No Effect of Consumption of Green and Black Tea on Plasma Lipid and Antioxidant Levels and on LDL Oxidation in Smokers


Abstract—Intake of flavonoids is associated with a reduced cardiovascular risk. Oxidation of LDL is a major step in atherogenesis, and antioxidants may protect LDL from oxidation. Because tea is an important source of flavonoids, which are strong antioxidants, we have assessed in a randomized, placebo-controlled study the effect of consumption of black and green tea and of intake of isolated green tea polyphenols on LDL oxidation ex vivo and on plasma levels of antioxidants and lipids. Healthy male and female smokers (aged 34±12 years, 13 to 16 per group) consumed during a 4-week period 6 cups (900 mL) of black or green tea or water per day, or they received as a supplement 3.6 grams of green tea polyphenols per day (equivalent to the consumption of 18 cups of green tea per day). Consumption of black or green tea had no effect on plasma cholesterol and triglycerides, HDL and LDL cholesterol, plasma vitamins C and E, β-carotene, and uric acid. No differences were found in parameters of LDL oxidation. Intake of green tea polyphenols decreased plasma vitamin E significantly in that group compared with the control group (−11% P=.016) but had no effect on LDL oxidation ex vivo. We conclude that consumption of black or green tea (6 cups per day) has no effect on plasma lipids and no sparing effect on plasma antioxidant vitamins and that intake of a high dose of isolated green tea polyphenols decreases plasma vitamin E. Although tea polyphenols had a potent antioxidant activity on LDL oxidation in vitro, no effect was found on LDL oxidation ex vivo after consumption of green or black tea or intake of a green tea polyphenol isolate. (Arterioscler Thromb Vasc Biol. 1998;18:833-841.)

Key Words: tea ■ flavonoids ■ antioxidants ■ LDL oxidation ■ plasma cholesterol

The concept that oxidation of LDL in the vessel wall plays an important role in the development of atherosclerosis is supported by an increasing number of epidemiological and experimental studies.1 Oxidatively modified LDL has been found in atherosclerotic lesions of humans and experimental animals,2,3 and elevated plasma levels of autoantibodies to oxidized LDL have been reported in patients with atherosclerosis.4,5 In addition, the susceptibility of LDL to oxidation was found to be correlated with the severity of the atherosclerotic process.6–8 There is increasing evidence that antioxidants may protect LDL from oxidation and against the development of atherosclerosis.9 Most epidemiological studies demonstrate an association between the intake or plasma or tissue levels of dietary micronutrients vitamin E, β-carotene, and vitamin C (ascorbic acid) and a reduced cardiovascular risk.10–14 However, data from clinical studies with the isolated components are inconsistent.15–17 Dietary intake of some selected flavonoids, belonging to the family of the flavonoids (quercetin, myricetin, kaempferol) and flavones (apigenin and luteolin), has also been reported to be associated with reduced mortality from coronary heart disease18–20 and stroke,21 although data are not conclusive.22 Flavonoids are a large group of polyphenolic compounds with different antioxidant properties that are grouped in families based on structural differences; they occur naturally in vegetables and fruits and in beverages such as tea and red wine.23–26 Intake of the above-mentioned flavonoids exceeds that of the antioxidants β-carotene and vitamin E in the Netherlands.18 A major source of the flavonoids measured in the study of Hertog et al18 is black tea, which provides approximately 50% of the total intake. Consumption of tea in that study was also inversely related to coronary heart disease mortality.18 Among the flavonoids in tea, the catechins (belonging to the family of the flavonols) are the predominant polyphenols in green tea, whereas black tea contains thearubigins and theaflavins in addition to catechins.27 The catechins have been shown to possess strong antioxidant properties in vitro,28–30 inhibiting the peroxidation of phospholipids28 and the copper-ion and cell-mediated oxidation of LDL.29–31 Less is known of the more complex polyphenols, such as theaflavins, in black tea, but they have also been reported to be antioxidants.25,32–35
In addition to the antioxidative properties of tea, epidemiological data show an association between consumption of green and black teas and reduced plasma cholesterol and triglyceride levels, which may also explain the reduced cardiovascular mortality associated with tea consumption.

