its *trans* and *cis* isomeric forms (P-trend was 0.02 and 0.06, respectively), lycopene other than *trans*, 5-*cis*, and 7-*cis* isomers (P-trend =0.04), *cis* anhydrolutien (P-trend = 0.048), and *cis* β-cryptoxanthin (P-trend = 0.01).

The regular drinking of tea was marginally associated with higher concentrations of 15-F_{2t}-IsoP-M ($P = 0.06$) but not with F_2-IsoPs (Table 3). We showed that women with a higher urinary excretion of EGC, which is the most common tea polyphenol, were more likely to have higher concentrations of 15-F_{2t}-IsoP-M (P-trend = 0.03 for both unadjusted and adjusted analyses). In contrast, urinary excretion of quercetin, which is one major flavonoid polyphenol, was significantly and positively associated with both F_2-IsoP and 15-F_{2t}-IsoP-M concentrations after adjustment for confounding factors (P-trend = 0.01 and 0.03, respectively). Associations of urinary isoprostanes with epicatechin, which is another common tea polyphenol, metabolites of epicatechin and EGC, such as 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone, 5-(3′,4′,-dihydroxyphenyl)-γ-valerolactone, the methylated form of EGC (4′-MeEGC) and tea flavonoid kaempferol, were NS.

DISCUSSION

To our knowledge, this was the first study to investigate the relations of both F_2-IsoPs and 15-F_{2t}-IsoP-M with foods and dietary supplements rich in antioxidants, plasma antioxidants, and urinary tea polyphenols, although previous studies have extensively investigated the relation for F_2-IsoPs (15, 26, 27). In our study, higher circulating concentrations of α-tocopherol, β-carotenes, *trans* and *cis* β-carotenes, lycopene other than *trans*, 5-*cis*, and 7-*cis* isomers, *cis* anhydrolutien, and *cis* β-cryptoxanthin and the use of vitamin supplements (multivitamin and vitamin E) and ginseng were associated with lower urinary excretion concentrations of 15-F_{2t}-IsoP-M. Also, tea drinking; higher plasma concentrations of β-, γ-, and δ-tocopherols; and higher urinary excretion concentrations of tea polyphenol EGC and flavonol quercetin were related to higher urinary excretion concentrations of 15-F_{2t}-IsoP-M. In contrast, urinary F_2-IsoP concentrations were only related to circulating concentrations of *cis* β-carotene and urinary quercetin. These findings suggest that 15-F_{2t}-IsoP-M may be a more-sensitive biomarker of oxidative stress status than F_2-IsoPs (28) in the assessment of the effects of antioxidants.

TABLE 1
Dose-response relations between urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations and daily intakes of fruit, vegetables, and antioxidant vitamins in the Shanghai Women's Health Study (n = 845)[1]

