The validity and reproducibility of food-frequency questionnaire-based total antioxidant capacity estimates in Swedish women

Susanne Rautiainen, Mauro Serafini, Ralf Morgenstern, Ronald L. Prior, and Alicja Wolk

ABSTRACT
Background: Total antioxidant capacity (TAC) provides an assessment of antioxidant activity and synergistic interactions of redox molecules in foods and plasma.

Objective: We investigated the validity and reproducibility of food-frequency questionnaire (FFQ)-based TAC estimates assessed by oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameters (TRAP), and ferric-reducing antioxidant power (FRAP) food values.

Design: Validity and reproducibility were evaluated in 2 random samples from the Swedish Mammography Cohort. Validity was studied by comparing FFQ-based TAC estimates with one measurement of plasma TAC in 108 women (54–73-y-old dietary supplement nonusers). Reproducibility was studied in 300 women (56–75 y old, 50.7% dietary supplement nonusers) who completed 2 FFQs 1 y apart.

Results: Fruit and vegetables (mainly apples, pears, oranges, and berries) were the major contributors to FFQ-based ORAC (56.5%), TRAP (41.7%), and FRAP (38.0%) estimates. In the validity study, whole plasma ORAC was correlated (Pearson) with FFQ-based ORAC (r = 0.35), TRAP (r = 0.31), and FRAP (r = 0.28) estimates from fruit and vegetables. Correlations between lipophilic plasma ORAC and FFQ-based ORAC, TRAP, and FRAP estimates from fruit and vegetables were 0.41, 0.31, and 0.28, and correlations with plasma TRAP estimates were 0.31, 0.30, and 0.28, respectively. Hydrophilic plasma ORAC and plasma FRAP values did not correlate with FFQ-based TAC estimates. Reproducibility, assessed by intraclass correlations, was 0.60, 0.61, and 0.61 for FFQ-based ORAC, TRAP, and FRAP estimates, respectively, from fruit and vegetables.

Conclusions: FFQ-based TAC values represent valid and reproducible estimates that may be used in nutritional epidemiology to assess antioxidant intake from foods. Further studies in other populations to confirm these results are needed.

INTRODUCTION
Many epidemiologic studies have reported an inverse association between a diet high in fruit and vegetables and the occurrence of chronic disease (1–3). The high content of antioxidants in fruit and vegetables has been proposed as one of the factors behind the protective mechanisms. Total antioxidant capacity (TAC) is a concept describing an antioxidant’s capacity for reducing oxidants; it takes into account the synergism between substances (4). TAC can also be applied to both foods and biological systems, and it is defined as the moles of radicals neutralized per liter (or gram) of tested sample (5); this calculation provides a measure of antioxidant capacity that reflects the synergistic interactions between redox molecules within the sample.

Food-frequency questionnaires (FFQs) can be used to assess TAC estimates by summarizing known TAC values of different food items (6) instead of assessing phytochemical intake from single phytochemicals, as many previous studies have done. The summarized TAC estimate may give a better estimate both because it reflects the synergism between compounds and because the total antioxidant intake is reflected through a single estimate. TAC food databases have been constructed by using a number of assays for measuring TAC in foods, including the oxygen radical absorbance capacity (ORAC) (7, 8), the total radical-trapping antioxidant parameters (TRAP) (9, 10), and the ferric-reducing antioxidant power (FRAP) (11) assays. These food databases include TAC data on commonly consumed foods, and the ORAC, TRAP, and FRAP assays can also be used for analyzing TAC in blood plasma. Only 2 epidemiologic studies have used FFQ-based TAC estimates in their analyses; one was a study of gastric cancer (FFQ-TRAP) (6), and the other was a study of mortality (FFQ-ORAC, -TRAP, and -FRAP) (12). Previously, because they have been proposed to be appropriate biomarkers of fruit and vegetable intakes, blood concentrations of carotenoids, vitamin C, and vitamin E were used to validate dietary assessments by FFQs (13–17). However, measuring concentrations of single antioxidants does not provide an indication of the total efficiency of food intake of all antioxidants in reducing free radicals, which can be obtained through TAC measurements.

The objective of the present study was to determine the validity and reproducibility of FFQ-based TAC, ORAC, TRAP, and FRAP estimates in random samples of women participating in...
the population-based Swedish Mammography Cohort (SMC). The validity of FFQ-based TAC estimates was evaluated by comparison with plasma TAC values, and we repeated measurements of the FFQ 1 y apart.

