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ORIGINAL CONTRIBUTION

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# Green tea extract decreases plasma malondialdehyde concentration but does not affect other indicators of oxidative stress, nitric oxide production, or hemostatic factors during a high-linoleic acid diet in healthy females

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**Abstract** *Background:* Green tea contains polyphenolic catechins which can act as antioxidants and thus decrease the risk for cardiovascular diseases.
*Aim of the study:* To investigate whether green tea extract differs from placebo in its effects on markers of antioxidant status, lipid peroxidation, nitric oxide production, thromboxane production, and blood coagulation during a controlled high linoleic acid diet in healthy subjects.
*Methods:* Twenty healthy nonsmoking females (23-50 years) participated in a 4-week controlled intervention study. The experimental diet was rich in linoleic acid (9 en%) and contained fat, protein, and carbohydrates: 27, 14, and 59 en%, respectively. In addition, the subjects ingested encapsulated green tea extract (3 g/d) or placebo mixture in a double-blind manner. Fasting blood samples and five 24-hour urines were collected before and at the end of the 4-week experimental period. Same samples were received from 10 control subjects.
*Results:* Green tea extract significantly decreased plasma malondialdehyde (MDA) concentration in comparison with the placebo treatment. The treatments did not differ in serum lipids, indicators of antioxidant status, urinary 8-iso-prostaglandin $F_{2\alpha}$, 2,3-dinor-thromboxane $B_2$, nitric oxide metabolites or coagulation indicators.
*Conclusions:* We conclude that an amount of green tea extract which corresponds to 10 cups of tea per day for 4 weeks does not have specific effects on several indicators related to risk of cardiovascular diseases in comparison with placebo treatment. The relatively small but significant decrease in lipid peroxidation indicated by decreased plasma MDA was not associated with changes in markers of oxidative stress (urinary 8-iso-prostaglandin $F_{2\alpha}$ and blood oxidized glutathione) or hemostasis.

**Key words** green tea – linoleic acid – antioxidants – lipid peroxidation – hemostasis

## Introduction

High tea consumption has been associated with decreased risk for cardiovascular diseases (CVD) in several (1-4) but not in all (5-7) epidemiological studies. Although part of the protective effects may be explained by the overall healthy lifestyle associated with tea consumption (8) the high content of antioxidant flavonoids in tea may provide biochemical protection against free radical-mediated degenerative diseases like atherosclerosis and cancer.

The low incidence of coronary heart disease in the Japanese cohorts in the Seven Countries Study has been explained by low intake of saturated fat and high intake of green tea flavonoids (3). Green tea is a very rich source of catechin flavonoids: (-)epicatechin, (-)epicatechin gallate, (-)epigallocatechin, and (-)epigallocatechin gallate which are condensed during manufacturing of black tea (9). Green tea catechins are powerful scavengers of oxygen radicals (9) and decrease LDL oxidation effectively in vitro (10, 11). Decreased LDL oxidation has been proposed as the mechanism of the protective effects of flavonoids like green tea catechins against CVD. However, although green tea ingestion may enhance overall plasma antioxidant activity (12, 13), ex vivo oxidation of LDL may not be affected by green tea consumption (12).

Alternatively, flavonoids could protect from CVD by inhibition of platelet function (14, 15). Flavonoids have been shown to inhibit platelet cyclooxygenase, thromboxane production, and aggregation in several in vitro studies (16-18). Green tea ingestion has been shown to diminish thromboxane production in rat platelets (19) and incubation of human platelets with physiological concentrations of epicatechin decreases platelet aggregation and unstimulated thromboxane production and protects platelets from oxidative stress (18). It has also been proposed that flavonoids bind to platelet thrombi and protect endothelial prostacyclin and nitric oxide (NO) production via their free radical scavenging properties (20). Red wine flavonoids cause endothelium-dependent vasorelaxation which is mediated by the NO-cyclic guanosine monophosphate pathway (21). No data is available on the effects of green tea catechins.

Human intervention data on the effects of flavonoids on oxidative stress and hemostasis is scarce. We studied the effects of green tea extract on antioxidant status, in vivo indicators of oxidative stress, platelet thromboxane production, blood coagulation, and NO metabolism. The study was a placebo-controlled double-blind parallel dietary intervention study with healthy female subjects. The subjects were on a strictly controlled linoleic acid-rich diet and were supplemented either with green tea extract or placebo mixture. In earlier studies oxidative stress of healthy subjects measured by lipid peroxidation-derived DNA adducts has been shown to be enhanced during high-linoleic acid diet (22, 23).

