JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Coffee Drinking Influences Plasma Antioxidant Capacity in Humans

FAUSTA NATELLA,* MIRELLA NARDINI, IRENE GIANNETTI, CRISTINA DATTILO, AND CRISTINA SCACCINI

Free Radical Research Group, National Research Institute for Food and Nutrition, via Ardeatina 546, 00178 Roma, Italy

Coffee and tea are widely consumed beverages, but only tea has been studied for its antioxidant capacity (AC) in vivo. The aim of this study was to compare the capacities of coffee and tea to affect plasma redox homeostasis in humans. The AC of plasma before and after supplementation with 200 mL of beverages (0, 1, and 2 h) was measured by the TRAP and crocin tests. The crocin test detected an increase in plasma AC only in subjects supplemented with coffee (+7% at peak time), whereas the TRAP method showed an increase in plasma AC after consumption of both coffee and tea (+6 and +4%, respectively, at peak time). Both beverages induced a significant increase in plasma uric acid (+5 and +7%, respectively). Uric acid strongly affects the results obtained by the TRAP test and does not affect those obtained by the crocin test. We can thus argue that uric acid is the main component responsible for the plasma AC increase after tea drinking, whereas molecules other than uric acid (probably phenolic compounds) are likely to be responsible for the increase in plasma AC after coffee drinking.

KEYWORDS: Coffee; tea; polyphenols; antioxidant capacity; human

INTRODUCTION

Some dietary plant constituents, such as flavonoids and related phenolics, are considered powerful antioxidants in vitro (*1*−*3*) and are assumed to be at least partially responsible for the inverse relationship between fruit and vegetable intake and risk of degenerative diseases (chronic heart diseases and cancer) (*4*). Polyphenols are endowed with biological activities, such as modulation of enzymes, activation of transcription factors, and general gene expression (*5*). However, their absorption, metabolic fate, and availability for antioxidant protection in humans have not yet been completely clarified.

To further complicate the picture, the definition "plant phenolics" includes thousands of compounds with different chemical structures corresponding to different antioxidant activities. In addition, the chemical structure (number of phenolic rings, aromatic substitution, glycosilation, conjugation with other phenolics or organic acids) is considered a putative determinant in their bioavailability. Moreover, the profile of phenolic compounds in plasma can be quite different from that in the original dietary source as a result of metabolization and biotransformation.

A number of beverages derived from vegetables have been tested for their in vitro and in vivo antioxidant activities (white and red wine, green and black tea, beer) (*6*−*13*). A straightforward analysis of these studies leads to two main conclusions: the capacity of a food to transfer its antioxidant activity is linked to several known and unknown chemical/biochemical/physiological characteristics (see the case of green and black tea with and without milk), and the effect of food phenolics on the redox balance in vivo cannot be simply extrapolated from their activities in vitro.

In the past 2 years, a number of studies have focused on the capacity of tea to elicit in vivo antioxidant protection in humans, giving results both contrasting and largely different in extent (*14*−*18*). However, a recent paper clearly demonstrated that the consumption of a single dose of black or green tea induces a significant rise in plasma antioxidant activity in vivo (*16*).

Although coffee is as rich as tea in phenolic antioxidants and is consumed equally in the world, its antioxidant activity in vivo has been never studied.

Coffee contains several phenolic components, other than tocopherols, that are endowed with an antioxidant capacity (AC), and the total polyphenols content ranges from 200 to 550 mg per cup (*19*). Among the phenolic compounds identified in coffeee are chlorogenic acids, a family of esters formed between quinic acid and several cinnamic acids such as caffeic, ferulic, and *p*-coumaric, with caffeoylquinic acid being by far the most abundant (*20*). On the basis of 10 g of coffee per cup of brew, a 1-cup content of chlorogenic acid can range from 15 to 325 mg. A value of 200 mg/cup has been reported for American coffee (*21*). Black tea contains catechins, thearubigins, and

* To whom correspondence should be addressed. Dr. Fausta Natella, National Research Institute for Food and Nutrition, via Ardeatina 546, 00178 Rome, Italy. E-mail address: natella@inran.it.

10.1021/jf025768c CCC: $22.00
Published on Web 09/11/2002

theaflavins, which are oxidation products of catechins formed during enzymatic oxidation by polyphenol oxidase in fresh tea leaves.

