NEWS & VIEWS

Are Dietary Antioxidant Intake Indices Correlated to Oxidative Stress and Inflammatory Marker Levels?

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Abstract

Epidemiologic and experimental studies have shown that a high intake of individual dietary antioxidants is associated with a reduced risk of cancers. Few studies, however, have investigated the influences of a combination of dietary antioxidants. We evaluated the association of two dietary antioxidant indices, the Dietary Antioxidant Quality Score (DAQS) and the Composite Dietary Antioxidant Index (CDAI), with 10 oxidative stress or inflammation biomarkers (urinary F2-isoprostanes [15-F₂-IsoP]; urinary F2-isoprostane metabolites [15-F₂-IsoPM]; urinary prostaglandin E2 metabolite [PGEM]; C-reactive protein [CRP]; interleukin-1 β [IL-1 β]; interleukin-6 [IL-6]; tumor necrosis factor- α [TNF- α]; soluble TNF-receptor 1 [sTNF-R1]; soluble TNF-receptor 2 [sTNF-R2]; and soluble GP130 [sGP130]) in 3853 participants of the Shanghai Women's Health Study (SWHS). We found the DAQS and CDAI to be highly correlated ($r=0.72$), and both were inversely associated with levels of IL-1 β ($p_{trend}=0.02$ and 0.03 , respectively) and TNF- α ($p_{trend}=0.005$ and 0.003 , respectively). In addition, IL-6 and sTNF-R2 levels were inversely associated with the DAQS score; β -coefficient ($\pm$ SE) for average-quality and high-quality group *versus* low-quality group were $-0.22(\pm 0.13)$ and $-0.30(\pm 0.13)$ ($p_{trend}=0.06$) for IL-6; $-0.06(\pm 0.04)$ and $-0.10(\pm 0.04)$ ($p_{trend}=0.01$) for sTNF-R2. Neither the DAQS nor CDAI score was significantly associated with oxidative stress or other inflammatory biomarkers. Our observations lead us to hypothesize that these two indices offer a potential aggregate method of measuring dietary anti-inflammation, but not anti-oxidation properties. *Antioxid. Redox Signal.* 22, 951–959.

Introduction

REACTIVE OXYGEN SPECIES (ROS) play an important role in signal transduction, cell proliferation, homeostasis, induction of apoptosis, and senescence (1). Under normal physiological conditions, ROS products (*i.e.*, superoxide anion [O₂ $\cdot^-$]; hydrogen peroxide [H₂O₂]; and hydroxyl radical [OH $\cdot$]) are produced in aerobic cells during various endogenous metabolic reactions *via* a mechanism known as a mitochondrial respiratory chain. Oxidative stress due to overproduction of ROS can cause significant damage to cell structure and function, which is linked to the pathogenesis of cancer, by causing DNA damage, genome instability, cell proliferations, and induction of angiogenesis and also to neurodegenerative disease (5). A major cause of ROS over-

Innovation

Most previous studies on the effects of dietary antioxidants on cancer risk or chronic disease have focused on one or a few individual dietary antioxidants. Ours is the first large population-based study that evaluates the association of aggregate dietary vitamin and mineral intake, as measured by two indices, with a wide range of oxidative stress and inflammatory biomarkers. As such, our study presents a novel method for assessing the underlying biological mechanism for the associations between aggregate dietary antioxidants and health outcomes.

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production is chronic inflammation, which, in turn, is caused by various biological, chemical, and physical factors, including viral and microbial infection; exposure to allergens, radiation, and chemicals; autoimmune and chronic diseases; alcohol consumption; tobacco smoke; and low intake of dietary antioxidants (5).

Epidemiologic and experimental studies have found that a high intake of dietary antioxidants (*e.g.*, vitamins A, C, E, selenium, *etc.*) is associated with reduced risk of several cancers (5), but recent intervention trials have failed to confirm these findings. Previous studies, however, have primarily focused on investigation of the influences of individual dietary antioxidants on cancer risk and other chronic diseases. Few studies have evaluated the aggregate effect of dietary antioxidant vitamins and minerals on health outcomes or their risk factors, such as oxidative stress and inflammatory biomarkers.

Two such indices, the Dietary Antioxidant Quality Score (DAQS), which sums up certain dietary antioxidants and assigns a score based on calculated quantity *versus* the FDA-recommended quantity (4), and the Composite Dietary Antioxidant Index (CDAI), which uses statistical modeling to rank an individual's intake in relation to population norms (7), have previously been proposed for evaluation of the overall impact of antioxidants on health outcomes. To our knowledge, the associations of these indices with inflammation or oxidative biomarkers have not been evaluated. In this report, we evaluated the DAQS and CDAI for their association with levels of oxidative stress and inflammatory biomarkers in an effort to objectively assess the preliminary validity of these two indices.

Results

Characteristics of the study participants by the DAQS and CDAI breakdowns are presented in Table 1. The DAQS and CDAI are highly correlated ($r=0.72$). For both measurements, women with higher scores were younger than those with lower scores ($p<0.0001$). Obesity is less prevalent among subjects with a high DAQS or CDAI. Subjects with higher scores also had a significantly higher total energy intake and consumed more red meat, vegetables, and fruits (Table 1) than those with lower scores.

