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Total Antioxidant Performance Is Associated with Diet and Serum Antioxidants in Participants of the Diet and Physical Activity Substudy of the Jackson Heart Study[1,2]

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Abstract

Total antioxidant performance (TAP) measures antioxidant capacities in both hydrophilic and lipophilic compartments of serum and interactions known to exist between them. Our objective was to assess TAP levels in a subset of Jackson Heart Study (JHS) participants and to examine associations with dietary and total (diet + supplement) intakes of α-tocopherol, γ-tocopherol (diet only), β-carotene, vitamin C, fruit, vegetables, and nuts, and serum concentrations of α-tocopherol, γ-tocopherol, and β-carotene. We conducted a cross-sectional analysis of 420 (mean age 61 y; 254 women) African American men and women participating in the Diet and Physical Activity Sub-Study of the JHS in Jackson, Mississippi. In multivariate-adjusted models, we observed positive associations between total α-tocopherol, total and dietary β-carotene, and total vitamin C intakes and TAP levels (P-trend < 0.05). Positive associations were also observed for vegetable, fruit, and total fruit and vegetable intakes (P-trend < 0.05). For serum antioxidant nutrients, α-tocopherol but not β-carotene was associated with serum TAP levels. There were inverse associations for serum γ-tocopherol and TAP levels. Associations for α-tocopherol were seen at intake levels much higher than the current Recommended Dietary Allowance. It may, therefore, be prudent to focus on increasing consumption of fruit, vegetables, nuts, and seeds to increase total antioxidant capacity. J. Nutr. 139: 1964–1971, 2009.

Introduction

Oxidative stress is an imbalance between the production of reactive oxygen radicals and the ability of the organism's natural protective mechanisms to cope with these radicals and to prevent adverse effects (1). Cells in the body are exposed to reactive oxygen species under normal circumstances via the leakage of electrons from the electron transport chain, phagocytic cells, and endogenous enzyme systems (2). The oxidation of lipids, nucleic acids, or protein by these reactive oxygen species is thought to be associated with the etiology of several age-related chronic diseases, including cancer (3), cardiovascular disease (4), cataract (5), and age-related macular degeneration (6). These chronic diseases account for a high percentage of morbidity and mortality and preventing them is a major public health priority.

Arrays of defense systems protect the body from the deleterious effects of oxidative stress. These include antioxidant enzymes and radical-scavenging antioxidants (7). Antioxidant nutrients such as vitamin E, carotenoids, and fruits and vegetables rich in such antioxidants have been associated with a lower risk of diseases caused by oxidative stress (8–10). Whereas individual actions of antioxidants have been reported, a large number of studies have indicated that cooperative/synergistic interactions exist among antioxidants in plasma (11). Therefore, studying the overall antioxidant status may be more biologically relevant than studying a single antioxidant (12). A relatively recent method called total antioxidant performance (TAP),[8] developed by Aldini et al. (13) and validated by Beretta

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[8] Abbreviations used: BODIPY 581/591, 4, 4-difluoro-5-(4-phenyl-1, 3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid; DPASS, Diet and Physical Activity Sub-Study; JHS, Jackson Heart Study; ORAC, oxygen radical absorbance capacity assay; TAP, total antioxidant performance.

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et al. (14), measures not only antioxidant capacity in both the hydrophilic and lipophilic compartments of the biological system, but also their synergistic/ cooperative interactions. Given the potential for nutrients and foods to contribute to the prevention of oxidative stress-associated chronic diseases and the unique ability of TAP to measure total antioxidant status, the objectives of our study were to: *1*) measure serum antioxidant capacity using the TAP assay in a subset of Jackson Heart Study (JHS) participants; *2*) examine associations between dietary and total intakes of α-tocopherol, γ-tocopherol (diet only), β-carotene, vitamin C, vegetable, fruit and nut intake, and TAP levels; and *3*) examine associations between serum α- and γ-tocopherol, β-carotene, and TAP levels.

Participants and Methods

Study population. The men and women in this cross-sectional analysis were participants of the Diet and Physical Activity Sub-Study (DPASS) of the JHS. The JHS is a single-site prospective epidemiological investigation of cardiovascular disease among African Americans from the Jackson, Mississippi metropolitan area. A detailed description of the original study has been published elsewhere (15).

Study sample selection. A subset of participants (n = 499) from the JHS cohort (n = 5301) was selected for the JHS DPASS. As participants were enrolled in the JHS, investigators recruited participants for DPASS to include an equal number of men and women from younger (34–64 y) and older (≥65 y) age groups, from lower and higher socioeconomic status, and from lower and higher physical activity groups. All eligible participants were invited to be part of DPASS until each of the enrollment strata were filled. The aim of DPASS was to provide data for validation of the diet and physical activity instruments used for the entire cohort of the JHS.

Dietary assessment. The Lower Mississippi Delta Nutrition Intervention Research Initiative conducted a telephone survey in the Delta region to collect representative dietary data using 24-h dietary recalls. These data were used to develop a new FFQ designed for use in the LMD region. Details regarding development of this regional FFQ are available elsewhere (16). This FFQ, called the Delta NIRI FFQ (283 items), and its shortened version, the Delta NIRI JHS FFQ (158 items), which was specifically developed for use in the JHS, were then used as dietary assessment tools in the DPASS. We have previously validated both FFQ against the mean of 4 24-h recalls (17), serum tocopherols (18), and carotenoids (19).