The aim of this study was to assess the capacity of coffee (brewed as a beverage by drip filter method) to affect the plasma redox homeostasis in humans in fasting conditions, using tea as control. The total antioxidant capacity and the concentrations of the main antioxidants were measured in plasma before and after supplementation with a standard cup of coffee or black tea. Metabolic parameters in plasma were also measured to control the eventual effect of acute coffee and tea consumption on lipid metabolism.

METHODS

Coffee brew was prepared by using a commercial automatic brewing machine (60 g of roasted and ground coffee per liter of water). The coffee brand was Lavazza Qualità Rossa. Tea (20 g/L) was prepared by a 5-min infusion in water at 100 °C. The tea brand was Twining Earl Gray.

1. Coffee and Tea Analyses. The total antioxidant capacities of the two beverages were measured using two different systems, the loss of fluorescence of *r*-phycoerithryn (TRAP test) (*22*) and the bleaching of a carotenoid, the crocin (crocin test) (*23*), triggered by the peroxyl radicals generated by thermal decomposition of 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH). TRAP (total radical trapping antioxidant parameter) is expressed as the amount of peroxyl radicals trapped by 1 L of plasma. The antioxidant capacity measured by the crocin test is expressed in terms of millimoles of Trolox equivalents.

Total phenols were measured by the Folin−Ciocalteu method (*24*) after deproteinization of the samples with ammonium sulfate.

Caffeine, theobromine, and theophylline were detected by HPLC as previously described (*25*).

2. Study Design. Ten healthy nonsmoking moderate coffee drinkers (2−4 cups per day) were recruited from among the staff of the National Research Institute for Food and Nutrition (INRAN). A standard amount (200 mL) of brewed coffee was administered under fasting conditions. In a different session (2 weeks apart), black tea was administered as a control. Beverages were ingested within 10 min after brewing. Subjects were asked to avoid antioxidant supplements and to have a diet low in "coffee, wine, chocolate, tea, fruit, and vegetable" in the 2 days preceding the experiments.

3. Plasma Analysis. Blood was collected in EDTA (1 mg/mL) before and at 1 and 2 h intervals after coffee/tea administration. The plasma total antioxidant capacity and ascorbic acid content were analyzed immediately after blood centrifugation. Plasma samples for metabolic and antioxidant analyses (total cholesterol, triacylglycerols, SH groups, and uric acid) were stored at −80 °C until the analysis was performed.

Plasma total cholesterol and triacylglycerol levels were measured by commercial kits (Sigma Chemical Co., St. Louis, MO). HDL cholesterol was measured after selective precipitation with dextran sulfate (*26*). The LDL cholesterol concentration was calculated using the Friedewald formula (*27*). Plasma total homocysteine was measured by HPLC after derivatization with *o*-phthaldialdehyde (*28*).

SH groups were measured according to the method of Ellmann (*29*). Ascorbic and uric acids were measured by EC−HPLC, according to the method of Kutnink (*30*); dehydroascorbic acid was indirectly estimated by conversion to ascorbic acid after reduction with DL-homocysteine (*31*).

Table 1. Antioxidant Capacity and Concentrations of Total Phenols, Caffeine, and Theobromine in Coffee and Tea Beverages

	coffee	tea
crocin test, mM TX equiv/cup	7.4 ± 0.3	1.4 ± 0.1[c]
TRAP, mM ROO• equiv/cup	10.1 ± 0.6	1.3 ± 0.1[c]
total phenols,[a] mg of GAE[b]/cup	161 ± 9	87 ± 9[d]
caffeine, mg/cup	181 ± 10	130 ± 7[d]
theobromine, mg/cup	28.9 ± 1.1	5.9 ± 0.4[c]

[a] Total phenols after deproteinization of the sample. [b] Gallic acid equivalents. [c] $p < 0.001$ from coffee by ANOVA. [d] $p < 0.005$ from coffee by ANOVA.

The plasma total antioxidant capacity was measured using the same methods employed for the analysis of beverages. TRAP is expressed as the amount of peroxyl radicals trapped by 1 L of plasma. The antioxidant capacity measured by the crocin test is expressed as the ratio between the rates of bleaching of crocin in the absence (V_0) and in the presence (V) of plasma. The higher the ratio V_0/V, the higher the antioxidant capacity of the sample.

