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Plasma total antioxidant capacity is associated with dietary intake and plasma level of antioxidants in postmenopausal women☆,☆☆

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Abstract

Increased plasma total antioxidant capacity (TAC) has been associated with a high consumption of fruits and vegetables. However, limited information is available on whether plasma TAC reflects the dietary intake of antioxidants and the levels of individual antioxidants in plasma. By using three different assays, the study aimed to determine if plasma TAC can effectively predict dietary intake of antioxidants and plasma antioxidant status. Forty overweight and apparently healthy postmenopausal women were recruited. Seven-day food records and 12-h fasting blood samples were collected for dietary and plasma antioxidant assessments. Plasma TAC was determined by vitamin C equivalent antioxidant capacity (VCEAC), ferric-reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) assays. TAC values determined by VCEAC were highly correlated with FRAP (r=0.79, P<.01) and moderately correlated with ORAC (r=0.34, P<.05). Pearson correlation analyses showed that plasma TAC values by VCEAC and ORAC had positive correlation with plasma uric acid (r=0.56 for VCEAC; r=0.49 for ORAC) and total phenolics (r=0.63 for VCEAC; r=0.36 for ORAC). However, TAC measured by FRAP was correlated only with uric acid (r=0.69). After multivariate adjustment, plasma TAC determined by VCEAC was positively associated with dietary intakes of γ-tocopherol (P<.001), β-carotene (P<.05), anthocyanidins (P<.05), flavones (P<.05), proanthocyanidins (P<.01) and TAC (P<.05), as well as with plasma total phenolics (P<.05), α-tocopherol (P<.001), β-cryptoxanthin (P<.05) and uric acid (P<.05). The findings indicate that plasma TAC measured by VCEAC reflects both dietary and plasma antioxidants and represents more closely the plasma antioxidant levels than ORAC and FRAP.

Keywords: Total antioxidant capacity; Dietary antioxidant; Plasma antioxidant; VCEAC; ABTS assay; ORAC assay; FRAP assay

1. Introduction

Considerable epidemiological evidence suggests an association between free-radical-induced oxidative stress and its biochemical consequences and the pathogenesis of many chronic diseases such as cardiovascular disease (CVD), cancer and Alzheimer's disease [1–3]. In order to assess body redox status, researchers in the past decades have studied a number of markers of oxidative stress and antioxidant defense which include measurement of antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidases (GPx) and catalase (CAT) and big molecules such as albumin, bilirubin as well as uric acid. Besides endogenous antioxidant components, exogenous antioxidants such as vitamin E, ascorbic acid, carotenoids and flavonoids are also well known as free radical scavengers [4]. These antioxidants work synergistically to regenerate each other. For example, reduced glutathione regenerates ascorbic acid which then regenerates α-tocopherol from its radical forms [5,6]. Therefore, plasma antioxidant status is the result of interaction and cooperation of various antioxidants. In 1993, a new test was introduced to measure the total antioxidant status *in vivo* [7]. Then, the concept of total antioxidant capacity (TAC) has been developed that considers the synergistic role of those antioxidants rather than the simple sum of individual antioxidants [8].

Different principle-based assays have been developed to measure plasma TAC such as Trolox equivalent antioxidant capacity (TEAC) [7], oxygen radical absorbance capacity (ORAC) [9] and ferric-reducing ability of plasma (FRAP) [10], which were commonly used in recent researches. Although there are some limitations of TAC assays due to their respective mechanisms of resistance to specific oxidation, such assays do provide a reliable measurement of the ability of body fluid to withstand the specific oxidative damage *in vivo*. Recently, Kim et al. [11] modified TEAC assay and developed a method named vitamin C

Abbreviations: AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); CVD, cardiovascular disease; FR, food record; FRAP, ferric-reducing ability of plasma; HPLC, high-performance liquid chromatography; ORAC, oxygen radical absorbance capacity; TAC, total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TG, triglyceride; TRAP, total radical-trapping antioxidant parameters; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VCE, vitamin C equivalent; VCEAC, vitamin C equivalent antioxidant capacity.

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equivalent antioxidant capacity (VCEAC) which shares the same principle with TEAC but used vitamin C instead of Trolox as standard, thus making the value of this method more straightforward for the public, and its weight-based expression enables researchers to link weight-based food consumption data to estimate TAC.