SUBJECTS AND METHODS

The SMC was established between 1987 and 1990 among women residing in Uppsala and Västmanland counties in Sweden. All women born between 1914 and 1948 were sent a questionnaire that asked about weight, height, and education level and that included an FFQ. The source population consisted of 90,303 women, 74% of whom returned the questionnaire. To update exposure data, an expanded questionnaire was sent to all cohort members who were alive in 1997 and still living in the study area of Uppsala and Västmanland counties. The second questionnaire was returned by 70% of the women.

A sample of 246 women 54–73 y old from the SMC agreed to participate in the validity study, which was performed between November 2003 and August 2004. Women completed questionnaires and donated blood samples for analyses of plasma TAC concentrations. The women in the validity group completed a 96-item FFQ, the same FFQ that they completed as part of the SMC in 1997. The 96-item FFQ has been previously described in 2 reproducibility and validity studies performed in subgroups of the SMC (18) and the Cohort of Swedish Men (19).

For the reproducibility study, another group of women, aged 56–75 y, was randomly chosen from the SMC (n = 595) between September 2004 and February 2005. Of these 595 women, 300 completed 2 additional FFQs, 1 at the time of their random selection and the other 1 y later (ie, between September 2005 and February 2006). The reproducibility group completed an extended 123-item FFQ that included additional questions regarding intakes of dairy products and meat as well as fruit (4 questions) and vegetables (2 questions). Both study groups provided information on age, level of education, alcohol consumption, and current cigarette smoking status. Height and weight were measured by a nurse at the time of blood collection. All participants provided written informed consent. The Ethics Committees of the Karolinska Institute and the Uppsala University Hospital approved the investigation.

Food-frequency questionnaire-based total antioxidant capacity estimates

To calculate ORAC estimates for each participant, we used a database containing the most commonly consumed foods in the United States, which were analyzed with the ORAC assay in one laboratory (7, 8, 20). To calculate TRAP estimates, we used Italian data from one laboratory (9, 10). FRAP estimates were based on American food analyzed in one Norwegian laboratory (11).

In the present study, we used several terms to describe antioxidant intake. To describe the intake of antioxidants from all foods, we used FFQ-based TAC_total, to express total antioxidant intakes from the fruit, vegetables, and fruit and vegetables, we used FFQ-based TAC_fruit&vegetables, FFQ-based TAC_fruit and FFQ-based TAC_vegetables. Information on coffee was not available in the ORAC database; therefore, for comparability, we did not include coffee in FFQ-TRAP_total and FFQ-FRAP_total estimates.

We included as many food items as corresponded to the items in the FFQs. If several similar food sources (eg, several types of apples) were analyzed, we used the mean value. Overall, in the validity and reproducibility studies, we were able to obtain TAC concentrations for almost all antioxidant-rich foods included in the FFQ-based TAC estimates. In the FFQ-ORAC_total, FFQ-TRAP_total, and FFQ-FRAP_total estimates, we included values for fruit, vegetables, beverages (ie, tea, wine, and fruit juices), grain products, chocolate, and nuts (7, 8, 20). In the FFQ-TRAP_total estimates, we also included values for legumes and snacks (9, 10). In the FFQ-FRAP_total estimates, we also included values available for legumes, dairy, snacks, salad dressing, and meat dishes (11).

We only included values of raw foods when creating FFQ-based TAC_fruit&vegetables estimates. In the validity study, we included 5 fruit items in all FFQ-based TAC_fruit&vegetables estimates, 12 vegetable items in the FFQ-ORAC_fruit&vegetables estimates, and 11 vegetable items in the FFQ-TRAP_fruit&vegetables and FFQ-FRAP_fruit&vegetables estimates. In the reproducibility study, we included 5 fruit items in all FFQ-based TAC_fruit&vegetables estimates; 15 vegetable items had ORAC and FRAP values and 16 vegetable items had TRAP values. The range of FFQ-TAC_fruit&vegetables values varied from 337 (tomatoes) to 7740 (blueberries) μmol trolox equivalent/100 g in ORAC values, from 130 (tomatoes) to 1048 (raspberries) μmol trolox equivalent/100 g in TRAP values, and from 34 (carrots) to 2334 (raspberries) μmol trolox equivalent/100g in FRAP values. The mean ORAC values in the database were based on 8 collected samples for each food item (8), TRAP values were based on 3 samples (9, 10), and FRAP values were based on 1–8 samples (11). More details on the amounts and range of foods that were measured for each item in the different databases were provided elsewhere (7–11, 20). The FFQ used in both the validity study and the SMC in 1997 contained 33 food items with available ORAC values, 35 food items with TRAP values, and 60 food items with FRAP values. The FFQ used in the reproducibility study had 46 foods with ORAC values, 48 foods with TRAP values, and 75 foods with FRAP values. Food items for which there were no available TAC values included meat dishes, dairy, margarine or oils, alcoholic beverages, and sweets or snacks.

