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Bioavailability of catechins from tea: the effect of milk

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Objectives: To assess the blood concentration of catechins following green or black tea ingestion and the effect of addition of milk to black tea.
Design: Twelve volunteers received a single dose of green tea, black tea and black tea with milk in a randomized cross-over design with one-week intervals. Blood samples were drawn before and up to eight hours after tea consumption.
Setting: The study was performed at the Unilever Research Vlaardingen in The Netherlands.
Subjects: Twelve healthy adult volunteers (7 females, 5 males) participated in the study. They were recruited among employees of Unilever Research Vlaardingen.
Interventions: Green tea, black tea and black tea with semi-skimmed milk (3 g tea solids each).
Results: Consumption of green tea (0.9 g total catechins) or black tea (0.3 g total catechins) resulted in a rapid increase of catechin levels in blood with an average maximum change from baseline (CVM) of 0.46 μmol/l (13%) after ingestion of green tea and 0.10 μmol/l (13%) in case of black tea. These maximum changes were reached after (mean (s.e.m.)) $t = 2.3$ h (0.2) and $t = 2.2$ h (0.2) for green and black tea respectively. Blood levels rapidly declined with an elimination rate (mean (CVM)) of $t_{1/2} = 4.8$ h (5%) for green tea and $t_{1/2} = 6.9$ h (8%) for black tea. Addition of milk to black tea (100 ml in 600 ml) did not significantly affect the blood catechin levels (areas under the curves (mean (CVM) of 0.53 h. μmol/l (11%) *vs* 0.60 h. μmol/l (9%) for black tea and black tea with milk respectively.
Conclusion: Catechins from green tea and black tea are rapidly absorbed and milk does not impair the bioavailability of tea catechins.
Descriptors: bioavailability; flavonoids; catechins; tea; milk; human study

Introduction

Several epidemiological studies suggest that black tea consumption is associated with a reduced risk of degenerative diseases such as cardiovascular disease (Hertog *et al*, 1993; Keli *et al*, 1996). There is increasing evidence from experimental studies that free radical-mediated damage may play a role in the aetiology of cardiovascular disease and that antioxidants may act in preventing this damage (Steinberg *et al*, 1989; Witztum & Steinberg, 1991; Esterbauer & Ramos, 1995). Tea is a rich source of flavonoids and the beneficial health effects of tea consumption have been related to the antioxidant activity of these tea flavonoids (Ho *et al*, 1993; Salah *et al*, 1995). The main group of flavonoids in green tea are catechins. Black tea is subjected to more extensive processing, during which a major part of the green tea catechins are converted into more complex condensation products, theaflavins and thearubigins. Interpretation of epidemiological data and findings on biological effects of tea consumption in animal and human trials is currently hampered by the limited amount of information on the bioavailability and pharmokinetics of tea flavonoids.

In addition to knowledge on the pharmokinetics of flavonoids from green and black tea, it is important to get insight into factors that affect the bioavailability of tea flavonoids because these may influence the potential health benefits of tea consumption. One aspect of interest in relation to tea is the addition of milk, which is common practice in several countries, for example, the United Kingdom. The consumption of black tea in the United Kingdom is high compared with other countries (5–6 cups *vs* 1–2 cups per day) and yet the incidence of coronary heart disease is not lower. In contrast to earlier epidemiological data from The Netherlands (Hertog *et al*, 1993; Keli *et al*, 1996), where plain black tea is consumed, a recent study in the United Kingdom showed that consumption of tea with milk was not inversely associated with mortality of ischemic heart disease (Hertog *et al*, 1997). A possible explanation may be that the bioavailability of flavonoids from tea is reduced by the addition of milk to black tea. Indirect evidence for this hypothesis was found by Serafini *et al* (1996) who showed that addition of milk to black tea abolished the increase of the antioxidant potential of plasma observed when tea was drunk without milk. However, direct evidence that milk inhibits the absorption of tea antioxidants in humans, based on measurement of actual blood levels of tea flavonoids, is currently lacking.

In this present study we assessed the blood concentrations of catechins following tea ingestion. Furthermore, we assessed the effect of the addition of milk to black tea on this blood response.

