Plasma and Lipoprotein Levels of Tea Catechins Following Repeated Tea Consumption (44366)

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Abstract. **Epidemiological studies suggest that antioxidant flavonoids in tea may reduce the risk of cardiovascular disease, possibly *via* protection of low-density lipoproteins (LDL) against oxidation. However, the extent of absorption of tea flavonoids and their accumulation in LDL during regular consumption of tea is not clear. Therefore we investigated plasma and lipoprotein levels of catechins during tea consumption and the impact on LDL oxidizability *ex vivo*. Eighteen healthy adults consumed, in an incomplete balanced cross-over design, green tea, black tea, black tea with milk or water, one cup every 2 hr (eight cups/day) for three days. Blood samples were obtained in the mornings and evenings of each day. Plasma total catechin concentration was determined in all blood samples, and the distribution of catechins among lipoproteins was determined at the end of the third day (t = 60 hr). The resistance of LDL to copper-induced oxidation *ex vivo* was assessed before tea consumption and at t = 60 hr. Repeated tea consumption during the day rapidly increased plasma total catechin levels whereas they declined overnight when no tea was consumed. There was a gradual increase in plasma levels in the mornings (respectively, 0.08 μ*M* vs. 0.20 μ*M* on first and last day of black tea consumption) and evenings (respectively, 0.29 μ*M* vs. 0.34 μ*M* on first and last day of black tea consumption). Green tea catechins were mainly found in the protein-rich fraction of plasma (60%) and in high-density lipoproteins (23%). Although present in LDL, the concentration of catechins in LDL was not sufficient to enhance the resistance of LDL to oxidation *ex vivo*. Addition of milk to black tea did not affect any of the parameters measured. In conclusion, the present study shows that catechin levels in blood rapidly increase upon repeated tea consumption. The accumulation of catechins in LDL particles is not sufficient to improve the intrinsic resistance of LDL to oxidation *ex vivo*.** [P.S.E.B.M. 1999, Vol 220]

Flavonoids are a large group of polyphenolic antioxidants naturally present in fruits, vegetables, and beverages such as wine and tea. Three recent epidemiological studies have shown an inverse association between intake of flavonoids and/or black tea and the risk of myocardial infarction or stroke (1–3). In contrast, one study in the United Kingdom showed a weakly positive association between black tea consumption and coronary mortality (4).

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It is believed that the possible protective effects against cardiovascular disease are due to the flavonoids present in tea. In the current concept of atherogenesis, oxidation of low-density lipoproteins (LDL) is thought to play an important role. LDL itself is not considered atherogenic, but appears to contribute to the formation of atherosclerotic lesions only after oxidative modification (5). LDL oxidation may occur in the artery wall where the lipoproteins are exposed to oxidative stress, and antioxidants can become depleted.

The possible involvement of oxidative processes early in atherogenesis has stimulated the concept that dietary antioxidants may protect against cardiovascular disease (6). Flavonoids derived from tea have been demonstrated to efficiently scavenge a variety of free radicals (7, 8). Catechins, theaflavins and quercetin, flavonoids present in

green and black tea, inhibit copper-induced or cell-mediated oxidation of LDL *in vitro* (reviewed in Ref. 9). However, the potential of dietary flavonoids to inhibit oxidation of LDL *in vivo* depends on their bioavailability and partitioning into the lipoprotein particle. It is currently not known if the steady state concentrations of tea flavonoids in plasma, LDL, or the arterial wall upon repeated consumption of green or black tea are sufficient to inhibit LDL oxidation, and the available data are inconsistent. We and others previously showed that in humans, consumption of ≈ 6 cups of green or black tea per day for 4 weeks had no effect on copper-mediated LDL oxidizability *ex vivo* (10–12). On the contrary, Ishikawa *et al.* (13) reported that copper-induced oxidizability of LDL was slightly decreased after 4 weeks of drinking black tea as compared to baseline values. The differing results may be due to the different amounts of tea flavonoids consumed or to other yet unknown factors.