Analysis of plasma total antioxidant capacity

A single plasma sample was collected from participants while in a fasting state. For the analysis of plasma TAC, blood was collected in evacuated tubes containing EDTA, and samples were centrifuged immediately in a dark room at 3000 × g for 10 min at 4 °C. Plasma samples were separated and stored for 1 to 3 y at –80 °C. The plasma TAC was analyzed with the ORAC assay described by Prior et al (7) at the Arkansas Children’s Nutrition Center (Little Rock, AR). In addition, plasma samples were analyzed with the TRAP assay described by Serafini et al (21) and the FRAP assay described by Benzie and Strain (22) at the laboratory of the Unit of Human Nutrition (National Institute for Food and Nutrition Research, Rome, Italy). The intraassay and the interassay CVs in the measures were <5% and <5% for the ORAC assay, <3% and <7% for the TRAP assay, and <2% and <5% for the FRAP assay. Information on batching and quality control for each assay was provided elsewhere (7, 21, 22). To remove the contribution that each protein makes to the antioxidant capacity measurement in the ORAC assay (23), we added perchloric acid; we then separated the lipophilic part from the hydrophilic part by using an extraction procedure based on the work of Aeschischer et al (24) and Prior et al (7). Perchloric acid...
was added to precipitate the proteins, and the mean ORAC value dropped by 88% for the hydrophilic part of plasma and by 95% for the lipophilic part of plasma. We used the terms plasma ORAC<sub>whole</sub>, plasma ORAC<sub>hydrophilic</sub>, plasma ORAC<sub>lipophilic</sub>, plasma TRAP, and plasma FRAP to denote the different compartments of the plasma.

### Statistical analysis

In the analyses of validity, we included 108 women. Of those women who filled in the FFQ, we excluded regular (n = 91) and occasional (n = 28) dietary supplement users and those with unknown dietary supplement status (n = 8), because we were unable to control for the TAC value of dietary supplements. We also excluded women who reported an energy intake outside the range of 855 to 2161 kcal (± 2.5 SD) (n = 10) and one woman with unreliable plasma TAC data. To examine whether the validity group is representative of the SMC cohort in baseline characteristics [i.e., age, energy intake, body mass index (BMI), FFQ-based TAC estimates, education, and smoking], we also chose a group of women (n = 7154) from the cohort who were in the same age range and were not using dietary supplements.

Characteristics of the women participating in the study were presented as means and SDs for age, BMI, FFQ-TAC estimates, and TAC values in blood plasma and as the percentages of smokers and those who had > 12 y of education. To test the distribution of FFQ-based TAC estimates and plasma TAC, we used residual and goodness-of-fit analyses; these analyses showed no evidence of departure from normality, and therefore we used untransformed variables in the analysis. To examine the difference in background characteristics between study groups, we used t tests and chi-square tests. To rank the food items by their contribution to FFQ-based TAC estimates, we calculated the percentage of contribution of each food item to each TAC estimate. To evaluate validity, we calculated the Pearson correlation coefficients. The Pearson's partial correlation coefficients were used to examine whether the FFQ had any effect on the results. We adjusted dietary FFQ-based TAC estimates in the validation study for energy by using residuals methods (25).

Deattenuated Pearson correlations were obtained by using data on within-person variation (25) in the FFQ-based TAC estimates from 300 women in the reproducibility group. In addition, we plotted the crude FFQ-ORAC<sub>fruit&vegetables</sub> estimates against whole plasma ORAC and plasma TRAP. We assessed the reproducibility of the 3 FFQ-TAC estimates by calculating the intraclass correlation between 2 identical FFQs completed 1 y apart. Statistical analyses were performed with SAS (version 9.1; SAS Institute, Cary, NC) and STATA (version 9.2; STATA Corp, College Station, TX) software.