In this study with healthy male and female smoking volunteers, we have assessed in a randomized, placebo-controlled study the effect of consumption of black or green tea or of intake of isolated green tea polyphenols on plasma lipids and antioxidants and on LDL oxidation ex vivo. As can be seen in Table 1, green tea contains 3.5-fold more catechins than black tea. To evaluate the effect of intake of an even higher amount of catechins, a group was included which was supplemented with a green tea polyphenol isolate that contained, on the basis of weight, three times the amount of green tea polyphenols as in the green tea group (vide infra). Smoking has been recognized as a major risk factor for the development of coronary artery disease because it accelerates the atherosclerotic process. Cigarette smoke contains a large amount of peroxyl radicals, which may deplete the antioxidative capacities of plasma and LDL and may modify LDL. An increase in circulating products of lipid peroxidation has been demonstrated in heavy smokers. Indeed, a decrease in plasma vitamin C and β-carotene levels has been reported in smokers. The present study was performed with heavy cigarette smokers to investigate whether consumption of tea and intake of green tea polyphenols may have a sparing effect on plasma levels of the latter antioxidants.

We found that consumption of black or green tea has no effect on plasma lipids and antioxidants and on resistance of LDL to oxidation, and that intake of a high dose of isolated green tea polyphenols decreases plasma vitamin E without having an effect on LDL oxidation.

Methods

Subjects

Healthy, normal weight subjects, who smoked at least 10 cigarettes per day were recruited through advertisements in local newspapers and in our hospital. All subjects underwent a medical screening, which included a medical history, physical examination, and routine hematological and biochemical analyses. Any apparent chronic disease; the use of any special diet; consumption of >4 alcoholic drinks per day; pregnancy; lactation; unexplained weight loss (≥2 kg) during the month preceding the study; the use of relevant medication, especially lipid-lowering drugs, estrogens (other than oral contraceptives), or antioxidant vitamins; and a serum cholesterol of ≥7 mmol/L were exclusion criteria. Sixty-four healthy volunteers (32 men and 32 women) were enrolled and randomized into 4 groups with respect to age, body mass index, gender, and habitual tea consumption. The study was conducted according to the principles of the “Declaration of Helsinki.” All participants in this study gave their informed consent. The study was approved by the Medical Ethics Committee of the Leiden University Hospital, Leiden, the Netherlands.

Interventions

To reduce variation in consumption of tea components caused by different brewing methods, freeze-dried extracts of green tea and black tea were supplied in small tea bags. Each bag contained 500 mg tea extract, which had to be dissolved in 150 mL heated water. The volunteers were asked to drink 6 cups (150 mL) of black tea, green tea, or control beverage daily. Thus, a daily dose of 3 g black or green tea solids was consumed. The green tea polyphenol isolate was provided in capsules. Each capsule contained 150 mg polyphenol isolate. The subjects were asked to use 6×4 capsules per day with 6×150 mL control beverage. Thus, a daily dose of 3.6 g green tea polyphenol isolate was used, which was equivalent to the amount of 9 g green tea solids (ie, equal to 18 cups per day). Green tea polyphenols were prepared by the Tea Research Institute, Hangzhou, China, through ethylacetate extraction of tea powder reconstituted in water. The ethylacetate was stripped and the extract reconstituted in water, and methylenechloride extraction was used to remove caffeine. The final extract was then freeze-dried. Black tea extract (US Tea Association blend Regular Black, freeze-dried), green tea extract (US Tea Association Regular Tea Solids, freeze-dried), and green tea polyphenol isolate were provided by T.J. Lipton Inc. The flavonoid composition of the green and black teas and of the green tea polyphenol isolate was determined by reverse phase HPLC (Table 1). The bags of tea and capsule boxes were labeled with each subject’s initials, a subject number, and a product code. The subjects were asked to stick their labels in a daily diary as a compliance check. The control beverage was mineral water, and antioxidant-free syrup was supplied with the mineral water.

