Proceedings of the Third International Scientific Symposium on Tea and Human Health: Role of Flavonoids in the Diet

Antioxidant Effects of Tea: Evidence from Human Clinical Trials[1]

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Tea remains the most consumed drink in the world after water, well ahead of coffee, beer, wine and carbonated soft drinks. An accumulated number of population studies suggests that consumption of green and black tea beverages may bring positive health effects (1). One hypothesis explaining such effects is that the high levels of flavonoids in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. Chemically, the flavonoids found in green and black tea are very effective radical scavengers. The tea flavonoids may therefore be active as antioxidants in the digestive tract or in other tissues after uptake. A substantial number of human intervention studies with green and black tea demonstrates a significant increase in plasma antioxidant capacity in humans ~1 h after consumption of moderate amounts of tea (1–6 cups/d). There are initial indications that the enhanced blood antioxidant potential leads to reduced oxidative damage to macromolecules such as DNA and lipids. However, the measurement of oxidative damage through biomarkers needs to be further established. In conclusion, tea flavonoids are potent antioxidants that are absorbed from the gut after consumption. Tea consumption consistently leads to a significant increase in the antioxidant capacity of the blood. Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important biomolecules. The scientific support is strongest for the protection of DNA from oxidative damage after black or green tea consumption. However, the quality of the studies now available is insufficient to draw firm conclusions. Therefore, further evidence from human intervention studies is required. J. Nutr. 133: 3285S-3292S, 2003.

KEY WORDS: • *green tea* • *black tea* • *antioxidants* • *flavonoids* • *plasma antioxidant capacity* • *oxidative damage* • *biomarkers*

The traditional tea (*Camellia sinensis*) infusion is characterized by a high content of flavonoids. Flavonoids are a large group of phenolic products of plant metabolism with a variety of phenolic structures that have unique biological properties and may be responsible for many of the health benefits attributed to tea. Many in vitro studies show that the flavonoids present in tea have strong antioxidant and metal-chelating properties and may therefore protect cells and tissues against free oxygen radicals. A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases. Reactive oxygen and nitrogen species are formed in the human body and endogenous antioxidant defenses are not always sufficient to counteract them completely. Diet-derived antioxidants may therefore be particularly important in protecting against chronic diseases (2,3). Tea is an important source of flavonoids in the diet with levels approaching 200 mg/cup for a typical brew of black tea (4). The flavonoids found in green and black tea are very effective antioxidants in vitro and may therefore be active as antioxidants in the body. In this review we will evaluate the human studies that investigated the antioxidant functions of tea in vivo. The uptake of tea flavonoids has been studied extensively as well as the changes in antioxidant capacity of plasma after tea consumption. Ultimately, it is necessary to determine whether tea consumption leads to reduced oxidative damage in the body. Products of oxidative damage to macromolecules have been identified in biological materials such as plasma, urine and blood cells and may serve as biomarkers for oxidative damage. Biomarkers have been studied in a number of human intervention trials to investigate the antioxidant effects of tea and tea flavonoids in vivo.

Bioavailability of tea flavonoids

Uptake of flavonoids from tea is most efficient for the monomeric catechins found in green tea and in low amounts

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[3] Abbreviations used: AAPH, 2′-azobis(2-amidinopropane) hydrochloride; EC, epicatechin; ECL, enhanced chemiluminescence; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; Fpg, formanidopyrimidine-DNA glycolase; FRAP, ferric-reducing antioxidant power; HRP, horseradish peroxidase; MDA, malondialdehyde; ORAC, oxygen radical absorbing capacity; PC, phosphatidylcholine; PCOOH, oxidized PC; R-PE, R-phycoerythrin; SCE, sister chromatid exchange; TBARS, thiobarbituric acid-reactive substances; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical trapping antioxidant parameter; WBC, white blood cells.

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in black tea. The results of a study on bioavailability and distribution in rats demonstrated that epigallocatechin-3-gallate (EGCG) behaves differently than epigallocatechin (EGC) and epicatechin (EC). The bioavailability of EGCG is lower and it is mainly excreted through bile whereas EGC and EC are excreted through urine and bile (5).

Recently, the uptake kinetics of catechins were studied in humans. In a randomized crossover study, 10 healthy volunteers ingested 1.5 mmol of ECG, EGC or EGCG with breakfast (6). Plasma and 24-h urine were analyzed for catechin levels. The study showed clear differences in the kinetics of appearance of the three catechins in the blood. ECG was slowest in rise with a peak at 4 h and showed the slowest decline with a half-life time ($t_{1/2}$) of almost 7 h. EGCG was intermediate with a peak at 2.9 h and decline with a $t_{1/2}$ of almost 4 h. The uptake of EGC was very fast with a peak at 1.4 h and decline with a $t_{1/2}$ of 1.7 h. The maximum concentrations were 5.0 ± 0.32, 1.3 ± 0.23 and 3.1 ± 0.35 μmol/L for EGC, EGCG and ECG, respectively. Up to 13.6% of the ingested EGC was excreted in the urine, a part of which was methylated. However, ECG and EGCG were not detected in urine.

