Intakes of Antioxidants in Coffee, Wine, and Vegetables Are Correlated with Plasma Carotenoids in Humans[1]

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ABSTRACT The consumption of fruits and vegetables reduces the risk of major chronic degenerative diseases. The active compounds and the mechanisms involved in this protective effect have not been well defined. The objective of this study was to determine the contribution of various food groups to total antioxidant intake, and to assess the correlations of the total antioxidant intake from various food groups with plasma antioxidants. We collected 7-d weighed dietary records in a group of 61 adults with corresponding plasma samples, and used data from a nationwide survey of 2672 Norwegian adults based on an extensive FFQ. The total intake of antioxidants was ~17 mmol/d with β-carotene, α-tocopherol, and vitamin C contributing <10%. The intake of coffee contributed ~11.1 mmol, followed by fruits (1.8 mmol), tea (1.4 mmol), wine (0.8 mmol), cereals (i.e., all grain containing foods; 0.8 mmol), and vegetables (0.4 mmol). The intake of total antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene. Among individual food groups, coffee, wine, and vegetables were significantly correlated with dietary zeaxanthin, β-carotene, and α-carotene. These data agree with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense. Surprisingly, the single greatest contributor to the total antioxidant intake was coffee. J. Nutr. 134: 562–567, 2004.

KEY WORDS: • *total antioxidants* • *coffee* • *wine* • *vegetables* • *carotenoids*

A diet rich in fruits and vegetables reduces the incidence of major diseases such as cancer, cardiovascular disease, diabetes, cataracts, and inflammatory disease (1–9). Recommendations to increase fruit and vegetable consumption have therefore been implemented in most countries (1–4). Because the active compounds and the mechanisms involved in this protective effect have not been well defined, the recommendations suggest that eating a variety of fruits and vegetables will provide the best protection (1–9).

A possible mechanism mediating the protective effect is related to bioactive compounds in fruits and vegetables that reduce oxidative stress, a condition that arises when the formation of reactive oxidants (most importantly reactive oxygen species, reactive nitrogen species, and reactive iron species) outstrips the antioxidant defense, and oxidative damage occurs (10–14). Fruits and vegetables contain at least several hundred different types of antioxidants (i.e., electron- or hydrogen-donating reductants) that may react directly with such reactive oxidants, forming products with much lower reactivity and therefore potentially protecting against oxidative damage (10–14). Another mechanism involves activation of genes encoding proteins in the antioxidant defense system and/or silencing of genes that may contribute to the oxidative stress (15–17). Analyses of promoter regions have suggested that several response elements may be involved in such transcriptional regulation, including activator protein-1 sites, nuclear factor-κB sites, and antioxidant/electrophilic response elements. Among several different bioactive compounds, dietary antioxidants are suggested to play a role in transcriptional regulation via these response elements (15–18). Antioxidants may therefore protect against oxidative damage both by directly neutralizing reactive oxidants, and by modulating gene expression contributing to oxidative stress.

Results from most intervention trials with single antioxidants in pharmacologic doses, however, have not supported a protective effect (19–24). One reason for the ineffective clinical trials may be that the protective effects of fruits and vegetables result from a concerted action of the numerous different antioxidants present in foods, i.e., antioxidants with different chemical characteristics may work in an integrated and complementary network. Such interaction has been proven in vitro for α-tocopherol, α-tocotrienol, vitamin C, lipoic acid, and thiols by Packer and colleagues (25), but the concept could have much broader validity as suggested by Buettner (26).

Recent studies suggest that the well-known antioxidants (e.g., α-tocopherol, vitamin C, and β-carotene) contribute a relatively small part of the total antioxidants in most dietary

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plants, whereas the majority is contributed by antioxidants such as other carotenoids, phenolic acids, sulfides, flavonoids, and lignans (27–29). If many of these antioxidants work in a network (25–26), the total amount of antioxidants derived from the combinations of individual antioxidants that occur naturally in foods may be a better concept than individual dietary antioxidants.