Table 2 shows the association between DAQS and CDAI with the prevalence of selected pre-existing metabolic-related diseases and conditions. We found that the prevalence of metabolic conditions was positively associated with CDAI quality score: odds ratio (OR)=1.22 (95% confidence interval [CI]: 1.01–1.47)—Tertile 2; 1.30 (95% CI: 1.02–1.66)—Tertile 3 ($p_{trend}=0.02$) comparing to Tertile 1. Both the DAQS and CDAI were positively associated with the prevalence of diabetes and coronary heart disease. Accordingly, the ORs for diabetes were 1.20 (95% CI: 0.74–1.95) for average-quality; 1.79 (95% CI: 1.11–2.88) for high-quality *versus* DAQS low-quality groups; 1.82 (95% CI: 1.24–2.66) for Tertile 2; and 3.06 (95% CI: 1.88–4.97) for Tertile 3 *versus* CDAI Tertile 1. These factors were adjusted for subsequent analyses. A similar positive association was observed between prevalence of hyperlipidemia and DAQS. No significant association was found for hypertension, stroke, or obesity (Table 2).

Results on the associations between DAQS and CDAI with 10 oxidative stress and inflammatory biomarkers are presented in

Table 3. After adjustment for potential confounders, both DAQS and CDAI were inversely associated with interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). The β -coefficients ($\pm$ SE) associated with the DAQS average-quality and high-quality groups were $-0.22(\pm 0.13)$ and $-0.28(\pm 0.13)$ for IL-1 β and $-0.16(\pm 0.08)$ and $-0.22(\pm 0.08)$ for TNF- α , respectively, compared with the DAQS low-quality group ($p_{trend}=0.02$ and 0.005, respectively); the corresponding estimates associated with the CDAI second and third tertiles were $-0.04(\pm 0.10)$ and $-0.25(\pm 0.13)$ for IL-1 β , and $-0.11(\pm 0.06)$ and $-0.27(\pm 0.08)$ for TNF- α , respectively, compared with the first tertile ($p_{trend}=0.03$ and 0.003, respectively). Significant or marginal significant inverse associations of interleukin-6 (IL-6) and soluble TNF-receptor 2 (sTNF-R2) levels with the DAQS were also observed [β -coefficient($\pm$ SE): $-0.22(\pm 0.13)$ and $-0.30(\pm 0.13)$ ($p_{trend}=0.06$) for IL-6; $-0.06(\pm 0.04)$ and $-0.10(\pm 0.04)$ ($p_{trend}=0.01$) for sTNF-R2; average-quality and high-quality group, respectively, *vs.* low-quality group] (Table 3). Additional analyses performed by grouping the CDAI using a distribution similar to the DAQS (*i.e.*, 11%, group 1; 22.6%, group 2; 66.2%, group 3) revealed a pattern comparable to that based on the tertile scale (data not shown).

In the stratified analyses, the observed inverse associations between DAQS and CDAI with IL-1 β and TNF- α , as well as between DQAS with IL-6 and sTNF-R2, did not vary by presence of metabolic condition, including hypertension, obesity, history of heart disease, BMI ≥ 30 , unspecified dyslipidemia (Table 4), or vitamin supplement use (data not shown).

Discussion

In this analysis of a large, population-based, cross-sectional study of 3853 Chinese women, we found that two correlated dietary antioxidant indices were inversely associated with levels of both IL-1 β and TNF- α . The DAQS was also inversely associated with IL-6 and sTNF-R2. These associations were not modified by metabolic condition or vitamin supplement use and were independent of a wide range of covariates, including baseline postmenopausal status and total energy intake. No significant association was found for DAQS or CDAI with oxidative stress biomarkers or other inflammatory biomarkers (*i.e.*, urinary 15-F2 $_t$ -IsoP, urinary 15-F2 $_t$ -IsoPM, urinary PGEM, CRP, sTNF-R1, or sGP130). To our knowledge, this is the first large population-based study that evaluates the association of aggregate dietary vitamin and mineral intake—*via* two separate antioxidant indices—with a wide range of oxidative stress and inflammatory biomarkers. However, several previous studies have examined the association between these indices and cancer (7) or bone mineral density (4). In one study, Rivas *et al.* (4) found that bone mineral density was higher in Spanish women with high *versus* low consumption of dietary antioxidants based on the DAQS, a finding that is held for all age groups (18–35, 36–45 and >45 years of age). In another study, Wright *et al.* (7) found that the risk of lung cancer was reduced when the CDAI increased across five quintiles (HR=1.0 [0.87–1.14], 0.91 [0.79–1.05], 0.79 [0.68–0.92] and 0.84 [0.72–0.98], $p_{trend}=0.02$ for second, third, fourth, and fifth quintiles *vs.* first quintile).