A good understanding of the bioavailability of tea flavonoids is a prerequisite for the interpretation of epidemiological studies and intervention studies. In the present study, we determined plasma levels and urinary excretion of catechins in humans drinking eight cups of green or black tea per day for three days. In addition, we assessed the distribution of catechins in the lipoprotein fractions and the effects on LDL oxidizability *ex vivo*.

Materials and Methods

Volunteers. Nonsmoking healthy subjects, between 18 and 65 years of age, were recruited among employees from the laboratory and inhabitants of Vlaardingen. The volunteers did not use vitamins E and C, carotenoids, selenium, or zinc supplements, a medically prescribed diet, or slimming regime and had been weight-stable for at least one month prior to the study. Pregnant or lactating females were excluded from the study. Volunteers gave their written informed consent before participation, and the study protocol was approved by the local Medical Ethical Committee.

Study Design. In an incomplete, balanced, crossover design, 18 adult volunteers received two out of four treatments: green tea, black tea, black tea with milk, or water. The volunteers consumed one cup of tea or water every 2 hr (eight cups per day) for 3 days (0.5 g tea solids/cup). The volunteers received a standard breakfast, lunch, and hot meal during the treatment days. Blood samples were obtained on the first day before starting tea consumption and at 4, 8, and 12 hr after. Additional blood samples were collected in the morning after an overnight fast and in the evening of the second and third days. The plasma concentration of total catechins was determined in all blood samples. Lipoproteins were isolated from samples obtained at t = 60 hr, after 3 days of green tea consumption, to determine the distribution of tea catechins among the different lipoprotein fractions. The resistance of LDL to copper-induced oxidation *ex vivo* was assessed before and at t = 60 hr. Urine was collected between 48 hr and 72 hr after the start of the intervention, and the concentration of catechins was measured.

Tea and Background Diet. Green tea and black tea (standard research blends) were provided by Lipton (Englewood Cliffs, NJ) as lyophilized tea solids. Tea was provided as a hot beverage at a concentration of 0.5 g tea solids/150 ml of water. In the case of black tea with milk, 135 ml of water and 15 ml of semiskimmed milk (10% milk; 1.5% fat, 3.5% protein) were used.

One day before and during the experimental days, the volunteers were instructed to avoid flavonoid-rich fruits, vegetables, and beverages (14). In addition, they excluded dairy products and products rich in other antioxidants, such as vitamin C, vitamin E, and carotenoids, from their diets on the experimental days. Ready-to-eat hot meals with low levels of flavonoids and other antioxidants were provided to replace their usual main meals, and breakfast and lunch were provided also. All meals were consumed at preset times during the experimental days.

Collection of Blood and Isolation of Lipoproteins. Venous blood samples were collected in EDTA-coated tubes from subjects in half-seated position. Plasma was prepared within 30 min after blood sampling by low-speed centrifugation (1500g for 10 min at 4°C). Plasma samples for catechin analysis were stored at −80°C until analysis. Lipoproteins were isolated from fresh plasma by discontinuous density gradient ultracentrifugation for 24 hr at 4°C (15). All gradient solutions contained 0.1 mM EDTA. The protein concentrations of VLDL ($d < 1.019$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), HDL ($1.063 < d < 1.21$ g/ml) and the bottom fraction ($d > 1.21$ g/ml) were determined using bovine serum albumin (Fraction V, Sigma, St Louis, MO) as a standard (16). An aliquot of the LDL fraction was used immediately for oxidation experiments. The residual LDL and the other lipoprotein fractions were stored at −80°C until analysis of catechins.

Collection of Urine. Volunteers were instructed to collect their urine quantitatively into plastic bottles provided by the laboratory from t = 48 hr to 72 hr after the start of the experiment. Urine from the first 12 hr was handed in at t = 60 hr and the second 12 hr at t = 72 hr, together with the volunteers' visits for blood sampling. Ascorbic acid/EDTA solution (20:0.1; v/v) was added to the bottles as a preservative, and volunteers stored the bottles in a cooled bag (≈ 4°C).

After receiving the urine, the volume was measured, and samples of the first and second 12 hr were frozen immediately and stored at −80°C until analysis.