The ferric reducing ability of plasma (FRAP)[4] assay (30) quantifies the total concentration of water- and fat-soluble antioxidants as the concentration of electrons or hydrogen atoms released in a redox reaction above a certain redox potential. The FRAP assay is based on the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. Because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants. By using this method, we observed recently that there is a >1000-fold difference between the total concentrations of antioxidants in various dietary plants (29). Interestingly, most berries, walnuts, sunflower seeds, ginger, and pomegranates are among the high antioxidant dietary plants, whereas many dietary plants contribute little to the total antioxidant intake (29).

We determined the contribution of different food groups in the Norwegian diet for the total antioxidant intake in a group of 61 adults (based on a 7-d dietary weighed record) and a nationwide survey of 2672 Norwegian adults (based on an extensive FFQ). In addition, to study the concordance of the concept of "total antioxidants" with plasma biomarkers, we tested whether plasma antioxidant vitamins and thiols were correlated with intake of total antioxidants.

SUBJECTS AND METHODS

The 7-d weighed dietary record study. The subjects ($n = 61$), all native Norwegians, were recruited consecutively in a general clinical practice at the time of their regular check-ups. Patients at high risk of cardiovascular disease dominate this practice. Eligible subjects were men and women ≥ 18 y old who had not previously undergone dietary intervention. A research assistant met the participants in groups of 2–6. At these group meetings, the participants filled in a short food questionnaire (31). A blood sample was taken from nonfasting subjects, and body weight and height were measured. The project was approved by the regional ethics committee, and written informed consent was obtained from all of the participants.

The individuals were between 30 and 82 y old with BMI between 18.9 and 49.5 kg/m^2 (**Table 1**). The men ($n = 28$) had a mean age of 57 y and the women ($n = 33$) had a mean age of 59 y. Most of the participants were nonsmokers and 48% used lipid-lowering agents (mainly statins).

The 7-d weighed dietary record was collected on consecutive days. The participants were provided with a record notebook and a digital scale with an accuracy of 1 g and a maximum capacity of 3000 g. They were given thorough practical and written instructions on how to weigh and describe in detail the consumption of foods and beverage. We stressed that the purpose of the study was to record their normal food intake and that any temptation to change the diet so as to lose weight or simplify the recording, should be resisted. After recording, the diet record was checked for completeness and then coded by a nutritionist and a research assistant. The data were double-checked for consistency and coding errors.

Estimates of basal metabolic rate (BMR) were calculated from standard formulas for men and women aged 30–59, 60–74 and >74 y old based on height, weight, age, and sex. A comparison of reported energy intake with estimates of BMR was used to calculate the number of respondents who underreported their energy intake. Based on estimates of BMR with 95% confidence limits and a diet recording period of 7 d, a ratio between measured energy intake (EI) and BMR (EI:BMR) < 1.10 for individual records may indicate underreporting (32); in 28 subjects this ratio was ≤1.10.

TABLE 1

Characteristics of the subjects in the 7-d weighed-record study[1]

Age, *y*	57.8 ± 9.4
Height, *cm*	169 ± 9
Weight, *kg*	80.9 ± 16.0
BMI, *kg/m²*	28.2 ± 4.7
EI:BMR	1.12 ± 0.24
	n (%)
User of lipid lowering agent	29 (48)
Men	28 (46)
User of dietary supplements	24 (39)
Current smokers	4 (7)

[1] Values are means ± SD, $n = 61$ or n (%).

Blood samples from nonfasting subjects were centrifuged at 1000 × g for 15 min and plasma was kept at −18°C for a maximum of 2 d before being stored at −70°C. Serum values from fasting subjects for cholesterol and triglycerides were obtained from the patient's recent medical records. FRAP assay was performed as described (29,30). Cholesterol and triglycerides were analyzed by Capio Laboratoriemedisin.

Nationwide survey. Total antioxidant intake was also assessed in the NORKOST2 study, a nationwide probability sample of 2672 Norwegian adults whose diets were characterized using an extensive, self-administered FFQ (33). No blood samples were obtained in NORKOST2.