Quartiles of daily intake from lowest to highest	n	F_2-IsoPs				15-F_{2t}-IsoP-M			
		Unadjusted	P-trend	Adjusted[2]	P-trend	Unadjusted	P-trend	Adjusted[2]	P-trend
		ng/mg creatinine		*ng/mg creatinine*		*ng/mg creatinine*		*ng/mg creatinine*	
Total vegetables			0.32		0.12		0.02		0.11
<185.5 g/d	211	1.58 (1.46, 1.72)[3]		1.58 (1.45, 1.73)		0.58 (0.54, 0.62)		0.58 (0.53, 0.62)	
185.5–267.4 g/d	212	1.58 (1.45, 1.71)		1.63 (1.49, 1.77)		0.56 (0.52, 0.61)		0.58 (0.54, 0.63)	
267.5–373.7 g/d	210	1.70 (1.57, 1.84)		1.66 (1.54, 1.82)		0.60 (0.55, 0.64)		0.59 (0.55, 0.64)	
≥373.8 g/d	212	1.62 (1.49, 1.75)		1.68 (1.54, 1.85)		0.52 (0.49, 0.56)		0.55 (0.51, 0.59)	
Total fruit			0.87		0.75		0.01		0.36
<139.8 g/d	213	1.75 (1.62, 1.90)		1.73 (1.58, 1.90)		0.62 (0.58, 0.67)		0.61 (0.56, 0.66)	
139.8–249.8 g/d	210	1.57 (1.45, 1.70)		1.58 (1.45, 1.73)		0.56 (0.53, 0.61)		0.58 (0.53, 0.62)	
249.9–373.7 g/d	210	1.49 (1.38, 1.62)		1.52 (1.39, 1.66)		0.53 (0.49, 0.57)		0.53 (0.49, 0.58)	
≥373.8 g/d	212	1.68 (1.55, 1.82)		1.73 (1.58, 1.90)		0.54 (0.50, 0.58)		0.56 (0.53, 0.61)	
Carotene			0.49		0.18		0.03		0.20
<1864.9 μg/d	212	1.57 (1.45, 1.68)		1.55 (1.42, 1.70)		0.58 (0.54, 0.64)		0.59 (0.54, 0.64)	
1864.9–2677.1 μg/d	211	1.66 (1.52, 1.80)		1.68 (1.54, 1.84)		0.56 (0.52, 0.61)		0.56 (0.52, 0.61)	
2677.2–3751.2 μg/d	211	1.63 (1.49, 1.77)		1.65 (1.51, 1.80)		0.59 (0.54, 0.65)		0.60 (0.55, 0.65)	
≥3751.2 μg/d	211	1.63 (1.49, 1.77)		1.68 (1.54, 1.84)		0.52 (0.49, 0.56)		0.55 (0.51, 0.59)	
Retinol			0.66		0.46		0.17		0.72
<85.9 μg/d	211	1.60 (1.48, 1.73)		1.58 (1.45, 1.73)		0.58 (0.53, 0.61)		0.56 (0.51, 0.61)	
85.9–142.2 μg/d	211	1.68 (1.55, 1.82)		1.77 (1.62, 1.91)		0.60 (0.55, 0.64)		0.61 (0.56, 0.66)	
142.3–217.2 μg/d	211	1.52 (1.40, 1.65)		1.51 (1.38, 1.63)		0.55 (0.52, 0.59)		0.57 (0.53, 0.62)	
≥217.2 μg/d	212	1.68 (1.55, 1.82)		1.73 (1.58, 1.90)		0.53 (0.49, 0.57)		0.55 (0.51, 0.60)	
Vitamin A			0.45		0.15		0.02		0.22
<458.2 μg/d	211	1.55 (1.43, 1.68)		1.54 (1.40, 1.68)		0.59 (0.55, 0.64)		0.58 (0.54, 0.64)	
458.2–608.1 μg/d	212	1.72 (1.58, 1.86)		1.70 (1.57, 1.86)		0.57 (0.53, 0.61)		0.59 (0.54, 0.64)	
608.2–828.7 μg/d	210	1.57 (1.45, 1.70)		1.62 (1.49, 1.77)		0.55 (0.51, 0.59)		0.55 (0.51, 0.60)	
≥828.8 μg/d	212	1.65 (1.54, 1.79)		1.72 (1.57, 1.86)		0.54 (0.51, 0.58)		0.57 (0.53, 0.62)	
Vitamin E			0.32		0.98		0.14		0.37
<9.7 mg/d	211	1.62 (1.49, 1.75)		1.60 (1.46, 1.75)		0.58 (0.54, 0.63)		0.59 (0.54, 0.64)	
9.7–12.6 mg/d	211	1.58 (1.46, 1.72)		1.60 (1.46, 1.76)		0.54 (0.50, 0.58)		0.55 (0.52, 0.60)	
12.7–16.3 mg/d	212	1.72 (1.57, 1.84)		1.70 (1.57, 1.86)		0.61 (0.56, 0.65)		0.61 (0.56, 0.66)	
≥16.4 mg/d	211	1.58 (1.46, 1.72)		1.66 (1.52, 1.82)		0.53 (0.49, 0.57)		0.55 (0.51, 0.60)	
Vitamin E α			0.86		0.60		0.03		0.31
<2.9 mg/d	212	1.62 (1.49, 1.75)		1.65 (1.51, 1.79)		0.61 (0.56, 0.65)		0.59 (0.54, 0.64)	
2.9–4.0 mg/d	211	1.72 (1.58, 1.84)		1.68 (1.54, 1.82)		0.56 (0.52, 0.61)		0.58 (0.53, 0.62)	
4.1–5.3 mg/d	210	1.51 (1.40, 1.63)		1.57 (1.43, 1.72)		0.54 (0.50, 0.58)		0.55 (0.51, 0.60)	
≥5.4 mg/d	212	1.66 (1.54, 1.80)		1.68 (1.54, 1.82)		0.55 (0.51, 0.60)		0.58 (0.53, 0.62)	
Vitamin E β and γ			0.17		0.79		0.09		0.24
<2.8 mg/d	211	1.58 (1.46, 1.72)		1.57 (1.43, 1.72)		0.56 (0.52, 0.60)		0.56 (0.52, 0.61)	
2.8–3.8 mg/d	212	1.66 (1.53, 1.86)		1.66 (1.52, 1.80)		0.59 (0.55, 0.64)		0.60 (0.55, 0.65)	
3.9–5.3 mg/d	210	1.68 (1.55, 1.82)		1.73 (1.58, 1.88)		0.57 (0.53, 0.65)		0.59 (0.54, 0.64)	
≥5.4 mg/d	212	1.57 (1.45, 1.70)		1.62 (1.48, 1.77)		0.53 (0.50, 0.58)		0.54 (0.50, 0.58)	
Vitamin E δ			0.21		0.78		0.35		0.54
<2.3 mg/d	209	1.55 (1.43, 1.68)		1.55 (1.42, 1.68)		0.56 (0.52, 0.60)		0.56 (0.52, 0.61)	
2.3–3.2 mg/d	214	1.72 (1.58, 1.86)		1.72 (1.57, 1.86)		0.59 (0.55, 0.64)		0.61 (0.56, 0.66)	
3.3–4.6 mg/d	210	1.58 (1.46, 1.72)		1.60 (1.48, 1.75)		0.55 (0.52, 0.60)		0.55 (0.52, 0.60)	
≥4.7 mg/d	212	1.63 (1.51, 1.77)		1.70 (1.49, 1.86)		0.55 (0.51, 0.59)		0.57 (0.53, 0.62)	
Vitamin C			0.36		0.18		0.05		0.31
<59.5 mg/d	212	1.63 (1.51, 1.77)		1.63 (1.49, 1.79)		0.58 (0.54, 0.62)		0.58 (0.54, 0.63)	
59.5–82.7 mg/d	211	1.68 (1.55, 1.82)		1.68 (1.54, 1.84)		0.57 (0.53, 0.61)		0.58 (0.53, 0.66)	
82.8–118.2 mg/d	211	1.51 (1.39, 1.63)		1.54 (1.42, 1.68)		0.56 (0.52, 0.61)		0.57 (0.52, 0.62)	
≥118.3 mg/d	211	1.67 (0.54, 1.82)		1.73 (1.58, 1.87)		0.54 (0.50, 0.58)		0.56 (0.52, 0.61)	

[1] F_2-IsoP, F_2-isoprostane; 15-F_{2t}-IsoP-M, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

[2] ANOVA estimate: values of log-transformed F_2-IsoPs and their major metabolite 15-F_{2t}-IsoP-M were adjusted for age (continuous); education; occupation; cigarette smoking (yes or no); BMI (continuous); multivitamin supplement use (yes or no); plasma total carotenoids, tocopherols, and retinol (continuous); and batch assays for F_2-IsoPs or 15-F_{2t}-IsoP-M (categories). P-trend values are from a linear regression model.

[3] Geometric mean; 95% CI in parentheses (all such values).