Study Design

The study had a single, blind, placebo-controlled, parallel design. During a run-in period of 2 weeks, the subjects drank 6 cups (150 mL) of the control beverage (mineral water) daily. A blood sample was collected in the morning after a 10-hour overnight fast. During the next 4 weeks, each subject used the intervention beverage that was assigned to him or her. Consumption of the intervention was evenly spread throughout the day: together with the three meals, between breakfast and lunch, between lunch and dinner, and in the evening. The addition of sugar to the tea was allowed, but addition of milk was prohibited. The subjects were instructed by a diettian to adhere as closely as possible to their normal eating habits during the intervention. They were not allowed to consume red wine or more than 2 oranges or 2 glasses (100 mL) of fruit juice per day or to drink tea apart from the prescribed 6 cups per day. The subjects visited the research center once a week to report side effects and indications to collect the intervention for the next week. After 4 weeks of treatment (on day 29), a fasting blood sample was collected in the morning between 1 and 3 hours after the last tea consumption or polyphenol intake of that morning. Food- and drink- frequency questionnaires were filled in twice to assess daily vitamin C, vitamin E, and β-carotene consumption and flavonoid intake, apart from intervention, before and during the study. For that purpose, the frequency with which each food item was consumed was multiplied by its antioxidant content as derived from The Netherlands Food Composition Table. Flavonoid contents of the inquired foods were derived by guest on November 9, 2017 http://atvb.ahajournals.org/ Downloaded from
from the data of Hertog et al. Blood was randomly collected from the study groups into EDTA-containing evacuated tubes (1 mg/mL) and immediately placed on ice and cooled to 4°C. Plasma was prepared, sucrose was added in a final concentration of 10% (wt/vol), and samples were frozen in liquid nitrogen in small portions (leaving as little empty space as possible in the tubes) and stored at −80°C, as described previously. This procedure was completed within 2 hours.

Preparation and Oxidation of LDL

The procedure for preparation and lipid peroxidation of LDL was adapted from the method described by Esterbauer et al., with major modifications as described previously in detail. Briefly, from each subject 3.5 mL of frozen plasma, stored at −80°C, was rapidly thawed and used for isolation of LDL by ultracentrifugation at 4°C in the presence of 10 μmol/L EDTA. To minimize the time between isolation and oxidation, the LDL was not dialyzed. By omitting dialysis we obtained a more stable LDL preparation, which can be stored in the dark at 4°C for several days without effect on resistance time and propagation rate. This improves the precision of the method, because each LDL preparation can be oxidized consecutively in triplicate. In a representative experiment, lag time was 90±2 minutes at 1 hour after LDL isolation in a LDL preparation that had not been dialyzed; 24 hours after LDL isolation, lag time was 91±3 minutes (n=3). Dialysis under nitrogen for 4 hours (2 changes) at 4°C against 1000 volumes of an oxygen-free buffer containing 150 mmol/L NaCl and 10 mmol/L sodium phosphate, pH 7.4, resulted in lag times of 52±5 minutes directly after dialysis and 23±3 minutes after storage of this LDL under nitrogen for 24 hours (n=3). In agreement with these observations, a loss of lipophilic antioxidants during dialysis was recently reported. Oxidation under hypersaline conditions (1.18 mol/L NaCl) results in a higher lag time than oxidation in physiological saline (0.15 mol/L NaCl; data not shown). Because of this and to overcome the 10 μmol/L EDTA background, 40 μmol/L CuSO4 was added to initiate lipid peroxidation.