Calculation of intakes of nutrients and total antioxidants. Daily intake of nutrients and total antioxidants was computed using a food database and software systems developed at the Institute for Nutrition Research, University of Oslo. The food database is based mainly on the official food composition table (34), and is continuously updated. The food database was supplemented with data on total antioxidant concentrations in foods measured by the FRAP assay [(29) and R. Blomhoff, unpublished results]. Cod liver oil and vitamin-mineral supplements were included in the calculations. In this study, we used the term $FRAP_{total}$ to describe the total intake of antioxidants, whereas the term $FRAP_{without\ coffee}$ expresses the total antioxidant intake minus antioxidants derived from coffee. The terms $FRAP_{coffee}$, $FRAP_{tea}$, $FRAP_{wine}$, $FRAP_{cereal}$, $FRAP_{fruit}$, and $FRAP_{vegetable}$ express total antioxidant intakes from the respective food groups. Cereals include all grain-containing foods.

Reagents for HPLC analysis. Lutein, α-carotene, β-carotene, lycopene, astaxanthin, BHT, tocol, glutathione, cysteine, homocysteine, and cysteinyl-glycine were supplied by Sigma. Tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol) were supplied by Calbiochem. Zeaxanthin was obtained from Carl Roth. The homocysteine HPLC kit (cat. no. 195-4075) was provided by Bio Rad. The water used was Milli-Q water with resistivity of 18.2 MΩ · cm.

HPLC analysis of carotenoids. The precipitating solution was prepared by mixing 20 μL internal standard (1 mg of astaxanthin in 4 mL benzene) in 10 mL of 2-propanol containing 10 mg/L BHT. A 200-μL aliquot of plasma sample was transferred to a 2-mL amber glass vial and 900 μL of the precipitating solution was added. The mixture was mixed on a vortex for 5 min and centrifuged for 15 min at 3000 × g at 4°C; 100 μL of the supernatant was injected into the HPLC system. A Waters pump was used to deliver the mobile phase to the analytical column (YMC Carotenoid S5μm, 4.6 × 250 mm from Waters) and detection was done by a Waters Tunable Absorbance Detector at 453 nm. The mobile phases used were A (100%

[4] Abbreviations used: BMR, basal metabolic rate; EI, energy intake; FRAP, ferric reducing ability of plasma.

TABLE 2

Daily dietary intake estimated from the 7-d weighed-record study[1]

	Mean ± SD	Median (P25, P75)[2]
Intake of energy, *MJ*	7.5 ± 1.9	7.6 (6.2, 8.8)
Energy from protein, %	16.2 ± 2.4	16.0 (14.5, 17.4)
Energy from fat, %	30.8 ± 6.0	30.6 (26.7, 35.1)
Energy from carbohydrates, %	50.8 ± 6.7	51.2 (45.9, 55.1)
Energy from added sugar, %	7.0 ± 4.3	6.4 (4.4, 9.3)
Fiber, *g*	21.3 ± 7.7	20.0 (15.7, 24.9)
β-Carotene, *μg*	3198 ± 2161	2769 (1603, 4218)
α-Tocopherol, *mg*	11.2 ± 5.8	9.2 (6.3, 15.3)
Vitamin C, *mg*	124 ± 59	109 (79, 162)
	mmol	
$FRAP_{total}$	17.3 ± 9.4	16.5 (10.5, 22.2)
$FRAP_{without\ coffee}$	6.2 ± 3.2	5.5 (3.9, 7.7)
$FRAP_{coffee}$	11.1 ± 9.3	10.5 (4.7, 15.7)
$FRAP_{tea}$	1.4 ± 2.4	0.7 (0.0, 1.6)
$FRAP_{wine}$	0.8 ± 1.3	0 (0, 1.3)
$FRAP_{cereal}$	0.8 ± 0.3	0.8 (0.6, 1.0)
$FRAP_{fruit}$	1.8 ± 1.2	1.8 (0.8, 2.7)
$FRAP_{vegetable}$	0.4 ± 0.3	0.4 (0.2, 0.6)

[1] Dietary intake values include supplements.
[2] P25, 25th percentile; P75, 75th percentile.

water), B (30% acetone in absolute ethanol), and C (100% acetone). A gradient elution was used with initial conditions of 10% A and 90% B at a flow rate of 2 mL/min. This was immediately followed by a linear increase to 100% B in 5 min. After elution with 100% B for 5 min, there was a linear increase to 100% solvent C in 1.5 min. After an additional 11 min, the system was returned to the initial conditions in 1 min and was equilibrated for 7 min before the next injection.

