Validation of an FFQ to assess short-term antioxidant intake against 30 d food records and plasma biomarkers

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Abstract

Objective: To validate a brief FFQ developed for capturing short-term antioxidant intake in a sample of US college students.
Design: A seventy-four-item antioxidant FFQ was developed based on major antioxidant sources in the American diet. The FFQ was validated against 30 d food records (FR) and plasma antioxidant concentrations. The reliability of the FFQ was evaluated by two FFQ administered at a 1-month interval.
Settings: University of Connecticut, CT, USA.
Subjects: Sixty healthy college students.
Results: Estimates of dietary antioxidants from the FFQ were moderately to highly correlated with those estimated from the 30 d FR ($r = 0.29$–0.80; $P < 0.05$) except for γ-tocopherol and β-cryptoxanthin. Total antioxidant capacity from diet only or from diet and supplements estimated by the 30 d FR and FFQ were highly correlated ($r = 0.67$ and 0.71, respectively; $P < 0.0001$). The FFQ categorized 91 % of participants into the same or adjacent tertiles of antioxidant intake as the 30 d FR. Most dietary carotenoids estimated from the FFQ were correlated with plasma levels ($P < 0.05$). Correlation coefficients for test–retest reliability ranged from 0.39 to 0.86. More than 94 % of the participants were classified in the same or adjacent tertiles between the two administrations of the FFQ.
Conclusions: The brief FFQ demonstrated reasonable validity for capturing a comprehensive antioxidant intake profile. This FFQ is applicable in epidemiological or clinical studies to capture short-term antioxidant intake or to simply document the variations of antioxidant intake in intervention trials. Cross-validation studies are warranted in other target populations.

Consumption of fruits and vegetables has been associated with low incidence and mortality rate from various degenerative diseases including CVD and cancer[1,2]. One of the major factors behind the protective mechanisms attributed to fruits and vegetables is their high content of vitamins and phytochemicals with antioxidant activity, such as ascorbic acid, tocopherols, carotenoids and flavonoids. Antioxidant intake has been indicated to possess greater daily variation than macronutrient intake and may not be adequately captured by commonly used 3 d or 7 d food records (FR)[3,4]. Alternatively, FFQ can act as an economical and practical method for monitoring dietary antioxidant variation in large populations because of the relatively low participant burden and ease in data processing[5].

A number of FFQ have been developed to estimate antioxidant intake; nevertheless, most FFQ have focused solely on certain antioxidants, such as vitamin C, vitamin E, carotenoids[6,7], isoflavones[8] and catechins in tea[9], and none of them efficiently captures the intake of vitamins and flavonoids comprehensively. Therefore, there is a need for a brief FFQ to effectively capture a wide range of antioxidant intake. Simply combining food items from previous FFQ may not be appropriate, since the resulting instrument may still lack certain important food items for certain antioxidant nutrients such as flavonoids or impose a considerable burden on participants because of its substantial detail[10]. A potentially useful approach to identify a comprehensive food list for assessing both vitamins and flavonoids is the use of dietary total antioxidant capacity (TAC), which can provide an integrated measurement of individual antioxidants[11]. Dietary TAC has been inversely associated with the risk of CVD[12], gastric cancer[12,13] and stroke[14,15], supporting that it may serve as a useful tool for investigating the health effects of antioxidants. Dietary TAC

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estimation in a representative US population has been reported via a newly validated 'theoretical' approach[16] which is not constrained by the limited food items in previously built TAC databases.

Accordingly, our research group has developed a brief FFQ to assess short-term antioxidant intake including both vitamins and flavonoids with antioxidant properties along with TAC estimates. The aims of the present study were to validate this newly developed FFQ against 30 d FR and corresponding plasma antioxidant concentrations and to test the reliability of this assessment instrument in healthy college students in Connecticut, USA.

Materials and methods

Participants and study design
A total of seventy-seven apparently healthy, non-smoking college students aged 18–25 years from the University of Connecticut in Connecticut, USA were recruited. Written informed consent was obtained from all participants. On the initial visit, a screening of anthropometric measurements (height, weight), blood pressure, lipid and glucose profile (Cholestech LDX; Cholestech Corporation, Hayward, CA, USA) and a medical history survey were performed to check the eligibility of participants. Participants who had chronic diseases such as diabetes mellitus, CVD, kidney disease, autoimmune disease, cancer and malnutrition or digestion problems were excluded. The first brief FFQ (FFQ$_1$) was administered to eligible participants by an expert dietitian and instructions were provided for a 30 d FR. At the second visit of 30 d apart, a 12 h fasting venous blood sample was taken to determine plasma antioxidant concentrations and the second FFQ (FFQ$_2$) was administered. In the present study, sixty volunteers were retained for the month of study with a dropout rate of 22 %.

