Development and Validation of a Food Frequency Questionnaire for the Assessment of Dietary Total Antioxidant Capacity[1,2]

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Abstract

The total antioxidant capacity (TAC) of the diet may be an important tool to monitor the protective effect of plant foods in epidemiological studies. We developed a semi-quantitative FFQ for the assessment of dietary TAC by 3 different assays, i.e., Trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP) and ferric reducing-antioxidant power (FRAP). The FFQ consists of 53 questions about the major sources of dietary TAC in Northern Italy and was validated against a 3-d weighed food record (3D-WR) in 285 individuals (159 males and 126 females) aged 35–88 y and living in the province of Parma (Italy). Plasma TAC was also evaluated in a subgroup of subjects using the TEAC and FRAP assays. The FFQ was associated with 3D-WR (quadratic-weighted $\kappa = 0.49$ for TEAC, 0.53 for TRAP, and 0.49 for FRAP; $P < 0.0001$) and proved reasonably accurate to classify individuals into quartiles of TAC intake. The FFQ had a good repeatability when readministered after 1 y in 55 subjects (quadratic-weighted κ for intertertile agreement = 0.66 for TEAC, 0.70 for TRAP and 0.68 for FRAP; $P < 0.0001$). With both dietary instruments, the main contributors to TAC intake were coffee and tea in women and alcoholic beverages in men, followed by fruits and vegetables in both sexes. Plasma TAC and dietary TAC were not associated. In conclusion, our FFQ has the potential for being used to rank subjects on the basis of their antioxidant intake as determined by dietary TAC in large epidemiological studies. The FFQ should be validated in external populations before being used for research purposes. J. Nutr. 137: 93–98, 2007.

Introduction

Epidemiological studies have found an inverse association between the intake of fruit, vegetables, and grains and the risk of cardiovascular disease and cancer (1,2). It is not known which dietary constituents are responsible for this association, but it is often assumed that antioxidants play a major role in this respect (3–5).

Plant foods contain many hundreds of compounds with antioxidant activity, including ascorbic acid and tocopherols, carotenoids, and a wide variety of antioxidant phytochemicals such as simple phenolics and flavonoids (6). Because the concentration of single antioxidants may not reflect the total antioxidant power of food, the concept of total antioxidant capacity (TAC)[8] was introduced (7).

TAC takes into account the antioxidant activity of single compounds present in food or biological samples as well as their potential synergistic and redox interactions. Several assays are available for measuring TAC, differing for chemistry (generation of different radicals and/or target molecules) and for the way endpoints are measured. To consider the major redox reactions that commonly happen in human body, 3 methods, i.e., Trolox equivalent antioxidant capacity (TEAC) (8), total radical-trapping antioxidant parameter (TRAP) (9), and ferric reducing-antioxidant power (FRAP) (10), were selected.

The great potential of TAC for epidemiological and clinical applications seems strengthened by the fact that dietary TAC may provide protection against gastric cancer and inflammatory processes (11,12). For dietary TAC to be used in such studies, simple and reliable instruments have to be developed for its assessment. The FFQ is the obvious choice for assessing food and

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[2] The number of individuals in cross-tabulations of the quartiles of FFQ and 3D-WR for TEAC, TRAP and FRAP intakes (Supplemental Tables 1–3) and the percentage contribution of food groups to TAC intake, as determined by TRAP and FRAP assays, in the 3D-WR and the FFQ (Supplemental Tables 4 and 5, respectively) are available with the online posting of this paper at jn.nutrition.org.

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[8] Abbreviations used: 3D-WR, 3-d weighed food record; FRAP, ferric reducing-antioxidant power; TAC, total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter.

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nutrient intake in epidemiological studies (13), and thus we developed and validated a FFQ for dietary TAC.