Increasing number of studies reported that an increase in plasma TAC is associated with intake of fruits and vegetables which are rich in antioxidants [12–14]. Nevertheless, there is limited information available on the association of plasma TAC with individual endogenous and exogenous antioxidant components in plasma or with dietary individual antioxidants. Thus, the aims of this study were to measure plasma TAC using three commonly used assays — VCEAC, FRAP and ORAC — in a group of overweight but otherwise healthy postmenopausal women, examine associations between dietary individual antioxidants and plasma TAC and investigate the relationship between individual antioxidants *in vivo* and plasma TAC.

2. Methods

2.1. Study population

A cross-sectional study was conducted in 40–70-year-old, overweight/obese [body mass index (BMI) 25–39.9 kg/m^2], nonsmoking postmenopausal women (n=40) without CVD and other inflammatory diseases. This population was specifically chosen for the following reasons: (1) obesity is a significant risk factor toward oxidative-stress-mediated CVD [15]; (2) obese individuals have a greater degree of chronic inflammation [16]; (3) obese individuals are more likely to have lower antioxidant status, likely due to their less frequent consumption of fruits and vegetables [17]; and (4) risk of CVD in women is significantly increased after menopause. Exclusion criteria included a diagnosis of CVD, diabetes or arthritis (excluding osteoarthritis); currently being treated for cancer (i.e., chemotherapy, radiation therapy); estrogen replacement therapy; slimming diets or alcohol consumption exceeding 2 drinks/day or total of 12 drinks/week.

2.2. Study design

Potential participants were recruited in the Hartford, Connecticut, area through printed flyers, email and newspaper advertisements. The potential subjects who were qualified for this study through a telephone interview were immediately invited to the General Clinical Research Center located in the University of Connecticut Health Center (UCHC) in Farmington, Connecticut, and completed the written informed consent form. Prior to the initiation of the project, its protocols and procedures were reviewed and approved by the Human Investigation Review Committees of the UCHC and University of Connecticut Storrs Campus. At the first screening visit, the subjects were given a brief physical examination by a nurse, including measured weight, height and blood pressures (systolic blood pressure; diastolic blood pressure), followed by an interview regarding their medical, dietary, smoking and alcohol consumption histories, and a fasting blood collection. The eligible participants were instructed to follow their usual dietary habits and on how to record 24-h food record (FR) by an experienced research staff and then asked to bring a completed 7-day FR when they visit the center 7 days after the initial screening.

2.3. Dietary assessment

Each subject's dietary data of all food sources from 7-day FR were input into the Nutrition Data System for Research software (Nutrition Coordinating Center, Minneapolis, MN, USA) for food composition analyses. Flavonoid and proanthocyanidin intakes were estimated by matching food consumption data with the nutrients in flavonoids and proanthocyanidins databases as described in our previous study [18]. The subject's individual antioxidant intake was estimated by multiplying the content of the individual antioxidants (flavonoid, proanthocyanidins, carotenoids and vitamins C and E) by the daily consumption of each selected food item. Individual antioxidant capacity was then determined by multiplying the individual amount of each antioxidant compound by its respective antioxidant capacity expressed as VCEAC [19]. Dietary TAC was determined by summating the individual antioxidant capacities as described in our previous publication. In this study, the TAC values of the subjects' diet were reported as mg VCE per day.

2.4. Blood collection and plasma antioxidant analyses

Twelve-hour fasting blood samples were collected in evacuated containers with EDTA or heparin. Samples were centrifuged immediately in a dark room at 500g for 15 min at 4°C. After plasma samples were isolated, they were immediately separated in small portions and stored at −80°C until analyzed after specific pretreatments for each assay.

The outcome measures included plasma TAC, glucose, lipid profiles [total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)], plasma antioxidant nutrients and enzymes. Plasma TAC was determined by the VCEAC, FRAP and ORAC assays. The VCEAC assay, which was developed by Miller et al. [7] and modified by Kim et al. [11], measures the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical chromogen at 734 nm, using 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) as a thermolabile water-soluble radical initiator. The reduction of ABTS radical chromogen is proportional to the TAC in plasma. The results were expressed as mg vitamin C equivalent (VCE) per liter plasma. The FRAP assay determines the ability of the sample to reduce ferric iron to ferrous iron in a low-pH environment. A colored ferrous-tripyridyltriazine complex is formed during this process and has a maximum absorbance at 593 nm [10]. The results are expressed as μmol Trolox per liter plasma. The ORAC assay determines the antioxidant capacity of plasma by measuring the oxidative degradation of a fluorescent molecule after being mixed and heated with AAPH; it was first developed by Cao et al. [9] and further developed by Huang et al. [20] using microplate fluorescence reader for analyses of large numbers of samples. The results are expressed as μmol Trolox per liter plasma. Plasma total phenolics were analyzed using the Folin–Ciocalteu method described by another study [21].