For the detection of theaflavins in plasma and urine, a HPLC-MS method was applied to study the uptake of theaflavin in two healthy volunteers after ingestion of 700 mg. After 2 h a maximum concentration of 1 μmol /L was reached. Simultaneously, a peak of 4.2 μmol/L was found in the theaflavin level of urine. Both plasma and urine levels are relatively low compared to the monomeric catechins. However, the uptake of theaflavins may be underestimated since the method will not detect theaflavin metabolites with modifications in the A- and C-ring (7). The absorption of the so-called catechin condensation products, the thearubigins, remains to be studied. Unabsorbed flavonoids and those excreted via bile into the small intestine are degraded by bacteria in the colon. Deconjugation and ring fission of the aglycones to phenolic acids are considered the major bacterial reactions (8).

The absorption of dietary quercetin, predominantly present in the glycoside form in tea, was analyzed in humans and estimated to range from 20 to 50%, depending on the nature of the glycoside chain. The presence of a glucose moiety enhances absorption and allows absorption as intact molecules (9).

The major catechins from tea are predominantly (>80%) found as conjugates in plasma and urine (10,11). These conjugates still contain intact catechol and gallate moieties and have been shown to scavenge superoxide with the same efficacy as their parent compounds (12). Flavonoids are metabolized predominantly in the colon and liver (13,14). Transformations in the liver include glucuronidation and sulfation of the phenolic hydroxyl groups and O-methylation of the catechol groups. Flavonoids not absorbed in the intestine or conjugates bile-excreted into the intestine are degraded by bacteria in the colon whereas hydrolysis of conjugates, and glycosides and ring fission of the aglycones to phenolic acids take place as major bacterial conversions, followed by (re)absorption (14). The bioavailability and metabolism of tea flavonoids have been reviewed in detail by Hollman et al. (8).

Activity of tea antioxidants in humans

In order to assess the modifying effect of tea flavonoids on plasma antioxidant status, a variety of methods has been employed. Commonly used is the ferric-reducing antioxidant power (FRAP) assay. This is a colorimetric assay that measures the ability of plasma to reduce the intense blue ferric tripyridyltriazine complex to its ferrous form, thereby changing its absorbance (15).

Another assay which has been applied in human plasma is the total radical trapping antioxidant parameter (TRAP) (16). In this assay, the rate of peroxidation induced by AAPH [2′-azobis(2-amidinopropane) hydrochloride] is monitored through the loss of fluorescence of the protein R-phycoerythrin (R-PE). In the TRAP assay the lag-phase induced by plasma is compared with that induced by Trolox in the same plasma sample.

In the oxygen radical absorbing capacity (ORAC) assay, basically the same principle is applied as in the TRAP assay (17). The ORAC assay is another commonly applied antioxidant assay based on the ability of a test substance to inhibit the oxidation of B-phycoerythrin by reactive oxygen species, relative to Trolox. Proteins interfere with the analysis, partially protecting R-PE when all plasma antioxidants are exhausted. Determination of the lag-phase TRAP and ORAC assays can be performed with different radicals and thus different results will be obtained depending on the selected radical. For these reasons, results obtained with the TRAP or the ORAC assay in plasma have to be interpreted with care.

Enhanced chemiluminescence (ECL) has been used to measure antioxidant capacity in biological fluids (18). The assay involves the chemiluminescent substrate luminol. Light emission occurs when the luminol is oxidized by hydrogen peroxide that is generated in a reaction catalyzed by horseradish peroxidase (HRP). This method can quantify the antioxidant capacity of a fluid because the reaction is sensitive to radical scavenging antioxidants that reduce the light output.

The Trolox equivalent antioxidant capacity (TEAC) assay is based on the ability of molecules to scavenge the stable free radical of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) in comparison with Trolox, a water soluble analogue of vitamin E (19). The activity of a compound is therefore expressed as TEAC. Of these assays, the ECL seems the least suitable to determine plasma antioxidant capacity because it relies on enzymatic acitivity. This technique has not been widely applied, which limits the possibility to compare results from different studies. All the other assays have been applied in plasma reproducibly.