## Methods

### Subjects and study design

Twenty healthy female volunteers were chosen after screening which included a questionnaire and urine glucose, serum lipid, blood pressure as well as body weight and height measurements. All subjects were non-smokers and used no oral contraceptives or medication. One subject received percutaneous estrogen replacement therapy. The subjects were randomized in two treatment groups: green tea group and placebo group. Mean age and BMI in the green tea group were (range in parenthesis) 32.8 (23-50) years and 22.3 (19.2-25.1) kg/m$^2$. The respective characteristics in the placebo group were 34.3 (26-45) years and 22.8 (19.2- 28.9) kg/m$^2$. Additional ten healthy normal weight (BMI 22.8 (18.2-29.4) kg/m$^2$) females aged 24-45 years (mean 34.5 years) were chosen as control subjects. They were also non-smokers and under no hormonal therapy. They gave the same blood and 24-hour urine samples as the subjects but kept their habitual lifestyle and diet throughout the study. The study design was approved by the Ethical Committee of the Faculty of Agriculture and Forestry, University of Helsinki. All subjects gave their informed consent after detailed explanations.

The study consisted of a 2-week pre-experimental period and a 4-week experimental period. The aim of the pre-experimental period was to decrease oxidative stress by replacing unsaturated fatty acids in the diet by saturated fatty acids. The subjects were councelled, e.g., to use butter instead of margarine or oil, high-fat milk products instead of low-fat products and meat instead of fish. The subjects visited the Division of Nutrition once weekly during the pre-experimental period. Butter as well as cakes baked with butter and cream were available.

The pre-experimental period was followed by a 4-week strictly controlled dietary intervention (experimental period). The subjects lunched every weekday at the Division of Nutrition. After lunch they took food for the rest of the day and for next morning and on Fridays for the whole weekend with them. The experimental foods were weighed separately for each subject and provided 90% of the daily energy intake (en%). The subjects had to choose the rest 10 en% from various low-fat foods: vegetables, fruits, berries, beverages, alcoholic drinks, cereal products, sweets and jams. These foods were scored for their energy content and the given sum of scores had to be consumed every day. The subjects recorded daily all freely chosen foods as well as possible uneaten foods in their diaries. Subjects also recorded coffee and tea intake, as well as any medications, symptons or illnesses which emerged during the study. They were asked to keep their physical activity and coffee, tea and alcohol intake on the pre-experimental level during the experimental period.

The experimental diet consisted of mixed natural foods and contained fat 27 en% (saturated, monounsaturated

and polyunsaturated fatty acids 8, 8, and 10 en%, respectively). The main polyunsaturated fatty acid in the diet was linoleic acid which came from sunflower oil (Raision Margariini, Finland) used in cooking and in salad dressing and from a sunflower oil-based spread (Van der Bergh Foods, Finland). Food intake for four energy levels (8, 9, 10, and 11 MJ/day) was calculated and the subjects were allocated on one of these levels according to their calculated energy expenditure. The subjects were weighed twice a week and energy levels were adjusted accordingly in order to keep their body weight constant during the experimental period. Double portions of the 10 MJ diet were collected and analyzed for nutrient composition.

In addition to the diet, the subjects took daily 10 gelatine capsules which contained either green tea extract powder or placebo. The Japanese green tea extract was manufactured by Itoen Co. (Tokyo, Japan) and contained 27% (w/w) polyphenols. Daily dose of the extract was 3 g/day which corresponds to 10 cups green tea per day, a dose which has been indicated to be effective for the reduction of risk of cardiovascular diseases and cancer in a Japanese cohort study (4, 24). The supplemented dose of green tea extract in the present study provided 90 mg/day of epicatechin, 300 mg/day of epigallocatechin, 30 mg/day of epicatechin gallate, 210 mg/day of epigallocatechin gallate, and 180 mg/day of other polyphenols. Caffeine intake from green tea extract was 150 mg/day (Kanji Suga, personal communication). The placebo mixture contained saccharose (4.4 g/day), microcrystalline cellulose (0.87 g/day), and cocoa (10 mg/day). Sugar intake in the green tea group was balanced to the same level as in the placebo group by adding sugar to the desserts.

The unopened capsules were unidentifiable and the subjects or the study personnel were not aware of the contents of the capsules. The capsules were always taken during meals (3 during breakfast, 4 during lunch and 3 during dinner) with ample fluids and the subjects were forbidden to open or bite the capsules. In a feed-back questionnaire, one subject reported that she accidentally saw the contents of the capsules but she could not identify the substance (green tea).