Plasma vitamin C and uric acid were determined as described [22] using high-performance liquid chromatography (HPLC, Agilent Technology 1200, Santa Clara, CA, USA) with a UV detector, separated with an Eclipse XDB-C18 column (5 μm; 250 mm×4.6 mm, Agilent Technology, Santa Clara, CA, USA) and a Zorbax C18 guard column (5 μm; 12 mm×4.6 mm, Agilent Technology, Santa Clara, CA, USA). The analytical conditions were modified in the present study. In order to preserve vitamin C, an aliquot of plasma was deproteinized with 10% perchloric acid. This sample was then centrifuged (15,000g, 5 min, 4°C), and the supernatant was kept at −80°C until analysis. All plasma samples were pretreated within an hour of blood collection. The mobile phase was 1 mM EDTA, 0.189 mM n-dodecyltrimethylammonium chloride mM, 36.6 mM tetraoctylammonium bromide, 0.05 M sodium hydroxide, 0.06 M acetic acid and 75% (vol/vol) acetonitrile in pH 4.7. Samples and standards were prepared with mobile phase and then analyzed by UV at 265 nm and 280 nm for vitamin C and uric acid, respectively, with a flow rate of 0.5 ml/min.

Vitamin E (as α- and γ-tocopherol) and carotenoids were measured together by an HPLC system (Agilent 1100, Hewlett Packard, Palo Alto, CA, USA) with a photodiode array detector and a C18 RP Symmetry analytical column (5 μm, 250 mm×4.6 mm, Agilent Technology, Santa Clara, CA, USA), as described previously [23,24] with minor modifications. In brief, 500 μl plasma was mixed with 500 μl 0.04% butylated hydroxytoluene prepared in ethanol (wt/vol), 20 μl internal standard and 1 ml purified water. Tocopherols and carotenoids were extracted with hexane (2 ml); the extract was dried under nitrogen and dissolved in 100 μl ethanol. The injected sample was separated isocratically (0.6 ml/min) on a Luna C18(2) column (3 μ; m, 150 mm×3 mm, Phenomenex, Torrance, CA, USA) and detected at 297 nm and 450 nm for vitamin E and carotenoids, respectively. The solvent gradient conditions of binary mobile phases [solvent A, acetonitrile; solvent B, methanol, tetrahydrofuran and methylene chloride (85:5:10)] for the HPLC analysis were as follows: 93% A/7% B from 0 to 7 min, 88.6% A/11.4% B from 8 to 15 min, 21% A/79% B from 16 to 17 min, 16% A/84% B from 18 to 50 min and 93% A/7% B from 51 to 55 min. The flow rate was set at 1 ml/min and changed to 1.8 ml/min at 16 min until 50 min.

SOD, CAT and GPx activities in plasma were determined by using commercially available kits (Cayman Chemical Company, Ann Arbor, MI, USA) [25–28]. One unit of SOD in plasma is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. One unit CAT in plasma is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C. One unit GPx in plasma is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP$^+$ per minute at 25°C.

2.5. Statistical analysis

Statistical Analysis System (SAS) software, release 9.2, 2009 (SAS Institute Inc., Cary, NC, USA) was used for statistical calculation. To test the distribution of plasma TAC estimates and plasma antioxidant nutrients, residual and goodness-of-fit analyses were used. If these analyses showed any evidence of departure from normality, we used log-transformed variables in the analysis. Pearson correlation was performed between VCEAC and FRAP, as well as VCEAC and ORAC. The same procedure was used to estimate the correlation between plasma TAC by each method and individual plasma antioxidant. To evaluate the association between dietary or plasma antioxidants and plasma TAC, participants were evenly divided into two groups according to plasma TAC levels. P values were tested across the median values of plasma TAC in each group by analysis of covariance using the general linear model procedure. To investigate the association between plasma TAC and plasma antioxidant levels, data were analyzed by two regression models. The simple model was adjusted for age, BMI, ethnicity, plasma cholesterol and uric acid (except uric acid itself). The simple model was further adjusted by self-reported hypertension and supplement use to exclude the confounding of self-awareness in health conditions. To investigate the associations between plasma TAC and dietary antioxidant intakes, data were also analyzed by two regression models. The simple model was adjusted for total energy intake. The complex model was adjusted for age, BMI, ethnicity, total energy and self-reported hypertension. Data of dietary intake were further adjusted for supplement use. Data were reported as