Our finding that both the DAQS and CDAI were inversely associated with IL-1 β and TNF- α levels is consistent with

TABLE 1. DIETARY ANTIOXIDANT QUALITY SCORE AND COMPOSITE DIETARY ANTIOXIDANT INDEX BY SELECTED DEMOGRAPHIC CHARACTERISTICS AND LIFESTYLE FACTORS IN THE SHANGHAI WOMEN'S HEALTH STUDY

Characteristics	Dietary Antioxidant Quality Score (N = 3853) (%)				Composite Dietary Antioxidant Index (N = 3853) (%)			
	1-2 (Poor quality) (n = 432)	3-4 (Average quality) (n = 870)	5-6 (Good quality) (n = 2551)	p-Value	Tertile 1 (n = 1284)	Tertile 2 (n = 1285)	Tertile 3 (n = 1284)	p-Value
Age at baseline (Mean ± SD)	59.0 ± 9.1	56.9 ± 9.1	54.3 ± 8.9	<0.0001	57.1 ± 9.4	55.0 ± 9.0	54.0 ± 8.8	<0.0001 (<i>P_{trend}</i>)
40-49	22.0	29.0	40.2	<0.0001	30.1	37.0	40.0	<0.0001
50-59	22.2	23.7	26.3		21.7	25.8	28.2	
60-69	54.2	46.0	33.0		46.7	35.5	31.7	
≥70	1.6	1.4	0.5		1.5	0.8	0.3	
Menopausal status-baseline	74.8	67.2	55.3	<0.0001	66.7	59.5	54.4	<0.0001
Passive smoking status	73.4	79.5	79.8	0.009	77.8	79.6	79.7	0.42
Education								
Elementary or less	51.2	36.2	21.7	<0.0001	39.1	25.5	20.2	<0.0001
Middle school	27.0	33.9	35.5		31.2	35.1	36.4	
High school	15.6	19.9	28.7		20.1	26.4	29.2	
Professional/college or higher	6.3	9.9	14.0		9.5	13.0	14.2	
Income								
Low	26.2	19.2	15.9	<0.0001	20.8	16.6	16.0	0.007
Lower-middle	38.2	40.8	38.4		38.8	40.1	37.9	
Upper-middle	23.8	26.6	27.4		26.1	26.3	28.1	
High	11.8	13.3	18.3		14.3	17.0	18.0	
Occupation								
Professional	63.2	58.7	47.7	<0.0001	59.0	49.8	47.0	<0.0001
Clerical	17.1	22.8	32.5		21.9	29.8	34.0	
Manual laborer/housewife	19.7	18.5	19.8		19.1	20.4	19.0	
BMI status								
Underweight (<18.5)	3.5	3.4	2.2	0.001	3.9	2.1	1.8	0.02
Normal (18.5-24.9)	52.1	52.9	58.9		55.1	58.0	57.2	
Overweight (25-29.9)	34.9	36.2	33.0		33.9	33.6	34.3	
Obese (≥30)	9.5	7.5	6.0		7.2	6.3	6.6	
Vitamin supplemental use	13.2	17.7	21.5	<0.0001	16.9	20.9	21.4	0.007
Use of antibiotics past week	5.1	6.8	7.7	0.14	6.1	7.6	7.9	0.16
Metabolic condition	41.0	40.1	36.2	0.04	38.2	38.2	36.4	0.53
Hypertension	32.4	30.7	26.8	0.01	30.2	28.9	25.7	0.03
Diabetes	6.9	6.5	5.6	0.42	6.3	6.1	5.6	0.75
Coronary heart disease	9.3	11.4	9.5	0.25	9.7	11.0	9.0	0.25
Stroke	3.0	1.3	0.8	0.0003	2.0	1.0	0.4	0.0004
Unspecified Dyslipidemia	2.1	2.1	2.9	0.31	2.3	2.8	2.7	0.73
Total energy intake (Kcal/day) (Mean ± SE) ^a	1316.5 ± 17.2	1471.7 ± 12.1	1814.6 ± 7.1	<0.0001 (<i>P_{trend}</i>)	1347.1 ± 8.0	1655.7 ± 8.0	2041.2 ± 8.0	<0.0001 (<i>P_{trend}</i>)
Red meat (mg/day) (Mean ± SE) ^b	47.8 ± 1.6	48.1 ± 1.1	51.4 ± 0.6	0.02 (<i>P_{trend}</i>)	49.2 ± 1.1	49.5 ± 0.9	52.1 ± 1.1	0.14 (<i>P_{trend}</i>)
Vegetable (mg/day) (Mean ± SE) ^b	147.0 ± 7.2	220.4 ± 4.9	349.8 ± 2.9	<0.0001 (<i>P_{trend}</i>)	159.0 ± 4.3	278.9 ± 3.6	455.6 ± 4.4	<0.0001 (<i>P_{trend}</i>)
Fruits (mg/day) (Mean ± SE) ^c	138.1 ± 7.9	194.7 ± 5.4	300.2 ± 3.2	<0.0001 (<i>P_{trend}</i>)	164.4 ± 5.1	252.1 ± 4.2	358.2 ± 5.3	<0.0001 (<i>P_{trend}</i>)

Metabolic condition: Participant had at least one of the following conditions: hypertension, obesity, history of heart disease, BMI ≥ 30, or unspecified dyslipidemia.

^aAdjusted by age.