Laboratory analyses. Participants provided blood samples on the day of the baseline clinic interview, which took place on the day of administration of the short FFQ. Blood samples from fasting (12 h) participants were collected in vacutainer tubes and centrifuged at 3000 × g for 10 min at 4°C. Samples were frozen at −70°C until analyzed. Serum TAP was determined by the method first developed by Aldini et al. (13) to measure total antioxidant capacity in both the hydrophilic and lipophilic compartments of serum and validated by Beretta et al. (14) for the application to high throughput studies. This method measures the rate of oxidation of 4, 4-difluoro-5-(4-phenyl-1, 3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (BODIPY 581/591), a lipid-soluble fluorescent probe, and uses the lipid-soluble radical initiator 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile). Oxidation is determined by monitoring the appearance of green fluorescence of the oxidation product of BODIPY (λ_{ex} = 500 nm, λ_{em} = 520 nm) using a 1420 multilabel counter (Wallac Victor 2, Perkin Elmer Life Sciences). The results are expressed as TAP values, which represent the percentage of inhibition of BODIPY oxidation in human serum with respect to that occurring in a control sample consisting of BODIPY 581/591 in phosphatidylcholine liposomes. For the measurement of serum carotenoids and tocopherols, analyses were performed using HPLC, as described by Yeum et al. (20,21). After standard lipid extraction with chloroform:methanol (2:1) followed by hexane, samples were analyzed for carotenoids and tocopherols using a reverse phase HPLC system consisting of a 600S controller (Millipore), Waters 616 pump, Waters 717 autosampler, Waters 996 photodiode array detector, and C30 carotenoid column (3 μm, 150 × 4.6 mm, YMC). Millenium32 was the operating system. The programmable photodiode array detector was set at 445 and 455 nm for carotenoids and 292 nm for tocopherols. Carotenoids and tocopherols were quantified by determining peak areas in the HPLC chromatograms, calibrated against known standards. Serum cholesterol and uric acid concentrations for the cohort were determined according to methods described previously (22).

Other covariates. Information on covariates was obtained at either the initial home visit or the JHS baseline clinic visit. Age was computed from self-reported date of birth. Information regarding presence of self-reported hypertension was assessed from the medical history questionnaire. Smoking status was derived from a set of questions regarding cigarette use. Participant height and weight were measured by trained technicians at the clinic visit using physician quality measurement scales in an exam gown with no shoes. Detailed information regarding the procedures used for the anthropometric procedures conducted at the clinic visit have been published elsewhere (15). BMI was calculated as weight/height^2 (kg/m^2).

Statistical analyses. Several dietary assessment instruments were used in the DPASS of the JHS. However, we used data from the Delta NIRI JHS FFQ (158 item) for this analysis, as this questionnaire was administered on the day of the blood draw and is the only dietary questionnaire that was used in the main JHS cohort. We excluded participants who reported energy intake outside the plausible range of ≤600 or ≥4000 kcal/d[9] on the FFQ (n = 34) or whose FFQ had 5 or more food items blank (n = 2). DPASS participants without blood samples for the TAP analysis (n = 43) were also excluded, leaving a sample of 420 individuals. For analyses with serum nutrients, due to missing data, n = 416 for β-carotene and 417 for α-tocopherol. Two participants had γ-tocopherol concentrations below detectable levels; therefore, the number of observations for those analyses was 418.

We examined associations between TAP levels and sample characteristics. Variables included dietary intakes of antioxidants, fruit and vegetables, and nuts and serum measures of dietary antioxidants, uric acid, and cholesterol. Servings of vegetables per day were estimated from questions on the FFQ. These included vegetable groups such as orange vegetables, sweet potato, tomato and tomato products, green leafy vegetables, root vegetables, and other vegetables but did not include white potato and white potato products. Also, vegetables that were part of mixed dishes, e.g. mixed dishes with meat were not

[9] 1 kcal = 4.184 kJ.

included. Servings of fruit were also estimated from questions on the FFQ. These included all citrus and noncitrus fruit and fruit juice, but not fruit drinks.

Associations of TAP with nuts, including peanuts, peanut butter, and pecans, were also examined. As mentioned previously, the FFQ was developed using 24-h recall data from the Delta region. The serving sizes are therefore reflective of this region.

As dietary and biological measures of antioxidant nutrients and foods were skewed, they were log transformed prior to analysis. Dietary intake data were energy adjusted using the residual method (23). Log-transformed dietary measurements were regressed on log-transformed total energy intake to compute residuals. The predicted value of the dietary measurement for the mean of total energy intake was added back to each residual, and the antilogarithm of this value was taken. We tested for interactions between the main dietary and serum predictors (in continuous form) and sex with respect to TAP levels. As none were significant, we present the results for men and women together. We estimated least squares means for TAP levels by quartile of dietary as well as total (diet + supplement) intake of α-tocopherol (mg/d), γ-tocopherol (diet only, mg/d), β-carotene (μg/d), vitamin C (mg/d), fruit, vegetables, and nuts (all servings per day). Because TAP is a new measure of serum antioxidant status, we examined several variables that were available for the cohort as potential covariates and confounders of the association with antioxidants, fruit and vegetables, and nuts and serum measures of dietary antioxidants and adjusted for these in the models. After analysis with a simple model, a second model was further adjusted for age (y), sex, BMI (kg/m^2), energy intake (kJ/d), supplement use (yes/no), current smoking status (yes/no), and self-reported hypertension (yes/no). Serum uric acid, which is mainly produced endogenously but is also affected by dietary intake, was strongly associated with TAP levels. Serum uric acid was also a significant negative confounder between total α- tocopherol intake and TAP. Therefore, for the analyses with α-tocopherol, we examined the association between a residual measure of TAP after adjustment for serum uric acid and α-tocopherol intake.