Eleven human studies can be found in which antioxidant parameters were tested in relation to black or green tea consumption and they will be discussed here. An overview of the studies and their design parameters can be found in **Table 1**. In a crossover study with 24 volunteers, each person received different treatments on separate days in a randomized order (20). The treatments consisted of a 300-mL single dose of black or green tea or an equal volume of hot water. Each 300 mL of tea was prepared with 2 g of lyophilized tea solids, equivalent to three normal cups. Ingestion of dissolved green or black tea solids significantly increased the plasma FRAP value by 2–3%. The study was repeated with 24 volunteers to confirm the findings. A single dose ingestion of green or black tea again resulted after 60 min in a significant increase of catechins in plasma ($P < 0.001$) (**Fig. 1**). As anticipated from the higher catechin concentration in green tea, the rise in plasma total catechins was significantly higher following consumption of green tea as compared with black tea ($P < 0.001$). Consumption of black and green tea also resulted in a significant increase in plasma antioxidant activity relative to consumption of water (**Fig. 2**). Given the large difference in plasma catechin levels, one would anticipate a similar difference in the FRAP values after green and black tea consumption if the increase relative to water were determined by the catechins only. However, the much smaller difference found

TABLE 1

Overview of cited studies: general design parameters and results of human intervention studies

Study	*n*	Design	Control	Tea	Dose *g*	Dose *ml*	Time	Parameters[1]	Results
Benzie, 1999	10	CO	W	GT	20	500	SI	Plasma FRAP	>4% at t = 20–40′
								Urine total phenolics	>45% at t = 60′
								Urine FRAP	>28% at t = 60–90′
Cherubini, 1999	8	—	—	BTE	3.6	500	SI	CEOOH	—
Duffy, 2001								FMD	>60%
								Plasma ORAC	>11% (ns)
								Plasma FRAP	>12% (ns)
								Lipid profile	—
								Vitamin C	—
								Glucose	—
Freese, 1999	20	P10	NT	GTE	3/d	caps	4W	MDA	<21%
								Lipid profile	—
								Vitamin C	—
								Vitamin E	—
								GSH/GSSG	—
								Coagulation factors	—
								Urine NO_3^- and NO_2^-	—
								Urine 8-iso-PGFα2	—
								Urine thromboxane	—
Hodgson, 2000	20	CO	W	GT, BT	7.6	400	SI	*ex vivo* LDL oxidation	lag time > 3%
								TRAP	>4% GT (ns), >3% BT (ns)
								Urine 4-OMGA	>7-fold GT, >22-fold BT
Hodgson, 2001	22	P11	W	BT	10	1250/D	4W	FMD	>40%
								Lipid profile	—
								Urinary 4-OMGA	>18 fold
Ishikawa, 1997	22	P8/14	W	BT	11	750/D	4W	*ex vivo* LDL oxidation	lag time > 15%
								Vitamin E	—
								Lipid profile	—
								Apolipoprotein B	—
								LDL composition	—
	3	—	—	Catechins	0.34	—	SI	Catechins (t = 120′)	468 ± 256 nM EGCG, 85 ± 31 nM ECG
Langley, 2000	8	CO	NT	BT	19.5	1200	1D	FRAP	>76%
Leenen, 2000	21	CO	W	GTE, BTE	2	300	SI	FRAP	+3% GT, +2% BT
								Uric acid	—
								Vitamin C	—
								Bilirubin	—
								Total catechins (t = 90′)	1.8 μM GT, 0.4 μM BT
Maxwell, 1996	10	—	—	BT	5	500	SI	AOX capacity	—
McAnlis, 1997	5	—	—	BT	3.3	600	SI	*ex vivo* LDL oxidation	—
								AOX capacity	—
	10	CO	Coffee	BT	20	1800	1D	Lipid profile	—
								ex vivo LDL oxidation	—
								AOX capacity	—
Miura, 2000	22	P11	?	GTP	0.6	?	1W	*ex vivo* LDL oxidation	lag time > 23%
								Lipid profile	—
								TBARS	—
								EGCG (after 1 week)	56.0 ± 14.4 nM
Nakagawa, 1999	18	—	—	GTE	0.254	caps	SI	EGCG (t = 60′)	267 ± 126 nM
								PCOOH	<40%
								Lipid profile	—
								Carotenoids	—
								Tocopherols	—
Princen, 1998	64	P13-16	W	GTE, BTE	3	900/D	2W	*ex vivo* LDL oxidation	—
				GTP	3.6/d	caps	2W	Vitamin E	<5% GTP
								Lipid profile	—
								Vitamin C	—
								β-carotene	—
								Uric acid	—

here suggests that other flavonoids from black tea are taken up and contribute to increased FRAP. Peak levels in FRAP were reached 60 min after consumption of black or green tea. Plasma uric acid concentration dercreased over time (**Fig. 3**), but this response was apparent in all treatments. Contributions of uric acid to plasma FRAP activity were evaluated by analysis of covariance. After adjustment for the contribution of uric acid, the effects of tea consumption on the FRAP were still significant.