### RESULTS

Characteristics of participants in the validity and the reproducibility study are presented in Table 1. The validity group was composed of 108 women who were similar to the SMC comparison group (n = 7154) with regard to age, FFQ-based TAC estimates, and percentage of smokers. The women in the validity group had a slightly higher BMI, were better educated, and had a lower energy intake (P < 0.05 for all) than did the SMC comparison group. The validity group was not significantly different from the excluded women (regular and occasional supplement users) in background characteristics (i.e., age, energy intake, BMI, smoking, and education) except for FFQ-based ORAC<sub>total</sub> and FFQ-based TRAP<sub>total</sub> estimates, which were 13% and 4% (P < 0.05 for both) lower, respectively, in the validity group. In comparison with the group from the SMC (n = 7154), participants in the reproducibility group were older, had a higher energy intake, were less likely to be smokers, and were more educated (P < 0.05 for all). The reproducibility group also had TAC estimates higher than those of the comparison group from the SMC (P < 0.05).

Women participating in the reproducibility study who filled in both the first and second FFQs did not differ significantly from nonresponders to the 2 FFQs (n = 295) in background characteristics from the baseline SMC questionnaire (i.e., BMI, dietary supplement use, smoking, and education) (P > 0.05 for all). Responders were significantly different in few characteristics: they were 1.7 y younger (P = 0.05) and had 124 kcal greater energy intake (P < 0.05).

All food items contributing ≥2% to FFQ-ORAC<sub>total</sub>, FFQ-TRAP<sub>total</sub>, and FFQ-FRAP<sub>total</sub> estimates in the validity group are shown in Table 2. Fruit and vegetables were the major contributors to all 3 FFQ-based TAC estimates—FFQ-ORAC<sub>total</sub>, fruit, 34.8%; vegetables, 21.6%; FFQ-TRAP<sub>total</sub>, fruit, 23.0%; vegetables, 18.8%; and FFQ-FRAP<sub>total</sub>, fruit, 18.1%; vegetables, 19.9%. In Table 2, we have identified the 5 major fruit and vegetables contributing to each FFQ-based TAC estimates. In the reproducibility study, we also identified the top 5 fruit and vegetable contributors to the 3 different FFQ-based TAC total estimates—ORAC<sub>total</sub> estimates: apples or pears, 12.5%; berries, 5.7%; oranges, 5.5%; potatoes, 4.0%; and bananas, 2.6%; TRAP<sub>total</sub> estimates: oranges, 5.9%; berries, 4.9%; lettuce, 2.1%; prunes, 1.9%; and spinach, 1.7%; FRAP<sub>total</sub> estimates: oranges, 6.6%; berries, 5.5%; potatoes, 4.0%; apples or pears, 3.2%; and broccoli, 2.9%.

We observed significant Pearson correlation coefficients (P < 0.05) between different FFQ-based TAC estimates and plasma TAC concentrations that ranged from 0.20 to 0.35 (data not shown). These values, deattenuated for within-person variation in FFQ-based TAC estimates, are shown in Table 2. Pearson correlation coefficients (P < 0.05) between energy-adjusted FFQ-based TAC estimates and plasma TAC concentrations ranged between 0.20 and 0.32 (data not shown). We observed that the different FFQ-based TAC estimates (both crude and energy-adjusted) correlated with plasma ORAC<sub>whole</sub>, plasma ORAC<sub>lipophilic</sub>, and plasma TRAP concentrations. The highest positive correlation for plasma ORAC<sub>whole</sub> was with FFQ-ORAC<sub>vegetables</sub>; that for plasma ORAC<sub>lipophilic</sub> was with FFQ-ORAC<sub>fruit&vegetables</sub>; and that for plasma TRAP was with FFQ-ORAC<sub>vegetables</sub>. All 3 plasma TAC concentrations (ORAC<sub>whole</sub>, ORAC<sub>lipophilic</sub>, and TRAP) had significantly positive Pearson correlations with FFQ-based TAC<sub>total</sub> and FFQ-based TAC<sub>fruit&vegetables</sub> estimates (Table 3). The plasma ORAC<sub>hydrophilic</sub> and plasma FRAP concentrations did not correlate with any of the FFQ-based TAC estimates. The season in which women completed the FFQ had no effect on the correlation coefficients. In visual evaluation of scatter plots of crude values, we identified 2 possible outliers: after exclusion of these participants, the Pearson correlation coefficients were lower between plasma ORAC<sub>whole</sub> and the FFQ-ORAC<sub>fruit&vegetables</sub> (r = 0.18, P = 0.07).
### TABLE 1
Characteristics of the women participating in the validity study, the comparison group of the Swedish Mammography Cohort (SMC), and the reproducibility study.