Subjects and methods

Volunteers

Volunteers were recruited among employees of Unilever Research Vlaardingen in The Netherlands. They were

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eligible for participation if they were healthy as assessed by medical investigation, had a Quetelet Index between 19 and 30 kg/m^2, and were non-smoker. The volunteers did not use vitamin C, vitamin E, carotenoid, calcium, iron or fibre supplements, a medically prescribed diet or slimming regime and were regular consumers of tea ($\geq$1 cup/d, mean intake $\pm$s.d.: 3.75 $\pm$ 2 cups/d). Female volunteers were not pregnant or lactating.

Study design
In a cross-over design with one-week intervals, twelve healthy adult volunteers (5 males, 7 females, aged 23–54 y) consumed after an overnight fast a single amount of green tea, black tea or black tea with milk (3 g lyophilized tea solids (TJ Lipton Inc, USA)). Green or black tea solids were dissolved in 600 ml wake or in 500 ml water plus 100 ml semi-skimmed milk. All subjects received a standard breakfast and lunch and abstained from flavonoid-rich, antioxidant-rich and dairy products one day before and during the day of tea consumption. Blood samples were collected before and 0.5, 1, 2, 4, 6 and 8 h after tea consumption and blood concentrations of catechins were determined. The study protocol was approved by our local Medical-Ethical Committee and volunteers gave their written informed consent before participation.

Catechin analysis in blood
Catechins were determined as described previously (Kivits *et al*, 1997). This colorimetric method comprises a solid phase extraction of blood with aluminium oxide followed by complexation of catechins with dimethyl amino cinnamaldehyde (DMACA). The complexation is specific for flavones containing meta-oriented hydroxy groups in the A-ring and a single bond in the 2,3-position of the heterocyclic ring. The method shows, on a molar base, the same response factor for each of the catechins present in tea (namely, catechin, epicatechin, epigallocatechin, epigallocatechin gallate and epichatechin gallate). Theaflavins and thearubigins are also detected, but their response factor is lower than that of catechins. The method is rapid, specific and sensitive but does not separate individual catechins as do other methods which require sophisticated equipment (Lee *et al*, 1995). For this paper, the total response of the spectrophotometric measurement is referred to as the concentration of total catechins.

Venous blood samples were collected into Na_2EDTA tubes, rapidly frozen and stored at $-80°C$. One ml of thawed blood was thoroughly mixed with 3.0 ml methanol containing 1 g/L butylated hydroxytoluene. After centrifugation, the supernatant was added to 100 mg aluminium oxide (Super I; ICN Biomedicals, Eschwege, Germany). The mixture was vortexed, centrifuged and the supernatant was discarded. The residual aluminium oxide was washed with diethyl ether. Complexation of catechins was initiated by the addition of 0.5 ml of DMACA (Merck, Darmstadt, Germany), 6 mmol/L in methanol/perchloric acid/water (8 : 1 : 1; v/v). After 6 min, the absorption spectrum of the clear supernatant was measured from 500–750 nm using DMACA reagent as reference. The molar concentration of total catechins was determined by using an external calibration curve of human control plasma spiked with catechin (0–5 μmol/L; Sigma, St Louis, MO, USA). The intra assay variation of this method is 3.5% and the inter assay variation is 9.9%.

Catechin analyses in tea
The total catechin content of green and black tea was determined as above, omitting the solid phase extraction. The amount of individual catechins in green and black tea was determined by reversed phase HPLC. After addition of propyl gallate as internal standard, the green or black tea extract was injected on a RP-C18 column (Merck, Darmstadt, Germany). Water/acetonitril/tetrahydrofurane/acetic acid was used as mobile phase with a linear concentration gradient (975/25/3/5; v/v) to (535/400/70/5; v/v) at a flow rate of 0.8 ml/min and a column temperature of 20°C. The eluent was monitored by UV-Vis detection at 280 nm.