Identification of major antioxidant food sources
Inclusion of items in the FFQ was determined by identifying the regularly consumed food sources contributing most to TAC in the American diet, which was documented in our recent publication[16]. In brief, a flavonoid/proanthocyanidin database was created based on recent US Department of Agriculture (USDA) data sets: the USDA database for the flavonoid content of selected foods (2007 update)[17], the USDA–Iowa State University database on the isoflavone content of foods (2008 update)[18] and the USDA proanthocyanidins database released in 2004[19]. To calculate the antioxidant intake from food sources, we matched the food consumption data of 8809 US adults in the National Health and Nutrition Examination Survey (NHANES) 1999–2000[20] and 2001–2002[21] with the flavonoid/proanthocyanidin database. Daily individual flavonoid/proanthocyanidin intake from selected foods was determined by multiplying the content of the individual flavonoids or proanthocyanidins (mg aglycone equivalent/100 g food) by the daily

consumption (g/d) of the selected food item. Data on individual participants' daily dietary intakes of antioxidant vitamins were available in the NHANES 2001–2002[20,21]. Participants in the NHANES were questioned specifically about their use of vitamin and mineral supplements. To determine TAC scores of food items or dietary supplements, a 'theoretical' method was used in the present study[16]. The antioxidant power of individual antioxidant nutrients, expressed as vitamin C equivalent (VCE) measured by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay, was documented in our previous study[22]. Concisely, antioxidant capacities of forty-three major antioxidant nutrients were measured by the ABTS assay conducted according to Kim *et al.*[23,24]. These antioxidants included thirty flavonoids, four proanthocyanidins, seven carotenoids, two forms of vitamin E and vitamin C. The ABTS assay utilizes quantitative concepts in reference to the familiar vitamin C to measure both hydrophilic and lipophilic antioxidant activities, and its weight-based expression enables researchers to link weight-based food consumption data to estimate TAC[23,24]. Therefore, individual antioxidant intake from diet and supplements was determined by multiplying the content of the individual antioxidant (flavonoids, proanthocyanidins, carotenoids, vitamin C and vitamin E) by the daily consumption of each selected food item. Sum of individual antioxidant intake was then calculated by summing individual antioxidant levels from all food sources reported in NHANES and dietary supplement use. Antioxidant capacity of each antioxidant consumed daily was calculated by multiplying the consumption data of each antioxidant by its respective antioxidant capacity. TAC score from the specific foods consumed daily was assessed by summing individual antioxidant capacities. The 'theoretical' method was validated and applied in several previous studies[22,25,26]. Estimation of theoretical TAC in fifty popular antioxidant-rich food items in the US diet by this approach was proved to be highly correlated with the TAC values of matching food items determined analytically by ABTS and 1,1-diphenyl-2-picrylhydrazyl assays ($r = 0.83$ and $r = 0.70$, respectively)[22]. It was also found to be positively associated with TAC values of forty-four food items from the USDA oxygen radical absorbance capacity database ($r = 0.48$)[22]. According to dietary TAC estimation of the American diet by this method, teas, dietary supplements, fruit and fruit juices, and wine were the major foods or food groups contributing to TAC based on the 24 h dietary recall[16].

Development of the FFQ
The food list for the FFQ was extracted according to the percentage of TAC contributed by each food as follows:

Contribution to TAC (%)

$$= \frac{\text{TAC of specific food item or dietary supplement}}{\text{TAC from both diet and supplements}}.$$

The top food items contributing most to TAC in this food list were selected to cover at least 83 % of the cumulative TAC, and were translated into seventy questions. The final FFQ contained eight food groups (fifteen vegetables and vegetable products, eighteen fruits, twenty-one beverages, two breads and cereals, six dairy and eggs, four oils and seasonings, two sweets and desserts, and two others such as nuts or seeds). Since the TAC scores of vitamin C, α-tocopherol and β-carotene from dietary supplements contributed almost 25 % of the TAC from diet and supplements in Americans according to the previous study[16], vitamin C, α-tocopherol, β-carotene and multivitamins were included in the four dietary supplement questions with dosage information nested. Because limited information is available on supplementary flavonoid composition and the flavonoid intake from supplements was documented to be less than 2 % in US adults[16,27], flavonoid intake from supplements was not included. Food frequency was coded as daily, weekly and monthly, and from 0 to 7 occasions such as none, once monthly and daily, and the FFQ was intended to cover the previous month's consumption of food and supplements. Portion sizes were estimated using three different scales (small, medium and large). Photographic figures for a medium serving size were included to illustrate the portion size.

Estimation of antioxidant intake and total antioxidant capacity from the FFQ

To calculate antioxidant intake and TAC from data collected by the FFQ, a 'medium' serving size of each food item in the FFQ was set as one 'unit' and a 'unit' antioxidant database was created through combining the dietary nutrient profile for individual food items from the Nutrition Data System for Research (NDSR) software release 2010 (University of Minnesota, Minneapolis, MN, USA) with the Flavonoid and Proanthocyanidin Provisional Table developed by the Nutrition Coordinating Center (NCC; University of Minnesota). This NCC provisional table provided a way for NDSR users to link the USDA data with NDSR data via NDSR food identification numbers[28]. Frequency of intake on the FFQ was converted proportionally to daily units. Consequently, the daily antioxidant intake from food was calculated as follows:

Daily antioxidant intake from food

$$= \sum (\text{Unit nutrient profile} \times \text{Frequency} \times \text{Portion size}).$$

Vitamin C, α-tocopherol and β-carotene intakes from dietary supplements were determined from the addition of single-nutrient supplements and from multivitamin use. The default dietary supplements database in NHANES 2007–2008 was applied to explore the strength of the supplements[29]. The mean dose of multivitamins per day was calculated by the frequency of intake.

TAC from food or from dietary supplements was obtained by multiplying the daily antioxidant intake from food or dietary supplements by the individual antioxidant capacity analysed through ABTS assay.

30 d Food records

The 30 d FR was chosen as the reference method because it is reliable in measuring antioxidant intake with a high day-to-day variation[3] and because its measurement errors are usually not correlated with those in FFQ[30]. The study dietitian trained the participants to include all foods, beverages and dietary supplements consumed during the thirty consecutive days and reviewed the records to check for errors or omissions every day. Dietary intake data were collected and analysed by using the NDSR and the NCC Flavonoid and Proanthocyanidin Provisional Table. Dietary supplement data were estimated through NDSR updated with an NCC enhanced version of the NHANES Dietary Supplement Database 2007–2008[28]. TAC from diet and TAC from supplements were obtained by multiplying antioxidant profiles by the antioxidant capacities. Antioxidants estimated from 30 d FR were divided by 30 to generate daily average intake data.