Analysis of Total Catechins in Tea, Plasma, and Lipoproteins. Total catechins in plasma and lipoproteins were determined spectrophotometrically with 4-dimethylamino cinnamaldehyde (DMACA, Merck, Darmstadt, Germany) a complexing reagent decribed previously (17). Briefly, plasma or lipoproteins were precipitated with 3 volumes methanol containing 1 g/l butylated hydroxytoluene. Catechins were extracted by solid phase extraction with

aluminum oxide (super I, ICN Biomedicals, Eschwege, Germany). Following centrifugation, the aluminum oxide was washed with diethyl ether and dried. Complexation was initiated by the addition of 0.5 ml DMACA reagent, 6 mmol/l dissolved in methanol/perchloric acid/water (8:1:1; v/v). The absorbance of the colored complex was determined at 637 nm exactly 6 min after initiation of the reaction. The concentration of catechins was calculated from an external calibration curve of human control plasma spiked with (+)catechin. The within- and between-day coefficients of variation of this assay were 1.1% and 2.6%, respectively. Total catechins in green and black tea extract were determined as described above, except that the solid phase extraction with aluminum oxide was omitted.

Analysis of Individual Catechins in Tea, Plasma, and Urine. The distribution of catechins in tea was determined by reversed-phase HPLC using UV-Vis detection (18). Analysis of individual catechins in urine was performed as described by Lee *et al.* (19). This method measures epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) only. Urine samples (50 µl) were thawed and incubated with 150 µl of a 0.4 *M* sodium phosphate buffer (pH 3.5), 20 µl vitamin C-EDTA solution (20:0.1; v/v), β-glucuronidase (500 units), and sulfatase (40 units). Released catechins were extracted with ethyl acetate and evaporated to dryness. Catechins were separated on an NBS C18 column (5 µm, 150 × 4.6 mm; ESA Inc., Bedford, MA). The column was eluted at 35°C with a linear gradient from 96% of solvent A (30 m*M* NaH_2PO_4 containing 2.37% acetonitrile and 0.12% tetrahydrofurane, pH 3.35) and 4% of solvent B (30 m*M* NaH_2PO_4 containing 40% acetonitrile and 6.65% tetrahydrofurane, pH 3.45) to 76% of solvent A and 24% of solvent B in 24 min at a flow rate of 1 ml/min. From 24 to 35 min the gradient was changed linearly to 5% of solvent A and 95% of solvent B. The eluent was monitored by a coulochem electrode array system (ESA Inc.) with potential settings at −90, −10, 70, and 150 mV. The peak height was used to calculate the urine concentration using an external calibration curve of spiked control urine.

Oxidation of LDL *ex vivo* Following Tea Consumption. The resistance of LDL to oxidation *ex vivo* was determined using copper as prooxidant essentially as described by Princen *et al.* (20). Briefly, the oxidation of 50 µg LDL protein was initiated with 50 µ*M* $CuCl_2$ at 30°C, and the formation of conjugated dienes was monitored spectrophotometrically at 234 nm. The lag phase and maximum rate of oxidation were determined from the oxidation profile as described previously (21).

***In vitro* Studies of Relation Between Catechin Levels in LDL and LDL Oxidizability.** Human plasma was incubated for 15 min at room temperature with green tea or black tea at a concentration of 0–200 mg tea solids/l. LDL was isolated from plasma by discontinuous gradient ultracentrifugation (15), and the concentration of LDL protein was determined (16). The resistance of LDL to copper-induced oxidation and the concentration of catechins were determined as decribed above.

Statistical Evaluation. The areas under the concentration curves of plasma total catechin concentrations and the 24 hr urinary excretions of catechins were calculated. Plasma concentrations of total catechins and the areas under the curves were log-transformed to minimize correlation between mean values and standard deviations within treatments. Differences among the treatments were tested for significance using analysis of variance (ANOVA) with treatment, period, sex, and subject (within sex) as factors. Bonferroni correction was applied and P-values < 0.01 were considered significant. Comparison of plasma levels of total catechins at different time points in the morning or evening within a treatment was performed as above with time point as repeated measures.

Descriptive variables are presented as means with standard deviation whereas other variables are shown as means with standard error or coefficient of variation (CVM) in case log-transformation was applied.