The kinetics of the LDL oxidation were followed by continuously monitoring the change of absorbance at 234 nm (see Figure). Absorbance curves of LDL preparations obtained from four subjects (one from each intervention group and from the water group) before and at the end of the intervention period were determined in parallel. Each LDL preparation was oxidized in three consecutive oxidation runs on the same day. The values shown for lag time and propagation rate are means of the values thus obtained. The intra-assay coefficients of variation for lag time and propagation rate were 2.6% and 3.1%, respectively, on oxidation of the same LDL in 3 consecutive oxidation runs on one day. The inter-assay coefficients of variation for lag time and propagation rate were 4.9% and 7.4%, respectively, and were obtained by determining the oxidation of LDL of the same subject prepared on different days. In every oxidation run one reference LDL, prepared from a reference plasma stored at −80°C, was used as a control. Oxidation runs with a >10% deviation from the mean lag time and propagation rate of former measurements of this reference LDL were repeated. By using this highly standardized method, we found no differences in lag time and propagation rate between LDL prepared from plasma frozen in liquid nitrogen and that prepared from freshly collected plasma from the same subject. In addition, no differences in these parameters were found on storage of plasma at −80°C up to 18 months. In a representative experiment, the lag time and propagation rate of a reference LDL prepared from freshly collected plasma were 91±2 minutes and 8.7±0.3 nmol/mg per minute (n=5), respectively. After freezing of the plasma in liquid nitrogen, storage for 3 hours at −80°C, and rapid thawing at 37°C, these data were 90±3 minutes and 8.8±0.3 nmol/mg per minute (n=5) on oxidation in the same oxidation run. After storage of the same plasma for 18 months at −80°C, the lag time and propagation rate were 92±4 minutes and 8.9±0.5 nmol/mg per minute, respectively (n=4 independent oxidations on different days).

In separate experiments, the effect of different concentrations of green tea polyphenols on LDL oxidation was assessed after addition of the polyphenols directly to the oxidation assay, with use of the copper ion–induced oxidation assay described above and oxidation mediated by AAPH (2,2′-azo-bis-[2-aminopropane]hydrochloride; Polysciences Inc), as described by Frei and Gaziano. Kinetics of LDL oxidation and bar graphs showing the effect of green polyphenols on LDL oxidation after addition directly in the assay. A, Kinetics of copper ion–induced LDL oxidation. Representative profiles of an oxidation of a reference LDL are shown that contain no (line without symbols), 50 μg/L (△), 150 μg/L (○), 500 μg/L (◇), and 1000 μg/L (♦) green tea polyphenols. A234 indicates absorbance at 234 nm. B, Effect on oxidation resistance in the copper-induced oxidation assay. C, Effect on oxidation resistance in the AAPH-induced oxidation assay. Assay mixture contained 4 mmol/L AAPH and 0.1 mmol/L EDTA. Values are the mean±SD of 3 to 7 oxidations in B and C. *Significant difference between the indicated concentration and the LDL oxidations to which no green tea polyphenols were added (by Student’s paired t test).
TABLE 2. Characteristics of Subjects by Treatment Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Water (n=15)</th>
<th>Black Tea (n=16)</th>
<th>Green Tea (n=15)</th>
<th>Green Tea Polyphenols (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35±12</td>
<td>34±12</td>
<td>32±13</td>
<td>33±10</td>
</tr>
<tr>
<td>BMI</td>
<td>24±4</td>
<td>24±3</td>
<td>22±3</td>
<td>23±3</td>
</tr>
<tr>
<td>Cigaretts/d</td>
<td>21±5</td>
<td>22±6</td>
<td>25±11</td>
<td>21±7</td>
</tr>
<tr>
<td>Tea, cups/d*</td>
<td>3±4</td>
<td>1±1</td>
<td>2±4</td>
<td>2±2</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>7/8</td>
<td>8/8</td>
<td>8/7</td>
<td>7/6</td>
</tr>
</tbody>
</table>

Values are mean±SD. BMI indicates body mass index (body weight in kilograms divided by the square of the height in meters).

*Habitual tea consumption before the start of the study.

Analytical Measurements

Cholesterol and triglyceride concentrations were determined enzymatically using commercially available reagents (CHOD-PAP kit No. 236.691 and triglyceride kit No. 701.904, Boehringer-Mannheim). HDL cholesterol was measured after precipitation of VLDL, IDL, and LDL by use of the precipitation method with sodium phosphotungstate/Mg2+ as previously reported.40 LDL cholesterol concentrations were calculated by the formula of Friedewald.39 Cholesterol and triglyceride concentrations were determined enzymatically using commercially available reagents (CHOD-PAP kit No. 236.691 and triglyceride kit No. 701.904, Boehringer-Mannheim). HDL cholesterol was measured after precipitation of VLDL, IDL, and LDL by use of the precipitation method with sodium phosphotungstate/Mg2+ as previously reported.40 LDL cholesterol concentrations were calculated by the formula of Friedewald.39

Fatty acid composition of LDL was determined by gas-liquid chromatography as previously described,53 with use of a Carlo Erba Mrga 2 series gas chromatograph equipped with a CP-Sil88 column (50 m×0.25 mm ID) and a flame ionization detector.