For serum antioxidant analysis, we examined the associations between TAP and serum β-carotene and α and γ-tocopherol levels. Two models were examined, the first adjusting for age (y), sex, BMI (kg/m^2), serum cholesterol concentrations (mmol/L), and serum uric acid concentrations (μmol/L) and the second model further adjusted for current smoking status (yes/no) and presence of self-reported hypertension (yes/no).

We also present the *P*-values for test for trend, which were calculated by assigning subjects the median value of the category of nutrient or food group or serum antioxidant nutrient being considered and including this as a continuous variable in the model. All statistical tests were 2-sided with a significance level of 0.05. SAS version 9.1 (SAS Institute) was used for all statistical analyses.

Results

Age was positively associated with serum TAP levels (*P*-trend $<$ 0.01) (**Table 1**). A higher percentage of women were in the lower quartile of TAP (75 compared with 45% in the highest quartile; $P < 0.0001$). BMI was positively associated with TAP (*P*-trend $<$ 0.05). Although there was a difference in supplement use across the TAP quartiles (with an increasing trend up to quartile 3), the percentage of supplement users in the highest quartile was 46% compared with 50% in the lowest quartile. Smoking status did not differ across TAP quartiles. A higher percentage of participants in the highest quartile of TAP reported hypertension (66 compared with 48% in the lowest quartile; $P < 0.001$). Serum β-carotene, γ-tocopherol, and cholesterol concentration were not associated with TAP. Serum uric acid and α-tocopherol were associated across TAP levels (*P*-trend $\leq$ 0.0001). There were no associations between intakes of energy, total or dietary α- or γ- tocopherol, and serum TAP levels. Total and dietary β-carotene intake and vegetable and fruit intake were positively associated with serum TAP levels. There were weak associations between dietary and total vitamin C intake and serum TAP level.

Unlike dietary α - and γ-tocopherols and vitamin C intakes, the highest quartile of dietary β-carotene intake with a median intake of 4.0 mg/d had ~4% higher serum TAP compared with the lowest quartile of intake, with a median intake of 1.75 mg/d; tests for trend were also significant (**Table 2**). The highest quartile of total β-carotene intake, with a median intake of 4.3 mg/d, was also associated with 3.2% higher serum TAP levels compared with the lowest quartile with a median intake 1.9 mg/d and tests for trend were significant. The highest quartile of total vitamin C intake was also associated with an ~2.5% increase in TAP levels compared with the lowest.

Serum β-carotene and TAP levels were not associated (**Table 3**). Individuals in the highest quartile of serum α-tocopherol concentrations had significantly higher serum TAP compared with those in the lowest quartile (~5.6% higher). Tests for trend were also significant. γ-Tocopherol concentrations and TAP levels were inversely associated. No associations were seen for a further adjusted model.

The highest quartile of vegetable intake, with a median intake of 1.7 servings/d, was associated with ~4% higher TAP values compared with the lowest quartile, with a median intake of 0.8 servings/d (**Table 4**). Although serum TAP did not differ between quartiles of fruit intake, tests for trend were significant ($P <$ 0.05). The strongest associations were for combined fruit and vegetable intake (for both models), with significant associations between the lowest quartile of intake and the subsequent quartiles, as well as for trend. Nuts, which are a good source of vitamin E, were also examined. However, only small amounts of nuts were consumed in this population and there were no associations with TAP levels.

Discussion

A variety of assays have been developed to measure antioxidant capacity in plasma. These include the oxygen radical absorbance capacity assay (ORAC) (24), the Randox Trolox-equivalent antioxidant capacity assay (25) and the ferric reducing ability of plasma assay (26). However, most of these assays use either a hydrophilic or a lipophilic approach, thereby not capturing valuable information on the interactions that exist between antioxidants of the 2 compartments (27).

The TAP assay measures the activity of both the hydrophilic and lipophilic antioxidants in serum/plasma along with their interactions (14). The results of this study show that, in African Americans, total α-tocopherol, total vitamin C, total and dietary β-carotene, fruit, vegetable, and fruit plus vegetable intakes are associated with serum TAP levels. Serum α-tocopherol was also associated with this measure of overall antioxidant status.

α-Tocopherol in serum is a potent lipid-soluble antioxidant (28). In this study, we did not find an association between increasing quartiles of dietary α-tocopherol intake and TAP

TABLE 1 Sample characteristics of the JHS diet and physical activity substudy participants by quartiles of TAP[1]