TABLE 2
Dose-response relations between urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations and plasma lipophilic antioxidants in the Shanghai Women's Health Study (n = 717)[1]

Quartiles from lowest to highest	n	F_2-IsoPs				15-F_{2t}-IsoP-M			
		Unadjusted[2]	P-trend	Adjusted[2]	P-trend	Unadjusted[2]	P-trend	Adjusted[2]	P-trend
		ng/mg creatinine		*ng/mg creatinine*		*ng/mg creatinine*		*ng/mg creatinine*	
Total tocopherols			<0.01		0.21		0.94		0.39
<9150.3 ng/mL	180	1.79 (1.65, 1.95)[3]		1.68 (1.55, 1.86)		0.56 (0.52, 0.62)		0.58 (0.52, 0.63)	
9150.3–10,769.6 ng/mL	178	1.68 (1.54, 1.84)		1.65 (1.51, 1.80)		0.60 (0.55, 0.66)		0.61 (0.56, 0.66)	
10,769.7–12,946.3 ng/mL	179	1.57 (1.43, 1.72)		1.60 (1.46, 1.73)		0.55 (0.51, 0.60)		0.55 (0.50, 0.59)	
≥12,946.4 ng/mL	180	1.54 (1.40, 1.68)		1.62 (1.48, 1.77)		0.58 (0.53, 0.62)		0.56 (0.52, 0.61)	
α-Tocopherol			<0.01		0.10		0.11		0.05
<6816.3 ng/mL	179	1.75 (1.62, 1.91)		1.68 (1.54, 1.84)		0.59 (0.54, 0.64)		0.59 (0.54, 0.64)	
6816.3–8108.8 ng/mL	180	1.77 (1.63, 1.96)		1.73 (1.58, 1.90)		0.59 (0.55, 0.64)		0.59 (0.55, 0.64)	
8108.9–10,231.0	178	1.54 (1.42, 1.68)		1.58 (1.45, 1.73)		0.56 (0.52, 0.61)		0.56 (0.54, 0.61)	
≥10,231.1	180	1.51 (1.38, 1.63)		1.57 (1.43, 1.72)		0.55 (0.51, 0.60)		0.55 (0.51, 0.59)	
β- + γ-Tocopherols			0.76		0.45		<0.01		<0.01
<1518.5	180	1.60 (1.48, 1.75)		1.60 (1.46, 1.75)		0.51 (0.44, 0.55)		0.52 (0.49, 0.58)	
1518.5–2024.2	178	1.65 (1.51, 1.80)		1.63 (1.49, 1.77)		0.55 (0.52, 0.61)		0.56 (0.52, 0.61)	
2024.3–2614.8	179	1.70 (1.55, 1.86)		1.68 (1.55, 1.84)		0.59 (0.54, 0.64)		0.58 (0.53, 0.62)	
≥2614.9	180	1.62 (1.48, 1.77)		1.65 (1.51, 1.80)		0.65 (0.60, 0.70)		0.63 (0.58, 0.68)	
δ-Tocopherol			0.98		0.49		<0.01		<0.01
<252.8	179	1.65 (1.51, 1.79)		1.62 (1.48, 1.76)		0.49 (0.45, 0.53)		0.49 (0.46, 0.53)	
252.8–349.3	179	1.63 (1.51, 1.79)		1.63 (1.49, 1.79)		0.57 (0.53, 0.61)		0.58 (0.53, 0.62)	
349.4–457.0	180	1.68 (1.54, 1.82)		1.68 (1.54, 1.84)		0.61 (0.56, 0.66)		0.61 (0.56, 0.66)	
≥457.1	178	1.62 (1.49, 1.75)		1.65 (1.51, 1.79)		0.63 (0.58, 0.68)		0.62 (0.58, 0.68)	
Total retinol			<0.01		0.14		0.63		0.46
<508.2	179	1.79 (1.63, 1.95)		1.72 (1.60, 1.88)		0.60 (0.56, 0.65)		0.61 (0.56, 0.66)	
508.2–602.8	180	1.75 (1.50, 1.91)		1.73 (1.58, 1.90)		0.55 (0.51, 0.60)		0.55 (0.52, 0.60)	
602.9–714.2	178	1.46 (1.34, 1.60)		1.48 (1.35, 1.63)		0.58 (0.53, 0.62)		0.57 (0.53, 0.62)	
≥714.3	180	1.60 (1.46, 1.73)		1.65 (1.51, 1.80)		0.56 (0.52, 0.61)		0.55 (0.51, 0.61)	