## Blood samples and analyses

Venous blood samples were collected at the end of the pre-experimental and experimental periods. The samples were taken in the morning (7.30-9.30 am) after overnight fasting from the antecubital vein with minimal stasis. The subjects were in sitting position. Vacuum tubes (Terumo Europe, Leuven, Belgium and Beckton Dickinson, Meylan Cedex, France) and 20 gauge needles were used. The anticoagulants and tubes used were (in sampling order) 10 mL serum, 4.5 mL trisodium citrate, 10 ml EDTA, and 10 mL lithium heparin.

Blood for lipid analyses was centrifuged (3000 x g for 10 min) at room temperature and serum was stored in frozen aliquots at -20 ºC. Serum total cholesterol and triglycerides were analyzed with commercial reagents (Merckotest, Merck, Darmstadt, Germany). The intra-assay coefficient of variation was 1.4% for total cholesterol and 2.1% for total triglycerides.

Plasma triglyceride fatty acids were analyzed in order to check the compliance to the diet. Fatty acids were extracted by the method of Folch (25) and lipid classes were separated by thin-layer chromatography with hexane: diethyl ether 4:1. Methylation was carried out by the method described by Stoffel (26). Fatty acids were analyzed by gas-liquid chromatography as reported (27) using standards purchased from Nu Check Prep (USA). The fatty acid compositions are expressed as % of total integrated fatty acids.

Vitamin E was analyzed from serum samples by the HPLC method of Grossi et al. (28). Samples for vitamin C analysis were taken in lithium heparin. The blood was centrifuged within 20 minutes from blood sampling and 200 µL of plasma was immediately pipetted in 1.8 mL of 5% metaphosphoric acid. Samples were stored at –20 ºC. Vitamin C analysis was based on the HPLC-method described by Parviainen et al. (29). The analyses were carried out at the Department of Medical Chemistry, University of Helsinki.

Samples for analyses of plasma malondialdehyde (MDA) as well as whole blood reduced (GSH) and oxidized (GSSG) glutathione were taken in EDTA. For MDA analyses butylated hydroxytoluene (BHT) was added in 400 µL of blood to reach final concentration 1 mmol/L. For GSH and GSSG analyses 200 µL of perchloric acid or 100 µL of sulfosalicylic acid was added in 400 µl of blood, respectively. Samples were mixed, centrifuged (4000 rpm for 10 min) and aliquots of were stored at -40 ºC.

In MDA analysis, the amount of thiobarbituric acid reactive material in plasma after protein precipitation (to decrease non-specific color reaction) was assayed as described by Wade and van Rij (30). In the assay, intermediate peroxides were peroxidated by the addition of iron (1 mmol/L $FeCl_3$) and the assay without iron was used as a background to prevent a non-specific color reaction from interfering with the analysis. Also, the sample without added BHT or iron was measured for MDA. The actual MDA measurement was conducted as described (31). Although the method is not specific in its original set-up, the modifications, including protein precipitation, addition of antioxidant, and the use of iron increase the specificity by eliminating the interfering substances (32).

Blood glutathione concentrations were measured in the supernatant fraction of hemolyzed whole blood. The oxidized form GSSG was separated from the reduced form GSH by Sep-Pak C18 cartridges and GSH and GSSG were assayed by a colorimetric method using 5,5´-dithiobis-(2-nitrobenzoic acid) (DTNB) as a substrate (33).

Blood for coagulation factor analyses was taken in buffered sodium citrate after serum samples. Blood samples were centrifuged within 30-60 minutes after sampling (3 000 x g for 20 min) and plasma samples were immediately frozen in liquid nitrogen. The samples were stored at -40 °C for 6 months before analyses of coagulation indices at the Coagulation Laboratory of Finnish Red Cross Blood Transfusion Service, Helsinki. Plasma fibrinogen concentration was determined with STA®-Fibrinogen and antithrombin III (AT-III) activity with Stachrom® AT III (Diagnostica Stago, Asnieres, France) with STA Compact-analyzer according to the manufacturer. A normal plasma pool calibrated with the International Standard for Antithrombin, Plasma (National Institute for Biological Standards and Control, Hertfordshire, U.K.) and Unicalibrator from Diagnostica Stago were used as standards, respectively. Thrombin-antithrombin-III complex (TAT) was determined with Enzygnost® TAT micro (Behring, Marburg, Germany) covering the concentration range from 1 to 60 µg/L and prothrombin fragment 1+2 (F1+2) with Enzygnost® F 1+2 micro (Behring) according to the manufacturer. D-dimer was determined with Asserachrom® D-Di (Diagnostica Stago).