Variables	TAP[2] *(% protection)*				
	Q1[3]	Q2	Q3	Q4	*P*[4]
Participants' characteristics					
Age, *y*	59.2 ± 0.92	60.0 ± 0.92	62.4 ± 0.92	62.1 ± 0.92	0.007
Women, *%*	75.2	60.0	62.0	44.8	0.0001
BMI,[5] *kg/m²*	29.6 ± 0.66	29.9 ± 0.64	31.7 ± 0.64	31.4 ± 0.64	0.013
Current smoker, *%*	5.7	10.5	16.2	8.6	0.282
Supplement user, *%*	49.5	57.1	69.5	45.7	0.003
Self reported hypertension, *%*	47.6	64.8	78.1	66.4	0.001
Serum β-carotene,[5] μ*mol/L*	0.64 ± 0.06	0.73 ± 0.06	0.67 ± 0.06	0.61 ± 0.06	0.421
Serum α-tocopherol,[5] μ*mol/L*	27.0 ± 1.38	31.0 ± 1.34	34.4 ± 1.33	32.6 ± 1.33	0.001
Serum γ-tocopherol,[5] μ*mol/L*	5.78 ± 0.41	5.39 ± 0.40	5.25 ± 0.40	6.39 ± 0.40	0.585
Serum uric acid,[5] μ*mol/L*	275 ± 7.38	324 ± 7.14	361 ± 7.15	430 ± 7.11	0.0001
Serum cholesterol,[5] *mmol/L*	5.18 ± 0.10	4.97 ± 0.10	5.06 ± 0.10	5.29 ± 0.10	0.986
Food/nutrient intakes					
Energy,[6] *kJ/d*	8198 ± 306	8440 ± 296	8045 ± 297	8168 ± 295	0.663
Total α-tocopherol,[6,7] *mg/d*	55.6 ± 11.8	89.4 ± 11.4	96.0 ± 11.4	64.4 ± 11.4	0.249
Dietary α-tocopherol,[6] *mg/d*	6.98 ± 0.21	6.67 ± 0.20	6.59 ± 0.20	7.27 ± 0.20	0.568
Dietary γ-tocopherol,[6] *mg/d*	13.9 ± 0.45	13.3 ± 0.44	13.3 ± 0.43	14.4 ± 0.43	0.562
Total β-carotene,[6,7] μ*g/d*	2928 ± 113	3115 ± 109	3302 ± 109	3317 ± 109	0.005
Dietary β-carotene,[6] μ*g/d*	2666 ± 106	2811 ± 103	2937 ± 103	3085 ± 102	0.002
Total vitamin C,[6,7] *mg/d*	184 ± 19.1	178 ± 18.5	220 ± 18.5	208 ± 18.4	0.06
Dietary vitamin C,[6] *mg/d*	112 ± 6.57	109 ± 6.36	120 ± 6.37	124 ± 6.33	0.07
Vegetables,[6,8] *servings/d*	1.15 ± 0.04	1.13 ± 0.04	1.12 ± 0.04	1.30 ± 0.04	0.009
Fruit,[6,9] *servings/d*	1.39 ± 0.09	1.40 ± 0.09	1.55 ± 0.09	1.61 ± 0.09	0.021
Vegetables + fruit,[6,8,9] *servings/d*	2.54 ± 0.10	2.53 ± 0.10	2.67 ± 0.10	2.91 ± 0.10	0.002
Nuts,[6,10] *servings/d*	0.29 ± 0.04	0.24 ± 0.04	0.23 ± 0.04	0.27 ± 0.04	0.332

[1] Values are means ± SE or %, n = 420, 416 (serum β-carotene), 417 (serum α-tocopherol and uric acid), 418 (serum γ-tocopherol) or 419 (hypertension).
[2] Median total antioxidant performance values for Q1 through Q4 were: 64.0, 71.7, 76.5, and 81.2% protection, respectively.
[3] Quartile.
[4] Continuous variables were examined using linear regression (SAS Proc GLM) with test for trend across median values of total antioxidant performance in each quartile. Categorical variables were examined with chi-square analysis.
[5] Adjusted for age and sex and, for serum antioxidant concentrations, also for serum cholesterol. Values for serum antioxidant, cholesterol, and uric acid concentrations are presented in original scale.
[6] Adjusted for sex, age, and energy intake (except for energy intake itself). Dietary, total nutrient, and food intakes are presented in original scale.
[7] Total = dietary + supplement intake.
[8] Vegetable intakes were calculated from specific questions about vegetable consumption on the FFQ. We were unable to include the vegetables part of mixed recipes in this calculation.
[9] Fruit intakes were calculated from specific questions asking about fruit and fruit juice consumption on the FFQ.
[10] Nut intakes were calculated from specific questions asking about nuts and nut butters on the FFQ.

levels, but we did see significantly greater TAP levels in the highest vs. lowest quartile of total α-tocopherol intake (including supplements) and a linear trend across quartiles. This association remained after adjustment for several covariates. The median intake of total α-tocopherol intake in the highest quartile was 285 mg/d, whereas the mean daily dietary intake of α-tocopherol in this population was ~7 mg/d, which is considerably lower than the Estimated Average Requirement of 12 mg/d for adults (29). The highest quartile of total α-tocopherol intake in this population, therefore, represents supplement use. In accordance with the correlation between plasma TAP and α-tocopherol in a human plasma model (13) and in plasma samples from a small number of participants previously reported by Beretta et al. (14), there was an association between α-tocopherol and TAP values but only for those taking supplements.

TAP levels did not differ across γ- tocopherol intake quartiles. The inverse association between serum γ- tocopherol and TAP levels for 1 of the statistical models examined was surprising. A study that examined the effect of smoking and environmental tobacco exposure on plasma antioxidant status in smokers, passive smokers, and nonsmokers found that whereas several other plasma antioxidant concentrations were significantly lower in smokers and passive smokers compared with nonsmokers, the levels of γ-tocopherol were elevated even after adjusting for dietary intakes (30). These results warrant further investigation into the role of γ- tocopherol in oxidative stress.