<table>
<thead>
<tr>
<th>Background variable</th>
<th>Validity study (n = 108)</th>
<th>SMC cohort (n = 7154)</th>
<th>Reproducibility study (n = 300)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>63 ± 5&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>62 ± 6</td>
<td>65 ± 6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td>26.7 ± 4.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.6 ± 4.0</td>
<td>25.2 ± 3.6</td>
</tr>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>1520 ± 334&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1594 ± 313</td>
<td>1768 ± 503&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Smoking status (%)</strong></td>
<td>15&lt;sup&gt;e&lt;/sup&gt;</td>
<td>24</td>
<td>13&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt;12 y of education (%)</td>
<td>33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14</td>
<td>39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TAC all foods (μmol/d)</strong></td>
<td>FFQ-ORAC&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>12 127 ± 6180</td>
<td>12 079 ± 5913</td>
</tr>
<tr>
<td></td>
<td>FFQ-TRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>3165 ± 1659</td>
<td>3237 ± 1379</td>
</tr>
<tr>
<td></td>
<td>FFQ-FRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>2793 ± 1622</td>
<td>2866 ± 1479</td>
</tr>
<tr>
<td><strong>TAC, fruit and vegetables (μmol/d)</strong></td>
<td>FFQ-ORAC&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>6091 ± 3608</td>
<td>6523 ± 3374</td>
</tr>
<tr>
<td></td>
<td>FFQ-TRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>1542 ± 885</td>
<td>1416 ± 849</td>
</tr>
<tr>
<td></td>
<td>FFQ-FRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>1047 ± 559</td>
<td>983 ± 535</td>
</tr>
<tr>
<td><strong>Plasma TAC (μmol/L)</strong></td>
<td>Plasma-FFQ-ORAC&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>11 194 ± 2504 (6962–16 967)</td>
<td>16 288 ± 7266&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Plasma-ORAC&lt;sub&gt;lipophilic&lt;/sub&gt;</td>
<td>584 ± 210 (219–1091)</td>
<td>5673 ± 3382&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Plasma-ORAC&lt;sub&gt;hydrophilic&lt;/sub&gt;</td>
<td>1348 ± 285 (8069–2055)</td>
<td>3985 ± 2121&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Plasma-TRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>1172 ± 162 (859–1566)</td>
<td>8349 ± 4487&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Plasma-FRAP&lt;sub&gt;96-item&lt;/sub&gt;</td>
<td>842 ± 140 (592–1216)</td>
<td>2157 ± 1206&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. FFQ, food-frequency questionnaire; TAC, total antioxidant capacity; ORAC, oxygen radical absorbance capacity; TRAP, total radical-trapping antioxidant parameters; FRAP, ferric-reducing antioxidant power.
2. Because of the greater number of questions in this FFQ, higher values for energy intake and FFQ-based TAC estimates were obtained than with the 96-item FFQ used in the validity study and the SMC.
3. ± SD (all such values).
4. Significantly different from the SMC comparison group, P < 0.05 (t tests and chi-square tests).
5. FFQ-based TAC estimate from all foods.
6. FFQ-based TAC estimate from fruit and vegetables.
7. ORAC value of whole plasma.
8. Minimum and maximum in parentheses (all such values).
9. ORAC value of the lipophilic part of plasma.
10. ORAC value of the hydrophilic part of plasma.
11. TRAP value of whole blood plasma.
12. FRAP value of whole blood plasma.

and FFQ-TRAP<sub>fruit&vegetables</sub> (r = 0.15, P = 0.04) and FFQ-FRAP<sub>fruit&vegetables</sub> (r = 0.17, P = 0.01) estimates. Correlations between plasma ORAC<sub>lipophilic</sub> and the FFQ-ORAC<sub>fruit&vegetables</sub> (r = 0.31, P = 0.001) and FFQ-TRAP<sub>fruit&vegetables</sub> (r = 0.22, P = 0.03) estimates remained significant after the exclusion. In contrast, after exclusion, stronger correlations were observed between plasma TRAP and the

### TABLE 2
Food contributors, to 3 food-frequency questionnaire (FFQ) total antioxidant capacity (TAC) estimates, based on the 96-item FFQ completed by the validation group including 108 women.