RESULTS AND DISCUSSION

1. Antioxidant Capacities of Coffee and Tea. The measurements of the antioxidant capacities of the two beverages, using both the TRAP and crocin test methods (**Table 1**), clearly indicate that coffee is more powerful in scavenging peroxyl radicals than tea, at least in a hydrophilic environment. In fact, coffee is endowed with an antioxidant capacity 5−8 times higher than that of tea, depending on the method utilized. These results are in agreement with a previous in vitro study indicating that coffee protects LDL against oxidation 3−14 times better than tea, depending on the method of beverage preparation (*6*).

Total phenols, expressed as gallic acid equivalents, are still higher in coffee than in tea, but the difference is not as dramatic (+40%) as for the antioxidant capacity. Thus, the antioxidant capacity of the beverages cannot be explained by the mere measurement of total phenols. Coffee and tea contain quite different patterns of phenolic compounds. Coffee is rich in simple phenolic acids, whereas tea contains large amount of catechins. As the chemical structures of the individual phenolic antioxidants strongly affect their antioxidant activities (*3*, *32*), the higher AC of coffee with respect to tea could be linked to the higher ACs of the specific phenolic compounds.

In addition, phenolic compounds could have synergist or antagonist effect when present in complex mixtures. Thus, as the antioxidant capacity represents the total effect of the single or combined activities of antioxidants, the different polyphenolic compositions of the two beverages are likely to magnify the observed difference in antioxidant capacities.

To further characterize the two beverages, we measured the concentrations of 1,3,7-trimethylxanthine (caffeine), 3,7-dimethylxanthine (theobromine), and 1,3-dimethylxanthine (theophylline). The amount of caffeine in a cup of coffee (200 mL), as administered in the in vivo study, corresponded to 181 mg, whereas 200 mL of tea contained 130 mg of caffeine. The figures for theobromine were 28.9 and 5.9 mg/200 mL, respectively, for coffee and tea. In both samples, theophylline was below the detection limit of our method.

Caffeine has been reported to scavenge hydroxyl radicals in an ESR study (*33*). In addition, caffeine inhibits lipid peroxidation of rat liver microsomes when present at millimolar concentrations, but the extent of inhibition against peroxidation induced by peroxyl radicals is quite low (*34*). A recent paper (*35*) clearly demonstrated that, at physiological concentrations, caffeine and its dimethylxanthine metabolite do not have any

Table 2. Plasma Concentration of Metabolic Parameters[a,b]

	coffee			tea		
	baseline	1 h	2 h	baseline	1 h	2 h
total cholesterol, mmol/L	3.94 ± 0.23	4.04 ± 0.26	4.01 ± 0.26	3.83 ± 0.26	3.78 ± 0.28	3.81 ± 0.28
HDL cholesterol, mmol/L	1.24 ± 0.08	1.30 ± 0.08	1.27 ± 0.08	1.30 ± 0.08	1.30 ± 0.08	1.30 ± 0.08
LDL cholesterol, mmol/L	2.28 ± 0.23	2.38 ± 0.26	2.38 ± 0.23	2.20 ± 0.23	2.18 ± 0.23	2.18 ± 0.23
triacylglycerol, mmol/L	0.88 ± 0.06	0.81 ± 0.08	0.78 ± 0.05	0.75 ± 0.09	0.73 ± 0.09	0.75 ± 0.09
homocysteine, μmol/L	9.8 ± 2.2	–	9.7 ± 2.2	9.6 ± 2.5	–	10.0 ± 3.0

[a] Mean ± standard error of nine subjects. [b] No statistically significant differences by ANOVA.