Plasma antioxidant and total antioxidant capacity measurement

A 12 h fasting blood sample for plasma antioxidant analysis was collected in Vacutainer tubes containing heparin sodium at the second visit. Samples were centrifuged immediately at 3000 **g** for 10 min at 4°C. Plasma was separated and stored at −80°C until further measurements. Plasma vitamin C and uric acid were measured on deproteinized plasma by HPLC with UV detection as described by Ross[31]. In order to preserve vitamin C, an aliquot of plasma was deproteinized with 10 % (w/w) perchloric acid. This sample was then centrifuged (15 000 **g**, 5 min, 4°C) and the supernatant was kept at –80°C until analysis. Plasma α-tocopherol and γ-tocopherol were analysed using HPLC[32]. The slightly modified method described by Karppi et al.[33] was used for carotenoid analyses. Plasma TAC was determined by the ABTS assay developed by van den Berg et al.[34] and modified by Kim et al.[23,24]. Lipid profiles including total cholesterol, TAG and glucose were measured using the Cobas C111 analyser (Roche Diagnostics, Indianapolis, IN, USA).

Statistical analyses

All statistical analyses were carried out with the SAS statistical software package version 9·2. Descriptive statistics were computed to describe sociodemographic characteristics, daily antioxidant intakes estimated from the 30 d FR and the two FFQ, daily TAC from food and supplements, plasma antioxidant/TAC levels and lipid profiles. Antioxidant intakes, TAC from diet and TAC from both diet and supplements were reported in the same units as the 30 d FR and FFQ. Crude data from the FFQ

and 30 d FR were not log-transformed since the distribution test through residual and goodness-of-fit analyses did not show improvement of normality. Spearman rank correlation coefficients were calculated between the dietary nutrients estimated from FFQ_2 and the 30 d FR (validity) and between dietary nutrients estimated from FFQ_1 and FFQ_2 (test–retest reliability). Spearman rank correlation coefficients were also examined between the respective plasma and dietary nutrients after adjusting for age, BMI, ethnicity, gender and (except for vitamin C) plasma cholesterol and plasma TAG. Attenuated correlation coefficients were not corrected given that a relatively large repetitive FR was collected[3]. Percentage agreement was assessed by calculating the percentages of participants classified into the same or adjacent tertiles of antioxidant intake by the 30 d FR and FFQ_2 or by FFQ_1 and FFQ_2. Misclassification was reported as the percentage of participants categorized into the extreme opposite tertiles. Significance was set to a value of $P < 0.05$ for two-sided testing.

Ethical approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Connecticut Institutional Review Board.

Results

The majority of college students who participated in the present study were non-Hispanic white (Table 1). Fifteen participants reported taking a daily multivitamin. Eight students took a daily supplement containing vitamin C only and none of the students reported ever taking supplements with either vitamin E only or β-carotene only. The levels of plasma α-tocopherol, carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein+zeaxanthin, lycopene) and TAC are also shown in Table 1.

Daily antioxidant intakes

Mean total energy intake was 8694 (SD 2791) kJ/d (2078 (SD 667) kcal/d) based on the 30 d FR (Table 2). Daily consumption of vitamin C, vitamin E, carotenoids, flavonoids and proanthocyanidins estimated from FFQ_2 accounted for 78.0% (78.5% including vitamin C supplements), 46.7% (53.6% including vitamin E supplements), 79.9%, 111.2% and 30.1% of those estimated from the 30 d FR, respectively. TAC from diet and TAC from both diet and supplements estimated by FFQ_2 were almost equal to those estimated from the 30 d FR (104.8% and 102.8%, respectively; Table 2).

Validity

To test the validity, daily antioxidant intakes or TAC values from diet and from diet and supplements estimated from FFQ_2 were compared with those obtained from the 30 d FR (Table 3). Spearman rank correlations to

Table 1 Demographic characteristics and plasma antioxidant concentrations: college students aged 18–25 years who completed a 30 d FR and two FFQ (*n* 60), Connecticut, USA

Characteristic	%	
Gender		
Male	33.3	
Female	66.7	
Ethnicity		
Non Hispanic white	80.0	
Non-Hispanic black	5.0	
Hispanic	3.3	
Asian or Pacific Islander	11.7	
Supplement use		
Multivitamins	25.0	
Vitamin C	13.3	
Vitamin E	0.0	
	Mean	**SD**
Age (years)	19.8	1.8
BMI (kg/m²)	23.1	2.7
Plasma antioxidants (μmol/l)		
α-Tocopherol	23.6	7.1
Vitamin C	70.2	10.8
β-Carotene	0.32	0.36
α-Carotene	0.19	0.19
β-Cryptoxanthin	0.24	0.16
Lutein+zeaxanthin	0.03	0.01
Lycopene	0.03	0.02
Plasma TAC (mg VCE/l)†	295.7	21.8

30 d FR, 30 d food record; TAC, total antioxidant capacity; VCE, vitamin C equivalent; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid.
†Plasma TAC was measured by ABTS assay and expressed as mg VCE/l.

estimate validity fell within the range of 0.29 to 0.80 ($P < 0.05$) except for γ-tocopherol and β-cryptoxanthin, while TAC from diet and TAC from diet and supplements were highly correlated between FFQ_2 and the 30 d FR ($r = 0.67$ and 0.71, respectively; $P < 0.0001$). The proportion of participants categorized in the same or adjacent tertile of dietary intake averaged 53% or 37%, respectively (Table 3). Therefore, on the basis of all antioxidants, on average 9% were misclassified into the opposite tertiles.