Materials and Methods

Participants and study design. A total of 422 workers and previous workers of the Barilla food company (Parma, Italy), recruited in 1981 for a survey on diabetes and cardiovascular disease and evaluated again in 1993 (14), were invited to join the present study. Three hundred and twenty-five subjects (77%) accepted the invitation and 299 of them (92%) underwent the screening visit. Exclusion criteria were: *1*) diabetes mellitus, *2*) recent cardiovascular disease (i.e., acute coronary event, stroke, or surgery for peripheral arterial disease <6 mo), *3*) kidney disease [plasma creatinine >1.4 mg/dL,[9] measured by use of a kinetic Jaffé procedure with a Beckman-2 creatinine analyzer (Beckman Instruments) in the Hospital Central Laboratory according to the manufacturer's instructions], *4*) history of autoimmune disease and *5*) cancer. Recruitment of subjects was performed between September 2002 and June 2003. The protocol was approved by the local Ethics Committee and all subjects gave their written informed consent.

On subjects' initial visit, a fasting venous blood sample was taken to determine antioxidant markers, and an oral glucose tolerance test was administered, providing 75 g of glucose, to exclude a diagnosis of diabetes (15). During this 1st visit, the FFQ was administered to the subjects by an expert dietician to assess the 3-mo food habits preceding the beginning of the study. Subjects were then instructed to fill in a 3-d weighed food record (3D-WR).

FFQ design and validation. A food list for the major dietary sources of TAC in the Italian diet was obtained by analyzing 1125 24-h dietary recalls from the Varese cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) (16). These recalls listed a total of 1081 different foods. For 713 of these items, a TAC value was then assigned as based on previously published databases (17,18) or laboratory data. Then, the food list for the FFQ was extracted with the aim of covering at least 95% of the variance of TAC intake and energy intake in the original population. The percentage of TAC contributed by each food (f) was calculated as follows:

$$\%\,\text{contribution of TAC(f)} = \frac{\text{contribution of TAC (f)}}{\text{contribution of TAC (all foods)}}.$$

The above equation was estimated by the following, as proposed by Block et al. (19):

$$\%\,D_f = \frac{\sum_{J=1}^{1.125} Q_f D_f}{\sum_{J=1}^{1.125}\sum_{i=1}^{1.081} Q_{J_i} D_i},$$

where Q = g of food consumed, D = TAC/g of food, j = 1…1125 subjects, and i = 1…1081 foods.

The final food list comprised 150 foods and was translated into 53 questions. Food frequency was coded as daily, weekly, and monthly, and from 1 to 6 occasions (e.g., twice a day, 5 times per week, 6 times per month) and the FFQ was intended to cover a period of the previous 3 mo of food intake. Portion sizes were estimated using 3 different pictures (A, B, and C; with A illustrating the smallest portion and C the biggest portion) for 33 foods or courses from a photographic atlas developed for the Italian population (20).

For food groups whose single items have remarkably different antioxidant capacity (e.g., fruits and vegetables) and for which daily intake is usually overestimated (13,21), a question about overall intake was asked and then qualitative questions (never, sometimes, often) related to the frequency of consuming single items were additionally asked. The intake of single food items in specific food groups was obtained by weighing the overall intake as determined by answers to qualitative questions for single items.

In addition, the questionnaire included questions on the quality of selected foods; for instance, caffeinated vs. decaffeinated coffee and whole-grain vs. refined cereals. Moreover, additional questions were related to complex dishes rich in TAC (i.e., pasta and rice with tomato sauce or with vegetable sauce, vegetable soup, soup with beans, chickpeas, or lentils, tomato/cheese and vegetarian pizza), whose recipes were compiled on the basis of 24-h dietary recalls from the Varese cohort. The TAC of recipes were estimated on the basis of the TAC of the single ingredients and their actual weight in the recipe.

The FFQ was administered by an expert dietitian who entered the data directly into a Microsoft Excel application. The application was designed to calculate TAC directly during FFQ compilation. To obtain this, a database of TAC values of raw foods was linked to the FFQ application. Approximately 150 items among fruits, vegetables, oils and beverages (17), and among cereals and cereal products, pulses, nuts and processed foods (18) were analyzed. The TAC values were obtained applying 3 different assays: *1*) TEAC assay (22), *2*) TRAP assay (9), and *3*) FRAP assay (10), as previously described (17). The computer output consisted of the mean daily TAC of the overall diet and was also split into 20 food categories (fruits, vegetables, pulses, alcoholic beverages, coffees and teas, cereals, oils and fats, salad dressings, sweets and dairy, pizza, fruit juices, nuts, chocolates, breads, biscuits, potatoes, spices, cakes, soft drinks and sauces).