### Urine samples and analyses

All subjects collected five 24-hour urine samples at the end of the pre-experimental and experimental periods using urine collecting aliquot cups (Daisho co. Ltd, Osaka, Japan). With this equipment 1/21 of the total urine volume was sampled and the rest was discarded. The volumes of the collected urine samples were measured and aliquots were stored at -20 °C.

Before analyses the separate 24-hour urine samples from the pre-experimental and experimental periods were pooled in the ratios of the total urinary volumes. Creatinine was analyzed from the pooled samples with commercial reagents (Mercotest, Merck, Darmstad, Germany). For 2,3-dinor-$TXB_2$ measurement 10 mL of the pooled sample was purified by Sep-pak C18 columns (Waters Co, Milford, Massachusetts, USA) and analysis was carried out enzymoimmunologically as reported (27) using 2,3-dinor-$TXB_2$ antiserum, tracer and standard purchased from Cayman Chemicals (Ann Arbor, MI, USA). The results are expressed as ng/mmol creatinine. Urinary nitrate and nitrite ($NO_3^-$+$NO_2^-$) were analyzed as an overall measure of body nitric oxide production (34). Colorimetric assay kit from Cayman Chemicals (#78001, Ann Arbor, MI, USA) was used. The results are expressed as µmol/mmol creatinine.

Urinary 8-iso-$PGF_{2\alpha}$ was analyzed from the 24-hour urine samples by a newly developed radioimmunoassay (35). In brief, an antibody was raised in rabbits by immunization with 8-iso-$PGF_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by 1,1´-carbonyldiimmidazole method. The cross reactivities of the antibody with 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, $TXB_2$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, and 8-iso-$PGF_{3\alpha}$ were 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8, and 0.6%, respectively. The detection limit of the assay were about 8 pg/mL (23 pmol/L). Unextracted urine samples of 50 µL were used in the assay. The results are expressed as ng/mmol creatinine.

In most analyses, the pre-experimental and experimental samples were analyzed in the same assay in order to eliminate the effects of inter-assay variation in the results. Only serum vitamin C and plasma vitamin E were analyzed separately from the samples collected during the pre-experimental and experimental periods.

### Statistical analysis

The differences between pre-experimental and experimental values were tested within groups by non-parametric Wilcoxon signed ranks test. Differences with p-value 0.05 or less were considered significant. If the control values differed significantly in the same direction as in the treatment groups, a period effect was presumed and the possible changes in the treatment groups can thus not be considered significant.

The difference between the treatment effects in the treatment groups and the changes in the control group were tested by calculating the difference and 95% confidence intervals for the difference of the deltas in the three groups. A Bonferroni adjustment (0.05/3) was made because three groups were compared.

## Results

All 20 subjects completed the study and none reported any side effects during the experimental period. The weight of the subjects remained constant during the study (data not shown). Diet composition data (Table 1) shows the mean intakes of energy and nutrients based on double portion analysis and study diaries. Mean energy intake was higher in the green tea group but the proportions of energy from fat, protein, alcohol, and carbohydrates did not differ. Consumption of coffee and tea during the experimental period was calculated from study diaries and were similar in the treatment groups. Coffee intake was [mean(range)] 3.1 (0-9.0) dL/day in the green tea group and 2.6 (0-6.8) dL/day in the placebo group; and tea consumption in the groups was 1.2 (0-3.8) and 2.0 (0-5.6) dL/day, respectively. The subjects consumed almost exclusively black tea.

The proportion of linoleic acid in plasma triglyceride fatty acids increased markedly in both treatment groups during the experimental period. In the green tea group the proportion was [mean(SD)] 7.3 (2.7) and 17.9 (5.0)% of the integrated fatty acids at the beginning and at the end

**Table 1** Mean daily energy and nutrient intakes in the study groups during the experimental period

| Nutrient | | Green tea group | Placebo group |
|---|---|---|---|
| Total energy | (kJ/day) | 10.8 | 9.8 |
| Fat | (g/day) | 76.6 | 71.0 |
| | (en%) | 27.0 | 27.4 |
| Fatty acids SFA, MUFA, PUFA* | (g/day) | 22.0, 23.2, 27.5 | 19.6, 22.1, 25.8 |
| | (en%) | 7.8, 8.2, 9.7 | 7.5, 8.5, 10.0 |
| Linoleic acid | (g/day) | 25.6 | 24.2 |
| | (en%) | 9.1 | 9.4 |
| Oleic acid | (g/day) | 21.8 | 20.9 |
| | (en%) | 7.7 | 8.0 |
| Protein | (g/day) | 92.4 | 84.7 |
| | (en%) | 14.6 | 14.6 |
| Carbohydrates | (g/day) | 359.3 | 330.7 |
| | (en%) | 56.7 | 57.2 |
| Alcohol | (g/day) | 6.1 | 2.6 |
| | (en%) | 1.7 | 0.8 |
| Cholesterol† | (mg/day) | 154.3 | 117.1 |
| Vitamin C† | (mg/day) | 123.4 | 130.3 |
| Vitamin E† | (mg/day) | 19.2 | 18.0 |
| Calcium† | (mg/day) | 1130 | 1040 |
| Iron† | (mg/day) | 12.4 | 10.8 |

*SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids
† Nutrients from freely chosen foods not included

of the experimental period, respectively. In the placebo group the respective values were 8.4 (2.3) and 18.4 (4.3)%. The difference between pre-experimental and experimental values were significant in both study groups and no differences between the treatments were found. In the control group no changes in serum triglyceride fatty acids were seen (data not shown). Serum total cholesterol and triglyceride concentrations decreased in both treatment groups during the experimental period and no differences between the groups were seen (Table 2). Cholesterol levels remained constant but serum total triglyceride concentration increased in the control group.

Serum vitamin E decreased in all three groups during the experimental period which indicates a possible period effect (Table 2). When vitamin E was standardized with serum cholesterol the treatment groups showed increased and the control group decreased values. No changes in vitamin C, GSH, or GSSG levels were seen in the treatment groups but vitamin C increased and GSSG decreased in the control group. Both treatment groups differed from the control group in respect of vitamin E/cholesterol. Otherwise no significant differences between the groups were found (Table 2).

Plasma MDA decreased in the green tea group during the experimental period and the treatment groups differed in this respect (Table 2). Urinary excretion of 8-iso-$PGF_{2\alpha}$ decreased in the placebo group and tended to decrease (p=0.093) in the green tea group (Table 3). No changes in urinary creatinine, $NO_3^-$+$NO_2^-$, or 2,3-dinor $TXB_2$ were seen in the treatment groups. Urine excretion increased from the pre-experimental level in the green tea group. No differences in the urinary indices were seen between the treatment groups. $NO_3^-$+$NO_2^-$, excretion decreased significantly in the control group (Table 3).

Plasma fibrinogen, AT-III activity, TAT-complexes, and D-dimer remained unchanged during the experimental period in all groups (Table 4). F1+2 decreased in the green tea group but was not changed in the other groups. No differences between the groups were seen.

## Discussion

The present study was a controlled dietary intervention which included double blind supplementation with either green tea extract or placebo. The results suggest that in the present group of 20 female subjects green tea extract had no specific effects on serum lipids and antioxidants, plasma coagulation factors or on urinary indicators of oxidative stress (8-iso-$PGF_{2\alpha}$) and thromboxane and NO formation. However, plasma MDA was decreased in the green tea group which suggests decreased lipid peroxidation in comparison with the placebo treatment and might also suggest decreased oxidative stress in the drinkers of green tea.

Adherence to the diet and supplementation was good. The proportion of linoleic acid increased markedly in serum triglyceride fatty acids which indicates a substantial increase in linoleic acid intake among the subjects (36). The drop in serum cholesterol and triglyceride concentrations in both treatment groups further indicates good compliance to the high linoleic acid diet (37). There were no differences between the treatment groups in the habitual coffee or tea consumption. Basal BMIs were similar in the two groups and body weight of the subjects remained stable during the experimental period in both groups which indicates that the higher energy intake in the green tea group was due to slightly higher physical activity in this group. Because of technical problems, data of polyphenol concentrations in plasma samples of the subjects is not available. However, in a pilot study carried out at Saitama Cancer Cener, 4-week supplementation of subjects with the green tea extract (2.25 g/day) markedly increased fasting serum concentrations of green tea polyphenols already within one week. We conclude that in the present intervention subjects in both groups complied well with the intervention and possible differences between the treatment groups are due to the supplements.