^bAdjusted by age and total energy intake.

BMI, body mass index; SE, standard error; SD, standard deviation.

TABLE 2. ASSOCIATION OF DIETARY ANTIOXIDANT QUALITY SCORE AND COMPOSITE DIETARY ANTIOXIDANT INDEX WITH PREVALENCE OF SELECTED METABOLIC-RELATED DISEASES AND CONDITIONS IN SHANGHAI WOMEN'S HEALTH STUDY

	Yes	No	Dietary Antioxidant Quality Score (OR, 95% CI) ^a			Composite Dietary Antioxidant Index (OR, 95% CI) ^a		
			3-4 (Average quality)	5-6 (High quality)	P _{trend}	Tertile 2	Tertile 3	P _{trend}
Metabolic condition ^b	1449	2404	1.11 (0.87-1.43)	1.17 (0.91-1.50)	0.30	1.22 (1.01-1.47)	1.30 (1.02-1.66)	0.02
Hypertension	1090	2763	1.05 (0.81-1.37)	1.08 (0.84-1.40)	0.83	1.09 (0.90-1.33)	1.02 (0.79-1.32)	0.22
Diabetes	231	3622	1.20 (0.74-1.95)	1.79 (1.11-2.88)	0.009	1.82 (1.24-2.66)	3.06 (1.88-4.97)	<0.0001
Coronary heart disease	382	3471	1.44 (0.97-2.15)	1.55 (1.04-2.30)	0.05	1.45 (1.08-1.95)	1.45 (0.99-2.14)	0.02
Stroke	44	3809	0.61 (0.26-1.42)	0.82 (0.35-1.94)	0.43	0.96 (0.43-2.15)	0.63 (0.18-2.17)	0.84
Obese	258	3595	0.89 (0.58-1.36)	0.82 (0.54-1.25)	0.14	1.08 (0.76-1.52)	1.24 (0.80-1.93)	0.57
Hyperlipidemia	110	3752	1.09 (0.48-2.48)	1.97 (0.91-4.28)	0.03	1.50 (0.86-2.62)	1.88 (0.93-3.80)	0.22

^aModel adjusted for: age, education, occupation, income, baseline menopausal status, total energy intake, vitamin supplemental use; Poor quality score (1-2) as reference group for analysis of Dietary Antioxidant Quality Score and Tertile 1 as reference group for analysis of Composite Dietary Antioxidant Index.

^bMetabolic condition: Participants had at least one of the following conditions: hypertension, obesity, history of heart disease, BMI ≥ 30 , or unspecified dyslipidemia. Values in bold are statistically significant ($p < 0.05$).

CI, confidence interval; OR, odds ratio.

results found in previous clinical trials and population-based studies of the effect of vitamin and mineral supplementation on the levels of various interleukins. For example, in a 12-week double-blind trial of supplementation of daily antioxidants (*i.e.*, 200 μ g selenium, 3 mg manganese, 30 mg D- α -tocopheryl succinate, 450 μ g β -carotene and retinol, and 90 mg ascorbic acid), Trebble *et al.* (6) found significant reductions in IL-6 and TNF- α in peripheral blood mononuclear cells.

In a previous report (3), we showed that while dietary vitamin E was associated with neither 15-F_{2t}-IsoP nor 15-F_{2t}-IsoPM; plasma concentrations of several antioxidants (*i.e.*, β -carotene, 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 both 15-F_{2t}-IsoP and 15-F_{2t}-IsoPM. These results suggest that a combination of dietary and plasma measurements may be needed to improve the measurement of an individual's antioxidant capacity.

Our study has several strengths. First, this is the first study to assess two measurements of aggregate dietary antioxidant intake for their associations with oxidative stress and inflammatory biomarkers. The consistent results from these two indices support their validity. Second, our study utilized resources from a well-designed, population-based, prospective cohort study with an extremely high response rate (92.7%); therefore, selection bias is reduced. Third, dietary intakes in our study were evaluated quantitatively using validated questionnaires. Because of the standardized dietary measurements, dietary vitamins and minerals in our study could be quantified by intake in grams per day.

A major limitation of this study is the presence of pre-existing inflammatory-related diseases/conditions and the use of drugs that might influence the biomarker levels. This concern is minimized by excluding participants who had taken antibiotics within the 24 h before sample collection and adjusting for metabolic condition, diabetes, obesity, stroke, CHD, and hypertension. Analyses stratified by presence of metabolic condition(s) did not reveal any interaction. Furthermore, because a cross-sectional study design was applied, no causal association can be established. Finally, because most participants of this study had a healthy lifestyle (only 2.8% were smokers and less than 8% were obese), there was a low background level of inflammation or oxidative stress, which may have compromised the statistical power of this study.

In summary, we found that both the DAQS and CDAI are highly correlated and are inversely associated with levels of IL-1 β and TNF- α . The DAQS was additionally associated with the reduction of IL-6 and sTNF-R2 levels. Our observation sheds light on the possible biological mechanisms underlying the associations between dietary antioxidants and health outcomes (*i.e.*, cancer, mortality, and cardiovascular diseases).