The FFQ repeatability was evaluated 1 y after the initial administration in 55 subjects.

3D-WR. The 3D-WR was chosen as the reference method because it is reliable in measuring food intake and because its estimation errors are usually not correlated with those made in the FFQ (21). The study dietitian trained participants to complete the 3D-WR, which included all foods, beverages, and supplements consumed during 2 nonconsecutive working days plus a weekend day. Subjects were asked to weigh all food and drinks consumed and to provide a detailed description of each food, including methods of preparation and recipes whenever possible. All subjects completed the 3D-WR within 1 wk of their first visit. The study dietitian reviewed the 3D-WR with participants to check for errors or omissions and to estimate the portions of foods eaten outside the home using a book of photographs and standard household measures. Nutrient and TAC intake was calculated by a Microsoft Access application linked to the food database of the European Institute of Oncology (EIO), covering >700 Italian foods (23) and our TAC database (see above). To assign a value to TAC-containing foods not directly analyzed, we applied the following criteria: *1*) for dry and canned foods, the TAC values of fresh foods were converted on the basis of the moisture content specified by the EIO database and vice versa and, *2*) if no data were available for a given food item, the data for a similar food item (e.g., same botanical group) were used as proxy. The computer output of 3D-WR gave the mean daily TAC, the TAC from the 20 food categories described above for the FFQ, and the macro- and micronutrient content of the diet for each subject.

Plasma TAC assessment. Blood samples for plasma antioxidant analysis were collected in vacutainers containing EDTA-Na and were centrifuged at 1000 × *g* for 10 min at 4°C. Plasma was transferred to Eppendorfs and stored at –80°C until measurement, which was performed the day immediately after collection. TAC was measured by means of TEAC and FRAP high throughput assays, as described by Bompadre et al. (24) and Benzie and Strain (10), respectively. The plasma samples were not analyzed using the TRAP assay due to the lack of an available high throughput technique, which did not allow analyzing samples immediately after collection.

Statistical analysis. Of the 299 subjects who agreed to participate in the study, 285 (95%) had all the data needed for analysis. For plasma TAC analysis, 282 samples were analyzed for TEAC and 244 for FRAP. Characteristics of subjects were given as medians and interquartile ranges (IQR) because of very skewed distributions. Wilcoxon's test, Spearman's ρ, quadratic-weighted κ values were calculated to measure interquartile agreement between FFQ and 3D-WR measures of TEAC, TRAP and FRAP assays. To measure the value of gross misclassification, the percentage of subjects into the same quartile or within one quartile was calculated (25–27). The same procedure was used to test the relation

[9] To convert the values for creatinine to μmol/L, multiply by 88.4.

between plasma values of TEAC and FRAP and dietary intake as assessed by the FFQ and 3D-WR. We also tested whether gender, age, BMI, and energy intake influenced the probability of being classified by the FFQ in the same or within one quartile of the 3D-WR using a logistic regression model where age, BMI, and energy were evaluated as quartiles. To assess repeatability of the FFQ after 1 y, Spearman's ρ inter-tertile agreement using quadratic-weighted κ was performed. Significance was set to a value of $P < 0.05$ for all tests. Statistical analysis was performed using STATA 9.1 (STATA Corp.).

Results

Measurements of the study subjects. The age of the subjects (159 males and 126 females) ranged from 35 to 88 y and there was a wide variation in BMI and energy intake (**Table 1**).

FFQ repeatability. As determined by Spearman's ρ, test and retest correlation values after 1 y were 0.66 for TEAC, 0.70 for TRAP, and 0.68 for FRAP ($P < 0.0001$). The corresponding values of quadratic-weighted κ for inter-tertile agreement were 0.57, 0.68, and 0.59, respectively ($P < 0.0001$).