Total carotenoids			0.40		0.55		<0.01		0.18
<1012.5	179	1.62 (1.48, 1.76)		1.60 (1.46, 1.75)		0.62 (0.57, 0.68)		0.60 (0.54, 0.64)	
1012.5–1250.8	179	1.68 (1.55, 1.77)		1.70 (1.55, 1.84)		0.58 (0.59, 0.62)		0.56 (0.52, 0.62)	
1250.9–1557.1	180	1.73 (1.60, 1.90)		1.77 (1.62, 1.91)		0.59 (0.55, 0.64)		0.60 (0.55, 0.65)	
≥1557.2	179	1.54 (1.40, 1.66)		1.52 (1.39, 1.66)		0.51 (0.47, 0.55)		0.53 (0.49, 0.58)	
Carotenes									
β-Carotene			0.05		0.07		<0.01		0.07
<152.3	180	1.77 (1.62, 1.93)		1.79 (1.63, 1.95)		0.65 (0.60, 0.70)		0.62 (0.58, 0.69)	
152.3–225.9	179	1.63 (1.51, 1.79)		1.63 (1.49, 1.77)		0.58 (0.54, 0.63)		0.58 (0.53, 0.62)	
226.0–312.1	178	1.60 (1.45, 1.72)		1.59 (1.46, 1.73)		0.54 (0.50, 0.58)		0.54 (0.50, 0.59)	
≥312.2	180	1.58 (1.45, 1.73)		1.58 (1.45, 1.73)		0.53 (0.49, 0.57)		0.55 (0.51, 0.59)	
trans β-Carotene			0.05		0.10		<0.01		0.02
<140.9	179	1.77 (1.63, 1.93)		1.79 (1.63, 1.95)		0.64 (0.59, 0.70)		0.62 (0.51, 0.67)	
140.9–211.5	180	1.63 (1.49, 1.79)		1.62 (1.49, 1.77)		0.59 (0.54, 0.64)		0.57 (0.54, 0.63)	
211.6–290.2	179	1.58 (1.45, 1.72)		1.58 (1.43, 1.72)		0.54 (0.50, 0.59)		0.55 (0.51, 0.59)	
≥290.3	179	1.60 (1.46, 1.77)		1.60 (1.46, 1.75)		0.52 (0.48, 0.56)		0.55 (0.51, 0.59)	
cis β-Carotene			0.02		0.04		<0.01		0.06
<10.2	180	1.75 (1.60, 1.90)		1.75 (1.60, 1.91)		0.67 (0.61, 0.72)		0.64 (0.59, 0.69)	
10.2–14.8	178	1.65 (1.51, 1.79)		1.65 (1.51, 1.80)		0.57 (0.53, 0.62)		0.57 (0.53, 0.62)	
14.9–21.3	180	1.63 (1.49, 1.79)		1.63 (1.51, 1.79)		0.55 (0.51, 0.60)		0.55 (0.51, 0.59)	
≥21.4	179	1.55 (1.42, 1.70)		1.55 (1.42, 1.70)		0.52 (0.48, 0.56)		0.54 (0.50, 0.59)	
trans α-Carotene			0.88		0.93		0.48		0.81
<15.2	179	1.79 (1.65, 1.95)		1.77 (1.62, 1.93)		0.62 (0.58, 0.68)		0.61 (0.56, 0.66)	
15.2–21.2	180	1.52 (1.39, 1.65)		1.52 (1.39, 1.66)		0.58 (0.53, 0.62)		0.58 (0.53, 0.62)	
21.3–32.4	178	1.63 (1.49, 1.77)		1.65 (1.52, 1.80)		0.55 (0.51, 0.59)		0.56 (0.52, 0.61)	
≥32.5	180	1.65 (1.51, 1.79)		1.63 (1.49, 1.77)		0.54 (0.51, 0.59)		0.55 (0.51, 0.60)	
Total lycopene			0.52		0.73		0.04		0.18
<68.0	180	1.72 (1.57, 1.86)		1.68 (1.54, 1.84)		0.63 (0.58, 0.68)		0.61 (0.56, 0.66)	
68.0–108.9	178	1.60 (1.46, 1.73)		1.59 (1.46, 1.72)		0.57 (0.52, 0.62)		0.58 (0.54, 0.62)	
109.0–179.9	179	1.62 (1.49, 1.77)		1.65 (1.51, 1.79)		0.56 (0.52, 0.61)		0.56 (0.52, 0.61)	
≥180.0	180	1.65 (1.49, 1.79)		1.65 (1.52, 1.80)		0.54 (0.50, 0.58)		0.55 (0.51, 0.59)	