Both dietary and total β-carotene and total vitamin C intakes were associated with serum TAP. Intervention studies have shown that β-carotene and vitamin C supplements alone or in combination with other known antioxidants have been shown to improve antioxidant capacity (31) or lower markers of oxidative stress (32). Although, we did see significantly higher TAP values with higher β-carotene intake and vitamin C (total intake only) intakes, results were not reflected in serum β-carotene and TAP associations. Because serum vitamin C has not been estimated in the cohort, we were not able to examine its associations with

TABLE 2 Adjusted mean total antioxidant performance of JHS DPASS participants by antioxidant nutrient intakes[1–3]

	Model	
Antioxidant nutrient[4]	Basic	Adjusted[5]
Total α-tocopherol intake, *mg/d*		
4.6	72.2 ± 0.53[a]	72.0 ± 0.73[a]
7.4	72.0 ± 0.53[a]	72.0 ± 0.69[a]
23.7	73.7 ± 0.53[ab]	73.4 ± 0.74[a,b]
285	75.2 ± 0.54[b]	74.7 ± 0.76[b]
P-trend[6]	<0.0001	0.01
R^2	0.06	0.09
Dietary α-tocopherol intake, *mg/d*		
4.7	72.8 ± 0.55	72.4 ± 0.65
5.7	73.4 ± 0.55	73.1 ± 0.65
6.6	73.5 ± 0.55	73.2 ± 0.66
8.1	73.1 ± 0.55	72.7 ± 0.69
P-trend[6]	NS[7]	NS
R^2	0.002	0.07
Dietary γ-tocopherol intake,[2] *mg/d*		
7.8	72.7 ± 0.74	72.3 ± 0.85
10.8	74.1 ± 0.74	74.1 ± 0.86
13.6	72.1 ± 0.74	72.6 ± 0.87
17.1	73.8 ± 0.74	74.6 ± 0.88
P-trend[6]	NS	NS
R	0.01	0.14
Total β-carotene intake, $\mu g/d$		
1920	72.0 ± 0.74[a]	72.0 ± 0.89[a]
2767	73.2 ± 0.71[a,b]	73.4 ± 0.85[a]
3389	74.0 ± 0.71[a,b]	73.8 ± 0.87[a]
4319	74.5 ± 0.71[b]	74.3 ± 0.87[a]
P-trend[6]	0.01	0.03
R^2	0.02	0.13
Dietary β-carotene intake, $\mu g/d$		
1750	71.3 ± 0.74[a]	71.8 ± 0.86[a]
2351	73.5 ± 0.74[a,b]	73.7 ± 0.86[a]
3063	73.8 ± 0.74[a,b]	73.8 ± 0.87[a]
4010	74.3 ± 0.74[b]	74.1 ± 0.86[a]
P-trend[6]	0.01	0.04
R^2	0.02	0.14
Total vitamin C intake, *mg/d*		
69.0	73.1 ± 0.74[a,b]	73.3 ± 0.83[a,b]
108	72.1 ± 0.74[a]	72.2 ± 0.90[a]
155	72.8 ± 0.74[a,b]	72.6 ± 0.89[a,b]
498	74.9 ± 0.74[b]	75.0 ± 0.90[b]
P-trend[6]	0.01	0.01
R^2	0.02	0.14
Dietary vitamin C intake, *mg/d*		
58.4	72.7 ± 0.74	73.0 ± 0.83
86.0	73.3 ± 0.74	73.7 ± 0.87
123	73.2 ± 0.74	73.1 ± 0.87
173	73.7 ± 0.74	74.0 ± 0.91
P-trend[6]	NS	NS
R^2	0.002	0.13

[1] Values are means ± SE. Within category and column, means with superscripts without a common letter differ after Tukey's adjustment for multiple comparisons, $P < 0.05$.
[2] TAP values for α-tocopherol (total and dietary) are residuals, adjusted for serum uric acid.
[3] n = 420 (basic model) and n = 418 (adjusted model).
[4] Median value of energy adjusted nutrient for quartile.
[5] Adjusted for age, sex, BMI, energy intake, supplement use, current smoker, and self-reported hypertension.
[6] Test for trend was calculated across median values of intake in each quartile.
[7] Not significant.

TABLE 3 Adjusted mean total antioxidant performance of JHS DPASS participants by serum antioxidant concentrations[1,2]

	Model	
Serum antioxidant[3]	Adjusted 1[4]	Adjusted 2[5]
Serum α-tocopherol, $\mu mol/L$		
19.4	71.4 ± 0.55[a]	71.6 ± 0.61[a]
25.3	72.5 ± 0.53[a,b]	72.2 ± 0.60[a]
31.7	73.7 ± 0.54[b]	73.3 ± 0.60[a,b]
45.4	75.7 ± 0.55[b]	75.1 ± 0.66[b]
P-trend[6]	<0.0001	<0.0001
R^2	0.51	0.51
Serum γ-tocopherol, $\mu mol/L$		
1.88	75.1 ± 0.55[a]	74.2 ± 0.66
3.82	73.0 ± 0.54[a]	72.5 ± 0.60
6.21	72.0 ± 0.54[a]	72.0 ± 0.60
9.75	73.0 ± 0.57[a]	73.1 ± 0.63
P-trend[6]	0.02	NS[7]
R^2	0.49	0.50
Serum β-carotene, $\mu mol/L$		
0.215	72.4 ± 0.57[a]	72.4 ± 0.60
0.376	73.5 ± 0.55[a]	73.2 ± 0.60
0.638	72.8 ± 0.55[a]	72.4 ± 0.60
1.17	74.5 ± 0.58[a]	73.8 ± 0.66
P-trend[6]	0.02	NS
R^2	0.48	0.49