Plasma and dietary antioxidants and TAC were compared (Table 4). Significant Spearman rank correlation coefficients were observed between dietary carotenoids estimated from FFQ_2 (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin from diet) and their corresponding plasma levels in the range of 0.27 to 0.50 ($P < 0.05$). Adjustment for age, gender, BMI, plasma cholesterol and plasma TAG did not appreciably change the significance of these correlations. Neither TAC from diet nor TAC from diet and supplements was correlated with plasma TAC level. These associations found with FFQ_2 were comparable to those from the 30 d FR, while the plasma–diet correlation regarding α-tocopherol was relatively stronger in the 30 d FR than in FFQ_2.

Test–retest reliability

Correlation coefficients to evaluate the reliability of the FFQ by comparing the two FFQ collected at 1-month interval

Table 2 Daily dietary antioxidant intakes and TAC estimated from the 30 d FR and FFQ₂: college students aged 18–25 years who completed a 30 d FR and two FFQ (*n* 60), Connecticut, USA

	Daily intake					
	30 d FR			FFQ₂		
Nutrient	Mean	SD	Median	Mean	SD	Median
Energy (kJ/d)	8694	2791	8552	–	–	–
Energy (kcal/d)	2078	667	2044	–	–	–
Diet vitamin E (mg/d)	28·30	13·34	24·01	13·21	11·77	9·05
Diet+supplement vitamin E (mg/d)†	31·26	15·08	26·95	16·77	13·93	12·75
Diet α-tocopherol (mg/d)	13·02	10·19	9·86	4·43	3·56	3·36
Diet+supplement α-tocopherol (mg/d)	15·62	12·88	10·47	7·98	8·40	4·80
Diet γ-tocopherol (mg/d)	12·30	5·23	11·65	6·65	6·93	4·19
Diet vitamin C (mg/d)	113·42	54·26	108·48	88·48	64·00	75·05
Diet+supplement vitamin C (mg/d)	154·15	138·34	117·38	120·95	144·10	91·05
Diet carotenoids (μg/d)	13 448	8579	11 144	10 750	8580	8040
β-Carotene (μg/d)	4154	2882	3481	2780	2450	1930
α-Carotene (μg/d)	702	644	524	510	590	300
β-Cryptoxanthin (μg/d)	185	196	161	80	90	50
Lutein+zeaxanthin (μg/d)	2546	1959	2003	2900	3980	1320
Lycopene (μg/d)	5862	6138	4059	4490	6320	2460
Diet flavonoids (mg/d)	131·09	174·12	69·39	145·80	167·03	78·69
Isoflavones (mg/d)	4·13	5·30	2·32	2·18	4·44	0·28
Anthocyanins (mg/d)	17·06	31·02	8·93	26·43	59·86	14·80
Flavan-3-ols (mg/d)	84·07	157·04	31·67	88·08	141·52	23·69
Flavanones (mg/d)	10·44	11·67	5·91	23·35	28·47	11·34
Flavones (mg/d)	1·56	1·36	1·13	1·14	1·35	0·73
Flavonols (mg/d)	13·83	9·59	12·09	15·76	14·04	10·16
Diet proanthocyanidins (mg/d)	99·89	103·55	72·13	30·05	23·30	26·89
Diet TAC (mg VCE/d)	456·15	431·47	334·08	478·12	463·35	292·82
Diet+supplement TAC (mg VCE/d)	497·69	451·65	355·14	511·88	483·63	320·36

30 d FR, 30 d food record; TAC, total antioxidant capacity; VCE, vitamin C equivalent.
†'Diet+supplement' means the antioxidants were estimated from both food and dietary supplements.

were moderate to high ($r = 0\cdot39\text{--}0\cdot86$; $P < 0\cdot01$; Table 3). The two FFQ categorized the majority of participants into the same (average 62%) or adjacent (average 32%) tertile of antioxidant intake, while only 6% on average were misclassified into the opposite tertile (Table 3).

Discussion

In the present study, most dietary vitamins and flavonoids estimated from the newly developed brief antioxidant FFQ were significantly correlated with those estimated from the 30 d FR and plasma biomarkers, which suggested the FFQ to effectively capture major antioxidant intake in these college students residing in Connecticut, USA. Thus the developed FFQ could be used to assess a comprehensive range of antioxidant intakes during a short period in epidemiological or clinical settings or to simply monitor variations of antioxidant intakes in intervention trials.

In theory, during the development of an FFQ for a wide range of antioxidants, the selection of foods not only incorporates those food items that are rich in specific antioxidants, but also involves regularly consumed antioxidant sources, as well as those that may substantially contribute to dietary antioxidant variations[10]. The present study identified such food sources that coincide with the

aforementioned criteria through ranking dietary TAC scores from food items consumed most in the USA. Food items high in dietary TAC thus represented an antioxidant source that was either commonly consumed or richest in certain or total antioxidants. Furthermore, the new 'theoretical' approach to assess dietary TAC was used in the present study, which added diverse individual antioxidant capacities of food items consumed daily[16]. It was validated by positive linking with TAC values obtained from the USDA oxygen radical absorbance capacity database[22].