Notes

Study population

Participants of this study were drawn from the Shanghai Women's Health Study (SWHS), an ongoing population-based cohort study in seven communities of urban Shanghai, as previously described (9). Briefly, between 1996 and 2000, 74,492 women aged 40-70 years were recruited to the study.

TABLE 3. ASSOCIATION OF DIETARY ANTIOXIDANT QUALITY SCORE AND COMPOSITE DIETARY ANTIOXIDANT INDEX WITH OXIDATIVE STRESS AND INFLAMMATORY BIOMARKERS IN THE SHANGHAI WOMEN'S HEALTH STUDY^a

	Dietary Antioxidant Quality Score			Composite Dietary Antioxidant Index		
	3–4 (Average quality)	5–6 (High quality)	<i>p</i> _{trend}	Tertile 2	Tertile 3	<i>p</i> _{trend}
F2-isoprostanes (15-F _{2t} -IsoP) (pg/ml)						
<i>n</i>	268	871		439	437	
β -Coefficient $\pm$ SE	−0.09 $\pm$ 0.32	−0.23 $\pm$ 0.33	0.50	−0.04 $\pm$ 0.19	0.03 $\pm$ 0.26	0.73
<i>p</i> -Value	0.78	0.49		0.83	0.90	
F2-isoprostane metabolites (15-F _{2t} -IsoPM) (pg/ml)						
<i>n</i>	260	815		414	409	
β -Coefficient $\pm$ SE	−0.37 $\pm$ 0.31	−0.33 $\pm$ 0.32	0.54	−0.003 $\pm$ 0.19	0.41 $\pm$ 0.26	0.65
<i>p</i> -Value	0.23	0.31		0.99	0.13	
PGEM (pg/ml)						
<i>n</i>	488	1466		754	734	
β -Coefficient $\pm$ SE	0.06 $\pm$ 0.14	−0.02 $\pm$ 0.14	0.54	0.01 $\pm$ 0.10	0.04 $\pm$ 0.13	0.61
<i>p</i> -Value	0.65	0.87		0.93	0.74	
C-reactive protein (mg/L)						
<i>n</i>	495	1453		721	749	
β -Coefficient $\pm$ SE	− 0.39 $\pm$ 0.15	−0.22 $\pm$ 0.15	0.45	0.07 $\pm$ 0.12	0.17 $\pm$ 0.15	0.67
<i>p</i> -Value	0.01	0.15		0.54	0.25	
IL-1 β (pg/ml)						
<i>n</i>	223	554		276	289	
β -Coefficient $\pm$ SE	− 0.22 $\pm$ 0.13	− 0.28 $\pm$ 0.13	0.02	−0.04 $\pm$ 0.10	− 0.25 $\pm$ 0.13	0.03
<i>p</i> -Value	0.09	0.03		0.71	0.06	
IL-6 (pg/ml)						
<i>n</i>	231	577		294	301	
β -Coefficient $\pm$ SE	− 0.22 $\pm$ 0.13	− 0.30 $\pm$ 0.13	0.06	− 0.19 $\pm$ 0.10	− 0.22 $\pm$ 0.13	0.09
<i>p</i> -Value	0.08	0.02		0.06	0.08	
TNF- α (pg/ml)						
<i>n</i>	246	618		316	320	
β -Coefficient $\pm$ SE	− 0.16 $\pm$ 0.08	− 0.22 $\pm$ 0.08	0.005	−0.11 $\pm$ 0.06	− 0.27 $\pm$ 0.08	0.003
<i>p</i> -Value	0.04	0.005		0.08	0.0007	
Soluble TNF-receptor 1 (pg/ml)						
<i>n</i>	240	588		301	305	
β -Coefficient $\pm$ SE	−0.05 $\pm$ 0.05	−0.09 $\pm$ 0.05	0.18	−0.01 $\pm$ 0.04	−0.06 $\pm$ 0.05	0.34
<i>p</i> -Value	0.29	0.07		0.74	0.24	
Soluble TNF-receptor 2 (pg/ml)						
<i>n</i>	170	402		215	206	
β -Coefficient $\pm$ SE	−0.06 $\pm$ 0.04	− 0.10 $\pm$ 0.04	0.01	−0.02 $\pm$ 0.03	−0.05 $\pm$ 0.04	0.14
<i>p</i> -Value	0.17	0.02		0.54	0.18	
Soluble GP130 (pg/ml)						
<i>n</i>	240	588		302	304	
β -Coefficient $\pm$ SE	−0.02 $\pm$ 0.06	−0.08 $\pm$ 0.06	0.13	−0.06 $\pm$ 0.05	−0.06 $\pm$ 0.06	0.59
<i>p</i> -Value	0.71	0.18		0.19	0.30	

Metabolic condition: Participants had at least one of the following conditions: hypertension, obesity, history of heart disease, BMI $\geq$ 30, or unspecified dyslipidemia.

^aModel adjusted for age, education, occupation, income, baseline menopausal status, total energy intake, vitamin supplemental use, metabolic condition, and assay batch.

Values in bold are statistically significant ($p < 0.05$).