[1] Values are mean ± SE. Within category and column, means with superscripts without a common letter differ after Tukey's adjustment for multiple comparisons, $P < 0.05$.
[2] α-Tocopherol n = 416 (Model 1) and 415 (2); γ-tocopherol n = 417 (Model 1) and 416 (2); β-carotene n = 415 (Model 1) and 414 (2).
[3] Median value of serum antioxidant concentrations are in original scale.
[4] Adjusted for age, sex, BMI, serum cholesterol, and uric acid.
[5] Adjusted for above plus current smoker and self reported hypertension.
[6] Test for trend was calculated across the median value of serum antioxidant concentration in each quartile.
[7] Not significant.

serum TAP levels. Considering that TAP was significantly (positively) associated with intake of fruit and vegetables and that this remained after adjustment for dietary intakes of β-carotene and vitamin C (data not shown), the association of these food groups with TAP is not solely because of these well-documented antioxidant vitamins. Intervention studies have demonstrated increases in overall antioxidant status with increases in fruit and vegetable intakes (33–35). On the other hand, single or combinations of antioxidant supplement interventions have not shown improvements of antioxidant status or oxidative stress measures (36–38). It is interesting to note that observational studies have also suggested that higher intake of foods that are rich sources of these phytochemicals are associated with lower risk of chronic disease morbidity and mortality (39–42).

We are not aware of any other study examining associations between this particular measure of antioxidant status (serum TAP levels) and dietary intake of antioxidants and antioxidant-rich foods. The results from our study are consistent with others that have examined the associations between diet, dietary estimates of total antioxidant capacity, and other biological estimates of total antioxidant capacity. In a recent study, Rautiainen et al. (43) concluded that fruit and vegetables were major contributors to FFQ-based total antioxidant capacity measures such as ORAC, total radical-trapping antioxidant

TABLE 4 Adjusted mean total antioxidant performance of JHS DPASS participants by fruit and vegetable intakes[1–3]

	Model	
Food group[3]	Basic	Adjusted[4]
Vegetable intake, *servings/d*		
0.82	72.0 ± 0.74[a]	72.5 ± 0.84[a]
0.96	72.6 ± 0.74[a]	72.6 ± 0.86[a,b]
1.20	73.2 ± 0.74[a,b]	73.4 ± 0.88[a,b]
1.66	75.1 ± 0.74[b]	75.2 ± 0.87[b]
P-trend[6]	0.001	0.003
R^2	0.02	0.14
Fruit intake, *servings/d*		
0.75	72.1 ± 0.74[a]	72.3 ± 0.85[a]
1.11	73.0 ± 0.74[a]	73.4 ± 0.85[a]
1.57	73.7 ± 0.74[a]	73.6 ± 0.89[a]
2.22	74.1 ± 0.74[a]	74.6 ± 0.89[a]
P-trend[6]	0.03	0.04
R^2	0.01	0.13
Vegetable + fruit intake, *servings/d*		
1.72	71.3 ± 0.73[a]	71.6 ± 0.83[a]
2.28	73.1 ± 0.73[a,b]	73.5 ± 0.85[a,b]
2.81	73.6 ± 0.73[a,b]	73.5 ± 0.89[a,b]
3.63	75.0 ± 0.73[b]	75.2 ± 0.87[b]
P-trend[6]	0.0007	0.0006
R^2	0.03	0.15
Nut intake, *servings/d*		
0.006	73.1 ± 0.74	72.9 ± 0.88
0.05	73.1 ± 0.74	73.1 ± 0.89
0.18	73.3 ± 0.74	73.4 ± 0.85
0.51	73.4 ± 0.74	73.8 ± 0.86
P-trend[6]	NS	NS
R^2	0.0003	0.12

[1] Values are mean ± SE. Within category and column, means with superscripts without a common letter differ after Tukey's adjustment for multiple comparisons, $P < 0.05$.
[2] n = 420 (basic model) and n = 418 (multivariate model).
[3] Median value of energy adjusted food for quartile.
[4] Adjusted for age, sex, BMI, energy intake, supplement use, current smoker, and self-reported hypertension.
[5] Test for trend was calculated across median values of intake in each quartile.

parameters, and ferric reducing ability of plasma assay. These dietary estimates were also positively correlated with plasma measures of ORAC and total radical-trapping antioxidant parameters. Results from the ATTICA epidemiologic study (44) demonstrated that, in an apparently healthy population, plasma total antioxidant capacity was positively associated with consumption of fruit and vegetables. Our results disagree with a recent crossover intervention study examining the effects of an intervention of a high and low total antioxidant capacity diet on markers of antioxidant status, systemic inflammation, and liver dysfunction (45). This study did not observe significant differences in serum total antioxidant capacity measures associated with the 2 interventions. Unlike our study, where there was a wide variation in antioxidant and fruit and vegetable intakes, the intervention study had fewer participants and a similar range of fruit and vegetable intake due to its study protocol. This may have prevented them from detecting differences in serum total antioxidant capacity between the 2 study groups.

In the past there has been criticism leveled against the concept of "total antioxidant capacity" (46). Most assays use either a hydrophilic or lipophilic approach and are unable to capture interactions between fat- and water-soluble antioxidants that exist or antioxidant enzymes that contribute to the antioxidant defense systems in the body. Yeum et al. (47) recently demonstrated that the TAP assay captures the synergistic protective associations between both water- and fat-soluble antioxidants. However, it is important to note that, similar to the shortcomings of other assays, TAP cannot capture direct reactive oxygen or nitrogen scavenging activities.