Similar findings have been reported in other human studies. In a study with three groups of five volunteers drinking water, black tea or green tea, Serafini demonstrated a significant and

TABLE 1 (*continued*)

Overview of cited studies: general design parameters and results of human intervention studies

Study	*n*	Design	Control	Tea	Dose *g*	Dose *ml*	Time	Parameters[1]	Results
Serafini, 1996	15	P5	W	GT, BT	6	300	SI	TRAP	>34% GT, >29% BT
Serafini, 2000	5	CO	W	GT, BT	6	300	SI	TRAP	>40% GT, >52% BT
Van het Hof, 1997	45	P15	W	GTE, BTE	3	900	1D	*ex vivo* LDL oxidation	—
								AOX capacity	>3% GT, no effect BT
								Vitamins E and C	—
								β-carotene	—
								AOX enzyme activity	—
								Lipid profile	—
								MDA	—
								Uric acid	—
Van het Hof, 1999	18	CO	W	GTE, BTE	4	1200/D	3D	*ex vivo* LDL oxidation	—
								Total catechins (t = 8 hrs)	1.0 μM GT, 0.3 μM BT
								LDL catechins	77 nM
								Urine catechins (mg/24 hrs)	24.3 GT, 3.3 BT, 3.4 BT Milk

[1] All parameters have been measured in plasma, unless mentioned otherwise. Abbreviations: Dose, Total dose (g) of leaf or extract for tea preparation in given volume (ml) or capsule; Time, Duration of the study; FRAP, ferric-reducing antioxidant power; CEOOH, cholesterol ester hydroperoxide; FMD, flow mediated dilation; ORAC, oxygen radical absorbing capacity; MDA, malondialdehyde; GSH, reduced glutathione; GSSG, oxidized glutathione; TRAP, total radical trapping antioxidant parameter; AOX, antioxidant; ns, not significant; P#, Parallel study, number per group; CO, Crossover study; DR, Dose-response; SI, Single intake; W, Water; NT, No treatment; GT, Green tea; BT, Black tea; GTE, Green tea extract; BTE, Black tea extract; GTP, Green tea polyphenols; D, Day; W, Week; M, Month; 8-OMGA, 8-*O*-methylgallic acid.

strong increase in TRAP value in the tea groups between 30 and 60 min after a single consumption of 300 mL of either green or black tea (21). The scavenging capacity returned to its initial level after 80 min. There was no significant difference between the green and the black tea groups.

In a small crossover study with 10 healthy subjects, tea consumption resulted in a 4% increase in FRAP 40 min after green tea ingestion (22). No increase in FRAP was observed after water intake. Also, in urine a significant increase in FRAP value was found. Plasma FRAP values were back at baseline levels after 120 min. In this study, tea was prepared from 20 g of dry tea leaves, about eight times more than commonly used per cup of tea.

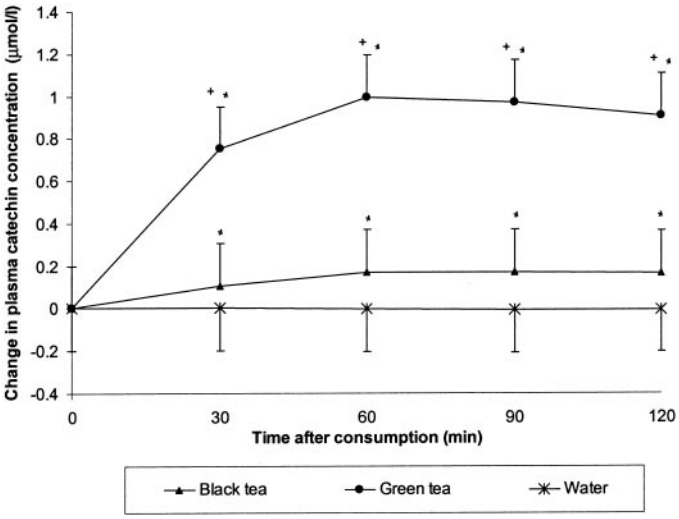

FIGURE 1 Changes in total catechin concentration in plasma (μmol/L ± SEM) over 120 min after a single dose of black tea, green tea and water. *$P < 0.001$, tea versus water; +$P < 0.001$, green tea versus black tea.

In a parallel intervention study by Van het Hof, healthy subjects consumed 6 cups of green tea, black tea or water per day for 4 wk (23). The total antioxidant capacity of plasma was measured with the TEAC assay. A small but significant increase in the total antioxidant capacity of plasma could be observed after 4 wk of green tea but not black tea consumption. Because ingestion of a green tea polyphenolic fraction in mice was found to be associated with an increased activity of antioxidant enzymes (24), Van het Hof tested the activity of antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase in erythrocytes from human plasma. The activity of the enzymes did not change during the intervention.