(Continued)

TABLE 2 (*Continued*)

Quartiles from lowest to highest	*n*	F_2-IsoPs				15-F_{2t}-IsoP-M			
		Unadjusted[2]	*P*-trend	Adjusted[2]	*P*-trend	Unadjusted[2]	*P*-trend	Adjusted[2]	*P*-trend
trans, 5-*cis*, and 7-*cis* Lycopene			0.58		0.81		0.05		0.21
<46.4	180	1.73 (1.58, 1.88)		1.70 (1.55, 1.86)		0.62 (0.58, 0.68)		0.61 (0.56, 0.66)	
46.4–74.3	178	1.65 (1.46, 1.75)		1.58 (1.46, 1.72)		0.56 (0.52, 0.61)		0.57 (0.53, 0.62)	
74.4–124.2	179	1.62 (1.48, 1.75)		1.63 (1.49, 1.77)		0.56 (0.52, 0.61)		0.57 (0.53, 0.66)	
≥124.3	179	1.63 (1.49, 1.79)		1.66 (1.52, 1.80)		0.54 (0.50, 0.58)		0.54 (0.50, 0.59)	
Other than *trans,* 5-*cis*, and 7-*cis*			0.39		0.55		0.02		0.04
<21.3	179	1.72 (1.57, 1.88)		1.70 (1.55, 1.86)		0.62 (0.58, 0.68)		0.62 (0.58, 0.66)	
21.3–34.1	180	1.63 (1.49, 1.79)		1.63 (1.49, 1.77)		0.58 (0.53, 0.62)		0.57 (0.53, 0.62)	
34.2–52.9	178	1.60 (1.46, 1.75)		1.62 (1.48, 1.75)		0.55 (0.51, 0.60)		0.55 (0.51, 0.60)	
≥53.0	180	1.62 (1.48, 1.77)		1.63 (1.49, 1.79)		0.53 (0.49, 0.58)		0.54 (0.50, 0.59)	
Other carotenoids									
trans Lutein + zeaxanthin			0.56		0.69		0.24		0.72
<313.3	179	1.62 (1.49, 1.77)		1.58 (1.49, 1.79)		0.58 (0.49, 0.62)		0.58 (0.53, 0.62)	
313.3–389.5	179	1.60 (1.46, 1.75)		1.60 (1.48, 1.75)		0.57 (0.53, 0.62)		0.56 (0.52, 0.62)	
389.6–475.9	179	1.73 (1.60, 1.90)		1.73 (1.58, 1.87)		0.55 (0.51, 0.60)		0.55 (0.51, 0.60)	
≥476.0	180	1.60 (1.46, 1.75)		1.60 (1.48, 1.75)		0.59 (0.55, 0.64)		0.59 (0.55, 0.64)	
cis Lutein + zeaxanthin			0.60		0.55		0.16		0.35
<85.8	179	1.54 (1.40, 1.66)		1.52 (1.40, 1.65)		0.58 (0.53, 0.62)		0.56 (0.52, 0.61)	
85.8–103.6	180	1.77 (1.62, 1.93)		1.75 (1.60, 1.91)		0.60 (0.55, 0.65)		0.60 (0.55, 0.65)	
103.7–126.5	178	1.70 (1.57, 1.86)		1.72 (1.58, 1.86)		0.56 (0.52, 0.61)		0.56 (0.52, 0.61)	
≥126.6	180	1.60 (1.45, 1.72)		1.58 (1.45, 1.73)		0.55 (0.51, 0.60)		0.55 (0.52, 0.61)	
trans Anhydrolutein			0.64		0.48		<0.01		0.83
<48.0	179	1.58 (1.45, 1.73)		1.62 (1.48, 1.73)		0.61 (0.56, 0.66)		0.59 (0.55, 0.64)	
48.0–65.6	180	1.70 (1.55, 1.84)		1.61 (1.54, 1.82)		0.56 (0.52, 0.61)		0.55 (0.51, 0.59)	
65.6–83.4	178	1.70 (1.55, 1.86)		1.70 (1.54, 1.86)		0.58 (0.53, 0.62)		0.59 (0.54, 0.63)	
≥83.5	180	1.57 (1.45, 1.73)		1.57 (1.45, 1.72)		0.54 (0.50, 0.59)		0.56 (0.51, 0.61)	
cis Anhydrolutein			0.26		0.11		<0.01		0.048
<24.2	180	1.70 (1.55, 1.86)		1.70 (1.55, 1.84)		0.64 (0.59, 0.69)		0.61 (0.55, 0.66)	
24.2–31.1	179	1.57 (1.43, 1.72)		1.58 (1.45, 1.73)		0.56 (0.52, 0.61)		0.56 (0.52, 0.61)	
31.2–40.1	178	1.79 (1.63, 1.93)		1.77 (1.63, 1.93)		0.57 (0.53, 0.62)		0.58 (0.54, 0.62)	
≥40.2	180	1.54 (1.40, 1.68)		1.52 (1.40, 1.66)		0.53 (0.49, 0.57)		0.55 (0.51, 0.59)	
trans α-Cryptoxanthin			0.85		0.75		<0.01		0.12
<19.8	179	1.55 (1.42, 1.68)		1.52 (1.39, 1.66)		0.59 (0.54, 0.64)		0.55 (0.51, 0.60)	
19.8–25.2	180	1.63 (1.49, 1.77)		1.63 (1.51, 1.79)		0.59 (0.54, 0.64)		0.61 (0.56, 0.66)	
25.3–31.7	178	1.75 (1.60, 1.91)		1.77 (1.63, 1.93)		0.56 (0.51, 0.61)		0.56 (0.52, 0.61)	
≥31.8	180	1.63 (1.51, 1.79)		1.63 (1.49, 1.79)		0.56 (0.51, 0.61)		0.57 (0.53, 0.62)	
trans β-Cryptoxanthin			0.70		0.60		0.01		0.66
<71.1	180	1.77 (1.62, 1.91)		1.77 (1.62, 1.93)		0.65 (0.60, 0.70)		0.62 (0.57, 0.67)	
71.1–122.1	178	1.58 (1.46, 1.73)		1.60 (1.46, 1.75)		0.55 (0.51, 0.60)		0.56 (0.52, 0.61)	
122.2–241.1	180	1.62 (1.62, 1.77)		1.62 (1.48, 1.77)		0.55 (0.51, 0.60)		0.56 (0.53, 0.61)	
≥241.2	179	1.60 (1.46, 1.73)		1.60 (1.46, 1.75)		0.54 (0.50, 0.59)		0.55 (0.51, 0.60)	
cis β-Cryptoxanthin			0.53		0.42		0.02		0.01
<30.9	180	1.70 (1.55, 1.86)		1.70 (1.55, 1.86)		0.66 (0.61, 0.71)		0.63 (0.58,0.68)	
30.9–42.9	179	1.62 (1.48, 1.77)		1.60 (1.48, 1.75)		0.58 (0.54, 0.63)		0.58 (0.54, 0.63)	
43.0–70.9	178	1.68 (1.54, 1.84)		1.70 (1.55, 1.86)		0.54 (0.49, 0.58)		0.54 (0.51, 0.59)	
≥71.0	180	1.57 (1.43, 1.72)		1.55 (1.42, 1.70)		0.53 (0.49, 0.60)		0.54 (0.50, 0.59)	

[1] *P*-trend values are from a linear regression model. F_2-IsoP, F_2-isoprostane; 15-F_{2t}-IsoP-M, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

[2] ANOVA estimate: values of log-transformed F_2-IsoPs and their major metabolite 15-F_{2t}-IsoP-M were adjusted for age (continuous), education, occupation, cigarette smoking (yes or no), BMI (continuous), multivitamin supplement use (yes or no), fruit and vegetable intakes (continuous), and batch assays for F_2-IsoPs or 15-F_{2t}-IsoP-M.

[3] Geometric mean; 95% CI in parentheses (all such values).

Holt et al (15) reported inverse associations between urinary F_2-IsoPs and intakes of total fruit, vegetables, vitamin C, and β- carotene in adolescents aged 13–17 y. In our study, we showed that high intakes of fruit, vegetables, vitamin C, and carotenes were related to lower 15-F_{2t}-IsoP-M concentrations but not F_2-IsoP concentrations in unadjusted models. However, none of these associations remained significant after adjustment for potential confounding factors. We showed that the use of a multivitamin or vitamin E supplement was associated with lower urinary excretion rates of isoprostanes, particularly of 15-F_{2t}-IsoP-M. In one study, a suppressive effect of vitamin E supplementation on plasma F_2-IsoPs was observed only at a dose of 800 IU/d after 16 wk of use, and this effect was most apparent when the dose became very high (1600–3200 IU/d) (29). Another trial showed that a high dose of vitamin E (800 IU/d) or vitamin C (1000 mg/d) reduced concentrations of

TABLE 3
Urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations in healthy women according to tea drinking and urinary excretion of tea polyphenols in the Shanghai Women's Health Study[1]