In the present study, information on nutritional intake was obtained by FFQ. In the past there have been several criticisms levied against this method of dietary assessment (48). However, the FFQ is the method of choice for dietary assessment in large epidemiological studies for ranking nutritional intakes of individuals (49). Also, the FFQ used in the present study was region specific and designed to capture the intake patterns of the study population and had been previously validated for total α-tocopherol and β-carotene intakes using corresponding measures in serum as biomarkers (18,19). Our study participants were from a population-based cohort and were not disease free. TAP may be influenced by the presence of disease and hence final models were adjusted for presence of self-reported hypertension, because it was one of the most commonly reported heath conditions. Although we adjusted for a number of covariates, all observational studies may have residual confounding.

In recent years, several antioxidant supplement trials have shown lack of benefit and, in some cases, potential harm (50,51). This has shifted the focus to overall dietary patterns and food intake rather than single nutrient intakes (52). Although higher total antioxidant status was associated with antioxidant supplement use, our study also clearly demonstrates that modest increases in fruit and vegetable intake (1 serving/d of vegetables or 2 servings/d of fruit and vegetables) were positively associated with total antioxidant status. Antioxidant nutrients that are associated with increased fruit and vegetable consumption include carotenoids and vitamin C (53). Beneficial effects of increased fruit and vegetable consumption have been attributed in part to their antioxidant flavonoids. A review of intervention studies examining the effect of fruit and vegetables on measures of antioxidant capacity attributed postintervention increases to a spike in serum/plasma concentrations of uric acid. According to the authors, fructose intake (mainly due to fruit consumption) results in purine nucleotide degradation from fructose catabolism, resulting in increased uric acid concentrations and, thereby, increased antioxidant capacity measures (54).

In general, we found ~4% greater serum TAP levels across extreme quartile categories of intakes of total α-tocopherol, β-carotene (total and dietary), total vitamin C, fruit and vegetables, and serum measures of α-tocopherol. This seemingly small increase may be due to the fact that endogenous serum components (e.g. protein, uric acid, etc.) contribute greatly to overall antioxidant capacity, and dietary micronutrients may, thus, play a relatively small role. The clinical importance of this increase in serum TAP levels is not presently known. Further studies measuring TAP in other populations and examining its associations with clinical outcomes are warranted.

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A.T. supervised data collection for the JHS; H.A.T. and K.L.T. were responsible for funding acquisition. S.A.T. and K.L.T. were responsible for final content. All authors made critical comments during the preparation of the manuscript and fully accept responsibility for the work.