TABLE 3. Effect of Consumption by Treatment Group of Black and Green Tea and Intake of Green Tea Polyphenols on Concentrations of Plasma Cholesterol, HDL Cholesterol, Plasma Triglycerides, LDL Cholesterol, and Fatty Acid Composition of LDL

<table>
<thead>
<tr>
<th></th>
<th>Water (n=15)</th>
<th>Black Tea (n=16)</th>
<th>Green Tea (n=15)</th>
<th>Green Tea Polyphenols (n=13)</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>5.42±1.16</td>
<td>5.33±1.05</td>
<td>5.35±1.14</td>
<td>5.06±0.90</td>
<td>.834</td>
</tr>
<tr>
<td>∆</td>
<td>-0.16±0.36</td>
<td>+0.00±0.50</td>
<td>-0.12±0.56</td>
<td>-0.30±0.57</td>
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</tr>
<tr>
<td>%∆</td>
<td>-2±6</td>
<td>-0±10</td>
<td>-3±10</td>
<td>-6±12</td>
<td>.461</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.06±0.31</td>
<td>1.04±0.25</td>
<td>1.26±0.34</td>
<td>1.11±0.30</td>
<td>.190</td>
</tr>
<tr>
<td>∆</td>
<td>+0.01±0.10</td>
<td>+0.01±0.12</td>
<td>-0.05±0.21</td>
<td>+0.01±0.14</td>
<td></td>
</tr>
<tr>
<td>%∆</td>
<td>+2±7</td>
<td>+2±13</td>
<td>-3±16</td>
<td>+1±12</td>
<td>.720</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.70±1.27</td>
<td>1.65±0.79</td>
<td>1.31±0.66</td>
<td>1.46±0.61</td>
<td>.611</td>
</tr>
<tr>
<td>∆</td>
<td>-0.12±0.44</td>
<td>-0.10±0.36</td>
<td>+0.03±0.43</td>
<td>-0.13±0.36</td>
<td></td>
</tr>
<tr>
<td>%∆</td>
<td>-6±27</td>
<td>-3±26</td>
<td>+7±33</td>
<td>-4±26</td>
<td>.560</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.59±1.17</td>
<td>3.55±1.07</td>
<td>3.49±0.95</td>
<td>3.30±0.88</td>
<td>.892</td>
</tr>
<tr>
<td>∆</td>
<td>-0.11±0.36</td>
<td>+0.04±0.52</td>
<td>-0.09±0.45</td>
<td>-0.26±0.51</td>
<td></td>
</tr>
<tr>
<td>%∆</td>
<td>-2±10</td>
<td>+2±19</td>
<td>-3±12</td>
<td>-8±17</td>
<td>.372</td>
</tr>
<tr>
<td>PUFA/total FA*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.50±0.06</td>
<td>0.51±0.04</td>
<td>0.51±0.04</td>
<td>0.53±0.05</td>
<td>.508</td>
</tr>
<tr>
<td>∆</td>
<td>+0.01±0.03</td>
<td>+0.00±0.03</td>
<td>+0.01±0.02</td>
<td>-0.00±0.03</td>
<td></td>
</tr>
<tr>
<td>%∆</td>
<td>+1±6</td>
<td>+1±6</td>
<td>+1±5</td>
<td>-0±5</td>
<td>.798</td>
</tr>
</tbody>
</table>

Blood was collected before the start and at the end of the intervention period. Values are mean±SD for all subjects (men and women). Basal indicates baseline values; ∆ indicates absolute change between treatment and baseline values; and %∆ indicates percent change with respect to baseline values. For ANOVA, the first P value indicates significance of differences in basal values and the second indicates significance of differences in %∆. No significant changes occurred in the indicated parameters during the course of the study in one of the three intervention groups compared with the water group (Mann-Whitney) or within the treatment groups (Wilcoxon).

*Ratio of polyunsaturated fatty acids and the total amount of fatty acids in LDL.