**Table 2** Serum lipids, antioxidants, plasma malondialdehyde, and whole blood GSH and GSSG before and after the experimental period in the study groups

| Variable | | Study Group | | | Difference (95%Cl)* | | |
|---|---|---|---|---|---|---|---|
| | | Green tea | Placebo | Control | Green tea vs. placebo | Green tea vs. control | Placebo vs. control |
| Cholesterol (mmol/L) | PRE† | 5.64 (1.01)‡ | 5.32 (1.00) | 5.07 (0.74) | -0.40 | -1.46 | -10.7 |
| | EXP | 4.37 (0.83)§ | 4.45 (1.13)§ | 5.22 (0.69) | (-0.97 to 0.18) | (-1.85 to -1.07) | (-1.52 to -0.62) |
| Triglycerides (mmol/L) | PRE | 0.80 (0.14) | 0.85 (0.30) | 0.66 (0.16) | 0.08 | -0.31 | -0.32 |
| | EXP | 0.63 (0.10)§ | 0.68 (0.18)§ | 0.86 (0.28)§ | (-0.22 to 0.23) | (-0.69 to 0.08) | (-0.71 to 0.08) |
| Vitamin C (µmol/L) | PRE | 60.40(17.30) | 53.67(11.55) | 50.00 (8.06) | -6.70 | -6.70 | 0.00 |
| | EXP | 60.70(11.45) | 60.67(12.59) | 57.00 (8.34)§ | (-20.62 to 7.22) | (-19.0 to 5.55) | (-11.00 to 11.00) |
| Vitamin E (µmol/L) | PRE | 14.40 (1.33) | 13.85 (2.91) | 13.86 (2.44) | -0.40 | 0.74 | 1.14 |
| | EXP | 12.97 (1.54)§ | 12.82 (2.72)§ | 11.69 (1.62)§ | (-1.72 to 0.92) | (-0.85 to 2.33) | (-0.18 to 2.48) |
| Vitamin E/Chol. (µmol/mmol) | PRE | 2.65 (0.38) | 2.61 (0.29) | 2.73 (0.24) | 0.04 | 0.86 | 0.82 |
| | EXP | 3.02 (0.61) | 2.94 (0.43)§ | 2.24 (0.10)¤ | (-0.41 to 0.49) | (0.45 to 1.27) | (0.51 to 1.14) |
| GSH (mg/L) | PRE | 337.7 (89.0) | 328.9 (86.7) | 324.0 (78.7) | -83.0 | -39.8 | 43.2 |
| | EXP | 326.6 (148.1) | 400.8 (106.6) | 352.7 (91.1) | (-274.6 to 108.7) | (-208.6 to 129.0) | (-113.2 to 199.6) |
| GSSG (mg/L) | PRE | 495.0 (167.5) | 568.1 (111.3) | 585.5 (125.2) | 87.8 | 220.7 | 132.2 |
| | EXP | 380.7 (205.8) | 365.9 (170.9) | 250.5 (84.9)§ | (-175.8 to 351.4) | (-14.1 to 455.4) | (-75.3 to 341.0) |
| Malondialdehyde (mol/L) | PRE | 6.21 (0.92) | 5.89 (1.27) | 6.23 (1.28) | -3.74 | -0.65 | 3.09 |
| | EXP | 4.87 (0.85)§ | 8.37 (4.48) | 5.54 (1.51) | (-7.40 to -0.08) | (-2.92 to 1.62) | (-0.86 to 7.04) |

* Difference between deltas in the study groups
† PRE=pre-experimental period, EXP=experimental period
‡ Values are mean (SD)
§ Difference between PRE and EXP values within treatment group (Wilcoxon $p<0.05$)

**Table 3** Urine excretion and urinary creatinine, $NO_3^-+NO_2^-$, 2,3-dinor-$TXB_2$, and 8-iso-$PGF_{2\alpha}$ before and after the experimental period in the study groups

| Variable | | Study Group | | | Difference (95%Cl)* | | |
|---|---|---|---|---|---|---|---|
| | | Green tea | Placebo | Control | Green tea vs. placebo | Green tea vs. control | Placebo vs. control |
| Urine (L/24h) | PRE† | 2.32 (0.99)‡ | 1.61 (0.50) | 1.62 (0.40) | 0.26 | 0.69 | 0.44 |
| | EXP | 2.81 (1.27)§ | 1.83 (0.39) | 1.42 (0.40) | (-0.28 to 0.80) | (0.14 to 1.25) | (-0.18 to 1.05) |
| Creatinine (mmol/L) | PRE | 3.43 (1.78) | 5.47 (3.67) | 4.53 (1.57) | 0.43 | -0.88 | -1.32 |
| | EXP | 3.00 (1.79) | 4.60 (1.34) | 4.98 (2.00) | (-1.85 to 2.71) | (-2.43 to 0.66) | (-3.74 to 1.11) |
| $NO_3^-+NO_2^-$ (µmol/mmol crea) | PRE | 236.4 (299.5) | 119.6 (56.9) | 134.6 (55.1) | -75.7 | -49.7 | 26.0 |
| | EXP | 135.6 (101.6) | 94.5 (47.5) | 83.6 (48.1)§ | (-261.4 to 110.1) | (-232.9 to 133.5) | (-47.9 to 99.8) |
| 2,3-dinor-$TXB_2$ (ng/mmol crea) | PRE | 16.70 (12.22) | 17.01 (6.75) | 16.74 (6.34) | 4.00 | -1.46 | -5.46 |
| | EXP | 17.64 (11.49) | 13.96 (5.04) | 19.15 (12.89) | (-4.69 to 12.68) | (-14.39 to 11.47) | (-19.74 to 8.82) |
| 8-iso-$PGF_{2\alpha}$ (ng/mmol crea) | PRE | 553.7 (433.6) | 318.8 (117.5) | 310.4 (121.8) | -104.4 | -164.1 | -59.7 |
| | EXP | 345.5 (185.7) | 215.0 (51.2)§ | 266.4 (63.5) | (-373.1 to 164.3) | (-422.7 to 94.4) | (-199.3 to 79.9) |