HPLC analysis of total thiols. Total thiols were determined using the homocysteine kit from BioRad with further validation of the method for total glutathione, total cysteine, and total cysteinylglycine. The internal standard solution, the sample preparation, and HPLC method used were as described by the manufacturer.

HPLC analysis of tocopherols and tocotrienols. The precipitating solution was prepared by mixing 125 μL of internal standard (5.8 mg tocol dissolved in 10 mL absolute ethanol) with 20 mL of 2-propanol containing 10 mg/L BHT. To a 200-μL aliquot of the plasma sample, 600 μL of the precipitating solution was added. The mixture was mixed on a vortex and centrifuged for 15 min at 3000 × *g* at 4°C; 20 μL of the supernatant was injected into the HPLC system. A Waters pump was used to deliver the mobile phase (acetonitrile:methanol:dichloromethane:water, 60:30:5:5 by vol) at 1.0 mL/min to the analytical column (Suplex pKb-100, 5 μm, 250 mm × 4.6 mm id). Detection was done using a Shimadzu fluorescence detector with excitation at 294 nm and emission at 330 nm.

Statistical methods. Because the intake data and plasma metabolite concentrations were not normally distributed, these values are presented both as sample means with SD and medians with percentiles. Furthermore, the intake data and the plasma concentrations of carotenoids and tocopherols were natural log transformed to normalize their distribution before calculation of the Pearson correlation coefficients. The correlations are presented both unadjusted and adjusted for age, BMI, serum triglycerides, and serum cholesterol (Pearson's partial correlation coefficients). The difference in plasma concentration of triglycerides, cholesterol, carotenoids, thiols, and tocopherols between men and women was tested with an unpaired *t* test. All of the statistical analyses were performed using SPSS 11.0. A significance level of 5% was used.

RESULTS

Contribution of different food groups to intake of antioxidants. Daily intakes of total antioxidants, β-carotene, α-tocopherol, vitamin C, and energy in the 7-d weighed dietary records are presented in **Table 2**. The estimated intake of total antioxidants was 17.3 ± 9.4 mmol/d. The mean intakes of β-carotene, α-tocopherol, and vitamin C corresponded to 0.01, 0.05, and 1.41 mmol antioxidants (i.e., electron- or hydrogen-donating reductants), respectively, because these molecules can donate 1, 2, and 2 electrons or hydrogens, respectively, in a redox reaction. Thus, in total they contributed ~1.47 mmol compared with a total intake of 17.3 mmol.

The intake of coffee contributed ~11.1 mmol or 64% of the total antioxidant intake, followed by fruits and berries (1.8 mmol), tea (1.4 mmol), wine (0.8 mmol), cereals (0.8 mmol), and vegetables (0.4 mmol). Small amounts were also contributed by edible fat (0.3 mmol), cakes (0.2 mmol), and potatoes (0.1 mmol); <0.1 mmol total antioxidants was contributed by each of fish, milk, meat, sweets, and beer. Of the noncoffee antioxidants, on average, β-carotene, α-tocopherol, and vitamin C intake contributed 23.7%.

Similar results were obtained when the contribution of different food groups to total antioxidant intake was estimated using a representative nationwide dietary survey in Norway that is based on an extensive, self-administered FFQ (**Table 3**). Thus, these data sets demonstrated that coffee was a major contributor to total antioxidant intake. Furthermore, of the noncoffee antioxidants, fruits (including berries), tea, cereals, wine, and vegetables contributed ~30, 24, 12, 10, and 6% (mean of the two studies) of total antioxidants, respectively.

Correlations between total antioxidant intake and intakes of β-carotene, α-tocopherol and vitamin C. In the 7-d weighed dietary records, the intake of vitamin C was significantly correlated with the intake of total antioxidants from fruits and vegetables ($r = 0.82$ and $r = 0.46$, respectively) (**Table 4**). Furthermore, intake of β-carotene was also significantly correlated with total intake antioxidants in vegetables ($r = 0.55$).