		F_2-IsoPs				15-F_{2t}-IsoP-M			
Tertiles from lowest to highest	*n*	Unadjusted[2]	*P*	Adjusted[2]	*P*	Unadjusted[2]	*P*	Adjusted[2]	*P*
		ng/mg creatinine		*ng/mg creatinine*		*ng/mg creatinine*		*ng/mg creatinine*	
Tea drinking	845		0.48		0.72		0.18		0.06
Never	605	1.60 (1.54, 1.68)[3]		1.62 (1.54, 1.70)		0.55 (0.53, 0.58)		0.56 (0.53, 0.59)	
Ever	240	1.66 (1.54, 1.79)		1.65 (1.52, 1.77)		0.59 (0.55, 0.63)		0.61 (0.56, 0.66)	
Polyphenols	700								
Epicatechin			0.82		0.55		0.38		0.42
0 μmol/g creatinine[4]	325	1.60 (1.51, 1.70)		1.62 (1.52, 1.73)		0.56 (0.53, 0.59)		0.58 (0.54, 0.62)	
>0–0.13 μmol/g creatinine	142	1.54 (1.39, 1.68)		1.57 (1.42, 1.73)		0.55 (0.50, 0.61)		0.56 (0.51, 0.62)	
>0.13 μmol/g creatinine	233	1.72 (1.58, 1.84)		1.73 (1.58, 1.87)		0.59 (0.55, 0.63)		0.59 (0.53, 0.61)	
Epigallocatechin			0.18		0.41		0.03		0.03
<0.03 μmol/g creatinine	228	1.61 (1.48, 1.72)		1.60 (1.48, 1.78)		0.55 (0.50, 0.59)		0.55 (0.52, 0.60)	
0.03–0.28 μmol/g creatinine	238	1.52 (1.41, 1.63)		1.55 (1.41, 1.65)		0.56 (0.52, 0.60)		0.56 (0.52, 0.61)	
≥0.29 μmol/g creatinine	234	1.80 (1.66, 1.93)		1.79 (1.66, 1.95)		0.59 (0.55, 0.64)		0.59 (0.55, 0.65)	
Polyphenol metabolites									
4′-MeEGC			0.21		0.45		0.52		0.25
0 μmol/g creatinine[4]	334	1.66 (1.55, 1.77)		1.68 (1.57, 1.80)		0.57 (0.54, 0.61)		0.58 (0.55, 0.62)	
>0–0.03 μmol/g creatinine	131	1.60 (1.45, 1.76)		1.60 (1.45, 1.79)		0.54 (0.50, 0.60)		0.54 (0.49, 0.60)	
>0.03 μmol/g creatinine	235	1.58 (1.46, 1.72)		1.62 (1.49, 1.75)		0.58 (0.54, 0.63)		0.58 (0.53, 0.62)	
5-(3′,4’,5′-rihydroxyphenyl)-γ-Valerolactone			0.84		0.27		0.28		0.30
0 μmol/g creatinine[4]	493	1.57 (1.49, 1.66)		1.62 (1.52, 1.70)		0.56 (0.54, 0.59)		0.57 (0.54, 0.60)	
>0–0.004 μmol/g creatinine	104	1.72 (1.55, 1.90)		1.73 (1.55, 1.91)		0.59 (0.55, 0.65)		0.61 (0.54, 0.67)	
>0.004 μmol/g creatinine	103	1.88 (1.57, 2.05)		1.68 (1.45, 1.95)		0.52 (0.45, 0.59)		0.54 (0.47, 0.62)	
5-(3′,4′-dihydroxyphenyl)-γ-Valerolactone			0.62		0.95		0.14		0.18
<0.89 μmol/g creatinine	233	1.57 (1.45, 1.72)		1.58 (1.46, 1.72)		0.59 (0.55, 0.64)		0.60 (0.55, 0.64)	
0.89–7.91 μmol/g creatinine	233	1.62 (1.49, 1.73)		1.65 (1.52, 1.79)		0.55 (0.52, 0.59)		0.60 (0.51, 0.59)	
>7.91 μmol/g creatinine	234	1.70 (1.57, 1.84)		1.70 (1.57, 1.86)		0.56 (0.52, 0.60)		0.57 (0.53, 0.62)	
Flavonoid polyphenols									
Kaempferol			0.59		0.72		0.33		0.29
<0.04 μmol/g creatinine	228	1.60 (1.48, 1.72)		1.58 (1.46, 1.73)		0.54 (0.51, 0.58)		0.55 (0.51, 0.60)	
0.04–0.28 μmol/g creatinine	236	1.60 (1.48, 1.73)		1.65 (1.52, 1.79)		0.55 (0.52, 0.59)		0.56 (0.52, 0.59)	
>0.28 μmol/g creatinine	236	1.68 (1.55, 1.80)		1.70 (1.55, 1.84)		0.61 (0.56, 0.65)		0.61 (0.56, 0.65)	
Quercetin			0.06		0.01		0.24		0.03
<0.06 μmol/g creatinine	217	1.46 (1.35, 1.58)		1.51 (1.39, 1.65)		0.54 (0.51, 0.59)		0.54 (0.50, 0.58)	
0.06–0.25 μmol/g creatinine	249	1.63 (1.52, 1.77)		1.63 (1.52, 1.77)		0.55 (0.52, 0.59)		0.56 (0.52, 0.60)	
>0.25 μmol/g creatinine	234	1.77 (1.63, 1.93)		1.79 (1.65, 1.93)		0.61 (0.56, 0.65)		0.61 (0.57, 0.66)	

[1] *P* values were from ANOVA for variables with 2 categories, and *P*-trend values were from a linear regression model for variables with >2 categories. F_2-IsoP, F_2-isoprostane; 4′-MeEGC, methylated form of epigallocatechin; 15-F_{2t}-IsoP-M, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

[2] ANOVA estimate: values of log-transformed F_2-IsoPs and their major metabolite 15-F_{2t}-IsoP-M were adjusted for age (continuous); education; occupation; cigarette smoking (yes or no); BMI (continuous); multivitamin supplement use (yes or no); fruit and vegetable intakes (continuous); plasma total carotenoids, tocopherols, and retinol (continuous); and batch assays for F_2-IsoPs or 15-F_{2t}-IsoP-M (categories) and urinary tea polyphenols.

[3] Geometric mean; 95% CI in parentheses (all such values).

[4] The limits of detection for epicatechin, epigallocatechin, kaempferol, and quercetin are 1 nmol/L, whereas the limits of detection for polyphenol metabolites are 100 nmol/L.