Results

Volunteers and Compliance. Table I shows the descriptive characteristics of the participants. All volunteers completed the study. The compliance to tea drinking, as assessed by collection of unused tea bags and interviews of the participants, was more than 99%.

Table I. Descriptive Characteristics of Volunteers who Consumed Water, Green Tea, Black Tea, or Black Tea with Milk[a]

Characteristic	Water	Green tea	Black tea	Black tea with milk
Males/females (n)	3/6	3/6	3/6	3/6
Age (y)	35.3 ± 11.0 (25–50)	45.8 ± 9.0 (33–63)	41.1 ± 13.0 (25–63)	39.1 ± 10.0 (25–50)
Body weight (kg)	69.8 ± 11.6 (53.5–91.8)	71.4 ± 11.0 (59.0–93.8)	72.1 ± 10.6 (59.0–91.8)	70.0 ± 11.6 (53.5–93.8)
Body height (m)	1.71 ± 0.09 (1.59–1.92)	1.68 ± 0.06 (1.59–1.81)	1.73 ± 0.09 (1.63–1.92)	1.70 ± 0.09 (1.60–1.89)
Body mass index (kg/m^2)	23.6 ± 2.4 (20.9–27.4)	25.1 ± 2.9 (21.3–29.1)	24.0 ± 2.0 (21.3–27.4)	24.1 ± 3.0 (20.9–29.1)

[a] Values are shown as mean ± SD and (range).

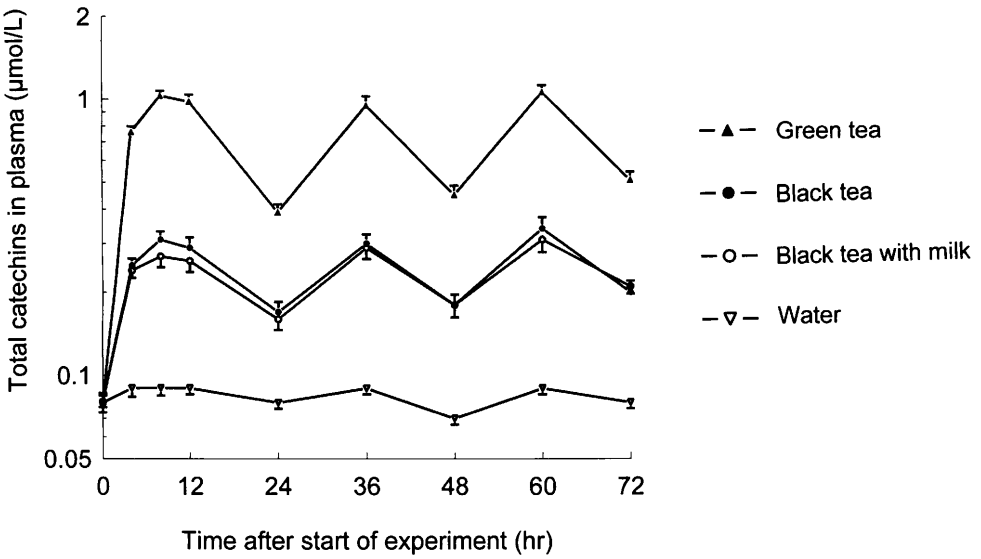

Figure 1. Total catechin concentration in plasma (mean ± CVM) during 3 days of regular consumption of water, green tea, black tea, or black tea with milk (one cup every 2 hr, 8 cups per day). Volunteers refrained from tea drinking from t = 14–24 hr, t = 38–48 hr, and t = 62–72 hr (n = 9/treatment).

Catechin Levels in Plasma and Lipoproteins. Figure 1 shows the plasma concentration of total catechins during repeated consumption of tea or water, one cup every 2 hr. As expected, no change was found during consumption of water, whereas green tea or black tea consumption induced a significant increase.