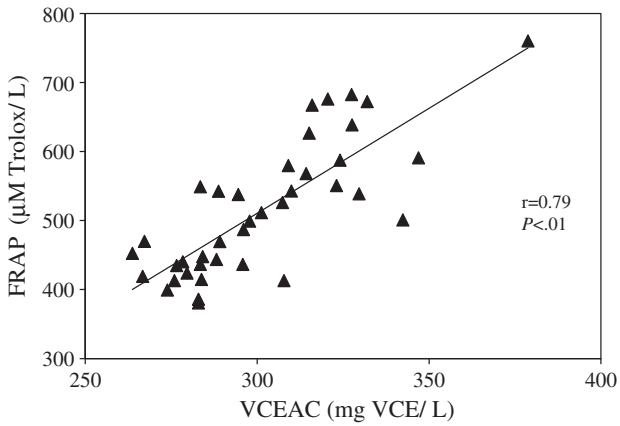

Fig. 1. Pearson correlation between VCEAC and FRAP.

geometric least square means and 95% confidence intervals (CIs) if strongly skewed. The level of statistical significance was set at $P<.05$.

3. Results

Figs. 1 and 2 showed that plasma TAC determined by VCEAC was significantly correlated with FRAP ($r=0.79$, $P<.01$) and ORAC ($r=0.34$, $P<.05$). Pearson correlations shown in Table 1 indicated that plasma TAC determined by VCEAC and ORAC was moderately correlated with plasma uric acid ($r=0.56$; $r=0.49$) and total phenolics ($r=0.63$; $r=0.36$); differently, TAC determined by FRAP was only correlated with uric acid ($r=0.69$), but not with phenolics or other plasma antioxidants. Considering the advantage of the VCEAC method, the results from this method were used in the following analysis.

The participants were evenly divided into two groups according to their plasma TAC assessed by VCEAC. The demographic and clinical characteristics of the participants are summarized in Table 2. The participants in the low and high plasma TAC groups had similar demographic and clinical conditions except that participants in the high plasma TAC group had higher mean BMI ($P<.05$) and moderately higher TG ($P=.05$) which is still within the healthy range.

Individual antioxidant intakes from both diet and supplement were analyzed with respect to the association with plasma TAC determined by VCEAC assay. After adjustments for multiple confounders, plasma TAC determined by VCEAC was positively associated with intakes of γ-tocopherol ($P<.001$), β-carotene ($P<.05$), anthocyanidins ($P<.05$), flavones ($P<.05$), proanthocyanidins ($P<.01$) and TAC ($P<.05$) (Table 3). Some negative correlations were found in α-tocopherol ($P<.05$) and ascorbic acid ($P<.05$).

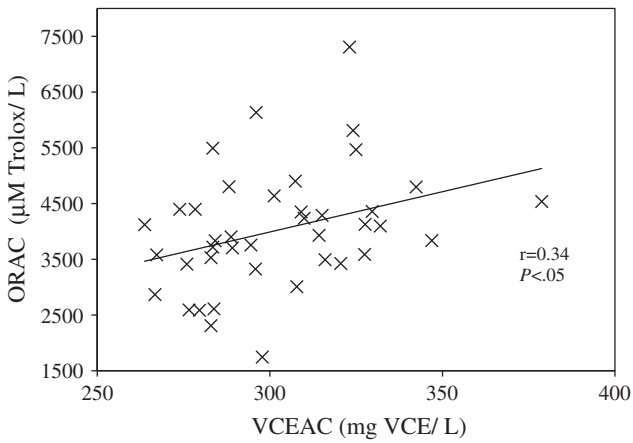

Fig. 2. Pearson correlation between VCEAC and ORAC.

Table 1
Pearson correlations between plasma nutrient profile and plasma TAC measured by VCEAC, FRAP and ORAC assays in the study subjects ($n=40$)