Table 3. Plasma Concentration of Some Parameters of Antioxidant Status[a]

	coffee			tea		
	baseline	1 h	2 h	baseline	1 h	2 h
SH groups, μM	398 ± 16	412 ± 14	409 ± 17	432 ± 15	437 ± 14	413 ± 12
uric acid, μM	369 ± 18	387 ± 24[b]	381 ± 24[b]	363 ± 24	387 ± 24[c]	375 ± 24
ascorbic acid[d]	98 ± 1	98 ± 1	97 ± 1	99 ± 1	97 ± 1[c]	95 ± 1[c]
crocin test, V_0/V	1.41 ± 0.02	1.47 ± 0.03	1.42 ± 0.03	1.44 ± 0.02	1.43 ± 0.03	1.40 ± 0.02[b]
TRAP, mM ROO• equiv	1.45 ± 0.07	1.53 ± 0.07[c]	1.51 ± 0.06	1.49 ± 0.05	1.56 ± 0.05	1.52 ± 0.05

[a] Mean ± standard error of ten subjects. [b] $p < 0.005$ from baseline by paired t-test. [c] $p < 0.05$ from baseline by paired t-test. [d] Reduced form as percentage of total.

antioxidant activity against peroxyl radicals. Finally, using our methods (TRAP and crocin tests), caffeine, theobromine, and theophylline did not show any antioxidant activity at concentrations up to 1000 times their concentrations in the two beverages (data not shown).

Thus, we can postulate that the higher antioxidant activity of coffee with respect to tea is not related to its major content of xanthines, but is probably linked to its different pattern of antioxidant compounds.

Finally, α-tocopherol was present in negligible amounts in coffee and was absent in tea (data not shown). Thus, we can exclude the participation of α-tocopherol in the beverages' ACs.

2. Effects of a Single Dose of Coffee or Tea on Lipid Metabolism. Recently, the relation between coffee consumption and plasma levels of cholesterol and homocysteine has been investigated (*36*−*38*). The component of coffee and the method of coffee brewing responsible for such variations are still unidentified, but associations between coffee polyphenols and plasma homocysteine (*36*) and coffee terpenoids and plasma cholesterol (*38*) have been proposed.

In our study, the acute supplementation of small doses of coffee or tea (200 mL) did not produce any modification in the plasma levels of the above-mentioned metabolic parameters (**Table 2**). Significant modifications in cholesterol and homocysteine plasma concentrations were found in other studies, where very strong doses of coffee or coffee components (chlorogenic acid) (*36*, *37*) were chronically administered for 2 or more weeks.

3. Effects of a Single Dose of Coffee or Tea on the Total Plasma Antioxidant Capacity in Humans. Table 3 reports the effect of tea or coffee drinking on some plasma antioxidants. Ascorbic acid (expressed as percentage of the reduced form) decreased significantly from 99 ± 1 to 95 ± 1% ($p < 0.05$) 2 h after tea drinking, whereas it remained unchanged after coffee consumption. A statistically significant increase in uric acid was detectable after both coffee ($p < 0.005$ from baseline at time 1 and 2 h) and tea ($p < 0.01$ from baseline at time 1 h) drinking. A plasma uric acid increase after administration of phenol-rich foods/beverages has already been demonstrated (*10*, *12*, *39*, *40*) and could be ascribed to the "interference" of phenols with the secretion and reabsorption of uric acid (*41*). In contrast, Leenen et al. (*16*) reported no increase in uric acid concentration after supplementation with black and green tea in humans. At the moment, we are not able to interpret this divergence.

Finally, no significant increase in plasma SH groups was observed after either treatment.

At 1 h, the ingestion of 200 mL of coffee in bolus produced a statistically significant increase (5.5%, $p < 0.05$) in the plasma antioxidant capacity, measured by the TRAP method (**Table 3**), maintaining a 4% increase after 2 h. The 4.7% increase in TRAP 1 h after tea administration did not reach statistical significance.

In the case of coffee, the crocin test gave a similar trend in the modulation of antioxidant activity, even if the differences were not statistically significant. In the case of tea, the AC measured by the crocin test decreased significantly ($p < 0.005$) after 2 h, paralleling the decline in the reduced form of ascorbic acid.