<table>
<thead>
<tr>
<th>FFQ-ORAC</th>
<th>Percentage</th>
<th>FFQ-TRAP</th>
<th>Percentage</th>
<th>FFQ-FRAP</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit and vegetables&lt;sup&gt;2&lt;/sup&gt;</td>
<td>56.5</td>
<td>Fruit and vegetables&lt;sup&gt;2&lt;/sup&gt;</td>
<td>41.7</td>
<td>Fruit and vegetables&lt;sup&gt;2&lt;/sup&gt;</td>
<td>38.0</td>
</tr>
<tr>
<td>Grain products</td>
<td>19.7</td>
<td>Grain products</td>
<td>23.0</td>
<td>Tea</td>
<td>8.3</td>
</tr>
<tr>
<td>Tea</td>
<td>9.5</td>
<td>Tea</td>
<td>15.0</td>
<td>Grain products</td>
<td>7.4</td>
</tr>
<tr>
<td>Chocolate</td>
<td>4.9</td>
<td>Wine</td>
<td>6.0</td>
<td>Wine</td>
<td>7.1</td>
</tr>
<tr>
<td>Juice</td>
<td>3.9</td>
<td>Juice</td>
<td>4.5</td>
<td>Juicet</td>
<td>6.8</td>
</tr>
<tr>
<td>Wine</td>
<td>2.5</td>
<td>Juice</td>
<td>3.8</td>
<td>Chocolate</td>
<td>2.2</td>
</tr>
</tbody>
</table>

1. ORAC, oxygen radical absorbance capacity; TRAP, total radical-trapping antioxidant parameters; FRAP, ferric-reducing antioxidant power. Only food items contributing ≥2% are shown.
2. The top 5 fruit and vegetable contributors to FFQ-ORAC were apples or pears (16.1%), orange (6.2%), potatoes (5.8%), berries (5.0%), and banana (3.5%).
3. The top 5 fruit and vegetable contributors to FFQ-TRAP were orange (7.6%), apples or pears (6.4%), berries (4.7%), lettuce (3.5%), and spinach (2.7%).
TABLE 3
Validity of the food-frequency questionnaire–based total antioxidant capacity (TAC) estimates by comparison with plasma-TAC concentrations in a subgroup of 108 female dietary supplement nonusers from the Swedish Mammography Cohort.

<table>
<thead>
<tr>
<th>FFQ-based TAC estimates</th>
<th>Plasma-TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ORACwhole&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAC from all foods&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.27 (0.06, 0.46)</td>
</tr>
<tr>
<td>ORAC&lt;sub&gt;total&lt;/sub&gt;</td>
<td>0.22 (0.01, 0.42)</td>
</tr>
<tr>
<td>TRAP&lt;sub&gt;total&lt;/sub&gt;</td>
<td>0.24 (0.03, 0.43)</td>
</tr>
<tr>
<td>TAC from fruit and vegetables&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.35 (0.14, 0.53)</td>
</tr>
<tr>
<td>ORAC&lt;sub&gt;fruit&lt;/sub&gt; &amp; vegetables</td>
<td>0.31 (0.10, 0.51)</td>
</tr>
<tr>
<td>TRAP&lt;sub&gt;fruit&lt;/sub&gt; &amp; vegetables</td>
<td>0.28 (0.06, 0.47)</td>
</tr>
<tr>
<td>TAC from fruit&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.30 (0.07, 0.50)</td>
</tr>
<tr>
<td>ORAC&lt;sub&gt;fruit&lt;/sub&gt;</td>
<td>0.24 (0.01, 0.45)</td>
</tr>
<tr>
<td>TRAP&lt;sub&gt;fruit&lt;/sub&gt;</td>
<td>0.20 (−0.02, 0.42)</td>
</tr>
<tr>
<td>TAC from vegetables&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.36 (0.15, 0.55)</td>
</tr>
<tr>
<td>ORAC&lt;sub&gt;vegetables&lt;/sub&gt;</td>
<td>0.35 (0.14, 0.53)</td>
</tr>
<tr>
<td>TRAP&lt;sub&gt;vegetables&lt;/sub&gt;</td>
<td>0.31 (0.10, 0.51)</td>
</tr>
</tbody>
</table>

<sup>1</sup> ORAC, oxygen radical absorbance capacity; TRAP, total radical-trapping antioxidant parameters; FRAP, ferric-reducing antioxidant power. All results are Pearson correlation coefficients; 95% CIs in parentheses.
<sup>2</sup> ORAC value of whole plasma.
<sup>3</sup> ORAC value of the lipophilic part of plasma.
<sup>4</sup> ORAC value of the hydrophilic part of plasma.
<sup>5</sup> TRAP value of blood plasma.
<sup>6</sup> FRAP value of blood plasma.
<sup>7</sup> FFQ-based TAC total estimates including all items.
<sup>8</sup> FFQ-based TAC estimates from fruit and vegetables.
<sup>9</sup> FFQ-based TAC estimates from fruit.
<sup>10</sup> FFQ-based TAC estimates from vegetables.