Due to increased statistical power, a more precise description of the correlations was possible in the nationwide dietary survey (Table 4). β-Carotene intake estimated from NORKOST2 was correlated with total antioxidant intake from

TABLE 3

Contribution of different food groups to antioxidant intake in the 7-d weighed-record study and NORKOST2 study

Total intake of antioxidants,[2] *mmol*	7-d weighed-record study 17.3 ± 9.4	NORKOST2[1] 17.6 ± 10.6
	% of total antioxidant intake	
Cereals	5	4
Fruits and berries	11	7
Fruit juices	2	2
Vegetables	2	2
Coffee	64	68
Tea	8	9
Wine	5	2
Other foods	5	8

[1] The NORKOST2 used an extensive, self-administered FFQ developed by the National Nutrition Council (33).
[2] Values are means ± SD, *n* = 61 (weighed-record study) or 2672 (NORKOST2).

TABLE 4

Pearson correlation coefficients between total antioxidant intakes and intakes of β-carotene, α-tocopherol and vitamin C[1]

	7-d weighed-record study			NORKOST2		
	β-Carotene	α-Tocopherol	Vitamin C	β-Carotene	α-Tocopherol	Vitamin C
$FRAP_{total}$	0.08	0.33*	0.15	0.11**	0.03	0.11**
$FRAP_{without\ coffee}$	0.16	0.25	0.60**	0.22**	0.16**	0.47**
$FRAP_{coffee}$	0.00	0.24	−0.07	0.04	−0.03	−0.04
$FRAP_{tea}$	−0.05	−0.02	0.19	0.15	0.09**	0.11**
$FRAP_{wine}$	0.02	0.14	0.09	0.00	0.06*	0.05*
$FRAP_{cereal}$	0.14	0.25	0.00	0.11**	0.12**	0.20**
$FRAP_{fruit}$	0.15	0.10	0.82**	0.17**	0.15**	0.73**
$FRAP_{vegetable}$	0.55**	0.16	0.46**	0.57**	0.16**	0.52**

[1] In the 7-d weighed-record study, $n = 61$; for the nationwide dietary survey in Norway (NORKOST2), $n = 2672$. All variables were natural log transformed before computing correlation coefficients. * $P < 0.01$, ** $P < 0.001$.

cereals, fruits, and vegetables whereas α-tocopherol and vitamin C intake was correlated with total antioxidant intakes from tea, wine, cereals, fruits, and vegetables. Intake of total antioxidants from coffee was not correlated with intake of any of the single antioxidants.

Correlations between total antioxidant intakes and plasma antioxidants. The plasma concentrations of carotenoids, thiols, and tocopherols in the 7-d weighed dietary record study (**Table 5**) were all within normal ranges. There were no differences between men and women except for a significantly higher cholesterol concentration in women compared with men (data not shown).

The total intake of antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene (adjusted correlation coefficients 0.33, 0.40, and 0.31, respectively), whereas the noncoffee antioxidant intake was significantly correlated with plasma lutein and β-carotene (adjusted correlation coefficients 0.27 and 0.26, respectively). Intakes of coffee, wine, and vegetables were significantly correlated with zeaxanthin, β-carotene, and α-carotene (adjusted correlation coefficients 0.27, 0.31, and 0.28, respectively; **Table 6**).

TABLE 5

Plasma metabolite concentrations in 61 men and women in the 7-d weighed-record study

	Mean ± SD	Median (P25, P75)[1]
	mmol/L	
Cholesterol	5.3 ± 1.1	5.2 (4.4, 6.1)
Triglycerides	1.46 ± 0.84	1.30 (0.85, 1.80)
	μmol/L	
Lutein	0.209 ± 0.091	0.197 (0.148, 0.267)
Zeaxanthin	0.058 ± 0.039	0.063 (0.026, 0.084)
α-Carotene	0.125 ± 0.075	0.120 (0.066, 0.168)
β-Carotene	0.517 ± 0.312	0.425 (0.289, 0.704)
Lycopene	0.898 ± 0.856	0.563 (0.261, 1.324)
Glutathione	5.07 ± 1.41	5.00 (4.10, 6.00)
Cysteine	322.3 ± 44.8	315.0 (294.0, 343.0)
Homocysteine	11.0 ± 2.4	11.1 (9.3, 12.2)
Cysteinyl-glycine	31.2 ± 5.1	30.5 (27.4, 34.7)
Total antioxidants	891 ± 147	908 (764, 956)
β-Tocopherol	0.184 ± 0.296	0 (0, 0.250)
γ-Tocopherol	2.48 ± 1.82	2.20 (2.10, 3.10)
α-Tocopherol	32.5 ± 9.0	31.1 (25.8, 36.4)