Parameter	VCEAC (mg VCE/L)		FRAP (μmol Trolox/L)		ORAC (μmol Trolox/L)	
	ρ	P	ρ	P	ρ	P
Age (y)	0.1	.54	0.22	.18	0.12	.45
BMI (kg/m^2)	0.43	<.001	0.42	<.01	0.27	.09
Energy (kcal/d)	−0.14	.41	0.02	.88	−0.09	.6
Uric acid (μmol/L)	0.56	<.001	0.69	<.001	0.49	<.01
Vitamin C (μmol/L)	−0.09	.59	−0.18	.28	0.06	.70
Total phenolics (mg GE/L)	0.63	<.001	0.27	.10	0.36	<.05
α-Tocopherol (μmol/L)	0.22	.17	0.10	.55	0.22	.16
γ-Tocopherol (μmol/L)	0.16	.32	0.07	.66	0.09	.57
β-Carotene (μmol/L)	0.16	.33	0.04	.79	−0.16	.34
β-Cryptoxanthin (μmol/L)	0.28	.08	0.16	.33	−0.01	.95
Lutein (μmol/L)	−0.10	.53	0.06	.73	−0.06	.74
Zeaxanthin (μmol/L)	0.10	.52	−0.09	.57	−0.17	.30
Lycopene (μmol/L)	−0.21	.18	−0.31	.06	0.04	.83
Superoxide dismutase (U/ml)	−0.09	.57	−0.21	.20	−0.26	.10
Catalase (nmol formaldehyde /min/ml)	0.06	.71	−0.04	.80	−0.09	.57
Glutathione peroxidase (nmol NADPH/min/ml)	0.29	.07	0.27	.10	−0.057	.73
Albumin (mg/dl)	0.32	.05	0.15	.39	0.02	.92
Bilirubin (mg/dl)	−0.09	.58	0.08	.65	−0.10	.55

The association between individual plasma antioxidant and plasma TAC was also examined (Table 4). After adjustment for age, BMI, ethnicity, plasma cholesterol and uric acid (except uric acid itself), the participants in the high plasma TAC group had higher plasma antioxidant levels including total phenolics ($P<.05$), α-tocopherol ($P<.001$), β-cryptoxanthin ($P<.05$) and uric acid ($P<.05$) than the participants in the low group.

4. Discussion

In this study, plasma TAC determined by VCEAC was significantly correlated with that by FRAP and ORAC, which indicated that the

Table 2
Demographic, clinical and dietary characteristics according to plasma TAC levels of the study subjects

Variables	TAC (mg VCE/L)		P
	Low ($n=20$)	High ($n=20$)	
Ranges[a]	264–296 [283]	298–379 [322]	
Mean	282 ± 9.3	323 ± 18.3	
Age (y)	57 ± 7	59 ± 5	.24
BMI (kg/m^2)	29.0 ± 3.0	31.6 ± 2.7	<.01
Fasting glucose (mg/dl)	96.7 ± 7.7	97.8 ± 11.4	.79
TGs (mg/dl)	84.9 ± 36.3	108 ± 43.6	.05
LDL-C (mg/dl)	128 ± 33.7	144 ± 31.8	.17
HDL-C (mg/dl)	69.6 ± 14.9	64.8 ± 13.2	.29
Total:HDL-C ratio	2.9 ± 0.7	3.3 ± 0.7	.10
Albumin (mg/dl)	4.1 ± 0.2	4.2 ± 0.2	.13
Bilirubin, total (mg/dl)	0.41 ± 0.2	0.37 ± 0.11	.60
Cholesterol, total (mg/dl)	198 ± 33.0	209 ± 33.2	.30
Hypertension (self-reported) (%)	10	15	.48
Hypercholesterolemia (self-reported) (%)	15	13	.73
Antihypertensive (%)	8	5	.64
Aspirin (%)	18	23	.53
NSAID (%)	5	0	.15
Supplement use (%)	23	23	1.00
Energy intake (kcal/d)	1659 ± 297	1788 ± 335	.76

Values are mean ± S.D.
NSAID, nonsteroidal anti-inflammatory drug.
[a] Values within brackets are the medians.