* Difference between deltas in the study groups
† PRE=pre-experimental period, EXP=experimental period
‡ Values are mean (SD)
§ Difference between PRE and EXP values within treatment group (Wilcoxon $p<0.05$)

**Table 4** Blood coagulation factors and activation markers before and after the experimental period in the study groups

| Variable | | Study Group | | | Difference (95%Cl)* | | |
|---|---|---|---|---|---|---|---|
| | | Green tea | Placebo | Control | Green tea vs. placebo | Green tea vs. control | Placebo vs. control |
| Fibrinogen (g/L) | PRE† | 2.91 (0.28) ‡ | 3.01 (0.54) | 2.77 (0.48) | 0.09 | -0.02 | -0.10 |
| | EXP | 3.09 (0.50) | 3.06 (0.71) | 2.97 (0.82) | (-0.29 to 0.47) | (-0.83 to 0.80) | (-0.93 to 0.72) |
| Antithrombin III activity (%) | PRE | 109.7 (10.5) | 101.0 (5.9) | 101.8 (7.6) | -2.04 | -2.70 | -0.66 |
| | EXP | 109.1 (10.6) | 103.4 (7.7) | 103.9 (9.4) | (-8.23 to 4.14) | (-9.00 to 3.59) | (-6.22 to 4.91) |
| TAT complexes (µg/L) | PRE | 2.74 (3.22) | 3.88 (7.33) | 7.90 (20.11) | 1.30 | 5.51 | 4.21 |
| | EXP | 2.25 (1.78) | 2.02 (1.75) | 1.9 (0.98) | (-5.36 to 7.96) | (-11.27 to 22.28) | (-14.17 to 22.59) |
| F1+2 (nmol/L) | PRE | 0.90 (0.39) | 0.86 (0.46) | 1.04 (0.73) | -0.03 | 0.09 | 0.11 |
| | EXP | 0.82 (0.33)§ | 0.80 (0.28) | 0.87 (0.32) | (-0.37 to 0.31) | (-0.53 to 0.70) | (-0.57 to 0.80) |
| D-dimer (ng/mL) | PRE | 185.5 (89.2) | 186.8 (69.7) | 195.3 (113.7) | 43.4 | 80 | 36.6 |
| | EXP | 198.8 (118.0) | 156.7 (40.9) | 167.9 (88.7) | (-65.3 to 152.7) | (-89.7 to 249.8) | (-110.9 to 184.1) |

* Difference between deltas in the study groups
† PRE=pre-experimental period, EXP=experimental period
‡ Values are mean (SD)
§ Difference between PRE and EXP values within treatment group (Wilcoxon $p<0.05$)

Antioxidant status of the subjects quantified by plasma vitamin E, GSH, GSSG, and serum vitamin C was not affected by green tea extract in comparison with the placebo. We could thus not see any antioxidant sparing effects by green tea catechins which is in line with the results of an earlier human intervention with green tea (12). Vitamin E sparing effects have been seen in vitro with epicatechin and epicatechin gallate (18, 38).