[1] P25, 25th percentile; P75, 75th percentile.

There were no differences between gender-specific adjusted correlations (data not shown). Furthermore, when we divided the plasma concentration of carotenoids into tertiles of total antioxidant intake, there was an increase in the concentration of zeaxanthin ($P < 0.05$) and a trend for an increase in the concentration of lutein ($P = 0.09$) across tertiles. The use of lipid-lowering agents by 29 of the 61 participants did not substantially influence the correlations (data not shown).

We then evaluated the relations between antioxidant intake from various food groups and plasma α-, β-, γ-, and δ-tocopherols and thiols (glutathione, cysteine, homocysteine, cysteinyl-glycine). Correlations between total antioxidant intake from various food groups and plasma tocopherols and thiols were much lower than those with carotenoids.

DISCUSSION

In the present study, we determined the contribution of different food groups to the total antioxidant intake in a group of 61 adults (based on 7-d weighed dietary records) and a nationwide survey of 2672 Norwegian adults (based on an extensive FFQ). We were surprised to observe that coffee (mean daily intake ~480 mL) was the major contributor to the total intake of antioxidants (~66%). High antioxidant levels in coffee were reported recently in several studies (35,36), but this high contribution to the total dietary intake of antioxidants was not noted before. Chlorogenic acid (the ester of caffeic acid with quinic acid), the most abundant polyphenol in coffee, is likely responsible for a substantial part of coffee antioxidants.

When we analyzed antioxidants in foods to be included in our food composition table (R. Blomhoff, unpublished data) we observed that green and black coffee beans contain 15.9 and 22.6 mmol total antioxidants/100 g, respectively. This difference between green and black coffee beans agrees with previous data showing that although some antioxidants tend to be damaged during the roasting process, other are formed in so-called Maillard reactions (the browning reaction) (37,38).

Of the noncoffee total antioxidant intake, fruits (including berries), tea, cereals, wine, and vegetables contributed ~26, 25, 13, 10, and 6% (mean of the two studies) of total antioxidants, respectively. Furthermore, dietary β-carotene, α-tocopherol, and vitamin C contributed only 0.1, 0.3, and 8.5%, respectively, of the total intake of antioxidants. Of the noncoffee total antioxidant intake, β-carotene, α-tocopherol, and vitamin C together contributed ~24%.

A major issue is whether the antioxidants from coffee are

TABLE 6

Pearson correlation coefficients (r) and partial correlation coefficients (r_{par}) between antioxidant intakes and plasma concentrations of carotenoids in the 7-d weighed-record study[1]

	Lutein		Zeaxanthin		α-Carotene		β-Carotene		Lycopene	
	r	r_{par}	*r*	r_{par}	*r*	r_{par}	*r*	r_{par}	*r*	r_{par}
$FRAP_{total}$	0.25	0.33*	0.34**	0.40**	0.07	0.12	0.20	0.23	0.28*	0.31*
$FRAP_{without\ coffee}$	0.27*	0.27*	0.18	0.19	0.15	0.22	0.24	0.26*	0.08	0.13
$FRAP_{coffee}$	0.00	0.06	0.22	0.27*	−0.05	−0.07	0.07	0.08	0.22	0.22
$FRAP_{tea}$	0.15	0.15	0.03	0.04	0.10	0.12	0.14	0.14	0.05	0.07
$FRAP_{wine}$	0.07	0.08	0.06	0.05	0.11	0.09	0.31*	0.31*	0.07	0.04
$FRAP_{cereal}$	0.00	0.00	−0.13	−0.13	−0.09	0.03	−0.18	−0.14	−0.23	−0.20
$FRAP_{fruit}$	0.16	0.15	0.20	0.24	0.17	0.17	0.10	0.10	−0.05	0.06
$FRAP_{vegetable}$	0.21	0.21	0.06	0.03	0.16	0.28*	0.20	0.24	0.11	0.05