Table 3
Adjusted mean antioxidant intakes by plasma TAC in the study subjects

Intake	Model	TAC (mg VCE/L)				P^b
		Low (n=20)	95% CI	High (n=20)	95% CI	
Ranges[a]		264–296 [283]		298–379 [322]		
Mean		282 ± 9.3		323 ± 18.3		
α-Tocopherol (mg/d)	Model 1[c]	18.7	(10.2 34.1)	20.2	(11.1 36.9)	<.01
	Model 2[d]	21.8	(11.2 42.5)	17.3	(8.89 33.8)	<.05
γ-Tocopherol (mg/d)	Model 1	9.1	(7.9 10.5)	10.0	(8.7 11.4)	<.001
	Model 2	9.3	(8.0 10.8)	9.8	(8.4 11.4)	<.0001
Ascorbic acid (mg/d)	Model 1	114	(81.0 161)	146	(104 205)	<.05
	Model 2	130	(89.0 188)	128	(88.2 186)	<.05
Carotenoid intake (mg/d)	Model 1	12.4	(8.6 17.9)	16.4	(11.4 23.8)	.09
	Model 2	12.6	(7.6 17.7)	17.6	(11.5 26.9)	.37
β-Carotene (mg/d)	Model 1	3.9	(2.9 5.3)	5.0	(3.7 6.8)	<.05
	Model 2	3.9	(2.8 5.5)	5.0	(3.6 7.1)	<.05
α-Carotene (mg/d)	Model 1	0.257	(0.139 0.479)	0.231	(0.124 0.429)	.34
	Model 2	0.198	(0.106 0.372)	0.299	(0.160 0.561)	.2
β-Cryptoxanthin (mg/d)	Model 1	0.074	(0.052 0.104)	0.126	(0.089 0.177)	<.05
	Model 2	0.066	(0.045 0.096)	0.140	(0.097 0.204)	.12
Lutein+zeaxanthin (mg/d)	Model 1	2.20	(1.33 3.64)	2.74	(1.66 4.54)	.11
	Model 2	2.18	(1.22 3.89)	2.77	(1.55 4.93)	.3
Lycopene (mg/d)	Model 1	2.99	(1.15 7.76)	4.08	(1.57 10.6)	.73
	Model 2	2.48	(0.86 7.20)	4.92	(1.70 14.3)	.86
Flavonoid (mg/d)	Model 1	114	(71.5 182)	181	(113 288)	<.05
	Model 2	117	(69.4 197)	176	(105 297)	.2
Isoflavones (mg/d)	Model 1	1.17	(0.62 2.23)	1.29	(0.68 2.44)	.61
	Model 2	1.01	(0.49 2.07)	1.50	(0.73 3.08)	.73
Anthocyanidins (mg/d)	Model 1	15.4	(6.35 37.5)	53.5	(22.0 130)	<.05
	Model 2	13.0	(4.93 34)	63.7	(24.3 167)	<.05
Flavan-3-ols (mg/d)	Model 1	31.3	(14.2 68.7)	58.8	(26.8 129)	<.05
	Model 2	30.3	(12.3 74.3)	60.8	(24.8 149)	.17
Flavanones (mg/d)	Model 1	2.58	(1.19 5.6)	6.97	(3.21 15.1)	.2
	Model 2	3.23	(1.38 7.57)	5.57	(2.37 13.1)	.19
Flavones (mg/d)	Model 1	1.04	(0.638 1.68)	1.56	(0.96 2.53)	<.05
	Model 2	1.10	(0.671 1.81)	1.46	(0.89 2.41)	<.01
Flavonols (mg/d)	Model 1	13.8	(10.4 18.3)	17.8	(13.4 23.6)	.06
	Model 2	14.0	(10.1 19.4)	17.5	(12.6 24.3)	.29
Proanthocyanidins (mg/d)	Model 1	15.5	(10.1 24.0)	38.8	(25.2 59.7)	<.001
	Model 2	13.5	(8.4 21.8)	44.6	(27.7 71.7)	<.001
TAC (mg VCE/d)	Model 1	504	(347 732)	722	(497 1048)	<.01
	Model 2	536	(357 805)	679	(452 1020)	<.05

Values are geometric means (and 95% CIs).
[a] Values within brackets are the medians.
[b] Antioxidant data were log transformed for test for trend. P value was calculated across the median value of TAC from diet in each tertile using general linear model.
[c] Model was adjusted for total energy intake.
[d] Model was adjusted for age, BMI, ethnicity and total energy.

results obtained by the VCEAC assay are relatively consistent and reliable compared with the other two commonly used assays, though the principles of the three assays are not the same. The VCEAC and ORAC assays both reflect antioxidants' radical-scavenging capacity. In contrast, there is no free radical or chain reaction in the FRAP assay system; FRAP tests antioxidants' ferric-reducing ability. It is not surprising that phenolics as effective free radical scavengers due to their hydroxyl groups of the chemical structure were significantly correlated with VCEAC and ORAC, while uric acid, which has high concentration and relatively high reducing ability in plasma, significantly contributed to TAC by all the assays. Results by the VCEAC assay are time sensitive because of the rapid degradation of ABTS radical. In this method as well as in the FRAP assay, reaction is incomplete with respect to plasma at the recommended time [29]. At room temperature, ABTS radical reacts instantaneously with several antioxidants in a few minutes, such as Trolox, ascorbic acid, uric acid, cysteine, glutathione, quercetin and bilirubin, and slowly with albumin and most amino acids [30,31]. Similarly, albumin reacts very slowly and activity is low in the FRAP assay [10]. Cao and Prior [32] also found that FRAP cannot measure the SH-group containing antioxidants such as lipoic acid and some amino acids. Therefore, the VCEAC and FRAP assays are not sensitive to protein levels in plasma, which in fact is a good property because the results can reflect other antioxidants' contribution to the TAC without being interfered by variations in proteins. In contrast, reactions in ORAC assay are driven to the extent that all nonprotein antioxidants and most of the protein are oxidized by the peroxyl radical [9]. In the present study, neither albumin nor bilirubin was correlated with TAC determined by any assay. The possible reason might be that their concentrations or detecting sensitivities were low.