IL-1 β , interleukin-1beta; IL-6, interleukin-6; PGEM, prostaglandin E2 metabolite; TNF- α , tumor necrosis factor-alpha.

Study protocol included a baseline survey on socio-demographic characteristics, lifestyle and dietary habits, and medical history, as well as other exposures and anthropometric measurements, including weight, height, and circumferences of the waist and hips. Of the study participants, 75% ($n = 56,754$) donated a 10 ml-blood sample and 88% ($n = 65,755$) donated a spot urine sample (*i.e.*, collected using a sterilized 100-ml cup containing 125 mg ascorbic acid). Blood and urine samples were kept at 0°C–4°C and were processed within 6 h after collection. All processed samples were then stored at −70°C

for further analyses. Participants provided written informed consent, and the Institutional Review Boards of all participating institutions approved the study protocols.

The potential study participants of this cross-sectional analysis were 3869 SWHS participants who previously had at least 1 of 10 oxidative stress and circulating biomarkers (*i.e.*, urinary F2-isoprostanes [15-F_{2t}-IsoP]; urinary F2-isoprostane metabolites [15-F_{2t}-IsoPM]; urinary prostaglandin E2 metabolite [PGEM]; C-reactive protein [CRP]; IL-1 β ; IL-6; TNF- α ; soluble TNF-receptor 1 [sTNF-R1]; sTNF-R2;

TABLE 4. ASSOCIATION OF DIETARY ANTIOXIDANT QUALITY SCORE AND COMPOSITE DIETARY ANTIOXIDANT INDEX WITH OXIDATIVE STRESS AND INFLAMMATORY BIOMARKERS IN THE SHANGHAI WOMEN'S HEALTH STUDY, STRATIFIED BY METABOLIC CONDITION^a

	Dietary Antioxidant Quality Score			Composite Dietary Antioxidant Index		
	3-4 (Average quality)	5-6 (High quality)	p _{trend}	Tertile 2	Tertile 3	p _{trend}
Without metabolic condition						
F2-isoprostanes (15-F _{2t} -IsoP) (pg/ml)						
n	268	871		439	437	
β-Coefficient ± SE ^b	-0.33 ± 0.43	-0.33 ± 0.42	0.67	-0.15 ± 0.23	-0.002 ± 0.30	0.75
p-Value	0.45	0.43		0.53	0.99	
F2-isoprostane metabolites (15-F _{2t} -IsoPM) (pg/ml)						
n	260	815		414	409	
β-Coefficient ± SE ^b	-0.38 ± 0.47	-0.22 ± 0.46	0.82	0.22 ± 0.26	0.64 ± 0.34	0.28
p-Value	0.42	0.63		0.40	0.07	
PGEM (pg/ml)						
n	488	1466		754	734	
β-Coefficient ± SE ^b	0.13 ± 0.17	0.001 ± 0.16	0.77	-0.09 ± 0.13	-0.06 ± 0.17	0.55
p-Value	0.42	0.99		0.50	0.71	
C-reactive protein (mg/L)						
n	495	1453		721	749	
β-Coefficient ± SE ^b	-0.54 ± 0.21	-0.20 ± 0.20	0.65	0.16 ± 0.16	0.23 ± 0.20	0.69
p-Value	0.01	0.32		0.32	0.26	
IL-1β (pg/ml)						
n	223	554		276	289	
β-Coefficient ± SE ^b	-0.23 ± 0.17	-0.04 ± 0.17	0.02	0.006 ± 0.14	-0.45 ± 0.17	0.003
p-Value	0.18	0.03		0.97	0.01	
IL-6 (pg/ml)						
n	231	577		294	301	
β-Coefficient ± SE ^b	-0.31 ± 0.17	-0.37 ± 0.17	0.06	-0.26 ± 0.13	-0.32 ± 0.17	0.05
p-Value	0.07	0.03		0.05	0.06	
TNF-α (pg/ml)						
n	246	618		316	320	
β-Coefficient ± SE ^b	-0.23 ± 0.11	-0.35 ± 0.10	0.001	-0.14 ± 0.08	-0.39 ± 0.10	0.003
p-Value	0.03	0.001		0.09	0.0002	
Soluble TNF-receptor 1 (pg/ml)						
n	239	585		301	302	
β-Coefficient ± SE ^b	0.01 ± 0.07	-0.07 ± 0.07	0.20	-0.05 ± 0.05	-0.10 ± 0.07	0.43
p-Value	0.90	0.31		0.34	0.13	
Soluble TNF-receptor 2 (pg/ml)						
n	240	588		301	305	
β-Coefficient ± SE ^b	-0.07 ± 0.06	-0.15 ± 0.06	0.003	-0.08 ± 0.04	-0.12 ± 0.06	0.15
p-Value	0.20	0.008		0.08	0.04	
Soluble GP130 (pg/ml)						
n	240	588		302	304	
β-Coefficient ± SE ^b	-0.07 ± 0.10	-0.19 ± 0.09	0.03	-0.12 ± 0.08	-0.13 ± 0.10	0.33
p-Value	0.46	0.04		0.13	0.16	
With Metabolic Condition						
F2-isoprostanes (15-F _{2t} -IsoP) (pg/ml)						
n	268	871		439	437	
β-Coefficient ± SE ^b	0.51 ± 0.54	0.22 ± 0.60	0.99	0.16 ± 0.37	0.32 ± 0.55	0.50
p-Value	0.35	0.72		0.67	0.57	
F2-isoprostane metabolites (15-F _{2t} -IsoPM) (pg/ml)						
n	260	815		414	409	
β-Coefficient ± SE ^b	-0.41 ± 0.44	-0.41 ± 0.51	0.95	0.09 ± 0.37	0.50 ± 0.47	0.52
p-Value	0.36	0.43		0.81	0.30	
PGEM (pg/ml)						
n	488	1466		754	734	
β-Coefficient ± SE ^b	-0.14 ± 0.27	-0.19 ± 0.28	0.54	0.17 ± 0.17	0.28 ± 0.24	0.75
p-Value	0.61	0.49		0.31	0.25	
C-reactive protein (mg/L)						
n	495	1453		721	749	
β-Coefficient ± SE ^b	-0.20 ± 0.24	-0.22 ± 0.25	0.59	-0.004 ± 0.19	0.10 ± 0.24	0.91
p-Value	0.39	0.36		0.98	0.66	