[1] r_{par} values were adjusted for triglycerides, cholesterol, age and BMI. * $P < 0.05$, ** $P < 0.01$.

bioavailable and bioactive. Several studies demonstrated bioactivity of coffee that support coffee's contribution to antioxidant defense. Many epidemiologic studies found that coffee is associated with reduced plasma γ-glutamyl transpeptidase, a suggested biomarker for early oxidative stress (39). Furthermore, coffee is protective in models of experimental carcinogenesis (40) and is associated with reduced incidence of human bladder and colorectal cancer (41), gallstone (42), Parkinson's disease (43), liver cirrhosis (44), and type 2 diabetes (45) in epidemiologic studies. Our observation of a significant contribution of coffee to the total intake of antioxidants suggests a possible mechanism behind these potentially beneficial effects of coffee.

Coffee consumption has been shown to increase plasma homocysteine, and likely is also associated with a small increase in blood pressure after many years of consumption (46). Furthermore, coffee lipids contained in boiled coffee, but less so in filtered coffee, increase serum lipids (47). Thus, coffee may contain several bioactive compounds, some of which may be beneficial, whereas others may increase the risk of disease.

We observed in the present study that total intake of antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene. Because lutein, zeaxanthin, and lycopene are only minor contributors to the total intake of antioxidants, our data agree with the hypothesis that many antioxidants may interact in a network, i.e., that dietary antioxidants other than lutein, zeaxanthin, and lycopene may save, recharge, or salvage these carotenoids when they have been used in a redox reaction.

The various food groups contain overlapping but also significantly different varieties of antioxidants. Thus, it should be expected that antioxidants from different food groups and within food groups may have different bioavailability and bioactivity. Therefore, we analyzed antioxidants in different food groups separately. The antioxidant intake in individual food groups, coffee, wine, and vegetables was significantly correlated with zeaxanthin, β-carotene, and α-carotene, respectively. Thus, our study demonstrating correlations between intakes of total antioxidants in individual food groups and plasma carotenoids agrees with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense.

We did not observe any association between total intake of antioxidants, or food group antioxidants, and the level of plasma tocopherols or thiols. These observations suggest that the plasma tocopherols and thiols are not in complete equilibrium with plasma carotenoids. This could either be due to preferential chemical reactivities or to compartmentalization of the plasma pools (e.g., selective binding to the α-tocopherol transport protein). The individuals in our study were generally healthy with presumably low systemic oxidative stress. In clinical situations with prolonged systemic oxidative stress, more correlations or antioxidant networking might be expected.

The FRAP assay does not quantify dietary sulfur compounds because the redox potential for thiols is below the threshold used in the FRAP assay (26). Because thiols appear not to be well absorbed (48,49), we elected to use the FRAP assay in the assessment of dietary antioxidants to avoid the otherwise large "noise" of thiol antioxidants in foods. However, thiols have essential roles in many biochemical reactions in the body by virtue of their ability to be oxidized reversibly. Glutathione is the most important thiol-based redox buffer (49,50). In the response of a cell to oxidative stress, glutathione is first consumed in reactions that protect the cell (13–16,49,50). To counteract glutathione depletion, most cells increase their synthesis of glutathione. During persistent oxidative stress, cellular counteraction is not sufficient to replenish the consumption of glutathione, and total cell and plasma glutathione may be reduced. Plasma glutathione has therefore been used as a biomarker for systemic oxidative stress (13–16,49,50). It would be interesting to assess the role of total dietary antioxidants on the thiols in clinical situations with profound systemic oxidative stress.

Further studies are warranted to explore whether the concept of "total antioxidants" is valuable in the search for the protective compounds and mechanism behind the beneficial effects of dietary plants. The present results are encouraging and substantiate the hypothesis that antioxidants work as least in part in an integrated network in vivo.

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