Uric acid is a pitfall, and it drew concern of the researchers about its high contribution to plasma TAC. Some studies found that the antioxidant capacity of plasma was elevated after consumption of apple [33], wine [34], coffee [35] and other foods [36] that are high in phenolics and speculated that it is uric acid that was increased significantly after the consumption and contributed to plasma TAC rather than phenolics in the diet. The physiological function of uric acid is still inconclusive from numerous epidemiological studies. Hyperuricemia has been found to be positively associated with CVD [37,38]; on the other hand, as an important antioxidant, uric acid was reported to be inversely correlated with CVD [39] and to be associated with a reduced rate of cognitive decline in mild cognitive impairment patients [40]. To exclude the confounding of uric acid, when we examined the relationship of plasma antioxidants and plasma TAC, uric acid was adjusted.

α-Tocopherol acts as powerful chain-breaking antioxidant which is mainly located on the surface of the liposome [41]. The mean total

Table 4
Adjusted mean plasma antioxidant profiles according to plasma TAC in the study subjects

Variables	Model	TAC (mg VCE/L)				P^b
		Low (n=20)	95% CI	High (n=20)	95% CI	
Ranges[a]		264–296 [283]		298–379 [322]		
Mean		282 ± 9.3		323 ± 18.3		
Antioxidant enzymes						
Superoxide dismutase (U/ml)	Model 1[c]	12.9	(11.0 15.0)	13.7	(11.7 15.9)	.09
Catalase (nmol formaldehyde /min/ml)	Model 1	23.9	(17.8 32.0)	30.4	(22.7 40.7)	.56
Glutathione peroxidase (nmol NADPH/min/ml)	Model 1	111	(103 118)	111	(104 119)	.59
Antioxidant nutrients						
Ascorbic acid (μmol/L)	Model 2[d]	41.3	(34.2 49.8)	44.0	(36.5 53.1)	.26
Total phenolics (mg GE/L)	Model 1	2115	(2071 2160)	2173	(2127 2220)	<.01
α-tocopherol (μmol/L)	Model 1	30.6	(27.5 34.1)	32.8	(29.5 36.5)	<.001
γ-tocopherol (μmol/L)	Model 1	2.07	(1.49 2.87)	2.19	(1.58 3.04)	.87
β-carotene (μmol/L)	Model 1	0.101	(0.065 0.160)	0.175	(0.112 0.272)	.09
α-carotene (μmol/L)	Model 1	0.03	(0.015 0.060)	0.104	(0.051 0.209)	.19
β-cryptoxanthin (μmol/L)	Model 1	0.211	(0.166 0.270)	0.221	(0.174 0.280)	<.05
Lutein (μmol/L)	Model 1	0.196	(0.107 0.360)	0.125	(0.068 0.230)	.68
Zeaxanthin (μmol/L)	Model 1	0.117	(0.065 0.210)	0.076	(0.042 0.135)	.70
Lycopene (μmol/L)	Model 1	0.089	(0.066 0.120)	0.101	(0.075 0.136)	.30
Uric acid (μmol/L)	Model 2	236	(207 269)	279	(244 318)	<.05

Values are geometric means [and 95% CIs].
[a] Values within brackets are the medians.
[b] Plasma antioxidant data were log transformed for test for trend. P value was calculated across the median value of TAC from diet in each tertile using general linear model.
[c] Model was adjusted for age, BMI, ethnicity, plasma cholesterol and uric acid.
[d] Model was adjusted for age, BMI, ethnicity and uric acid.

α-tocopherol intake of 76 mg/d was much higher than the Estimated Average Requirement of 12 mg/d for adults [42], though our results showed that α-tocopherol intake was negatively correlated with plasma TAC. However, plasma α-tocopherol was strongly associated with plasma TAC in the multivariate model. These results were inconsistent with a previous study [12] that found that total α-tocopherol intake and its plasma level were positively associated with plasma TAC.