Results

Five subjects did not complete the study (3 dropped out during the run-in period and 2 during the intervention), all...
because of social circumstances. The characteristics of the remaining participants are given in Table 2. No adverse effects of any kind of intervention were reported. The compliance of the remaining participants was very good (as monitored by counts of unused bags of tea and capsules): 97% and 97% in the black tea and green tea groups, respectively, and 95% in the green tea polyphenol group. Dietary intake of antioxidants vitamin C, vitamin E, and flavonoids (quercetin, myricetin, and kaempferol, corrected for flavonoids from tea) was not different before and at the end of the study in all groups (data not shown).

**Effect on Plasma and LDL Lipids**

No changes were observed in plasma cholesterol between the treatment groups during the study as assessed by comparison of %Δ (Table 3). Plasma cholesterol (−6%; P = .087) and LDL cholesterol (−8%; P = .060) tended to be decreased after consumption of green tea polyphenol isolate compared with baseline values. However, no significant changes were observed when differences in this group were compared with differences in the water group (Mann-Whitney test). In addition, no changes were observed in plasma concentrations of HDL cholesterol and triglycerides (Table 3) or in triglyceride content and fatty acid composition of LDL after consumption of black or green tea or intake of green tea polyphenol extract (data not shown).

**Effect on Plasma and LDL Antioxidants**

Baseline levels of plasma β-carotene in the green tea polyphenol group and of LDL β-carotene in the green tea and in the polyphenol groups were higher than in the water group (Table 4), probably because of lower intake of β-carotene in the water group (data not shown). Plasma levels of the water-soluble antioxidants ascorbic acid (vitamin C) and uric acid, the latter quantitatively one of the most important water-soluble antioxidants in plasma, did not change during...

| Table 4. Effect of Consumption by Treatment Group of Black and Green Tea and Intake of Green Tea Polyphenols on Concentrations of Plasma Vitamin E, β-Carotene, Vitamin C, and Uric Acid and LDL Vitamin E and β-Carotene |
|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Antioxidants in plasma, μmol/L         | Water (n=15)    | Black Tea (n=16)| Green Tea (n=15)| Green Tea Polyphenols (n=13) | ANOVA     |
| Vitamin E                              |                 |                 |                 |                                | P          |
| Basal                                  | 27.9 ± 8.5      | 25.8 ± 5.9      | 25.4 ± 6.8      | 26.8 ± 5.2                  | .754       |
| %Δ                                     | −0.7 ± 3.0      | +0.2 ± 2.4      | −0.1 ± 3.3      | −3.7 ± 6.8†                 | .003       |
| β-Carotene                             |                 |                 |                 |                                |            |
| Basal                                  | 0.30 ± 0.11     | 0.40 ± 0.18     | 0.46 ± 0.25     | 0.47 ± 0.16*                 | .048       |
| %Δ                                     | −0.02 ± 0.05    | −0.03 ± 0.08    | −0.02 ± 0.09    | −0.10 ± 0.11†                |            |
| Vitamin C                              |                 |                 |                 |                                |            |
| Basal                                  | 40.7 ± 21.8     | 49.0 ± 21.9     | 55.5 ± 16.6     | 50.4 ± 20.0                  | .277       |
| %Δ                                     | −1.1 ± 12.6     | −1.3 ± 12.0     | +3.9 ± 17.5     | +2.0 ± 19.0                  |            |
| Uric acid                              |                 |                 |                 |                                |            |
| Basal                                  | 255 ± 76        | 259 ± 81        | 259 ± 49        | 268 ± 90                     | .972       |
| %Δ                                     | +2 ± 29         | +23 ± 50        | +6 ± 36         | +3 ± 27                      |            |

**Blood was collected before the start and at the end of the intervention period. Values are mean ± SD for all subjects (men and women). Basal indicates baseline values; Δ, absolute change between treatment and baseline values; %Δ, percent change with respect to baseline values. For ANOVA, the first P value indicates significance of differences in basal values and the second indicates significance of differences in %Δ.**

†Significant difference between the indicated intervention group and the water group (Mann-Whitney).