The 1-y reproducibility of the FFQ is shown in Table 4. The intraclass correlations for the FFQ-based TAC<sub>total</sub> and FFQ-based TAC<sub>fruit&vegetables</sub> estimates ranged between 0.55 and 0.68 (P < 0.0001).

DISCUSSION

In this study of middle-aged and elderly Swedish women, fruit and vegetables were the major contributors to all FFQ-based TAC, FFQ-ORAC<sub>total</sub>, FFQ-TRAP<sub>total</sub>, and FFQ-FRAP<sub>total</sub> estimates. We observed that plasma ORAC<sub>whole</sub> plasma ORAC<sub>lipophilic</sub> and plasma TAC concentrations correlated positively modestly with FFQ-based TAC<sub>total</sub> and FFQ-based TAC<sub>fruit&vegetables</sub> estimates, which indicated moderately good validity. No correlation was observed between any of the FFQ-based TAC estimates (FFQ-based TAC<sub>total</sub> and FFQ-based TAC<sub>fruit&vegetables</sub>) and the plasma ORAC<sub>hydrophilic</sub> or plasma FRAP concentrations. The intraclass correlations of all FFQ-based TAC estimates (FFQ-based TAC<sub>total</sub> and FFQ-based TAC<sub>fruit&vegetables</sub>) assessed by using 2 FFQs completed 1 y apart were relatively high, which indicated good reproducibility.

To the best of our knowledge, there is no previous study validating FFQ-based TAC estimates by comparing them with ORAC or TRAP plasma measurements. There is only one earlier study that estimated the validity of FFQ-based TAC estimates by using plasma FRAP concentrations and plasma trolox equivalent.
antioxidant capacity (TEAC) concentrations as reference methods (26). These authors (26) did not observe any significant correlation between FFQ-FRAP estimate and plasma FRAP concentrations \((r = 0.17)\), and they did not find a significant correlation with TEAC \((r = 0.11)\). Both FRAP and TEAC are based on single-electron transfer mechanisms, which have some limitations in the measurement of biological fluids. Both assays lack relevance under physiologic conditions \((5, 27)\), and the FRAP assay is not able to measure the contribution to TAC from thiol groups \((5)\). Our results are in agreement with the previous studies on FRAP and may suggest that plasma single-electron transfer assays are not good biomarkers for dietary TAC. In contrast, both the ORAC and TRAP assays, which are based on hydrogen atom transfer mechanisms, were positively correlated with FFQ-based TACtotal and FFQ-based TACfruit&vegetables concentrations. However, once proteins were removed from the plasma ORACwhole, no correlation was observed between FFQ-based TAC estimates and plasma ORAChydrophilic concentrations. FFQ-based TAC estimates were however, significantly correlated with plasma ORAClipophilic concentrations. Many hydrophilic antioxidants are bound to proteins and therefore may be lost during the extraction procedure \((28)\), but lipophilic antioxidants such as carotenoids and tocopherols will be extracted into the lipid fraction and were previously shown to correlate with dietary antioxidant intake \((17)\). These findings could explain why we see a modest positive correlation with plasma ORAClipophilic, but not with plasma ORAChydrophilic.

In general, our results are in agreement with those of the ATTICA Study, which also observed significant positive correlations between TAC concentrations of plasma analyzed with an immune diagnostic assay and fruit intake \((r = 0.34)\), vegetable intake \((r = 0.31)\), and the Mediterranean diet score \((r = 0.24)\) \((29)\). Furthermore, the observed significant Pearson correlation coefficients between FFQ-based TAC estimates and the plasma ORACwhole, plasma ORAClipophilic, and plasma TRAP concentrations in our study are of a range similar to that in previous validation studies in which fruit and vegetable intakes or single phytochemical estimates assessed with an FFQ were compared with estimates derived by using such biomarkers as vitamin C, vitamin E, and carotenoids \((13–17)\).

This study is the first to investigate the validity and reproducibility of FFQ-based TAC estimates by using data from available databases. Whereas the ORAC \((7, 8)\) and TRAP \((9, 10)\) databases are limited to fruit and vegetables, the FRAP database contains an extensive number of foods besides fruit and vegetables \((11)\). The concept of TAC has so far been applied in 2 observational epidemiologic studies \((6, 12)\). In the first, a study by Serafini et al \((6)\), high TAC intake (assessed by TRAP from fruit and vegetables) was inversely associated with gastric cancer. In the second, a study of the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition, researchers observed a lower risk of mortality in subjects with a high dietary TRAP intake from plant foods \((12)\).