(continued)

TABLE 4. (CONTINUED)

	Dietary Antioxidant Quality Score			Composite Dietary Antioxidant Index		
	3–4 (Average quality)	5–6 (High quality)	P _{trend}	Tertile 2	Tertile 3	P _{trend}
IL-1 β (pg/ml)						
<i>n</i>	223	554		276	289	
β -Coefficient $\pm$ SE ^b	-0.23 $\pm$ 0.20	-0.15 $\pm$ 0.21	0.53	-0.02 $\pm$ 0.16	0.09 $\pm$ 0.21	0.72
<i>p</i> -Value	0.25	0.46		0.88	0.66	
IL-6 (pg/ml)						
<i>n</i>	231	577		294	301	
β -Coefficient $\pm$ SE ^b	-0.07 $\pm$ 0.20	-0.18 $\pm$ 0.20	0.54	-0.11 $\pm$ 0.16	-0.08 $\pm$ 0.21	0.98
<i>p</i> -Value	0.72	0.38		0.48	0.69	
TNF- α (pg/ml)						
<i>n</i>	246	618		316	320	
β -Coefficient $\pm$ SE ^b	-0.02 $\pm$ 0.12	0.01 $\pm$ 0.12	0.94	-0.02 $\pm$ 0.09	-0.05 $\pm$ 0.12	0.42
<i>p</i> -Value	0.84	0.90		0.84	0.66	
Soluble TNF-receptor 1 (pg/ml)						
<i>n</i>	239	585		301	302	
β -Coefficient $\pm$ SE ^b	-0.11 $\pm$ 0.07	-0.10 $\pm$ 0.07	0.77	0.05 $\pm$ 0.05	0.03 $\pm$ 0.07	0.75
<i>p</i> -Value	0.10	0.16		0.38	0.67	
Soluble TNF-receptor 2 (pg/ml)						
<i>n</i>	240	588		301	305	
β -Coefficient $\pm$ SE ^b	-0.03 $\pm$ 0.06	-0.02 $\pm$ 0.06	0.96	0.06 $\pm$ 0.05	0.04 $\pm$ 0.06	0.58
<i>p</i> -Value	0.58	0.70		0.18	0.50	
Soluble GP130 (pg/ml)						
<i>n</i>	240	588		302	304	
β -Coefficient $\pm$ SE ^b	0.04 $\pm$ 0.05	0.06 $\pm$ 0.05	0.23	0.01 $\pm$ 0.04	0.03 $\pm$ 0.05	0.43
<i>p</i> -Value	0.45	0.27		0.85	0.55	

Metabolic condition: Participants had at least one of the following conditions: hypertension, obesity, history of heart disease, BMI $\geq$ 30, or unspecified dyslipidemia.

^aModel adjusted for age, education, occupation, income, baseline menopausal status, total energy intake, and assay batch.

Values in bold are statistically significant ($p < 0.05$).

and soluble GP130 [sGP130]) measured at baseline for various research projects.

Dietary assessment

A validated food frequency questionnaire (FFQ), which included 77 food items and food groups commonly consumed in urban Shanghai, China, was administered in the baseline survey to collect information on participants' usual dietary intake habits in the previous 12 months. Study participants were asked how frequently (five categories: daily, weekly, monthly, yearly, or never) they consumed the food or food group, followed by a question on the amount of food consumed in *liang* (1 *liang* = ~50 g) per unit of time during the previous 12 months. Daily nutrient intakes were then calculated from the FFQ using the nutrient content of each food based on the Chinese Food Composition Tables. The reproducibility and validity of the FFQs were evaluated using 12 monthly 24-h dietary recalls over a 1-year period, which covered the same period as the FFQs. The correlation coefficients were 0.59–0.66 for macronutrients, 0.41–0.59 for micronutrients, and 0.41–0.66 for major food groups in the SWHS.