Princen et al. (25) have analyzed plasma levels of antioxidant vitamins E and C, β-carotene and uric acid to determine

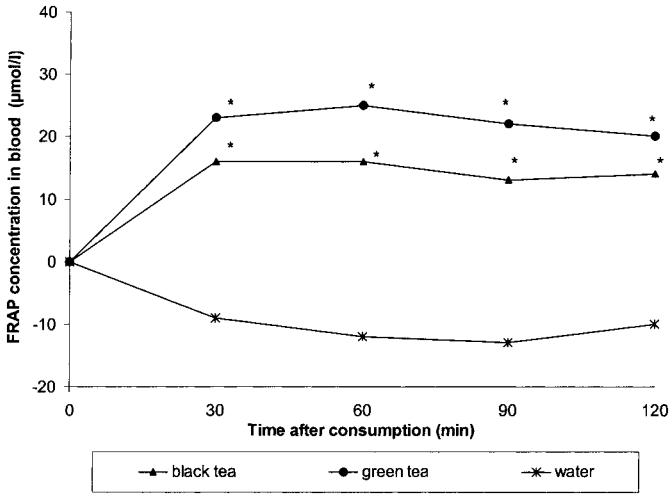

FIGURE 2 Changes in plasma FRAP activity (μmol/L) over 120 min after a single dose of black tea, green tea and water. *$P < 0.001$, tea versus water

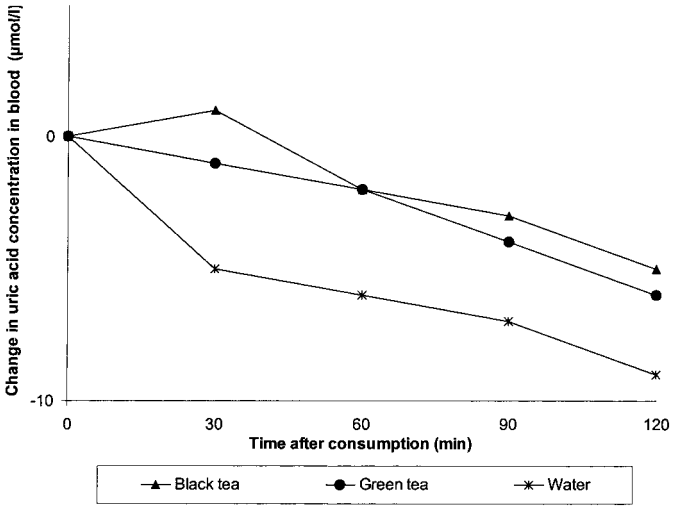

FIGURE 3 Changes in plasma uric acid concentration (μmol/L) over 120 min after a single dose of black tea, green tea and water.

whether tea polyphenols have a sparing or regenerating effect on these antioxidants, similar to the observed regeneration of vitamin E by vitamin C (10,26). In this placebo-controlled parallel study, 64 smokers were randomized into four groups and consumed either green tea, black tea, green tea polyphenol isolate or water for 4 wk. No significant differences were found in endogenous plasma antioxidant levels between tea and control groups, indicating that tea flavonoids do not have a sparing effect on these antioxidants.

Serum antioxidant activity was measured in 10 healthy subjects after ingestion of black tea by Maxwell (27). There was no significant change found in this study. The results in this study are based on the ECL assay which depends on enzymatic activity, in which it differs from the more commonly applied methods such as FRAP and TRAP. Also, the study did not include a control treatment. The chemiluminescence assay has been applied in one other tea study conducted by McAnlis (28), also in which no change in antioxidant capacity of plasma was found, consistent with the results described by Maxwell.

In contrast to the study by Maxwell, a very strong increase in plasma antioxidant activity was found in a randomized crossover study with black tea (29). The increase was measured using the FRAP assay 3 h after the first cup of tea. The antioxidant potential was further increased at 5 h after the first intake.

A more moderate increase in plasma antioxidant capacity was found in 10 young healthy subjects who received green tea on three occasions each separated by one week, with the amount of tea increasing stepwise from 150 to 300 and 450 mL (30). In the first week, a nonsignificant increase compared with baseline values was found. After doubling and tripling the amount of green tea, the increase became progressively significant. Thus, a positive dose-response relation was found. Apart from taking baseline blood samples, there was no control treatment in this study.

The plasma TRAP value was assessed in a randomized crossover study with black tea, green tea, water or water with caffeine treatments (31). A small nonsignificant increase in TRAP was found in both black and green tea groups. Consumption of caffeine in water did not change the plasma TRAP value.

Finally, a double-blind placebo-controlled crossover trial in 60 coronary artery disease subjects was performed by Vita and colleagues to determine the effect of tea consumption on antioxidant status and endothelial function (32). ORAC and FRAP were determined before and after beverage consumption. Both assays showed a nonsignificant increase in plasma antioxidant capacity.

In conclusion, a consistent increase in antioxidant capacity of plasma after tea ingestion has been demonstrated in independent studies with a variety of methods indicating that tea flavonoids enter the body and have an antioxidant function (**Table 2**).