Validation of FFQ against 3D-WR. FFQ estimates of TEAC, TRAP, and FRAP were higher than those given by the 3D-WR (Wilcoxon's test, $P < 0.0001$; see Table 1). The degree of association between FFQ and 3D-WR estimates was moderate (Spearman's $\rho = 0.52$ for TEAC; $\rho = 0.58$ for TRAP, and $\rho = 0.52$ for FRAP; $P < 0.00001$), as defined by Landis and Koch (28). The interquartile agreement, determined by quadratic-weighted κ, was 0.49 for TEAC, 0.53 for TRAP, and 0.49 for FRAP ($P < 0.0001$) (**Supplemental Tables 1–3**). When the FFQ was compared with the 3D-WR, using the percentage of subjects in the same or within one quartile, TEAC, TRAP, and FRAP identified correctly 81, 83, and 81% of cases, respectively, and the classification in opposite quartiles was rare (2, 1, and 1%, respectively). We also tested whether gender, age, BMI, and energy intake affected the probability of classification in the same or within one quartile using a logistic regression model where age, BMI, and energy were evaluated as quartiles (models not shown). None of these potential confounders was associated to the outcome of interest, with *P*-values for the likelihood ratio ranging from 0.0516 for age in the TRAP model to 0.8795 for age in the TEAC model.

Association between plasma and dietary TAC tools. There was no association between the 3D-WR values and those of plasma for TEAC ($\rho = 0.046$, $P = 0.44$, $n = 282$), and there was only a trivial association for FRAP ($\rho = 0.13$, $P = 0.04$, $n = 244$). Moreover, the quadratic-weighted κ values for interquartile agreement were not significant for TEAC ($\kappa = 0.01$, $P = 0.41$) and only marginally significant for FRAP ($\kappa = 0.12$, $P = 0.03$). The number of subjects within the same quartile or within one quartile was 64% for TEAC and 57% for FRAP.

There was no association between the FFQ values and those of plasma for TEAC ($\rho = 0.11$, $P = 0.07$, $n = 282$) and only a trivial association for FRAP ($\rho = 0.17$, $P = 0.008$, $n = 244$). Moreover, the quadratic-weighted κ values for interquartile agreement were not significant for TEAC ($\kappa = 0.08$, $P = 0.06$) and FRAP ($\kappa = 0.19$, $P = 0.06$). The number of subjects within the same quartile or within one quartile was 65% for TEAC and 59% for FRAP.

The daily intakes of food groups and their contribution to TAC intake. In the overall population, estimated intakes for 11 of 20 food groups in the FFQ were higher than those recorded in the 3D-WR (**Table 2**). Conversely, the FFQ estimate of fruit and pulses intake was lower than that estimated by 3D-WR and the intake of vegetables was comparable for both dietary tools. However, the percentage of contribution of the main food groups to TEAC, TRAP, and FRAP intake (**Table 3** and **Supplemental Tables 4 and 5**), as determined by both the 3D-WR and the FFQ, was similar. Coffee and tea beverages were the main contributors to TAC intake in women, followed by alcoholic beverages, fruits, and vegetables. In men, the main contributors to TAC intake were alcoholic beverages followed by coffee and tea, fruits, and vegetables.

TABLE 1 Characteristics and main measurements of study participants

	n	Median	IQR[1]	Min	Max
Age, *y*	285	60	8	35	88
BMI, *kg/m²*	285	27.0	4.7	18.7	43.5
3D-WR					
Energy, *kJ/d*	285	9025	2904	4322	16556
TEAC, *mmol/d*	285	5.3	2.6	1.3	13.9
TRAP, *mmol/d*	285	7.1	3.7	1.4	17.4
FRAP, *mmol/d*	285	15.5	7.3	4.1	37.5
FFQ, *mmol/d*					
TEAC	285	7.5*	3.8	1.9	33.8
TRAP	285	9.6*	5.2	2.3	43.4
FRAP	285	22.0*	10.9	5.7	89.8
Plasma, *μmol/L*					
TEAC	282	604	278	119	1337
FRAP	244	1007	228	575	1634

[1] IQR, interquartile ranges. *Different from the corresponding 3D-WR value, $P < 0.0001$.