The present study had several strengths. First, we used a random sample from a population-based cohort. Second, we used 3 parallel databases to assess FFQ-based TAC estimates and several plasma TAC measurements as biomarkers to assess the validity of the FFQ-based TAC estimates. Third, we included only supplement nonusers in our study to avoid possible contributions from dietary supplements to plasma TAC values. This limited cohort, however, may limit the generalizability of our results.

The study also has some limitations. First, we were not using data on Swedish foods when calculating TAC estimates. Available databases of TAC values of different foods are based on American \((7, 8, 11)\) and Italian \((9)\) foods. Different results are likely to be observed if we used TAC values obtained from laboratory analyses of foods consumed in Sweden, because the antioxidant content can vary with geographic location and growing conditions \((30)\). Factors influencing the antioxidant content may explain the difference in the rank order of contributors to the 3 TAC estimates. However, the difference in the rank order may also be explained by the fact that ORAC, TRAP, and FRAP are not based on the same chemistry. These differences in rank order were observed by Pellegrini et al \((9)\), who constructed 3 parallel databases using the same food items analyzed with the TRAP, FRAP, and TEAC assays; the ranking pattern they found was not similar.

Second, the 96-item FFQ used in the validation study asked about only a limited number of foods rich in antioxidants; however, these foods are the most commonly consumed in the study population. Because of a greater number of questions on fruit and vegetables in the 123-item FFQ, the reproducibility group differed in estimates of fruit and vegetable intakes from the SMC comparison group, which completed only a 96-item FFQ.

Third, one blood collection may not reflect the usual average dietary intake measured with the FFQ and does not provide information on random variation in biomarker concentrations within a person over time. One blood collection is common in many epidemiologic studies and may not reflect a person's long-term exposure. Collecting multiple blood samples per participant is one way to take into account an intraindividual variability over time in biomarkers \((31)\).

Fourth, the question could be raised as to whether plasma TAC measurement is a good reference method in validating FFQ-based TAC estimates—it may not be a direct measurement of dietary antioxidant intake. Plasma TAC is influenced by many factors, such as endogenous antioxidants; homeostatic control mechanisms of plasma antioxidants, stress, environment, pollution, inflammation and absorption; and the extent of the metabolism of dietary antioxidants. Uric acid is an antioxidant produced endogenously from dietary purines \((32)\), and it is one of the major antioxidants in human plasma. It has been shown that uric acid can provide as much as 60% of oxygen and free-radical scavenging in human serum \((33)\). Other dietary constituents such as fructose intake have also been shown to alter uric acid concentrations \((34)\). Uric acid has been shown to contribute 7% of the serum ORAC value, 20–60% of the plasma TRAP value, and 60% of the serum FRAP values \((35)\). We have observed (unpublished observations, 2006) that plasma concentrations of uric acid correlated significantly with plasma ORACwhole, plasma TRAP, and plasma FRAP, whereas uric acid correlated poorly with plasma ORAClipophilic, and plasma ORACwhole. Despite the fact that concentrations of endogenous antioxidants are tightly regulated in vivo, there is a need to validate dietary antioxidant intake with biomarkers of antioxidant efficiency, such as TAC, to understand the link between diet and the redox defense homeostasis in humans. Thus, the influence of other factors besides dietary intake on antioxidant biomarker values may partly explain the somewhat weak correlation coefficients in the present study and in other studies \((14, 36, 37)\). Finally, we measured TAC in plasma, which does not necessarily reflect changes
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in various tissues. Concentrations of antioxidants vary between different organs according to their requirements (38, 39).

In summary, our results indicate that FFQ-based TAC_{total} and FFQ-based TAC_{fruit/vegetables} estimates based on the ORAC, TRAP, and FRAP assays are relatively valid and reproducible estimates of dietary antioxidant exposures and that they may be used in nutritional epidemiology to assess total antioxidant intake. Further studies in other populations are needed to confirm our results.

The authors’ responsibilities were as follows—SR, MS, RLP, and AW: the study concept and design; AW: data collection; SR and MS: biochemical analyses; SR and AW: statistical analyses; SR, MS, RM, RLP, and AW: interpretation of results; SR: wrote the draft of the manuscript; SR, MS, RM, RLP, and AW: review and revision of the manuscript; and all authors: review of the final manuscript. None of the authors had a personal or financial conflict of interest.

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