Oxidative damage biomarkers

Products of free radical-mediated damage to lipids, protein and DNA have been identified in biological materials such as plasma, urine and blood cells and proposed as biomarkers for oxidative damage. These biomarkers can be used to analyze the protective effects of dietary antioxidants in vivo (33). The biomarker approach has been applied in a number of human intervention trials investigating the biological antioxidant effects of tea and tea flavonoids.

Oxidation of LDL has frequently been used as a marker for resistance to lipid oxidation. This assay has been particularly popular because it is hypothesized that oxidized LDL contributes to atherosclerosis. In this assay, the susceptibility of LDL to Cu^{2+}-induced oxidation is measured ex vivo, isolated from plasma samples or in plasma samples directly. Black tea and green tea are powerful in vitro antioxidants and effectively protect LDL from oxidation in vitro. However, intervention trials to determine the effect of black and green tea on ex vivo LDL oxidation in humans have failed to demonstrate a consistant effect of tea (**Table 3**).

Other approaches to assess (blood) lipid oxidation include the measurement of lipid oxidation products such as cholesteryl ester hydroperoxides, phosphatidyl-choline hydroperoxides, malondialdehyde (MDA) and F_2-isoprostanes (such as 8-iso-prostaglandin $F_2\alpha$), the latter being a product of arachidonic acid oxidation. F_2-isoprostanes are products of arachidonic acid peroxidation which are considered to be the most reliable and quantitative indicator for lipid peroxidation now available. A commonly applied method to study the formation

TABLE 2

Effect of tea consumption on plasma antioxidant capacity in human intervention studies

Study	Assay[1]	Plasma antioxidant capacity
Serafini, 1996	TRAP	+34% (GT), +29% (BT)
Maxwell, 1996	ECL	no effect (BT)
Van het Hof, 1997	TEAC	+3% (GT), no effect (BT)
McAnlis, 1998	ECL	no effect (BT)
Benzie, 1999	FRAP	+4% (GT)
Leenen, 2000	FRAP	+3% (GT), +2% (BT)
Serafini, 2000	TRAP	+40% (GT), +52% (BT)
Sung, 2000	TEAC	+12.7% (GT)
Langley-Evans, 2000	FRAP	+76% (BT)
Hodgson, 2000	TRAP	[+4% (GT), +3% (BT)] ns
Duffy, 2001	FRAP	[+12% (BT)] ns
	ORAC	[+11% (BT)] ns

[1] Abbreviations: TRAP, total radical trapping antioxidant parameters; ECL, enhanced chemiluminescence; TEAC, Trolox equivalent antioxidant capacity; FRAP, ferric-reducing antioxidant power; ORAC, oxygen radical absorbing capacity; BT, black tea; GT, green tea; ns, non-significant.

of lipid peroxides in a more general fashion is the measurement of thiobarbituric acid-reactive substances (TBARS). The TBARS assay has limited specificity and is susceptible to artificial oxidation.

Cherubini et al. studied the susceptibility of cholesteryl esters to ex vivo oxidation in eight volunteers after the consumption of a single dose of black tea (34). Cholesteryl ester hydroperoxides were measured with HPLC in plasma at different time points after free radical induction with the thermolabile inducer AAPH. No changes were observed in peroxidation lag phase or the subsequent peroxidation rate before and after the tea consumption.

An indirect method to evaluate the effects of antioxidants on oxidation of LDL is to determine the plasma level of oxidized derivatives of phosphatidylcholine (PC), the major phospholipid present in lipoproteins. Oxidized PC (PCOOH) was significantly reduced by 40% in 18 subjects 1 h after consumption of two cups of green tea, indicating that tea flavonoids do protect against lipid peroxidation in vivo (35) (Table 3). In this part of the study, no artificial oxidizing agents were added. Plasma TBARS, blood lipids including cholesterol, α- and γ-tocopherol, lycopene and β-carotene were not affected by the intake of the tea flavonoid supplement. These results should however be treated with caution because they have not been reproduced elsewhere and the change in lipid peroxide level is larger than can be expected on the basis of other tea studies. This may be due to artificial oxidation during sample preparation. In addition to that, the study was performed without control treatment.

The biological antioxidant properties of green tea extract have been demonstrated in 20 subjects consuming a controlled high linoleic acid diet (36) combined with a tea or placebo supplement. The consumption of green tea extract for 4 wk significantly decreased plasma MDA concentrations by 21% as compared with a 42% increase with placebo treatment. The urinary 8-iso-prostaglandin $F_2\alpha$ level was unaffected. The antioxidant status as indicated by the plasma levels of vitamin E, reduced and oxidized glutathione and serum level of vitamin C were not influenced by the tea intervention. This indicates that the catechins do not have a sparing effect on these endogenous antioxidants, consistent with earlier studies (23,25).

Hodgson et al. measured urinary F_2-isoprostane excretion after regular ingestion of 1 L/d of hot water containing caffeine, green tea or black tea for 7 d each in a crossover study with 13 subjects. Green and black tea in comparison with hot water containing caffeine did not change the excretion of F_2-isoprostanes (37).