Literature Cited

1. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol. 2001;54:176–86.
2. Lachance PA, Nakat Z, Jeong WS. Antioxidants: an integrative approach. Nutrition. 2001;17:835–8.
3. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J. 1996;313:17–29.
4. Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. Pharmacol Ther. 2001;89:187–206.
5. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J. 1995;9:1173–82.
6. Cai J, Nelson KC, Wu M, Sternberg P Jr, Jones DP. Oxidative damage and protection of the RPE. Prog Retin Eye Res. 2000;19:205–21.
7. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res. 1999;31:261–72.
8. Rimm EB, Stampfer MJ. Antioxidants and chronic disease: evidence from observational epidemiology. Bibl Nutr Dieta. 2001;80–91.
9. Hu FB. Plant-based foods and prevention of cardiovascular disease: an overview. Am J Clin Nutr. 2003;78:S544–51.
10. Blomhoff R. Dietary antioxidants and cardiovascular disease. Curr Opin Lipidol. 2005;16:47–54.
11. May JM, Qu ZC, Mendiratta S. Protection and recycling of alpha-tocopherol in human erythrocytes by intracellular ascorbic acid. Arch Biochem Biophys. 1998;349:281–9.
12. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med. 2000;29:1106–14.
13. Aldini G, Yeum KJ, Russell RM, Krinsky NI. A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. Free Radic Biol Med. 2001;31:1043–50.
14. Beretta G, Aldini G, Facino RM, Russell RM, Krinsky NI, Yeum KJ. Total antioxidant performance: a validated fluorescence assay for the measurement of plasma oxidizability. Anal Biochem. 2006;354:290–8.
15. Taylor HA Jr, Wilson JG, Jones DW, Sarpong DF, Srinivasan A, Garrison RJ, Nelson C,Wyatt SB. Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study. Ethn Dis. 2005;15:S6–4-17.
16. Tucker KL, Maras J, Champagne C, Connell C, Goolsby S, Weber J, Zaghloul S, Carithers T, Bogle ML. A regional food-frequency questionnaire for the US Mississippi Delta. Public Health Nutr. 2005;8:87–96.
17. Carithers TC, Talegawkar SA, Rowser ML, Henry OR, Dubbert PM, Bogle ML, Taylor HA Jr, Tucker KL. Validity and calibration of food frequency questionnaires used with African-American adults in the Jackson Heart Study. J Am Diet Assoc. 2009;109:1184–93.
18. Talegawkar SA, Johnson EJ, Carithers T, Taylor HA Jr, Bogle ML, Tucker KL. Total alpha-tocopherol intakes are associated with serum alpha-tocopherol concentrations in African American adults. J Nutr. 2007;137:2297–303.
19. Talegawkar SA, Johnson EJ, Carithers TC, Taylor HA, Bogle ML, Tucker KL. Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs. Public Health Nutr. 2008;11:989–97.
20. Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI, Russell RM. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. Am J Clin Nutr. 1996;64:594–602.
21. Yeum KJ, Ahn SH, Rupp de Paiva SA, Lee-Kim YC, Krinsky NI, Russell RM. Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. J Nutr. 1998;128:1920–6.
22. Carpenter MA, Crow R, Steffes M, Rock W, Heilbraun J, Evans G, Skelton T, Jensen R, Sarpong D. Laboratory, reading center, and coordinating center data management methods in the Jackson Heart Study. Am J Med Sci. 2004;328:131–44.
23. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol. 1999;149:531–40.
24. Cao G, Alessio HM, Cutler RG. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med. 1993;14:303–11.
25. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods Enzymol. 1994;234:279–93.
26. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70–6.
27. Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and beta-carotene. Am J Clin Nutr. 1995;62:S1322–6.
28. Jessup W, Kritharides L, Stocker R. Lipid oxidation in atherogenesis: an overview. Biochem Soc Trans. 2004;32:134–8.
29. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academy Press; 2000.
30. Dietrich M, Block G, Norkus EP, Hudes M, Traber MG, Cross CE, Packer L. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. Am J Clin Nutr. 2003;77:160–6.
31. Meydani M, Martin A, Ribaya-Mercado JD, Gong J, Blumberg JB, Russell RM. Beta-carotene supplementation increases antioxidant capacity of plasma in older women. J Nutr. 1994;124:2397–403.
32. Duthie SJ, Ma A, Ross MA, Collins AR. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res. 1996;56:1291–5.
33. Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr. 1998;68:1081–7.
34. Roberts WG, Gordon MH, Walker AF. Effects of enhanced consumption of fruit and vegetables on plasma antioxidant status and oxidative resistance of LDL in smokers supplemented with fish oil. Eur J Clin Nutr. 2003;57:1303–10.
35. Cao G, Russell RM, Lischner N, Prior RL. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. J Nutr. 1998;128:2383–90.
36. Castenmiller JJ, Lauridsen ST, Dragsted LO, van het Hof KH, Linssen JP, West CE. Beta-carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. J Nutr. 1999;129:2162–9.
37. Hininger IA, Meyer-Wenger A, Moser U, Wright A, Southon S, Thurnham D, Chopra M, Van Den Berg H, Olmedilla B, et al. No significant effects of lutein, lycopene or beta-carotene supplementation on biological markers of oxidative stress and LDL oxidizability in healthy adult subjects. J Am Coll Nutr. 2001;20:232–8.
38. McKay DL, Perrone G, Rasmussen H, Dallal G, Hartman W, Cao G, Prior RL, Roubenoff R, Blumberg JB. The effects of a multivitamin/mineral supplement on micronutrient status, antioxidant capacity and cytokine production in healthy older adults consuming a fortified diet. J Am Coll Nutr. 2000;19:613–21.
39. Steffen LM, Jacobs DR Jr, Stevens J, Shahar E, Carithers T, Folsom AR. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr. 2003;78:383–90.
40. Park Y, Subar AF, Kipnis V, Thompson FE, Mouw T, Hollenbeck A, Leitzmann MF, Schatzkin A. Fruit and vegetable intakes and risk of colorectal cancer in the NIH-AARP diet and health study. Am J Epidemiol. 2007;166:170–80.
41. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr. 2007;85:895–909.
42. Liu S, Serdula M, Janket SJ, Cook NR, Sesso HD, Willett WC, Manson JE, Buring JE. A prospective study of fruit and vegetable intake and the risk of type 2 diabetes in women. Diabetes Care. 2004;27:2993–6.
43. Rautiainen S, Serafini M, Morgenstern R, Prior RL, Wolk A. The validity and reproducibility of food-frequency questionnaire-based total

antioxidant capacity estimates in Swedish women. Am J Clin Nutr. 2008;87:1247–53.

44. Pitsavos C, Panagiotakos DB, Tzima N, Chrysohoou C, Economou M, Zampelas A, Stefanadis C. Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: the ATTICA study. Am J Clin Nutr. 2005;82:694–9.
45. Valtuena S, Pellegrini N, Franzini L, Bianchi MA, Ardigo D, Del Rio D, Piatti P, Scazzina F, Zavaroni I, et al. Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. Am J Clin Nutr. 2008;87:1290–7.
46. Sies H. Total antioxidant capacity: appraisal of a concept. J Nutr. 2007;137:1493–5.
47. Yeum KJ, Beretta G, Krinsky NI, Russell RM, Aldini G. Synergistic interactions of antioxidant nutrients in a biological model system. Nutrition. 2009;25:839–46.
48. Kristal AR, Peters U, Potter JD. Is it time to abandon the food frequency questionnaire? Cancer Epidemiol Biomarkers Prev. 2005;14:2826–8.
49. Willett WC, Hu FB. The food frequency questionnaire. Cancer Epidemiol Biomarkers Prev. 2007;16:182–3.
50. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007;297:842–57.
51. Lin J, Cook NR, Albert C, Zaharris E, Gaziano JM, Van Denburgh M, Buring JE, Manson JE. Vitamins C and E and beta carotene supplementation and cancer risk: a randomized controlled trial. J Natl Cancer Inst. 2009;101:14–23.
52. Lichtenstein AH, Russell RM. Essential nutrients: food or supplements? Where should the emphasis be? JAMA. 2005;294:351–8.
53. Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which plasma antioxidants are most related to fruit and vegetable consumption? Am J Epidemiol. 2001;154:1113–8.
54. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med. 2006;41:1727–46.