F_2-IsoPs after 8 wk of supplementation only for subjects with high basal plasma F_2-IsoP concentrations that were associated with high BMI (17). In a similar trial that used a high dose (800 IU/d) of vitamin E supplementation, plasma F_2-IsoPs was decreased in overweight middle-aged individuals (18). Another trial showed that the supplementation of vitamin E (130 mg/d) with other dietary supplements (β-carotene, vitamin C, zinc, selenium, and garlic) for 4 wk increased serum concentrations of vitamin E, as well as vitamin C and β-carotene concentrations, but did not reduce plasma F_2-IsoP concentrations (30). Although a small proportion of women used vitamin supplements in this study, our findings were generally consistent with those of previous studies. However, we could not exclude the possibility that the observed associations with 15-F_{2t}-IsoP-M for vitamin supplements were due to underlying confounding factors. Unlike randomized clinical trials, it is possible that supplement users may have other lifestyle factors that favor a reduced concentration of isoprostanes. A limited number of obese participants or vitamin-supplement users in our study prevented us from further examining whether associations between antioxidant vitamin supplements and urinary isoprostanes differed by obesity status, although BMI was positively associated with 15-F_{2t}-IsoP-M in our study population (13). Finally, because none of the previous clinical trials examined 15-F_{2t}-IsoP-M, it is unclear whether 15-F_{2t}-IsoP-M is a more-sensitive and accurate biomarker than F_2-IsoPs in the assessment of the effect of antioxidant supplementation on oxidative stress.

TABLE 4
Urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations in healthy women according to demographic and lifestyle characteristics in the Shanghai Women's Health Study (n = 845)[1]

		F_2-IsoPs		15-F_{2t}-IsoP-M	
Characteristics	%	Values	P	Values	P
		ng/mg creatinine		*ng/mg creatinine*	
Age group			0.02		<0.01
40–49 y	44.0	1.70 (1.60, 1.80)[2]		0.52 (0.49, 0.55)	
50–59 y	26.8	1.62 (1.49, 1.75)		0.59 (0.55, 0.64)	
60–70 y	29.2	1.52 (1.40, 1.63)		0.61 (0.57, 0.65)	
Education			0.003		0.05
More than high school	11.4	1.40 (1.26, 1.58)		0.53 (0.47, 0.59)	
High school	27.2	1.55 (1.45, 1.68)		0.54 (0.51, 0.58)	
Less than high school	61.4	1.70 (1.62, 1.79)		0.58 (0.55, 0.61)	
Occupation			0.04		0.04
Professional	240	1.49 (1.39, 1.62)		0.53 (0.50, 0.56)	
Clerical and administrative	187	1.68 (1.55, 1.84)		0.56 (0.52, 0.61)	
Manual worker	415	1.66 (1.57, 1.77)		0.58 (0.55, 0.61)	
Menopause status			0.33		<0.01
Premenopausal	47.6	1.65 (1.56, 1.75)		0.51 (0.49, 0.54)	
Postmenopausal	52.2	1.60 (1.51, 1.68)		0.61 (0.58, 0.65)	
Physically active in the past 10 y			0.42		0.11
No	66.7	1.65 (1.57, 1.73)		0.58 (0.55, 0.60)	
Yes	33.3	1.58 (1.48, 1.70)		0.54 (0.51, 0.58)	
Ever smoke cigarettes regularly			<0.01		0.09
No	97.3	1.60 (1.54, 1.66)		0.56 (0.54, 0.58)	
Yes	2.7	2.25 (1.77, 2.89)		0.68 (0.54, 0.86)	
Alcohol consumption regularly			0.45		0.94
No	96.9	1.63 (1.57, 1.70)		0.56 (0.54, 0.58)	
Yes	3.1	1.49 (1.18, 1.86)		0.57 (0.46, 0.70)	
Ever used multivitamins			0.049		0.01
No	94.9	1.63 (1.57, 1.70)		0.57 (0.55, 0.59)	
Yes	5.1	1.36 (1.19, 1.63)		0.46 (0.39, 0.54)	
Ever used vitamin E supplements			0.42		<0.01
No	88.9	1.62 (1.57, 1.70)		0.58 (0.55, 0.60)	
Yes	11.1	1.57 (1.39, 1.75)		0.47 (0.41, 0.52)	
Ever used ginseng supplement			0.03		0.30
No	73.8	1.66 (1.58, 1.73)		0.56 (0.53, 0.58)	
Yes	26.2	1.51 (1.39, 1.63)		0.58 (0.54, 0.63)	
BMI[3]			0.56		<0.01
<23.0 kg/m^2	25.2	1.65 (1.54, 1.77)		0.52 (0.49, 0.55)	
23.0–24.9 kg/m^2	24.4	1.60 (1.49, 1.73)		0.59 (0.54, 0.63)	
25.0–29.9 kg/m^2	25.3	1.60 (1.49, 1.72)		0.58 (0.54, 0.62)	
≥30.0 kg/m^2	25.1	1.62 (1.36, 1.90)		0.62 (0.53, 0.72)	
History of diabetes			0.28		0.92
No	95.0	1.62 (1.55, 1.68)		0.56 (0.54, 0.58)	
Yes	5.0	1.79 (1.49, 2.13)		0.57 (0.48, 0.67)	
History of cardiovascular diseases[4]			0.08		0.93
No	70.2	1.64 (1.54, 1.72)		0.56 (0.54, 0.59)	
Yes	29.8	1.46 (1.27, 1.64)		0.56 (0.53, 0.61)	

[1] ANOVA estimate: values of log-transformed F_2-IsoPs and their major metabolite 15-F_{2t}-IsoP-M were adjusted for age (except for age and menopausal status) and batch assays for F_2-IsoPs or 15-F_{2t}-IsoP-M (categories). P values were from ANOVA (for variables with 2 categories), and P-trend values (for variables with >2 categories) were from a linear regression model. F_2-IsoP, F_2-isoprostane; 15-F_{2t}-IsoP-M, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

[2] Geometric mean; 95% CI in parentheses (all such values).