Discussion

In the present study, we developed a FFQ to assess the dietary TAC of a group of healthy Italian adults. To the best of our knowledge, this is the first FFQ developed and validated to achieve this goal. This FFQ may be useful for investigating the relation between dietary antioxidant intake and the risk of chronic disease in epidemiological studies.

We validated our FFQ against a 3D-WR performed on different days of the week, which is considered a reference method for this kind of application (29). The FFQ estimate of TAC intake was significantly higher than that obtained from the 3D-WR. This may be partly due to under-reporting due to the 3D-WR (13). However, the FFQ and the 3D-WR estimates of TAC intake were significantly associated, regardless of the analytical method used to evaluate TAC in foods. More importantly, the classification in the same or within one quartile was satisfactory and the classification in opposite quartiles was rare. It should be pointed out that this degree of accuracy is comparable to that observed for most macronutrients (27). Contrary to what happens for most nutrients, we found no evidence that age, sex, BMI, and energy intake influence the agreement between FFQ and 3D-WR. Moreover, the test and retest study showed a good repeatability for the FFQ 1 y after its administration.

Even if this is the first validation study of a FFQ aimed at assessing dietary TAC, it has several limitations. First, a 3D-WR

TABLE 2 Daily intake of food groups as assessed by the 3D-WR and the FFQ[1]

	3D-WR			FFQ		
Food group	Women, $n = 126$	Men, $n = 159$	Total, $n = 285$	Women, $n = 126$	Men, $n = 159$	Total, $n = 285$
			g			
Alcoholic beverages	104.2 (156.7)	253.3 (250.0)	188.7 (211.7)	125.0 (231.8)	500.0 (327.1)	250.5 (410.9)
Biscuits	11.7 (25.0)	8.7 (28.0)	10.0 (26.7)	8.7 (28.0)	11.4 (23.7)	10.7 (26.6)
Breads	94.8 (57.7)	133 (70.7)	116.7 (68.3)	125.0 (101.7)	203.6 (110.0)	200.0 (110.8)
Cakes	28.0 (58.3)	16.7 (56.7)	23.3 (56.7)	10.6 (20.0)	6.3 (17.1)	7.8 (19.0)
Cereals	53.3 (36.7)	76.7 (50.7)	64.3 (46.7)	65.2 (47.2)	92.7 (62.5)	82.9 (57.6)
Chocolates	1.3 (6.7)	0.0 (1.7)	0.0 (3.3)	3.0 (9.3)	1.4 (4.3)	2.0 (6.0)
Coffees and teas	80.0 (80.0)	70.0 (103.3)	77.3 (91.7)	143.6 (150.7)	127.1 (136.7)	133.6 (141.7)
Fruit juices	0.0 (13.3)	0.0 (15.0)	0.0 (13.3)	0.0 (35.7)	0.0 (45.8)	0.0 (35.7)
Fruits	262.2 (163.3)	273.3 (200.0)	266.7 (171.3)	220.5 (149.2)	215.0 (193.2)	217.5 (163.4)
Nuts	0.0 (2.4)	0.0 (0.3)	0.0 (1.0)	1.0 (3.0)	1.0 (4.3)	1.0 (4.3)
Oils and fats	31.2 (16.7)	34.7 (16.7)	33.1 (16.7)	30.7 (19.8)	33.1 (24.3)	32.1 (22.8)
Pizza	0.0 (70.3)	0.0 (50.0)	0.0 (66.7)	20.0 (21.9)	14.3 (21.9)	20.0 (21.9)
Potatoes	19.2 (57.3)	19.3 (54.3)	19.3 (54.3)	17.9 (27.4)	22.9 (24.3)	17.9 (24.3)
Pulses	0.0 (30.0)	13.3 (40.0)	10.0 (31.0)	2.6 (3.3)	3.0 (5.7)	2.9 (4.3)
Salad dressings	5.0 (8.3)	5.0 (6.7)	5.0 (6.7)	8.7 (9.0)	8.4 (10.9)	8.5 (10.9)
Sauces	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	37.4 (43.3)	56.8 (53.6)	48.6 (53.3)
Soft drinks	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (11.0)	0.0 (11.0)	0.0 (11.0)
Spices	0.7 (1.3)	0.3 (1.3)	0.4 (1.3)	3.2 (2.4)	3.2 (3.0)	3.2 (2.7)
Sweets and dairy	20.8 (33.4)	24.2 (32.7)	23.3 (32.3)	38.6 (63.2)	25.8 (47.9)	30.0 (53.7)
Vegetables	181.0 (156.3)	218.0 (142.7)	200.0 (145.3)	208.7 (180.9)	201.0 (192.7)	204.5 (182.5)