The apparent lack of statistical significance in the increases in AC by the TRAP test for tea and by the crocin test for coffee disappears when differences between individuals are taken into account. In fact, analyzing individual data, we found that subjects did not reach the maximum value at the same time. For the most part, the peak time was 1 h. However, in the case of the measurement of AC by the crocin test, four subjects reached the maximum value 2 h after coffee drinking, and in the case of the measurement of AC by the TRAP test, three subjects reached the peak 2 h after tea drinking. This event can be linked to differences in the efficiency of absorption and/or metabolism of antioxidant compounds. Comparing the individual AC at time 0 with the AC at the peak time (1 or 2 h depending on the subjects) (**Figure 1**), we observed a significant increase in plasma AC using both methods after coffee drinking. The increase in plasma AC after tea drinking reached statistical significance only when measured by the TRAP method.

The two methods employed to measure AC differ in their capacity to be affected by uric acid. In fact, the plasma uric acid contribution to TRAP is about 60% (*22*), whereas its contribution to the crocin test is equal to zero (data not shown). Because coffee and tea drinking induced a significant increase in plasma uric acid (**Table 3**), we can speculate that the increase in plasma AC measured by the TRAP method was largely affected by the increase in plasma uric acid concentration. Indeed, Cao (*42*) reported that the main component responsible

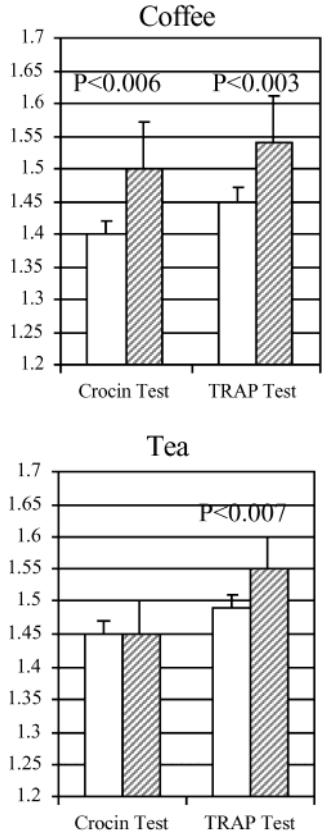

Figure 1. Effect of (A) coffee and (B) tea ingestion on plasma antioxidant capacity (AC). AC was measured by the TRAP and crocin tests at time 0 (white bar) and at peak time (stripped bar). Values are mean ± standard error of 10 subjects. Differences from time 0 were calculated by paired t-test.

for the increase in plasma AC after meal consumption is the increase in serum uric acid concentration. After coffee drinking, we observed a significant increase in AC also using the crocin test. Uric acid does not affect the crocin test (data not shown), so we can argue that molecules other than uric acid are responsible for the observed increase in AC after coffee consumption. As α-tocopherol did not increase after coffee drinking (data not shown) and xanthines do not affect crocin test, we can hypothesize that the components responsible for this increase are phenolic acids.

Caffeic acid is the most abundant phenolic compound in coffee brew, and it is endowed with strong antioxidant activity in vitro and in vivo (*43*−*47*). As caffeic acid is present in human plasma at micromolar concentrations after coffee drinking (*48*), we can assume that it is, at least in part, directly responsible for the increase in plasma antioxidant capacity observed in this study.

Therefore, the contribution of phenolic compounds from tea to the AC is essentially indirect; in fact, it influences the plasma uric acid level, even if a slight direct contribution cannot be ruled out. On the contrary, phenolic compounds from coffee could act both directly (*per se*) and indirectly (through the plasma uric acid level).

These results are probably linked to the different patterns of phenolic compounds present in the two beverages. Distinct phenolic compounds differ in their antioxidant activity, bioavailability, and metabolism and in the antioxidant activity of the resulting metabolites. Phenolic compounds in coffee have a higher antioxidant activity than those in tea (see **Table 1**) and are clearly absorbed (*48*). It is well-known that tea polyphenols are efficiently absorbed (*16*, *49*−*52*), but the data about their capacity to efficiently contribute to the plasma AC are contradictory (*15*, *17*, *53*, *54*). On this issue, our results seem to indicate that tea polyphenols do not contribute significantly to plasma AC. It is however worthwhile mentioning that tea polyphenols have a slower rate of absorption, which did not allow our study to measure their effect. In fact, after coffee supplementation, caffeic acid reaches its maximum concentration in plasma about 1 h after coffee drinking (*48*), whereas the peak concentration of total catechins occurs about 2−3 h after tea ingestion (*49*).