Volunteers finished tea consumption at about 22.00 hr in the evening and resumed tea drinking the following morning at about 8.00 hr after the first blood sample was drawn. This pattern is clearly reflected in the plasma concentration of total catechins. Repeated consumption of green tea or black tea rapidly increased the level of total catechins, and a steady state concentration was reached before the end of the day, after 5 cups of tea (t = 8 hr) (Fig. 1). Plasma total catechin levels dropped overnight while refraining from tea consumption, although there was a gradual and statistically significant increase in plasma levels of catechins in the first morning blood sample. The catechin concentration at the end of the day also increased gradually, and the level found at the end of the third day (t = 72 hr) was significantly higher than those of the 2 previous days.

In line with the difference in total catechin contents, which was 2.6-fold higher in green tea than black tea (Table II), the increase in plasma concentrations of catechins was significantly higher after drinking green tea as compared to black tea. Addition of milk to black tea did not affect the plasma levels of catechins. The area under the curve after consumption of black tea was not significantly different from black tea with milk (mean (CVM): 17.9 μM (9%) vs. 17.0 μM (8%), respectively).

The distribution of total catechins in lipoproteins at the end of the third day of green tea consumption (t = 60 hr) is shown in Figure 2. More than 50% of the catechins was recovered in the plasma protein fraction. In lipoprotein fractions, the concentration of catechins increased in the order VLDL < LDL < HDL. The concentration of catechins associated with LDL during green tea consumption was (mean (SEM)) 0.077 (0.021) μM, which accounted for less than 10% of the total amount in plasma.

Table II. Catechin Content[a] of Green Tea and Black Tea Extract

Component	Green tea	Black tea
Total catechins (g/g tea extract)	0.26	0.10
(+)-Catechin (%)	4	5
(+)-Gallocatechin (%)	4	4
(+)-Gallocatechin gallate (%)	1	0
(−)-Epicatechin (%)	13	11
(−)-Epigallocatechin (%)	24	18
(−)-Epicatechin gallate (%)	18	20
(−)-Epigallocatechin gallate (%)	36	42

[a] Total catechin content measured by spectrophotometry after complex formation with DMACA, individual catechins measured by HPLC and expressed as percentage of total catechins (see Methods section).

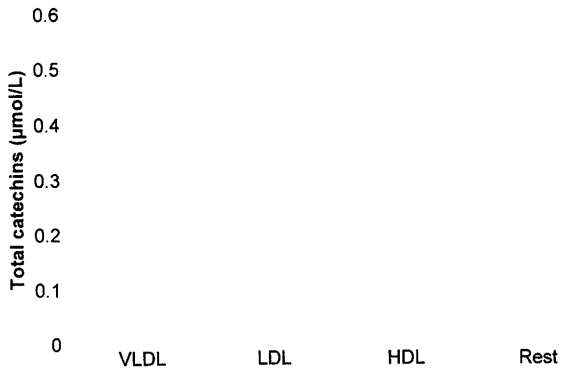

Figure 2. Distribution of catechins among different lipoprotein fractions in plasma after 3 days consumption of green tea (t = 60 hr, n = 3).

Urinary Excretion of Catechins. Volunteers collected 24 hr urine during the third day and night, from t = 48 hr to t = 72 hr. Table III shows the catechin levels in urine. In line with the changes observed in plasma total catechin levels, the amount of excreted catechins was significantly higher during consumption of green or black tea as compared to water. Green tea induced significantly higher levels than black tea, which agrees with the differences in catechin content of the teas (Table II). No significant difference was found between black tea and black tea with milk. Urine only contained EC and EGC; the levels of EGCG were below the detection limit of the assay (i.e., $< \approx$ 1 μg/24 hr) (Table III).

Oxidizability of LDL *ex vivo* following Tea Consumption. The resistance of LDL to copper-mediated oxidation was determined before and at the end of the third experimental day (t = 60 hr). Table IV shows that consumption of neither green nor black tea altered the lag phase of LDL oxidizability *ex vivo*. The change in maximum rate of oxidation was also not significantly different among the treatments.