In conclusion, lipid peroxidation measured as MDA levels or MDA equivalents (TBARS) in plasma was found to be reduced in two studies (35,36). Van het Hof did not find changes in plasma MDA in healthy nonsmoking subjects after consumption of green tea (23). Changes in urinary and plasma F_2 isoprostanes as a result of either green or black tea consumption were minor.

Oxidative DNA damage may lead to gene mutations, conformational changes in chromosomes and modulation of gene expression, events that have been associated with tumorigenesis and disease. Two epidemiological studies demonstrate an association between tea consumption and reduced DNA damage. In a case-controlled study, green tea drinking among chronic smokers was associated with a significantly lower level of smoking-induced micronuclei in peripheral white blood cells (WBC) (38).

In a latin square design study with 52 subjects, the effect of green tea on sister chromatid exchange (SCE) frequencies, a mutagenicity marker, was studied in smokers and nonsmokers. Smokers who consumed 2–3 cups of green tea per day for 6 mo had significantly lower levels SCE in mitogen-stimulated WBC than smokers who consumed 2–3 cups of coffee or other beverages (but no tea) per day (39). The SCE frequencies in tea-drinking smokers was similar to that in nonsmokers. These different approaches demonstrate a correlation between tea consumption and reduced DNA damage.

In an intervention study reported by Klaunig (40), 8-OHdG was measured in WBC and in urine as a marker for oxidative damage to DNA. As with MDA, the starting level of 8-OHdG was higher in smokers than in nonsmokers. After the green tea intervention, the 8-OHdG levels were significantly decreased in both WBC and urine. In a more recent study by Klaunig (41), consumption of black tea solids (2.4 g/d) for 2 wk reduced DNA strand breaks in white blood cells of smokers and nonsmokers, measured by the Comet assay (single cell gel electrophoresis). In this study, the effect of smoking and the potential antioxidant effect of tea on oxidative DNA damage in human WBC was examined. Oxidative DNA damage (8-hydroxyguanosine) was detected using Fpg (formamidopyrimidine-DNA glycolase) in alkaline comet assay. This enzyme recognizes and creates a strand break at oxidized bases in DNA. Oxidative DNA damage, evidenced by both comet tail length and comet tail moment, showed no apparent difference in smokers compared with nonsmokers. However, tea reduced oxidative DNA damage in both smokers and nonsmokers. The reduction in Fpg recognized sites in WBC positively correlated with plasma tea catechin concentration. These results confirmed that smoking causes DNA damage and that black tea consumption reduced this damage possibly through antioxidant properties.

TABLE 3

Ex vivo LDL and lipid oxidation studies

LDL oxidation studies	Tea[1]	Parameter	Ox. Lag time
Ishikawa, 1997	BT	ex vivo oxidation	>15%
Van het Hof, 1997	GT & BT	ex vivo oxidation	—
Princen, 1998	GT & BT	ex vivo oxidation	—
Van het Hof, 1999	GT & BT	ex vivo oxidation	—
McAnlis, 1998	BT	ex vivo oxidation	—
Miura, 2000	GTE	ex vivo oxidation	>23%
Hodgson, 2000	GT & BT	ex vivo oxidation In plasma	>3%
Lipid oxidation studies	**Tea**	**Parameter**	**Level**
Cherubini, 1999	BTE	ex vivo CEOOH	—
Nagakawa, 1999	GTE	PCOOH in plasma	<40%
Nakagawa, 1999	GTE	TBARS ex vivo	72% of control
Freese, 1999	GTE	MDA	<21%
Van het Hof, 1997	GT & BT	MDA	—

[1] Abbreviations: BT, black tea; GT, green tea; GTE, green tea extract; BTE, black tea extract; CEOOH, cholesterol ester hydroperoxide; PCOOH, phosphatidylcholine hydroperoxide; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

DISCUSSION

Flavonoid uptake. Enhanced plasma levels of catechins have consistently been found following the consumption of green and black tea, ranging from 0.63–1.8 μmol/L for green and 0.2–0.34 μmol/L for black tea. The plasma levels peaked after 1.5–2.6 h and were back to baseline within 24 h (20,42–

44). One study addressed the uptake of theaflavins. Both plasma and urine levels were measured with HPLC-MS and were found to be relatively low (1.8 and 7.4 nmol/L, respectively) compared with the monomeric catechins. However, the applied method does not detect theaflavin metabolites with modifications in the A- and C-ring (7).

No data are currently available addressing the uptake of thearubigins from black tea. The analysis of this group of compounds proves to be complicated due to technical limitations and the heterogeneity of the thearubigins. Plasma levels of catechins after consumption of black tea are different compared with green tea, as expected. However, the plasma antioxidant potential after green and black tea consumption differs much less (20). This supports the indication that a portion of the theaflavins and thearubigins in black tea are rapidly absorbed and contribute to the increased antioxidant potential.