CONCLUSIONS

The increase in AC after tea administration in humans seems to be essentially linked to uric acid increase (probably due to tea polyphenols), whereas the increase in AC after coffee administration could be linked to both the effect on plasma uric acid and the direct absorption of coffee polyphenols.

Moreover, our results confirm that the mere measure of "antioxidant capacities" in vitro can lead to confounding results in in vivo studies. In fact the antioxidant capacity of a food measured in vitro is not necessarily consistent with its effect in vivo. Many factors such us polyphenols composition, bioavailability, tissue distribution, metabolism, and effect on endogenous antioxidant compounds have to be taken into account.

Finally, the interpretation of data on dietary-induced changes in plasma antioxidant capacity can be made easier by using different methodological approaches.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; AC, antioxidant capacity; TRAP, total radical trapping antioxidant parameter.

LITERATURE CITED

(1) Sichel, G.; Corsaro, C.; Scalia, M.; Di Bilio, A. J.; Bonomo, R. P. In vitro scavenger activity of some flavonoids and melanins against O_2^-. *Free Radical Biol. Med.* **1991**, *11*, 1−8.

(2) Hiramoto, K.; Ojima, N.; Sako, K.; Kikugawa, K. Effect of plant phenolics on the formation of the spin-adduct of hydroxyl radical and the DNA strand breaking by hydroxyl radical. *Biol. Pharm. Bull*. **1996**, *19*, 558−563.

(3) Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol*. **1990**, *186*, 343−355.

(4) Hertog, M. G.; Feskens, E. J.; Hollman, P. C.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.

(5) Yoshioka, K.; Deng, T.; Cavigelli, M.; Karin, M. Antitumor promotion by phenolic antioxidants: inhibition of AP-1 activity through induction of Fra expression. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4972−4976.

(6) Richelle, M.; Tavazzi, I.; Offord, E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J. Agric. Food Chem.* **2001**, *49*, 3438−3442.

(7) Abu-Amsha, R.; Croft, K. D.; Puddey, I. B.; Proudfoot, J. M.; Beilin, L. J. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clin. Sci.* **1996**, *91*, 449−58.