LDL Catechin Levels and LDL Oxidizability following Preincubation with Tea *in vitro*. Plasma samples were preincubated with green tea or black tea in concentrations ranging from 0–200 mg tea solids/l. This latter amount is equivalent to 52 mg total catechin/l for green tea and 20 mg/l for black tea, respectively. In LDL isolated from plasma preincubated with tea, the concentration of catechins significantly increased with increasing concentrations of green or black tea. Approximately 1% of the amount of catechins present in plasma preincubated with green or black tea was recovered in LDL. The resistance of LDL to oxidation also enhanced with increasing tea concentrations. A significantly increased lag phase before LDL oxidation was found if plasma was incubated with ≥ 50 mg green tea extract/l (mean (SEM): 106 (1.7) min vs. 131 (3.0) min with no or 30 mg green tea extract) or ≥ 100 mg black tea extract/l (mean (SEM): 114 (6.6) min vs. 133 (5.2) min with no or 50 mg black tea extract). Measurement of total catechin levels in LDL showed that the concentration required to significantly increase the lag time was > 0.4 μ*M*.

Discussion

The present study shows that consumption of eight cups of green tea, black tea, or black tea with milk for 3 days results in a significant increase in the concentration of catechins in plasma, but does not affect the resistance of LDL to oxidation *ex vivo*.

Plateau levels of total catechins in plasma were ≈ 1.0 and 0.3 μ*M* for green and black tea, respectively, and were reached after drinking five cups of tea (Fig. 1). The difference between green and black tea reflects the catechin concentrations in the tea beverages, which in green tea is about 2.6-fold greater than that found in black tea (Table II). Black tea is subjected to more extensive processing than green tea, and part of the catechin fraction is converted into specific black tea flavonoids such as theaflavins and thearubigins (22). Currently, it is unclear to what extent these components are absorbed.

It has been suggested that addition of milk reduces the bioavailability of tea flavonoids due to binding to milk proteins (4, 23). Tea flavonoids strongly bind to milk proteins *in vitro* (24). However, we previously showed that addition of milk to black tea had no effect on the concentration of total catechins in plasma after single consumption (25). The present study confirms this observation and shows that there is no difference in plasma response or urinary excretion of catechins when regular consumption of black tea with milk is compared to plain black tea.

Although plasma levels increased rapidly during repeated tea consumption, they decreased significantly during the nights when no tea was consumed. The half-life of catechins in blood after single tea consumption varies from 4.8 hr for green tea to 6.9 hr for black tea (25). Volunteers consumed their last cup of tea 2 hr after the last blood sample at the end of the day and 10 hr before the next sample in the morning. In line with the difference in half-lives of green tea and black tea catechins, we found a larger reduction during green tea consumption (52%–60%) than during black tea consumption (38%–41%). These percentages were somewhat smaller than those extrapolated from the half-lives we calculated after a single dose of tea (25). The metabolism and thus the half-life of catechins may be

Table III. Urinary Excretion of Catechins[a] During Regular Consumption of Water, Green Tea, Black Tea, or Black Tea with Milk

	Water (n = 8)	Green tea (n = 9)	Black tea (n = 8)	Black tea with milk (n = 9)
Urinary excretion at t = 48–72 hr				
Epicatechin (mg/24 hr)	0.34 (0.10)[b]	11.0 (1.7)[c]	2.05 (0.27)[d]	2.22 (0.22)[d]
[% of intake/24 hr]	—	6.5%	6.5%	7.0%
Epigallocatechin (mg/24 hr)	0.06 (0.03)[b]	13.3 (1.6)[c]	1.21 (0.23)[d]	1.18 (0.17)[d]
[% of intake/24 hr]	—	4.2%	2.5%	2.4%
Epigallocatechin gallate (mg/24 hr)	nd[e]	nd[e]	nd[e]	nd[e]

[a] Values are presented as mean (SEM).
[b,c,d] Means in the same row with different superscript letters are significantly different.
[e] Not detectable (<1 μg/24 hr).

Table IV. Effect of Tea Consumption for 3 Days on LDL Oxidizability *ex vivo*[a]

	Water (n = 8)	Green tea (n = 9)	Black tea (n = 9)	Black tea with milk (n = 9)
Lag time (min)				
t = 0 hr	151 (10)	135 (10)	138 (11)	153 (7)
Change at t = 60 hr	−1.6 (4)	8.9 (13)	5.6 (4)	−3.1 (5)
Maximum rate (nmoles dienes/min/mg LDL protein)				
t = 0 hr	17.7 (1.2)	18.2 (0.7)	16.2 (0.9)	17.7 (0.7)
Change at t = 60 hr	0.1 (1.6)	0.6 (2.0)	1.7 (1.0)	−1.3 (1.1)

[a] Values are presented as mean (SEM).

affected by the time of day as the half-lives after single consumption of tea in our previous study were assessed during the day.