[1] Values are medians and (IQR).

is clearly less adequate than a 7D- or 14D-WR to evaluate usual food intake. However, we administered the 3D-WR to our subjects on different days of the week over 6 mo. Thus, we expect that the 3D-WR has given an acceptable estimate of the food intake of the population during this period. Second, we investigated the food intake during the previous 3 mo. We choose to do this because fruits and vegetables, which are among the most important contributors of TAC, undergo seasonal variation. Moreover, there is evidence of poor performance for FFQs in investigating fruit and vegetable intake for longer periods (21).

After analyzing the daily intake of food groups obtained from both dietary tools, some conclusions can be drawn. First, the consumption of alcoholic beverages (~75% as red wine) is quite high in our population, especially in men, although similar to data collected using a FFQ in the EPIC centers of northern Italy (30). Second, the daily intakes of other food groups recorded by

TABLE 3 The percentage contribution of food groups to TAC intake as determined by TEAC assay in the 3D-WR and the FFQ[1]

	3D-WR			FFQ		
Food group	Women, $n = 126$	Men, $n = 159$	Total, $n = 285$	Women, $n = 126$	Men, $n = 159$	Total, $n = 285$
Alcoholic beverages	15.3 (25.0)	34.0 (26.6)	25.5 (30.3)	12.4 (25.8)	40.3 (33.9)	27.9 (36.4)
Biscuits	0.3 (0.8)	0.2 (0.8)	0.3 (0.8)	0.3 (0.7)	0.2 (0.6)	0.2 (0.7)
Breads	3.8 (2.6)	3.9 (3.5)	3.9 (0.9)	3.6 (2.7)	4.2 (3.5)	4.0 (3.4)
Cakes	1.0 (2.4)	0.4 (1.7)	0.7 (2.0)	0.3 (0.6)	0.2 (0.5)	0.2 (0.6)
Cereals	0.4 (0.3)	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.5 (0.4)	0.5 (0.4)
Chocolates	0.9 (6.8)	0.0 (1.1)	0.0 (3.3)	2.9 (10.7)	1.1 (3.6)	1.8 (6.1)
Coffees and teas	38.2 (22.3)	27.0 (24.2)	31.7 (2.9)	37.9 (20.5)	26.5 (21.5)	31.3 (22.2)
Fruit juices	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (1.5)	0.0 (1.4)	0.0 (1.4)
Fruits	14.7 (15.5)	11.8 (16.7)	13.2 (0.0)	13.6 (10.4)	8.2 (7.8)	10.2 (9.0)
Nuts	0.0 (0.3)	0.0 (0.0)	0.0 (0.5)	0.6 (2.1)	0.5 (2.6)	0.5 (2.4)
Oils and fats	0.7 (0.6)	0.7 (0.5)	0.7 (0.4)	0.6 (0.5)	0.5 (0.6)	0.6 (0.6)
Pizza	0.0 (1.7)	0.0 (1.0)	0.0 (0.0)	0.3 (0.4)	0.2 (0.3)	0.3 (0.4)
Potatoes	0.3 (1.1)	0.3 (0.8)	0.3 (1.4)	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)
Pulses	0.0 (0.4)	0.1 (0.4)	0.0 (0.4)	0.0 (0.2)	0.0 (0.1)	0.0 (0.1)
Salad dressings	0.3 (0.5)	0.2 (0.3)	0.3 (0.4)	0.4 (0.3)	0.3 (0.3)	0.3 (0.3)
Sauces	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (1.0)	0.9 (1.0)	0.8 (1.0)
Soft drinks	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Spices	0.2 (0.6)	0.2 (0.5)	0.2 (0.5)	1.0 (0.6)	0.7 (0.6)	0.8 (0.7)
Sweets and dairy	0.5 (1.9)	0.2 (1.2)	0.3 (1.4)	0.7 (1.5)	0.3 (0.9)	0.4 (1.0)
Vegetables	6.3 (7.0)	5.9 (6.5)	6.1 (6.5)	8.0 (5.4)	6.1 (5.8)	7.1 (6.2)