(8) Day, A. P.; Kemp, H. J.; Bolton, C.; Hartog, M.; Stansbie, D. Effect of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. *Ann. Nutr. Metab.* **1997**, *41*, 353−357.
(9) Durak, I.; Avci, A.; Kacmaz, M.; Buyukkocak, S.; Cimen, M. Y.; Elgun, S.; Ozturk, H. S. Comparison of antioxidant potentials of red wine, white wine, grape juice and alcohol. *Curr. Med. Res. Opin.* **1999**, *15*, 316−320.
(10) Ghiselli, A.; Natella, F.; Guidi, A.; Montanari, L.; Fantozzi, P.; Scaccini, C. Beer increases plasma antioxidant capacity in humans. *J. Nutr. Biochem.* **2000**, *11*, 76−80.
(11) Karakaya, S.; El, S. N.; Tas, A. A. Antioxidant activity of some foods containing phenolic compounds. *Int. J. Food Sci. Nutr*. **2001**, *52*, 501−508.
(12) Natella, F.; Ghiselli, A.; Guidi, A.; Ursini, F.; Scaccini, C. Red wine mitigates the postprandial increase of LDL susceptibility to oxidation. *Free Radical Biol. Med*. **2001**, *30*, 1036−1044.
(13) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J. Nutr*. **1998**, *128*, 1003−1007.
(14) Andersen, M. L.; Outtrup, H.; Skibsted, L. H. Potential antioxidants in beer assessed by ESR spin trapping. *J. Agric. Food. Chem*. **2000**, *48*, 3106−3111.
(15) Hodgson, J. M.; Puddey, I. B.; Croft, K. D.; Burke, V.; Mori, T. A.; Caccetta, R. A.; Beilin, L. J. Acute effects of ingestion of black and green tea on lipoprotein oxidation. *Am. J. Clin. Nutr*. **2000**, *71*, 1103−1107.
(16) Leenen, R.; Roodenburg, A. J.; Tijburg, L. B.; Wiseman, S. A. A single dose of tea with or without milk increases plasma antioxidant activity in humans. *Eur. J. Clin. Nutr*. **2000**, *54*, 87−92.
(17) Hodgson, J. M.; Croft, K. D.; Mori, T. A.; Burke, V.; Beilin, L. J.; Puddey, I. B. Regular ingestion of tea does not inhibit in vivo lipid peroxidation in humans. *J. Nutr*. **2002**, *132*, 55−58.
(18) Liebler, D. C.; Valcic, S.; Arora, A.; Burr, J. A.; Cornejo, S.; Nair, M. G.; Timmerman, B. N. Antioxidant reactions of green tea catechins and soy isoflavones. *Adv. Exp. Med. Biol*. **2001**, *500*, 191−197.
(19) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev*. **1998**, *56*, 317−333.
(20) Clifford, M. N.; Wight, J. The measurement of feruloylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans. *J. Sci. Food Agric*. **1976**, *27*, 73−84.
(21) Viani, R. The composition of coffee. In *Caffeine, Coffee, and Health*; Garattini, S., Ed.; Raven Press Ltd.: New York, 1993; pp 17−41.
(22) Ghiselli, A.; Serafini, M.; Maiani, G.; Azzini, E.; Ferro-Luzzi, A. A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biol. Med*. **1995**, *18*, 29−36.
(23) Tubaro, F.; Ghiselli, A.; Rapuzzi, P.; Maiorino, M.; Ursini, F. Analysis of plasma antioxidant capacity by competition kinetics. *Free Radical Biol. Med*. **1998**, *24*, 1228−1234.
(24) Swain, T.; Hillis, W. E. The phenolic constituents of Pruna domestica. *J. Sci. Food Agric.* **1969**, *10*, 6368−6372.
(25) Blanchard, J.; Mohammadi, J. D.; Conrad, K. A. Improved liquid-chromatographic determination of caffeine in plasma. *Clin. Chem*. **1980**, *26*, 1351−1354.
(26) Warnick, G. R.; Benderson, J.; Albers, J. J. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem*. **1982**, *28*, 1379−1388.
(27) Friedewald, W. T.; Levy, R. I.; Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem*. **1972**, *18*, 499−502.
(28) Tcherkas, Y. V.; Denisenko, A. D. Simultaneous determination of several amino acids, including homocysteine, cysteine and glutamic acid, in human plasma by isocratic reversed-phase high-performance liquid chromatography with fluorimetric detection. *J. Chromatogr. A* **2001**, *913*, 309−313.
(29) Ellmann, G. L. Tissue sulphydryl groups. *Arch. Biochem. Biophys*. **1959**, *82*, 70−77.
(30) Kutnink, M. A.; Hawkes, W. C.; Schaus, E. E.; Omaye, S. T. An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components. *Anal. Biochem*. **1987**, *166*, 424−430.
(31) Behrens, W. A.; Madere, R. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Anal. Biochem*. **1987**, *165*, 102−107.
(32) Natella, F.; Nardini, M.; Di Felice, M.; Scaccini, C. Benzoic and cinnamic acid derivatives as antioxidants: structure−activity relation. *J. Agric. Food Chem.* **1999**, *47*, 1453−1459.
(33) Shi, X.; Dalal, N. S.; Jain, A. C. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem. Toxicol*. **1991**, *29*, 1−6.
(34) Devasagayam, T. P.; Kamat, J. P.; Mohan, H.; Kesavan, P. C. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys. Acta* **1996**, *1282*, 63−70.
(35) Lee, C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta* **2000**, *295*, 141−154.
(36) Olthof, M. R.; Hollman, P. C.; Zock, P. L.; Katan, M. B. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am. J. Clin. Nutr*. **2001**, *73*, 532−538.
(37) Post, S. M.; de Roos, B.; Vermeulen, M.; Afman, L.; Jong, M. C.; Dahlmans, V. E.; Havekes, L. M.; Stellaard, F.; Katan, M. B.; Princen, H. M. Cafestol increases serum cholesterol levels in apolipoprotein E*3-Leiden transgenic mice by suppression of bile acid synthesis. *Arterioscler. Thromb. Vasc. Biol*. **2000**, *20*, 1551−1556.
(38) Christensen, B.; Mosdol, A.; Retterstol, L.; Landaas, S.; Thelle, D. S. Abstention from filtered coffee reduces the concentrations of plasma homocysteine and serum cholesterol—a randomized controlled trial. *Am J Clin. Nutr*. **2001**, *74*, 302−307.
(39) Gasbarrini, A.; Addolorato, G.; Simoncini, M.; Gasbarrini, G.; Fantozzi, P.; Mancini, F.; Montanari, L.; Nardini, M.; Ghiselli, A.; Scaccini, C. Beer affects oxidative stress due to ethanol in rats. *Dig. Dis. Sci*. **1998**, *43*, 1332−1338.
(40) Caccetta, R. A.; Croft, K. D.; Beilin, L. J.; Puddey, I. B. Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability. *Am. J. Clin. Nutr*. **2000**, *71*, 67−74.
(41) Gutman, A. B.; Yu, T. F. Renal mechanisms for regulation of uric acid excretion, with special reference to normal and gouty man. *Semin. Arthritis Rheum.* **1972**, *2*, 1−46.
(42) Cao, G.; Prior, R. L. Postprandial increases in serum antioxidant capacity in older women. *J. Appl. Physiol*. **2000**, *89*, 877−883.
(43) Laranjinha, J. Redox cycles of caffeic acid with alpha-tocopherol and ascorbate. *Methods Enzymol*. **2001**, *335*, 282−295.
(44) Nardini, M.; Pisu, P.; Gentili, V.; Natella, F.; Di Felice, M.; Piccolella, E.; Scaccini, C. Effect of caffeic acid on *tert*-butyl hydroperoxide-induced oxidative stress in U937. *Free Radical Biol. Med*. **1998**, *25*, 1098−1105.
(45) Nardini, M.; Natella, F.; Gentili, V.; Di Felice, M.; Scaccini, C. Effect of caffeic acid dietary supplementation on the antioxidant defense system in rat: an in vivo study. *Arch. Biochem. Biophys*. **1997**, *342*, 157−160.
(46) Nardini, M.; D'Aquino, M.; Tomassi, G.; Gentili, V.; Di Felice, M.; Scaccini, C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biol. Med*. **1995**, *19*, 541−552.
(47) Laranjinha, J.; Cadenas, E. Redox cycles of caffeic acid, alpha-tocopherol, and ascorbate: implications for protection of low-density lipoproteins against oxidation. *IUBMB Life* **1999**, *48*, 57−65.
(48) Nardini, M.; Cirillo, E.; Natella, F.; Scaccini, C. Absorption of phenolic acids in humans after coffee consumption. *J. Agric. Food Chem*. **2002**, *50*, 5735−5741.