Daily intakes of carotenoids, vitamin C, flavonoids and TAC estimated from the 30 d FR and FFQ were generally comparable, with a difference of 10% to 30%. However, daily intakes of vitamin E and proanthocyanidins estimated from the FFQ were considerably lower than those estimated from the 30 d FR. Previous studies documented that dietary vitamin E in the US diet was derived mainly from grains, fat, oils and dressings, meat, poultry and fish[27], while proanthocyanidins were abundant in legumes and wines but not in vegetables and fruits[35]. Our FFQ was based on the food items most contributing to the TAC. Since the antioxidant capacities of various fats, meat or legumes are much lower than those of vegetables and fruits, the exclusion of the food items mentioned above may partially explain the underestimation.

However, different from the FR or dietary recall which is used for evaluating the absolute intake quantitatively,

Table 3 Spearman rank correlation coefficients and cross-classification between FFQ_2 and the 30 d FR (validity) and between FFQ_2 and FFQ_1 (reliability): college students aged 18–25 years who completed a 30 d FR and two FFQs (*n* 60), Connecticut, USA†

Nutrient	FFQ₂ v. 30 d FR (validity)				FFQ₂ v. FFQ₁ (reliability)			
		Cross-classification Percentage classified in				Cross-classification Percentage classified in		
	Spearman correlation coefficient	Same tertile	Adjacent tertile	Opposite tertile	Spearman correlation coefficient	Same tertile	Adjacent tertile	Opposite tertile
Diet vitamin E	0·29*	51	37	12	0·68***	62	33	5
Diet+supplement vitamin E‡	0·40**	49	43	8	0·73***	60	37	3
Diet α-tocopherol	0·45**	51	37	12	0·75***	55	40	5
Diet+supplement α-tocopherol	0·61***	48	37	15	0·77***	68	27	5
Diet γ-tocopherol	0·08	37	43	20	0·66***	66	27	7
Diet vitamin C	0·40*	51	37	12	0·56***	57	33	10
Diet+supplement vitamin C	0·56***	57	33	10	0·55***	53	37	10
Diet carotenoids	0·66***	55	40	5	0·75***	67	30	3
β-Carotene	0·73***	61	37	2	0·83***	71	27	2
α-Carotene	0·68***	60	33	7	0·86***	78	20	2
β-Cryptoxanthin	0·23	38	47	15	0·67***	57	40	3
Lutein+zeaxanthin	0·80***	67	33	0	0·71***	54	43	3
Lycopene	0·44***	53	37	10	0·64***	58	37	5
Diet flavonoids	0·69***	58	37	5	0·65***	62	33	5
Isoflavones	0·59***	63	30	7	0·72***	70	23	7
Anthocyanins	0·46***	52	40	8	0·39**	55	33	12
Flavan-3-ols	0·72***	71	27	2	0·74***	62	33	5
Flavanones	0·49***	45	47	8	0·63***	60	37	3
Flavones	0·31*	45	37	18	0·68***	69	23	8
Flavonols	0·66***	60	33	7	0·68***	57	40	3
Diet proanthocyanidins	0·46***	48	43	8	0·65***	62	33	5
Diet TAC	0·67***	58	37	5	0·62***	70	20	10
Diet+supplement TAC	0·71***	60	37	3	0·60***	57	33	10

30 d FR, 30 d food record; TAC, total antioxidant capacity.
Spearman's correlation coefficient between antioxidant intakes estimated from 30 d FR and FFQ_2, or from FFQ_1 and FFQ_2, was significantly different from zero: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
†FFQ_1 and FFQ_2 were administered at 1-month interval.
‡'Diet+supplement' means the antioxidants were estimated from both food and dietary supplements.

the FFQ is usually used to rank individuals from low to high intakes for associating dietary patterns with health outcomes[5]. In the present study, correlation coefficients for most individual antioxidant intakes and TAC values were above 0·4, which is considered reasonable and acceptable in FFQ validation studies[36,37]. Furthermore, correlation coefficients for antioxidant intakes in the present FFQ were comparable to those previously measured by either whole-food FFQ or brief FFQ for specific antioxidants. For instance, vitamin C correlation was relatively low but within the range of 0·27 to 0·71 reported by previous studies using FR as a reference[38–43] and was similar to or higher than those in FFQ with 1-month reference period. Correlation coefficients for carotenoid subclasses were generally higher than those from preceding FFQ[44,45]. Additionally, since the correlation analysis for testing validity has been questioned for its failure in measuring agreement[3,46], cross-classification was used in the present study to bridge this gap[47]. Cross-classification for the current FFQ indicated reasonable agreement across tertiles of antioxidant intake between the 30 d FR and FFQ and an acceptable low misclassification percentage, which further suggested that our FFQ could provide a similar

ranking of antioxidant intake as did the 30 d FR. The agreement or misclassification percentages were in accordance with the validation studies conducted by Dunn *et al.*[48] and Stiegler *et al.*[6]. To sum up, the correlation analysis along with the agreement categorization provided sufficient data to judge the overall ability of the FFQ on dietary antioxidant estimation and emphasized the reasonable validity of this new instrument.