[1] Values are medians and (IQR).

the FFQ developed are comparable with those reported in other Italian surveys (30,31). In addition, we determined the contribution of different food groups to TAC intake, which was similar for all 3 methods used to measure the TAC. However, a conclusion cannot be made regarding the most suitable assay for measuring the TAC of food because each assay measures different antioxidant mechanisms. In particular, the TEAC assay measures the ability of antioxidants to quench a radical cation in both lipophilic and hydrophilic environments (8), and the TRAP and FRAP assays evaluate the chain-breaking antioxidant potential (9) and the reducing power of the sample (10), respectively. Coffee and tea contributed between 37.9 and 54.3% of TAC intake in women and between 26.5 and 36.6% TAC intake in men, the variation of which depended upon the TAC assay and the tool used to assess food intake. The contribution of alcoholic beverages to TAC intake was between 11.3 and 15.3% in women and between 31.5 and 40.3% in men. The high contribution of the coffee and tea category to TAC in women is not surprising and is due to the high consumption of coffee in our study population. A large body of evidence supports the protective effect of coffee on the development of type 2 diabetes (32) and colon cancer in men as well as in women (33), and on the risk of premenopausal breast cancer (34). Moreover, the high contribution of coffee to TAC intake is in agreement with Svilaas et al. (35), who reported coffee as the major contributor (~64%) to TAC intake in 61 Norwegians. Conversely, wine intake contributed <5%. Interestingly, the mean intake of TAC reported in that group and expressed as FRAP (17.3 mmol/d) is in agreement with the TAC intake recorded by 3D-WR in our population.

We also measured plasma TAC by means of the methods used to measure food TAC (i.e., TEAC and FRAP). We did not find an association between dietary TAC (as determined by both the 3D-WR and the FFQ) and plasma TAC. These data clearly show that plasma values cannot be used as surrogate measurements for either short- or long-term dietary TAC intake. This result was not completely unexpected because the role of antioxidant-rich diets on the modulation of antioxidant plasma status is not yet clear. Many studies report the ability of diet to modulate plasma TAC after the acute consumption of antioxidant-rich foods (36–38). To our knowledge, to date, only one epidemiologic study demonstrated a significant association ($P < 0.01$) between plasma TAC (measured by a colorimetric test) and the adherence to a Mediterranean diet (39). The existence of homeostatic mechanisms of regulation and the physiological diversity in absorption and disposal of antioxidants are variables that might affect the ability of diet to modulate plasma TAC in vivo. Nevertheless, it cannot be excluded that an association could emerge in a group larger than that studied in our investigation.

Although both the accuracy and the repeatability of the FFQ were acceptable, the fact that it was developed using a sample of Italian subjects with traditional, albeit "westernized" dietary habits, does not imply that it will perform equally well in subjects from different countries and with different dietary habits. Thus, it is important that the FFQ be cross-validated in external populations before being used as research tool.

In conclusion, using a FFQ with a well-defined food list, we developed a simpler and less expensive tool than a 3-d weighed food record for assessing dietary TAC intake. Our FFQ has the potential for being used to rank subjects on the basis of their antioxidant intake as determined by dietary TAC. Based on the contribution of food groups to the daily TAC intake, the estimation of dietary TAC provides additional information to the daily intake of fruit and vegetables, because it also considers the contribution of antioxidant-rich beverages such as wine and coffee.

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