†Significant difference within the indicated intervention group with respect to baseline value (Wilcoxon).
the intervention period (Table 4). A significant influence of intervention was found on plasma levels of α-tocopherol (vitamin E) (ANOVA of %Δ; P = 0.003). Consumption of black or green tea had no effect on plasma vitamin E and β-carotene. However, plasma vitamin E decreased in the green tea polyphenol group compared with the control group (-11%; P = 0.016). β-Carotene tended to be lowered compared with the control group (-12%; P = 0.062). α-Tocopherol and β-carotene contents of LDL in the tea and polyphenol groups did not differ significantly from those in the water group after the intervention, although α-tocopherol in LDL tended to be lowered in the polyphenol group (-8%) compared with the control group (P = 102).

Effects on LDL Oxidation

Table 5 shows that baseline oxidation characteristics of all four groups did not differ significantly before the start of the intervention. Consumption of green tea, black tea, or green tea polyphenols did not significantly influence the lag time and propagation rate of LDL oxidized ex vivo in those groups compared with the control group.

The effect of green tea polyphenols on the resistance of LDL against modification was also assessed after the addition of the polyphenols directly to the oxidation assay. Representative oxidation curves are shown in Fig 1A. Addition of various concentrations of green tea polyphenols in the assay mixture showed a dose-dependent prolongation of the resistance time using two different procedures for oxidation, ie, copper ion and AAPH-mediated LDL oxidation. Lag time was already significantly increased by addition of 50 μg/L green tea polyphenols to the copper ion–induced oxidation (+6 minutes) and by addition of 500 μg/L to the AAPH-induced oxidation (+57 minutes) (Fig 1B and Fig 1C). No changes were found in the maximum rate of oxidation.

Discussion

In this article we have shown that consumption of black and green teas and intake of isolated green tea polyphenols has no significant effect on susceptibility of LDL to oxidation ex vivo. Plasma antioxidant and lipid concentrations were not changed by consumption of either tea. Intake of a high dose of green tea polyphenols did not affect plasma lipid levels but lowered plasma vitamin E without having an effect on the other measured plasma antioxidants.

Epidemiological studies18–20,22,36,38 have indicated that high intake of tea may reduce the risk of coronary heart disease, although data are not consistent. It has been suggested that the beneficial effect may result from inhibition of LDL oxidation.55 In the present study, we found that drinking 6 cups of green or black tea or the intake of green tea polyphenols equal to consumption of 18 cups of green tea per day did not affect resistance time and maximum rate of LDL oxidation. In agreement with other reports,56,30,31 we have shown that green tea polyphenols have strong antioxidant properties when added directly into the LDL oxidation assay. Since flavonoids are known to bind copper ions,56 it is conceivable that the green tea polyphenols may exert part of their effect by forming complexes with these ions, thereby preventing the formation of free radicals. This is unlikely, however, since a significant increase in resistance time was already observed at a concentration of 50 μg/L (approximately 0.1 to 0.2 μmol/L) after addition to the copper ion-induced LDL oxidation, an amount much lower than the effective copper ion concentration (20 μmol/L) in the assay. In addition, inhibition was found also in the AAPH-mediated oxidation of LDL, in which no metal ions are present.

To our knowledge, no data are available on the partitioning of tea polyphenols between the aqueous and lipid phases, but it seems unlikely that tea flavonoids are incorporated in the lipophilic LDL particle. Tea polyphenols have a high solubility in water, ie, they are extracted in hot water during tea preparation. Furthermore, our LDL oxidation assay appears to be sensitive enough to detect changes if the polyphenols are associated with the LDL particles. The lower detection limit for observation of significant inhibition of LDL oxidation was 50 ng/mL, which is about 6-fold lower than the levels of the major tea catechins observed in plasma after intake of a single dose of 1.2 g green tea (approximately 300

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<thead>
<tr>
<th>TABLE 5. Effect of Consumption by Treatment Group of Black and Green Tea and Intake of Green Tea Polyphenols on Oxidation of LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time, min</td>
</tr>
<tr>
<td>Basal</td>
</tr>
<tr>
<td>Δ</td>
</tr>
<tr>
<td>%Δ</td>
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</tbody>
</table>