Biochemical indicators of dietary intake are another useful approach to weigh FFQ validity, although this method is still prone to random and systematic errors[6]. In the present study, associations between questionnaire-derived antioxidant intakes and biomarkers were comparable to or stronger than those reported by the previous studies applying biomarkers[38,44,45,49–51]. For instance, the correlation between diet and plasma α-tocopherol level was as low as those reported previously[38,44,45,49–51]. The weak correlation was probably attributable to measurement errors of the FFQ as addressed by Dixon *et al.*[49], including under-reporting, poor assessments of fats and oils, and high variability of vitamin E content in the food composition databases. The positive diet–plasma relationships for several carotenoids were in accordance

Table 4 Spearman rank correlation coefficients between dietary antioxidants or TAC estimated from FFQ$_2$ or the 30 d FR and corresponding plasma antioxidant or TAC levels: college students aged 18–25 years who completed 30 d FR and two FFQs (n 60), Connecticut, USA

| | FFQ$_2$ | | 30 d FR | |
| | Spearman correlation | | Spearman correlation | |
Plasma v. diet	Coefficient	P value	Coefficient	P value
α-Tocopherol				
Unadjusted	0·28	0·043	0·18	0·164
Multivariate adjusted†	0·28	0·057	0·40	0·011
α-Tocopherol (including dietary supplements)				
Unadjusted	0·16	0·251	0·20	0·123
Multivariate adjusted†	0·14	0·353	0·36	0·006
γ-Tocopherol				
Unadjusted	−0·14	0·330	0·13	0·343
Multivariate adjusted†	−0·16	0·284	0·16	0·269
Vitamin C				
Unadjusted	0·07	0·594	0·02	0·890
Multivariate adjusted†	0·13	0·353	0·09	0·527
Vitamin C (including dietary supplements)				
Unadjusted	0·10	0·481	0·05	0·693
Multivariate adjusted†	0·14	0·316	0·12	0·388
β-Carotene				
Unadjusted	0·27	0·047	0·38	0·004
Multivariate adjusted†	0·37	0·011	0·47	0·0006
α-Carotene				
Unadjusted	0·50	0·0001	0·57	<0·0001
Multivariate adjusted†	0·57	<0·0001	0·60	<0·0001
β-Cryptoxanthin				
Unadjusted	0·38	0·004	0·28	0·043
Multivariate adjusted†	0·42	0·003	0·25	0·080
Lutein+zeaxanthin				
Unadjusted	0·31	0·021	0·21	0·116
Multivariate adjusted†	0·31	0·036	0·20	0·156
Lycopene				
Unadjusted	0·05	0·740	0·14	0·366
Multivariate adjusted†	0·05	0·752	0·10	0·569
TAC				
Unadjusted	0·21	0·100	0·32	0·037
Multivariate adjusted‡	0·09	0.540	0.15	0.319
TAC (including dietary supplements)				
Unadjusted	0.18	0.164	0.25	0.052
Multivariate adjusted‡	0.05	0.738	0.02	0.907

30 d FR, 30 d food record; TAC, total antioxidant capacity.
†Models were adjusted for age, gender, ethnicity, BMI and (except for vitamin C) plasma cholesterol, and plasma TAG. For associations between antioxidants from diet only and plasma concentrations, dietary supplement use was adjusted.
‡Models were adjusted for age, gender, ethnicity, BMI and plasma uric acid. For associations between TAC from diet only and plasma concentrations, dietary supplement use was adjusted.

with those reported by other FFQ validation studies[45,49] and quantifications of such a relationship remained within the range of previously reported correlation coefficients: from 0·31 to 0·56 for α-carotene[44,45,50], from 0·22 to 0·33 for β-carotene[44,45,49,50], from 0·28 to 0·62 for β-cryptoxanthin[45,49,50] and from 0·15 to 0·24 for lutein/zeaxanthin[45,49,50]. However, the diet–plasma correlation for lycopene was not within the range of 0·12 to 0·42[45,49,50], which might be attributable to the exclusion of certain mixed dishes that are rich in lycopene such as pizza or pasta from the current FFQ, although tomato sauces and ketchup were included. Nevertheless, validation regarding vitamin C varied and did not produce consistent correlations[40]. The use of vitamin C as a surrogate marker is limited due to its instability and 'threshold' effect[52,53]. Moreover, certain correlation coefficients between dietary and plasma TAC in the present study were

comparable to the results of Rautiainen et al.[54]. However, whether plasma TAC is a good reference to validate FFQ-based TAC estimates is still inconclusive[54,55]. Low bioavailability of flavonoids and proanthocyanidins might interfere the correlation[56]. Plasma TAC was found to increase immediately after a high-antioxidant diet but decrease to normal level after a few hours[57]. Besides, plasma TAC may be affected by plasma protein, uric acid and antioxidant enzymes rather than antioxidant nutrients and their metabolites[58]. As a result, plasma TAC may not be used as a surrogate measurement of dietary TAC[55]. Furthermore, the diet–plasma correlations of the FFQ and 30 d FR demonstrated comparable patterns, such as the significant associations between plasma and dietary carotenoids (except for lycopene). These results were indicative of a relatively strong association between the two dietary assessment tools.

There are several strengths of the present study. The FFQ captures intake of a wide range of vitamins and flavonoids with antioxidant properties with one administration, and is also useful to estimate the integrated TAC parameter. The food list derived from dietary TAC for commonly consumed food items covered the dietary sources richest in specific antioxidants, regularly consumed and most influencing antioxidant variations. Besides, involvement of supplements increased the ability of the FFQ for evaluating overall antioxidant status. Furthermore, a 30 d FR served as a reference for the FFQ for 1-month diet estimates.

Our study also had limitations. Although there was reasonable validity of the FFQ, the fact that it was validated in a small sample of American college students in Connecticut does not imply it will perform equally well in the other US populations. Cross-validation studies of the FFQ in external populations are important before its application. Additionally, a period of 1 month was used in the newly developed FFQ, considering the most accurate reference method selected (i.e. 30 d FR) to capture high variations of antioxidant intake. However, the short period may serve as a major drawback and restrict its application in epidemiological studies. As a result, whether it could be extended to adequately assess long-term habitual antioxidant intake needs further investigation. Moreover, whether the participation fatigue caused by the consecutive 30 d FR would affect the reliability of this reference may also need justification.