Statistical analyses
The average area under the concentration curve (change from baseline) the maximum concentration, the time to reach this maximum and the elimination rate of catechins in blood after ingestion of the tea were calculated. Except for the time to reach the maximum concentration, data were log-transformed to minimize correlation between mean values and standard errors within the treatments. Differences in these parameters between the treatments were tested for significance ($\alpha = 0.05$) by analysis of variance with persons, period and treatment as factors, using Student Newman Keuls-test. Means are presented with standard error or with coefficient of variation in case log-transformation was applied.

Results

All the volunteers received each treatment. Consumption of black tea without milk caused nausea in three volunteers (3 g tea extract equals six cups of tea). Statistical analysis of the data with and without the blood concentrations of these subjects revealed no difference in outcome. Therefore the results of the analysis of the data from all participants is presented.

Catechin concentrations
The composition of the green and black tea is shown in Table 1.

Figure 1 shows the blood concentrations of total catechins after consumption of green tea, black tea or black tea with milk. In agreement with the difference in catechin content of green and black tea (Table 1), consumption of green tea induced a significantly larger area under the catechin response curve than black tea (Table 2). The time to reach maximum concentration was not different for green and black tea, whereas the rate of elimination of catechins from blood was significantly higher after consumption of green tea as compared to black tea (Table 2).

Addition of milk to black tea had no effect on the total catechin levels in blood (Figure 1). There was no significant difference in either the area under the catechin concentration curves or the pharmokinetic parameters of the curves between black tea with and without milk (Table 2).

Discussion

The present study shows that consumption of green or black tea is followed by a rapid increase of the total catechin concentration in blood. The average maximum concentration of total catechins was 0.55 μmol/l after ingestion of green tea and 0.17 μmol/l after black tea. No significant effect was found of the addition of milk to black tea on the catechin levels in blood.

Table 1 Catechin content of green tea and black tea extracts

Component	*Green tea*	*Black tea*
Total catechins (g/g)[a]	0.31	0.10
Catechin (%)[b]	2	6
Epicatechin (%)[b]	11	22
Epigallocatechin (%)[b]	35	31
Epicatechin gallate (%)[b]	12	25
Epigallocatechin gallate (%)[b]	40	16

[a]Measured by spectrophotometry after complexion with DMACA (see Methods section).

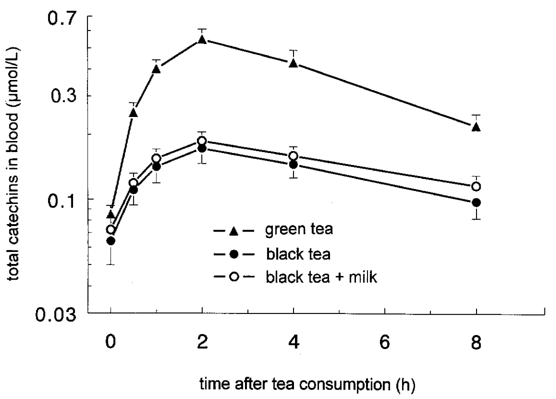

Figure 1 Total catechin concentration in blood after consumption of green tea, black tea or black tea with milk. Data represent mean ± CVM ($n = 12$).

Our data for total catechin concentrations after green tea consumption are comparable with the results from Lee *et al* (1995) who assessed the plasma response of individual catechins (namely, epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate) by HPLC after consumption of a single amount of 1.2 g green tea solids. In their study, the average calculated sum of catechin concentrations in plasma was 0.63 µmol/l after four hours. This is equivalent to about 0.3 µmol/l in whole blood. The average maximum concentration of total catechins in whole blood which we found after ingestion of 3 g green tea solids was 0.55 µmol/l. Taking into account the difference in amount of tea extract consumed, this concentration is of the same magnitude as the concentration found by Lee *et al* (1995).

Table 1 shows the time to reach the maximum catechin concentration and the elimination rate, based on a one compartment model. For green tea, the peak concentration of catechins in blood was reached on average 2.3 h after ingestion and the mean elimination half-life was 4.8 h. Hollman *et al* (1996) have reported the kinetics of the plasma response of the flavonol quercetin following consumption of a single portion of onions. They found, based on two subjects, that the maximum plasma response was reached after 3.3 h and that the elimination half-life was 16.8 h. Apparently, there are substantial differences in kinetics of uptake and elimination of different flavonoids.