(49) Van het Hof, K. H.; Kivits, G. A.; Weststrate, J. A.; Tijburg, L. B. Bioavailability of catechins from tea: the effect of milk. *Eur. J. Clin. Nutr*. **1998**, *52*, 356−359.
(50) Hollman, P. C.; Van het Hof, K. H.; Tijburg, L. B.; Katan, M. B. Addition of milk does not affect the absorption of flavonols from tea in man. *Free Radical Res*. **2001**, *34*, 297−300.
(51) Nakagawa, K.; Okuda, S.; Miyazawa, T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. *Biosci. Biotechnol. Biochem*. **1997**, *61*, 1981−1985.
(52) Kim, S.; Lee, M. J.; Hong, J.; Li, C.; Smith, T. J.; Yang, G. Y.; Seril, D. N.; Yang, C. S. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr. Cancer* **2000**, *37*, 41−48.
(53) Serafini, M.; Ghiselli, A.; Ferro-Luzzi, A. In vivo antioxidant effect of green and black tea in man. *Eur. J. Clin. Nutr*. **1996**, *50*, 28−32.
(54) Maxwell, S.; Thorpe, G. Tea flavonoids have little short-term impact on serum antioxidant activity. *Br. Med. J.* **1996**, *313*, 229.

Received for review June 27, 2002. Revised manuscript received August 12, 2002. Accepted August 12, 2002. This study was supported by grant from the Institute for Scientific Information on Coffee (ISIC) in La Tour-de-Peilz (Switzerland) and the Physiological Effects of Coffee Committee (PEC) in La Tour-de-Peilz (Switzerland).

JF025768C