Conclusions

Associations between dietary estimates from the FFQ, the 30 d FR and plasma antioxidant concentrations were in accordance with those reported by previous validation studies. The brief FFQ generally performed as well as the 30 d FR in estimating a comprehensive dietary antioxidant profile during a short time. This FFQ may be used in epidemiological or clinical studies to capture short-term antioxidant intakes or to simply document variations of antioxidant intakes in intervention trials.

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References

1. Genkinger JM, Platz EA, Hoffman SC *et al.* (2004) Fruit, vegetable, and antioxidant intake and all-cause, cancer, and cardiovascular disease mortality in a community-dwelling population in Washington County, Maryland. *Am J Epidemiol* **160**, 1223–1233.
2. Heidemann C, Schulze MB, Franco OH *et al.* (2008) Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. *Circulation* **118**, 230–237.
3. Willett W (2002) *Nutritional Epidemiology.* New York: Oxford University Press.
4. Davis CG (2010) Estimation of the number of days required to determine usual antioxidant intakes and assessment of the prevalence of nutrient inadequacy among college students. Master Thesis, University of Connecticut.
5. Andersen LF, Veierod MB, Johansson L *et al.* (2005) Evaluation of three dietary assessment methods and serum biomarkers as measures of fruit and vegetable intake, using the method of triads. *Br J Nutr* **93**, 519–527.
6. Stiegler P, Sausenthaler S, Buyken AE *et al.* (2010) A new FFQ designed to measure the intake of fatty acids and antioxidants in children. *Public Health Nutr* **13**, 38–46.
7. Cena H, Roggi C & Turconi G (2008) Development and validation of a brief food frequency questionnaire for dietary lutein and zeaxanthin intake assessment in Italian women. *Eur J Nutr* **47**, 1–9.
8. Patterson RE, Kristal AR, Tinker LF *et al.* (1999) Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* **9**, 178–187.
9. Hakim IA, Hartz V, Harris RB *et al.* (2001) Reproducibility and relative validity of a questionnaire to assess intake of black tea polyphenols in epidemiological studies. *Cancer Epidemiol Biomarkers Prev* **10**, 667–678.
10. Somerset SM & Johannot L (2008) Dietary flavonoid sources in Australian adults. *Nutr Cancer* **60**, 442–449.
11. Serafini M & Del Rio D (2004) Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? *Redox Rep* **9**, 145–152.
12. Puchau B, Zulet MA, de Echavarri AG *et al.* (2009) Dietary total antioxidant capacity is negatively associated with some metabolic syndrome features in healthy young adults. *Nutrition* **26**, 534–541.
13. Serafini M, Bellocco R, Wolk A *et al.* (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology* **123**, 985–991.
14. Rautiainen S, Larsson S, Virtamo J *et al.* (2012) Total antioxidant capacity of diet and risk of stroke: a population-based prospective cohort of women. *Stroke* **43**, 335–340.
15. Del Rio D, Agnoli C, Pellegrini N *et al.* (2011) Total antioxidant capacity of the diet is associated with lower risk of ischemic stroke in a large Italian cohort. *J Nutr* **141**, 118–123.
16. Yang M, Chung SJ, Chung CE *et al.* (2011) Estimation of total antioxidant capacity from diet and supplements in US adults. *Br J Nutr* **106**, 254–263.
17. Agricultural Research Service, US Department of Agriculture (2003) *Database for the Flavonoid Content of Selected Foods.* Beltsville, MD; available at http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.pdf
18. Agricultural Research Service, US Department of Agriculture (2002) *USDA–Iowa State University Database on the Isoflavone*

Content of Foods, Release 1.3. Beltsville, MD: USDA; available at http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html

19. Agricultural Research Service, US Department of Agriculture (2004) Database for the Proanthocyanidin Content of Selected Foods. Beltsville, MD: USDA; available at http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.html

20. National Center for Health Statistics (2002) National Health and Nutrition Examination Survey, 1999–2000 Data Files. Hyattsville, MD: Centers for Disease Control and Prevention; available at http://www.cdc.gov/nchs/nhanes/nhanes1999-2000/nhanes99_00.htm

21. National Center for Health Statistics (2004) National Health and Nutrition Examination Survey, 2001–2002 Data Files. Hyattsville, MD: Centers for Disease Control and Prevention; available at http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l36_b_doc.pdf

22. Floegel A, Kim D-O, Chung S-J et al. (2010) Development and validation of an algorithm to establish a total antioxidant capacity database of the US diet. Int J Food Sci Nutr 61, 600–623.

23. Kim D-O, Chun OK, Kim YJ et al. (2003) Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem 51, 6509–6515.

24. Kim D-O, Lee KW, Lee HJ et al. (2002) Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J Agric Food Chem 50, 3713–3717.

25. Wang Y, Yang M, Lee SG et al. (2012) Plasma total antioxidant capacity is associated with dietary intake and plasma level of antioxidants in postmenopausal women. J Nutr Biochem (Epublication ahead of print version).

26. Wang Y, Yang M, Lee SG et al. (2010) Total antioxidant capacity: a useful tool in assessing antioxidant intake status. In Natural Compounds and Apoptosis, pp. 265–292 [M Diederich, editor]. Springer: New York.

27. Chun OK, Floegel A, Chung S-J et al. (2010) Estimation of antioxidant intakes from diet and supplements in U.S. adults. J Nutr 140, 317–324.