Because green tea did not enhance antioxidant status in comparison with the placebo treatments, one can expect that indicators of oxidative stress would not be different in the treatment groups neither. Urinary 8-iso-$PGF_{2\alpha}$ can be considered a measure of oxidative stress in vivo because it is produced in the body from arachidonic acid by non-enzymatic mechanisms associated with lipid peroxidation (35, 39). Urinary 8-iso-$PGF_{2\alpha}$ tended to decrease in both treatment groups during the experimental period and no specific effect of green tea extract was seen. However, plasma MDA decreased specifically in the green tea group which indicates decreased lipid peroxidation. In our assay system intermediate lipid peroxides were peroxidated to MDA by iron. One could speculate that flavonoids could interfere with the assay by chelating iron. The chelating power of polyphenols in vitro is from 3:2 to 3:1 on a molar basis (40). Considering the amounts of polyphenols ingested and their distribution and excretion, it is unlikely that the effects found could be due to the possible small amounts of iron chelated in the assay. MDA is a nonspecific marker of lipid peroxidation (41) but the result shows at least that the large dose of catechins was not pro-oxidative. In some systems phenolic compounds may promote oxidation (42).

We could not find any difference between the treatment groups in urinary excretion of 2,3-dinor-$TXB_2$ which suggests that green tea catechins did not have specific effect on basal thromboxane production in vivo in our healthy subjects. Our results do thus not confirm the results of Ali et al. (19) who found that green tea ingestion decreased ex vivo thromboxane production in rats during blood clotting, an effect not seen with black tea; or the in vitro results of Polette et al. (18) who found decreased thromboxane formation in unstimulated human platelets in vitro after incubation with physiological concentrations (1 and 10 µM) of epicatechin.

Two recent human interventions have shown that other flavonoids, mainly quercetin, do not affect platelet aggregation or $TXB_2$ production ex vivo (16, 43). There are other mechanisms by which flavonoids may alter platelet function than cyclooxygenase inhibition. In earlier in vitro studies flavonoids have been shown to decrease lipoxygenase activity (14, 44). Flavonoids may also increase platelet cyclic adenosine monophosphate which inhibits platelet function (15). These mechanisms were not studied in the present study.

Blood coagulation factors were hardly affected by green tea. The only variable which slightly decreased in the green tea group was F1+2 but there were no difference between groups. Prothrombin fragments are cleaved from prothrombin when it is enzymatically activated to thrombin. Decreased F1+2 indicates, thus, decreased thrombin production which is naturally a favorable effect in the context of thrombosis. This possibility may be worth studying in more detail in the future. No effects were seen on AT-III activity or TAT complex which indicates that the possible anticoagulative effects of green

tea catechins are not mediated at the level of thrombin inhibition.

We could not see any changes in D-dimer concentrations which indicates that fibrinolysis was not affected by the treatments (45). Human studies on the effects of green tea on blood coagulation or fibrinolysis have not been reported earlier but there are indications that black tea ingestion could enhance fibrinolysis by decreasing the activity of plasminogen activator inhibitor 1 (PAI-1). This effect was, however, seen only in subjects who carry apolipoprotein E allele E2 (46). Fibrinogen and tissue plasminogen activator remained unchanged by black tea ingestion (46, 47). No effects on fibrinogen, factor VII coagulant activity, plasminogen, or PAI-1 activity were seen with other flavonoid-rich sources (onion and parsley) in another human study (16). In these studies, however, the prothrombin activation step was not studied.

Certain flavonoids are able to bind to platelet thrombi and may scavenge free radicals in the environment. At the site of injury, these antioxidative effects may lead to decreased oxidative stress in platelets and restore endothelial NO synthesis (20). In the present study, we evaluated NO production by measuring its breakdown products $NO_3^-$+$NO_2^-$ from urine (34) but could not find any differences between the treatment groups. Urinary NO metabolites may partly reflect protein intake and metabolism. Because the present study was a controlled intervention and intakes of protein and nitarte/nitrite were similar in the treatment groups this may not be a confounding effect between the groups. In some earlier studies flavonoids have been shown to affect NO synthesis or function (15, 21).

We saw no specific effects on serum lipids with green tea extract consumption. These findings are in line with those from other human tea interventions (10-12, 48) but do not support epidemiological (2, 49, 50) data showing decreased serum cholesterol concentrations associated with consumption of tea or tea components. However, in the present study, the cholesterol-lowering diet may have overruled the possible effects of green tea extract.

In summary, the present results show that an amount of green tea extract which corresponds to 10 cups of tea per day for 4 weeks has no specific effects on serum lipids, antioxidants, oxidative stress, thromboxane formation, blood coagulation, or NO synthesis when supplemented to a cholesterol-lowering diet in healthy female subjects. However, green tea extract decreased plasma MDA which indicates decreased lipid peroxidation. We conclude that green tea polyphenols at the dose applied in the present study at most modestly affect body oxidative status and biochemical markers associated with atherosclerosis and thrombosis. Nevertheless, effect after long-term consumption of green tea on the studied parameters can not be ruled out on the basis of the results of the present short-term study.

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