Blood was collected before the start and at the end of the intervention period. Values are mean±SD for all subjects (men and women). Basal indicates baseline values; Δ, absolute change between treatment and baseline values; and %Δ, percent change with respect to baseline values. For ANOVA, the first P value indicates significance of differences in basal values and the second indicates significance of differences in %Δ. No significant differences in baseline values were found and no significant changes occurred in the indicated parameters during the course of the study.
ng/mL; see Reference 57). In our study, the volunteers were supplemented with a 2.5-fold–lower dose of green tea (0.5 g consumed six times per day) and with a higher dose of green tea polyphenol isolate (equivalent to 1.5 g green tea, also consumed six times per day). We did not measure plasma levels of catechins in our study, because at the time of this study no selective method was available. The above arguments may provide an explanation for the absence of differences in LDL oxidation parameters among the treatment groups and the water group. Similarly, consumption of red wine, which also contains highly water-soluble antioxidant flavonoids belonging to different classes from those found in tea, has recently been shown not to increase LDL oxidation resistance ex vivo, although data are not consistent.

Whether tea polyphenols may protect LDL in vivo remains to be established. Both an increase and no change in total antioxidant activity in plasma or serum were found after consumption of black or green tea. Green tea polyphenols have been shown to be taken up and rapidly detectable in plasma, although plasma levels are low (ie, after a single high dose of green tea, transient peak levels of catechins were found about 1 hour after intake; these decreased to nearly undetectable levels 9 hours after consumption). With respect to the more complex polyphenols from black tea, there remains considerable uncertainty whether these compounds are also absorbed, and no methods are yet available to measure their plasma levels.

Cigarette smoke contains a large amount of free radicals, which increase products of lipid peroxidation in the circulation and decrease plasma levels of the antioxidants vitamin C, β-carotene, and total carotenoids and of LDL β-carotene and total carotenoids. This study was performed with heavy smokers to investigate whether tea consumption has a sparing effect on plasma and LDL antioxidants. Such an effect has been shown in vitro for the water-soluble antioxidant vitamin C, which regenerates vitamin E from the vitamin E radical. Similarly, it has been hypothesized that the presence of catechins near membrane surfaces may prevent consumption of vitamin E. However, no change was observed in plasma concentrations of water-soluble and lipophilic antioxidants in these cigarette smokers. Intake of green tea polyphenol isolate equivalent to 18 cups of green tea per day unexpectedly even decreased plasma vitamin E levels and tended to decrease vitamin E content in LDL and plasma β-carotene concentrations. It is possible that this large amount of polyphenols, administered as solid compounds, may have interfered with uptake of the lipophilic vitamin E because, at least in rats, a high concentration of tea catechins decreased the bile acid–induced micellar solubility and intestinal absorption of the lipophilic cholesterol. In our study, there was no effect of consumption of green tea polyphenol isolate on plasma and LDL cholesterol.

No significant changes in plasma lipids in this placebo-controlled study were found after consumption of black and green teas. A similar finding was reported by Aro et al in a shorter, noncontrolled study with black tea. This is in contrast to data from cross-sectional epidemiological studies that show an association between an increased consumption of green or black tea and decreased plasma cholesterol and triglyceride levels. However, confounding effects cannot be excluded in the latter studies.

In conclusion, this study demonstrates that consumption of black tea (equivalent to 6 cups per day), green tea (equivalent to 6 cups per day), and green tea polyphenols (equivalent to 18 cups per day) does not affect the susceptibility of LDL to oxidation ex vivo. It is possible that flavonoids, with regard to atherogenicity of LDL, are active via other (cellular) mechanisms, because the compounds have been reported to inhibit cyclo-oxygenase and lipoxygenase enzymes involved in pathways affecting platelet aggregation and vasomotor function of the vessel wall. Whether this and/or their antioxidant capacity may contribute to the reported reduced mortality from coronary heart disease associated with consumption of selected flavonols and flavones, including tea containing quercetin, awaits further research. This is true especially in the latter studies, in which the contribution of other individual constituents of fruit and vegetables or combinations thereof cannot be excluded.

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References

Tea Consumption and Plasma Lipids, Antioxidants, and LDL Oxidation


No Effect of Consumption of Green and Black Tea on Plasma Lipid and Antioxidant Levels and on LDL Oxidation in Smokers
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