We used two previously published methods to derive dietary antioxidant indices. The first method, the DAQS, was adapted from Rivas *et al.* (4). The DAQS was calculated based on intake of six antioxidant vitamins and minerals (vitamin A, vitamin C, vitamin E, selenium, manganese, and zinc) derived from the FFQ. We first calculated a ratio between the daily nutrient intake of each of these six nutrients/minerals and their respective daily re-

commended intake as determined by the US Food and Drug Administration (FDA). We then assigned this ratio a value of either 0 or 1, where 0 equaled a ratio of $< 2/3$ and 1 equaled a ratio of $\geq 2/3$. Thus, by assigning a score of 0 or 1 to each of the six nutrients/minerals, the summed DAQS would range from 0 (very poor-quality) to 6 (high-quality) (4). We further categorized the DAQS into three groups: 1–2 (low-quality), 3–4 (average-quality), and 5–6 (high-quality).

The second method, the CDAI, was developed by Wright *et al.* (7). To estimate CDAI, we standardized each of the same six dietary vitamins and minerals by subtracting the global mean and dividing by the global standard deviation. We then calculated the CDAI by summing the standardized intakes of these vitamins and minerals with equal weight, as described next:

$$CDAI = \sum_{i=1}^{n=6} \frac{\text{Individual Intake} - \text{Mean}}{SD}$$

The CDAI was then categorized into three groups using a tertile scale. We also categorized the CDAI results into three groups using a distribution similar to the one used for the DAQS (*i.e.*, 11.1%, 22.8%, and 66.1%). Since the results from the tertile scale and the later distribution scale were similar, here, we present results from the CDAI tertile scale only.

Measurements of oxidative and inflammatory biomarkers and their receptors

Urinary excretion of F2-isoprostanes (15-F_{2t}-IsoP) and urinary F2-isoprostane metabolite [15-F_{2t}-IsoPM] levels were

measured by gas chromatography/negative ion chemical ionization mass spectrometry as reported in our previous research (3). Urinary PGEM level was measured using the liquid chromatography/tandem mass spectrometric method (2). Plasma CRP level was measured using ACE High-Sensitivity C-reactive Protein Reagent (ACI-22) and the CRP (HS) Wide Range kit (Pointe Scientific) on an ACE Clinical Chemistry System (Alfa Wassermann, Inc.). Levels of IL-1 β , IL-6, and TNF- α were measured using the MILLIPLEX MAP High-Sensitivity Human Cytokine multiplex kit (Millipore Corporation), and levels of sTNF-R1, sTNF-R2, and sGP130 were measured using MILLIPLEX MAP Human Soluble Receptor Panel multiplex kits (Millipore Corporation), following the manufacturer's protocol (8).

Statistical analysis

Sixteen participants who had used antibiotics within 24 h before the samples were collected were excluded, leaving a final sample of 3853 for the current analysis. We evaluated the association between the DAQS and CDAI with prevalence of selected metabolic-related conditions using logistic regression. Metabolic condition was defined as having at least one of the following five conditions: hypertension, obesity, history of heart disease, BMI ≥ 30 , or unspecified dyslipidemia. In the multivariable logistic regression model, the following covariates were used: age at baseline (40–49, 50–59, 60–69, 70–79), education (elementary or less, middle school, high school, and professional/college or higher), income (low, lower-middle, upper-middle, high), occupation (professional, clerical, manual laborer/housewife), vitamin supplement use (yes/no), and total energy intake (continuous variable). Since provitamin A and preformed vitamin A are found in foods of animal source, and both vegetable and fruits are primary sources of vitamins C and E, we did not include red meat, vegetable, and fruit intakes in our final models to avoid over adjustments.

A generalized linear model was used to determine the association of DAQS and CDAI with 10 biomarkers, adjusting for a similar set of covariates, plus baseline menopausal status, metabolic condition, and assay batch. Log transformation was used to normalize the distribution of all 10 oxidative stress and inflammatory biomarkers. We calculated β -coefficients for these markers in association with average and high-quality groups or second and third tertile groups, respectively, using DAQS low-quality and CDAI first-tertile as reference groups. Tests for trend were performed by treating the ordinal variable as a continuous variable. We also conducted stratified analysis by metabolic condition. All statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Inc.). All tests were two sided, and $p < 0.05$ was considered statistically significant.

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Abbreviations Used

BMI = body mass index
CDAI = Composite Dietary Antioxidant Index
CHD = coronary heart disease
CI = confidence interval
CRP = C-reactive protein
DAQS = Dietary Antioxidant Quality Score
15-F_{2t}-IsoP = isoprostanes
15-F_{2t}-IsoPM = urinary F₂-isoprostane metabolites
FFQ = food frequency questionnaire
HR = hazard ratio

IL-1 β = interleukin-1beta
IL-6 = interleukin-6
OR = odds ratio
PGEM = prostaglandin E₂ metabolite
ROS = reactive oxygen species
SD = standard deviation
SE = standard error
sGP130 = soluble GP130
sTNF-R1 = soluble TNF-receptor 1
sTNF-R2 = soluble TNF-receptor 2
SWHS = Shanghai Women's Health Study
TNF- α = tumor necrosis factor-alpha