[3] Categories were adapted from the WHO (45).

[4] History of cardiovascular diseases including stroke, coronary heart diseases, and hypertension.

In the current study, higher circulating concentrations of β- and γ-tocopherols or δ-tocopherol were associated with increased 15-F_{2t}-IsoP-M excretion but not with F_2-IsoPs. Additional research is required to identify the underlying mechanisms of this positive association. We also showed that higher concentrations of several subgroups of carotenoids were related to lower 15-F_{2t}-IsoP-M but not F_2-IsoPs. Our findings were consistent with those of previous studies in which lower concentrations of urinary F_2-IsoP were associated with elevated concentrations of plasma β-carotene (27) and total plasma carotenoids, particularly α-carotene, in breast cancer survivors (31). In the Coronary Artery Risk Development study conducted

in young and healthy women and men aged 18–30 y, overall concentrations of serum α-carotene, β- carotene, zeaxanthin and lutein, β-cryptoxanthin, and lycopene were inversely associated with plasma F_2-IsoP concentration (32). None of these studies examined 15-F_{2t}-IsoP-M, and thus, replication is needed of these findings on 15-F_{2t}-IsoP-M. Our finding of a positive association between urinary 15-F_{2t}-IsoP-M and plasma β- and γ-tocopherols or δ-tocopherol agrees with a previous study in which a positive association was observed between urinary F_2-IsoPs and plasma γ-tocopherol in a healthy pediatric population (33). Again, additional research is needed to understand the mechanism for this potential relation.

One distinction of the current study is that it was conducted in Chinese women in Shanghai, which is a population with a high consumption of ginseng and tea rich in flavonoid polyphenols (eg, quercetin). Several studies in both bacterial and mammalian experiments have shown that some polyphenols, such as quercetin, are mutagenic and genotoxic (34), whereas other studies showed that quercetin inhibited catechol-*O*-methyltransferase enzyme activity and enhanced estradiol-induced tumorigenesis (35). These findings may indicate a possibility that certain polyphenols have detrimental side effects on mammary carcinogenesis at an excessive intake. In contrast, one alternative explanation is that free radicals play a critical role in the induction of apoptosis and senescence of cancer cells (36). Two previous clinical trials of tea drinking or tea-polyphenol supplementation showed a nonsignificant trend toward higher urinary or plasma F_2-IsoP concentrations (37, 38). In line with these findings, in our study, tea drinking or higher concentrations of EGC, which is the most abundant tea polyphenol, and flavonoid quercetin were associated with higher concentrations of F_2-IsoPs and/or 15-F_{2t}-IsoP-M. However, other studies showed an inverse association or nonassociation between tea consumption and isoprostanes (39, 40). Therefore, our findings need to be investigated further. To our knowledge, our data on ginseng are new. The regular use of ginseng was associated with a decreased concentration of F_2-IsoPs but not with 15-F_{2t}-IsoP-M. We could not find a biological explanation for these results because no previous experimental and observational studies have investigated the association between ginseng use and F_2-IsoPs and 15-F_{2t}-IsoP-M.

The current study has several notable strengths. Concentrations of F_2-IsoPs were measured together with their major metabolite15-F_{2t}-IsoP-M by using GC/NICI MS–based assay. The parent population-based cohort study had remarkably high rates of baseline participation (19), which minimized selection bias. One limitation of the study was that a single urine sample was used. However, reliability studies showed that, at a group concentration, F_2-IsoPs measured in a single urine sample did not significantly differ from the concentration measured by using multiple samples or a 24-h urine sample (41). Previous studies generated inconsistent results on the interday variation of urinary isoprostane concentrations (16), whereas our previous data in the same study population suggested that the major contributor to intraperson variation is seasonal fluctuation (25). Because interday variation is random, any residual interday variation may lead to nondifferential misclassification, which usually biases the result to the null. To the extent that residual interday variation concentrations existed in our data, the true associations could be stronger than the associations we observed. Another limitation was that urinary isoprostanes and plasma lipophilic antioxidants were measured at the same time. Thus, the temporal sequence was unclear for the observed associations. However, it is unlikely that intakes of dietary and supplemental antioxidants were caused by concentrations of endogenous isoprostanes (F_2-IsoPs and 15-F_{2t}-IsoP-M), although it is possible that concentrations of some plasma antioxidants, such as β-, γ-, and δ-tocopherols, may be the outcome of body regulations in response to high concentrations of oxidative stress (22, 27, 31).

Our findings potentially have very significant implications. F_2-IsoPs are generally considered the most reliable in vivo biomarker of oxidative stress and have been widely used in clinical trials and human studies (28, 31, 42). Results from clinical trials (17, 18, 29) that used F_2-IsoPs indicated that a very high dose of α-tocopherol supplementation is required to only moderately reduce F_2-IsoPs, whereas at this high dose, α-tocopherol was shown to increase risk of prostate cancer (43) and hemorrhagic stroke (44). The findings from the current study suggested that intakes of α-tocopherol or many other antioxidants were associated with lower concentrations of a major metabolite of F_2-IsoPs (15-F_{2t}-IsoP-M) but not with F_2-IsoPs. Therefore, 15-F_{2t}-IsoP-M may potentially be used to identify subjects at risk of oxidative stress or even marginal oxidative stress. Furthermore, 15-F_{2t}-IsoP-M may be a sensitive biomarker to assess the effect of antioxidants at moderate concentrations. Collectively, if our findings are confirmed in future studies, 15-F_{2t}-IsoP-M will be very critical in the personalized prevention and treatment of oxidative stress and free-radical–related diseases by avoiding possible adverse effects caused by an excessive dose of antioxidants.

Concentrations of several types of circulating lypophilic antioxidants, urinary tea polyphenol, and the use of some vitamin supplements may have stronger associations with urinary 15-F_{2t}-IsoP-M concentrations than with F_2-IsoPs, which suggested that 15-F_{2t}-IsoP-M is a more sensitive biomarker of oxidative stress in the assessment of the effects of antioxidants. Additional studies are warranted to confirm these findings.

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