28. Nutrition Coordinating Center, University of Minnesota (2010) Nutrition Data System for Research, Release 2010. http://www.ncc.umn.edu/products/ndsr.html.

29. National Center for Health Statistics (2010) National Health and Nutrition Examination Survey, 2007–2008 Data Documentation. Hyattsville, MD: Centers for Disease Control and Prevention; available at http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/DSDOC_E.htm

30. Marks GC, Hughes MC & van der Pols JC (2006) Relative validity of food intake estimates using a food frequency questionnaire is associated with sex, age, and other personal characteristics. J Nutr 136, 459–465.

31. Ross MA (1994) Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography. J Chromatogr B 657, 197–200.

32. Leonard SW, Bruno RS, Paterson E et al. (2003) 5-Nitro-γ-tocopherol increases in human plasma exposed to cigarette smoke in vitro and in vivo. Free Radic Biol Med 35, 1560–1567.

33. Karppia J, Nurmia T, Olmedilla-Alonsob B et al. (2008) Simultaneous measurement of retinol, α-tocopherol and six carotenoids in human plasma by using an isocratic reversed-phase HPLC method. J Chromatogr B 867, 226–232.

34. van den Berg R, Haenen GRMM, van den Berg H et al. (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem 66, 511–517.

35. Wang Y, Chung SJ, Song WO et al. (2011) Estimation of daily proanthocyanidin intake and major food sources in the US diet. J Nutr 141, 447–452.

36. Willett WC (1994) Future directions in the development of food-frequency questionnaires. Am J Clin Nutr 59, Suppl. 1, 171S–174S.

37. Jain M (1999) Culture specific food frequency questionnaires: development for use in a cardiovascular study. Can J Diet Pract Res 60, 27–36.

38. Andersen LF, Solvoll K, Johansson LR et al. (1999) Evaluation of a food frequency questionnaire with weighed records, fatty acids, and α-tocopherol in adipose tissue and serum. Am J Epidemiol 150, 75–87.

39. Bautista LE, Herran OF & Pryer JA (2005) Development and simulated validation of a food-frequency questionnaire for the Colombian population. Public Health Nutr 8, 181–188.

40. Block G, Woods M, Potosky A et al. (1990) Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 43, 1327–1335.

41. MacIntyre UE, Venter CS & Vorster HH (2001) A culture-sensitive quantitative food frequency questionnaire used in an African population: 1. Development and reproducibility. Public Health Nutr 4, 53–62.

42. Sauvaget C, Allen N, Hayashi M et al. (2002) Validation of a food frequency questionnaire in the Hiroshima/Nagasaki Life Span Study. J Epidemiol 12, 394–401.

43. Date C, Fukui M, Yamamoto A et al. (2005) Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol 15, Suppl. 1, S9–S23.

44. McNaughton SA, Marks GC, Gaffney P et al. (2005) Validation of a food-frequency questionnaire assessment of carotenoid and vitamin E intake using weighed food records and plasma biomarkers: the method of triads model. Eur J Clin Nutr 59, 211–218.

45. Satia JA, Watters JL & Galanko JA (2009) Validation of an antioxidant nutrient questionnaire in whites and African Americans. J Am Diet Assoc 109, 502–508.

46. Bland JM & Altman DG (1986) Statistical-methods for assessing agreement between 2 methods of clinical measurement. Lancet 1, 307–310.

47. Labonte ME, Cyr A, Baril-Gravel L et al. (2012) Validity and reproducibility of a web-based, self-administered food frequency questionnaire. Eur J Clin Nutr 66, 166–173.

48. Dunn S, Datta A, Kallis S et al. (2011) Validation of a food frequency questionnaire to measure intakes of inulin and oligofructose. Eur J Clin Nutr 65, 402–408.

49. Dixon LB, Subar AF, Wideroff L et al. (2006) Carotenoid and tocopherol estimates from the NCI diet history questionnaire are valid compared with multiple recalls and serum biomarkers. J Nutr 136, 3054–3061.

50. Hodge AM, Simpson JA, Fridman M et al. (2009) Evaluation of an FFQ for assessment of antioxidant intake using plasma biomarkers in an ethnically diverse population. Public Health Nutr 12, 2438–2447.

51. Malekshah AF, Kimiagar M, Saadatian-Elahi M et al. (2006) Validity and reliability of a new food frequency questionnaire compared to 24 h recalls and biochemical measurements: pilot phase of Golestan cohort study of esophageal cancer. Eur J Clin Nutr 60, 971–977.

52. Padayatty SJ & Levine M (2008) Fruit and vegetables: think variety, go ahead, eat! Am J Clin Nutr 87, 5–7.

53. Jenab M, Slimani N, Bictash M et al. (2009) Biomarkers in nutritional epidemiology: applications, needs and new horizons. Hum Genet 125, 507–525.

54. Rautiainen S, Serafini M, Morgenstern R et al. (2008) The validity and reproducibility of food-frequency questionnaire-based total antioxidant capacity estimates in Swedish women. Am J Clin Nutr 87, 1247–1253.

55. Pellegrini N, Salvatore S, Valtuena S et al. (2007) Development and validation of a food frequency questionnaire for the assessment of dietary total antioxidant capacity. J Nutr 137, 93–98.

56. Manach C, Williamson G, Morand C *et al.* (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**, Suppl. 1, 230S–242S.

57. Cao GH, Russell RM, Lischner N *et al.* (1998) Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J Nutr* **128**, 2383–2390.

58. Ghiselli A, Serafini M, Natella F *et al.* (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* **29**, 1106–1114.