De Vries *et al.* (26) determined plasma levels of quercetin after 3 days consumption of a similar amount of black tea (1.6 l/d vs. 1.2 l/d in the present study). The plasma concentration of catechins achieved following black tea consumption in the present study (0.3 μM) were higher than the plasma levels of quercetin found by De Vries *et al.* (0.1 μM). This difference can be explained by the fact that the amount of total catechins provided in the present study was also higher than the amount of quercetin (400 mg/d and 49 mg/d for catechins and quercetin, respectively). This comparison indicates that the contribution of catechins in black tea to the body's antioxidant status may be of equal importance to that of quercetin. Future studies on the health benefits of flavonoids should therefore not only focus on flavonols, such as quercetin, but also on catechins.

In line with previous observations (19), EC and EGC but not EGCG were detectable in urine. This indicates that catechins may vary in the extent to which they are absorbed and/or metabolized. It may be due to a lower absorption of EGCG compared to EC and EGC. Studies in rats showed that after intragastric administration of green tea, about 13.7% of EGC, 31.2% of EC, and only 0.1% of EGCG appeared in plasma (27). Furthermore, the results suggested that, in contrast to EC and EGC, EGCG is mainly excreted through the bile and not *via* urine.

Oxidation of LDL is thought to play an important role in the early stages of atherogenesis. *In vitro* studies show that green tea and black tea and purified tea catechins are effective inhibitors of LDL oxidation in transition metal ion-dependent (28) and -independent systems (8). However, data related to the effects of tea consumption on LDL oxidation *ex vivo* are currently inconclusive (10–13). In these *in vivo* studies, fasting blood samples were used and as plasma catechin levels decreased rapidly during refraining from tea consumption (Fig. 1), this may have influenced the outcome. Therefore, we took blood samples at the end of the third day of repeated tea consumption. We found no protective effect of tea consumption although catechins were present in LDL at low but detectable levels (0.077 μM during green tea consumption). *In vitro* experiments showed that the level of accumulation of catechins in LDL needed to enhance the intrinsic resistance of LDL to oxidation was ≈ 0.4 μM. It does not appear likely that such concentrations of tea catechins will be achieved in LDL during normal patterns of tea consumption.

It is plausible however, that *in vivo*, tea flavonoids are loosely associated with the protein on the outside of the LDL particle, as has recently been suggested for wine phenolics (29). In this location, tea flavonoids may, similar to vitamin C, have a sparing effect on fat-soluble antioxidants within the lipoproteins. This could be an important protective mechanism related to the beneficial effects of tea consumption. On the other hand, tea flavonoids may also interfere with other aspects of cardiovascular disease. There are indications that tea flavonoids may influence the final thrombotic event that leads to occlusion of the atherosclerotic coronary artery, as the epidemiological data on tea and cardiovascular disease are strongest for coronary heart disease mortality and not morbidity (reviewed in Ref. 30). In line with this hypothesis, flavonoid compounds have been shown to inhibit platelet aggregation *in vitro* (31), and recent experimental evidence suggests that tea inhibits *in vivo* platelet activity and prevents experimental coronary thrombosis in dogs (32). Previous studies (25, 26) and the present study show that tea flavonoids accumulate in blood after tea consumption. However, to help unravel the physiological actions of tea flavonoids *in vivo*, more data are required on the tissue localization of tea flavonoids and the extent of their cellular uptake.

In conclusion, the present study shows that catechin levels in blood rapidly increase upon repeated tea consumption. The migration of catechins into LDL particles is not sufficient to improve the intrinsic resistance of LDL to oxidation *ex vivo*. Furthermore, the present study confirms that addition of milk to black tea does not affect the bioavailability of catechins.

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