It is interesting to note that the peak concentration of catechins found in blood after tea consumption is of the same magnitude as the fasting plasma level of β-carotene or other carotenoids (Weststrate & van het Hof, 1995). Catechins therefore have the potential to significantly contribute to the total antioxidant status of the body. The rapid absorption and elimination of catechins in blood implies however, that frequent tea consumption is required to maintain a steady state level of catechins in blood. Based on an elimination half-life of 4.8 h, it can be calculated that after an overnight fast the concentration of catechins is only 19% of that of the steady state concentration during regular tea consumption. When measured in fasted blood samples the contribution of catechins to the antioxidant status may therefore be underestimated as compared to the value during the day.

As anticipated, the rise of catechins in blood was smaller after consumption of black tea compared to green tea (ratio of areas under the concentration curves, green *vs* black tea: 4.2 : 1). The main flavonoids in green tea are catechins whereas during production of black tea a major part of the catechins is transformed into theaflavins and thearubigins (Graham, 1992). The ratio of the total catechin content in green tea and black tea, when based on the spectrophotometric analysis, was 3.1 : 1. This method, however, is not only responsive to catechins but also to some extent to theaflavins and thearubigins. The fact that the ratio of catechins in green tea and black tea is higher than that in blood suggests that the bioavailability of theaflavins and thearubigins is relatively lower than that of catechins. This may be explained by the larger and more complex molecule structures of the former types of flavonoids. Although the time to reach the maximum concentration was the same for black and green tea, the elimination rate was significantly different after consumption of black tea as compared to green tea. This again suggests a difference between the different tea flavonoids in uptake, tissue distribution or metabolism.

Comparison of the blood response of catechins following black tea with milk and black tea without milk indicates that the addition of milk to black tea has no effect on the bioavailability of catechins. Both the total area under the curve as well as the parameters describing the kinetics of the catechin response in blood were not significantly different after consumption of black tea with milk as compared to black tea alone. This does not support the findings of Serafini *et al* (1996) who showed that the

Table 2 Pharmokinetic parameters of catechins in blood after consumption of a single amount of green tea, black tea or black tea with milk (means (s.e.m. or CVM in %); $n = 12$)

Parameter	*Green tea*	*Black tea*	*Black tea with milk*
Area under the curve (h. µmol/l)[1]	2.22 (16%)[a]	0.53 (11%)[b]	0.60 (9%)[b]
Maximum concentration (µmol/l)	0.55 (11%)[a]	0.17 (14%)[b]	0.18 (10%)[b]
T_{max} (h)[2]	2.3 (0.2)	2.2 (0.2)	2.0 (0.2)
Elimination rate ($t_{1/2}$ (h))	4.8 (5%)[a]	6.9 (8%)[b]	8.6 (10%)[b]

[1]Change from baseline concentration.
[2]Time to reach the maximum concentration after tea consumption.
[a,b]Means in the same row with different superscript letters are significantly different according to Student Newman Keuls-test at $P < 0.05$.
Note: Except for T_{max}, data were log-transformed before statistical analysis.

increase in antioxidant activity of plasma following black tea consumption was abolished by the addition of milk. This difference may be due to the larger amount of milk added to the tea in the study of Serafini *et al* (1996) (namely, 25% *vs* 16% of milk, v/v). However, the habitual amount of milk added to tea is estimated to be 10–15%, which is closer to the percentage used in the present study. Hollman (personal communication, 1996) recently found that the increase in the plasma concentration of quercetin following black tea consumption is also not affected by addition of milk to tea (16% of milk, v/v). However, it can not be excluded that the inhibitory effect of milk on plasma antioxidant activity, as observed by Serafini *et al* (1996) is due to a reduction of the absorption of antioxidants other than catechins or quercetin.

Conclusions

The present study shows that catechins from green and black tea are rapidly absorbed and that milk does not impair the bioavailability of the tea catechins.

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