The increase in plasma levels of catechins is transient in all studies. Chronic exposure may nevertheless lead to incorporation of polyphenols in tissues. To judge the full physiological consequences of tea intake, the metabolism of flavonoids, the properties of the derivatives as well as the tissue distributition of all tea flavonoids and metabolites should be known.

Antioxidant function. Tea is an important source of flavonoids in the diet and the flavonoids found in tea are known to be strong antioxidants. In vitro assessment of antioxidant power with the TEAC assay gives results closely similar with green and black tea. The majority of the human intervention studies in which biological antioxidant properties of tea polyphenols have been studied demonstrates an increase in plasma antioxidant potential after the consumption of green tea as well as black tea. Small but significant increases (2–4%) were found in studies by Leenen (FRAP), Benzie (FRAP) and Van het Hof (TEAC) after green tea consumption (20,22,23). Leenen found a small but significant increase (2%) with the FRAP assay after ingestion of black tea. A substantial increase in the antioxidant potential of plasma after consumption of green tea (50%) and black (40%) tea was found by Serafini with the TRAP assay (21). Langley-Evans reported an increase in plasma antioxidant capacity of 76% after black tea consumption, measured with the FRAP assay (29). An increase of 12% was found by Sung after ingestion of green tea measured with the TEAC assay (30). A small nonsignificant improvement of antioxidant status was found by Hodgson after green and black tea ingestion using the TRAP assay (31). Maxwell and McAnlis did not find any change in antioxidant potential after consumption of black tea (27,28). The study by Maxwell, however, was a low power study (10 subjects) without control treatment. In the study by McAnlis, coffee intake was used as a control. The high level of chlorogenic acids in coffee may explain the lack of results in this study.

Due to the nature of the applied assays and the different approaches to the expression of the results, it is at present difficult to make a quantitative comparison of the TEAC, TRAP, FRAP and ECL results. However, in qualitative terms all these assays, with the exception of ECL, indicate that tea consumption leads to a significant increase in the antioxidant capacity of the blood.

Tea dose. The amount of tea ingestion used to study changes in antioxidant potential are in the range of 1–6 cups/d of an average English infusion (0.6–2 g of tea per 100 mL of water) in most studies (Table 1). Van het Hof used 3 g of tea extract in 900 mL of water spread over the day which is approximately equivalent to six cups of leaf tea (23). These amounts could realistically be consumed. In the study by Benzie, subjects drank a very strong green tea prepared from 20 g of leaves in 500 mL of water, which is much more than is generally used for a green tea brew (22).

Ex vivo LDL oxidation. It has been suggested that susceptibility of LDL to oxidation plays an important role in atherogenesis. In vitro studies have consistently demonstrated that tea flavonoids can inhibit the oxidation of LDL (23,28,42), which has been taken as an indication that tea consumption may reduce the risk of coronary artery disease. Also, several epidemiological studies indicate that consumption of black tea in realistic amounts (~2 cups/d) is associated with decreased risk of coronary artery disease (1,43). However, the studies in humans summarized here have not provided sufficient support for such a function in vivo.

Lipid peroxidation. Frequently used biomarkers for lipid peroxidation are MDA and TBARS, which are considered as general markers with limited specificity. The value of these parameters has been questioned in the scientific community. F_2-isoprostanes are thought to be more specific and reliable as markers for lipid oxidation. A small number of studies looking at MDA and TBARS have reported contradictory results. There is however, a tendency toward an effect of tea on reduction of lipid oxidation in vivo based on the biomarker F_2-isoprostanes. Insufficient power may explain why the effects in these studies were directional but not statistically significant.

DNA oxidation. Significant reduction of DNA damage after consumption of green and black tea has been reported in population studies (38,39). Recently conducted intervention trials also indicate that tea consumption may lead to protection of DNA against oxidation (41). In this study, a significant reduction in DNA oxidation was found in smokers after black tea consumption.

Protein oxidation. Effects of tea consumption on protein oxidation has not been studied to date. Additional support may be obtained with dityrosine analysis, a biomarker for protein oxidation.

In conclusion, tea flavonoids are potent antioxidants that are absorbed from the gut after consumption and significantly increase the antioxidant capacity of the blood. Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important molecules. The scientific support is strongest for the protection of DNA from oxidative damage after black or green tea consumption. However, the quality of the studies now available is insufficient to draw a strong conclusion. Further evidence from human intervention studies is required. In addition to measurements of oxidative damage biomarkers, we recommend additional research focused on stimulation of the body's own antioxidant defense systems by tea flavonoids; increased gene expression of antioxidant responsive elements and subsequently enhanced expression and activity of detoxifying enzymes has been found in a number of animal studies.

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