In this study, γ-tocopherol intake was significantly correlated with plasma TAC after adjusting for confounders, but plasma γ-tocopherol concentration had no correlation with plasma TAC. Although γ-tocopherol has been much less frequently studied than α-tocopherol, as the major form of vitamin E in the US diet, it has been reported that dietary vitamin E (mainly γ-tocopherol) but not supplemental vitamin E (mainly α-tocopherol) was inversely associated with increased death of CVD [43]. γ-Tocopherol is also a chain-breaking antioxidant, though its plasma concentration is much lower than that of α-tocopherol. Probably, that is why it does not contribute significantly to plasma TAC. In contrast to our finding, Talegawkar et al. [12] found that serum γ-tocopherol was inversely associated with TAC level. One plausible reason is that plasma γ-tocopherol was suppressed by excessive α-tocopherol supplementation [44] among their α-tocopherol supplement users. Recently, γ-tocopherol has been drawing more attention than before [45]. These inconclusive findings in the relationship between γ-tocopherol and plasma TAC warranted further study on γ-tocopherol's role in antioxidant defense *in vivo*.

Ascorbic acid intake was slightly decreased in the high plasma TAC group than the low group, and its plasma level had no correlation with plasma TAC, probably because vitamin C plasma plateau is closely maximized at around 200 mg/d in healthy people [46]. Therefore, excessive vitamin C supplementation did not contribute to plasma vitamin C concentration. Cao et al. [36] found an acute augment in serum TAC after consumption of 1250 mg of vitamin C supplement in eight elderly women. An intervention study also found that vitamin C supplementation improved impaired antioxidant status in hemodialysis patient [47]. Several studies reported that plasma ascorbic acid contributed to plasma TAC [10,48,49], but it was not seen in the present study.

Carotenoids possess antioxidant and provitamin functions. As antioxidants, carotenoids have the ability to scavenge free radicals and protect cells from oxidative damage [50,51]. There was a positive correlation of intake of β-carotene with plasma TAC. A previous study has shown that β-carotene supplement improved plasma antioxidant capacity in older women [52]. Although we did not observe higher β-cryptoxanthin intake with higher plasma TAC, the result was reflected in plasma β-cryptoxanthin and TAC association. β-Cryptoxanthin had the lowest intake level among all carotenoids, though it had the highest plasma concentration which was positively associated with plasma TAC, indicating that β-cryptoxanthin has high bioavailability. This is in the same line with previous studies where β-cryptoxanthin was found to have the highest correlation between intake and plasma concentration among all the carotenoids [53,54].

There have been cumulative studies contributing to the high antioxidant capacity and cardiovascular protective effects of phenolic compounds [55,56]. Although dietary flavonoids intake was not found to be associated with plasma TAC, we did observe significantly positive associations between intakes of flavones (a subgroup of flavonoids) and proanthocyanidins with plasma TAC. According to the previous study, major food sources of total proanthocyanidin intake in the US diet were tea and legumes followed by wine [57]. Tea, which is high in catechins, is the major source of proanthocyanidin monomers and dimers. Participants in the high plasma TAC group consumed a higher amount of tea than those in the low group; this probably explained this association.

Plasma total phenol level has been found to parallel plasma TAC after consumption of fruits and vegetables [36], tea [58,59], red wine [60], chocolate [61] and nuts [62]. In the present study, we found a significant Pearson correlation between VCEAC and total phenolics as a previous study did [63], implying that phenolic compounds are the major contributors to the antioxidant properties in plasma. However, the method to measure total phenol using Folin–Ciocalteu reagent was doubted recently because not only all phenols but also proteins, thiols and many vitamin derivatives are reactive to Folin–Ciocalteu reagent [64]. Therefore, some researchers suggested that Folin–Ciocalteu assay should be seen as a method of TAC rather than total phenolics. Polyphenolics have different intake levels and bioavailabilities, as well as complex metabolites *in vivo*. Future studies need

to consider all the metabolites and develop an assay specific to phenolics.

We found a positive correlation between plasma TAC and intake of TAC which considers the antioxidant sources from both diet and supplement. Although acute studies found significant increase of plasma TAC 1 or 2 h after consumption of antioxidant-rich foods [35,36,62], chronic studies generated inconclusive results [14,65,66]. Long-term intervention studies that investigate antioxidant-rich food or food selection based on TAC in modifying plasma TAC are also warranted.

This study has several limitations. Firstly, this is a cross-sectional study; no cause and effect conclusion can be drawn. Secondly, misreporting can be the result of inaccurately estimating food intake or of undereating or overeating due to dietary restriction or recommendation, though all the subjects were instructed to follow their usual diet.

In conclusion, we found positive associations between plasma TAC determined by VCEAC and intake of antioxidants, as well as plasma antioxidants. among these obese postmenopausal women. VCEAC was more effective than ORAC or FRAP in reflecting plasma antioxidant status.

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