Regular Ingestion of Tea Does Not Inhibit In Vivo Lipid Peroxidation in Humans

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ABSTRACT Prospective studies suggest that tea may protect against cardiovascular disease. A potential mechanism for such an effect involves inhibition of lipid peroxidation by polyphenolic antioxidants derived from tea. Our objective was to determine whether regular ingestion of tea could inhibit in vivo lipid peroxidation. Two controlled intervention studies assessed the effects of regular ingestion of tea on lipid peroxidation determined by measurement of urinary F2-isoprostane excretion. Study 1: The effects of 1000 mL/d of green tea and black tea were compared with hot water containing caffeine in 13 subjects with elevated blood pressure using a randomized 3-period (7 d each) crossover design. Study 2: The effects of 1250 mL/d of black tea were compared with hot water in 22 subjects with mildly raised serum total cholesterol concentrations using a randomized 2-period (4 wk each) crossover design. F2-isoprostane excretion was not altered after regular ingestion of tea (273 ± 48 pmol/mmol creatinine; Study 1), or by regular ingestion of black tea (324 ± 71 pmol/mmol creatinine) in comparison with hot water (263 ± 47 pmol/mmol creatinine; Study 1), or by regular ingestion of black tea (274 ± 39 pmol/mmol creatinine) in comparison with hot water (355 ± 75 pmol/mmol creatinine; Study 2). These results do not support the suggestion that polyphenolic antioxidants derived from tea inhibit in vivo lipid peroxidation. J. Nutr. 132: 55–58, 2002.

KEY WORDS: tea • antioxidant • oxidative stress • isoprostanes • lipid peroxidation • humans

Dietary antioxidants are likely to be important in maintaining oxidative balance and inhibiting lipid peroxidation (3). Intake of black tea has been inversely associated with risk of cardiovascular disease in prospective population studies (4–6). A potential mechanism for any beneficial effects of tea on cardiovascular disease involves inhibition of lipoprotein oxidation in vivo by polyphenols found in tea (7). Tea is a rich dietary source of polyphenols (8), which have potent antioxidant activity in vitro (9). Tea inhibits lipoprotein oxidation in vitro (10), and we showed in a controlled intervention that black tea can inhibit ex vivo lipid peroxidation acutely (7). To demonstrate that tea can inhibit oxidation in vivo, studies using markers representative of oxidative stress in vivo are needed.

F2-isoprostanes are currently believed to be one of the best available markers of in vivo lipid peroxidation (11). They are present at elevated concentrations in conditions of increased oxidative stress (11,12), such as hypertension (13) and hyperlipidemia (14). F2-isoprostanes are formed by the nonenzymatic free radical oxidation of arachidonic acid (12) and reflect oxidative damage to tissues and lipoproteins (11,12). The results of human intervention studies that have examined the effects of dietary-derived antioxidants on F2-isoprostanes have been inconsistent (15–27). The effects of regular ingestion of tea on F2-isoprostanes have not been examined previously.

Our objective was to determine whether regular ingestion of tea could inhibit in vivo lipid peroxidation. We report here the results of two controlled intervention studies that assessed the effects of regular ingestion of tea on urinary excretion of F2-isoprostanes.

SUBJECTS AND METHODS

Study 1: subjects. Thirteen healthy subjects, 10 men and 3 postmenopausal women not taking hormone replacement therapy were recruited from the general population in response to media advertisements. Subjects had high normal range systolic blood pressure or mild systolic hypertension (systolic blood pressure 130–150 mm Hg), and diastolic blood pressure <100 mm Hg. Exclusion criteria included the following: smokers and exsmokers of <6 mo; those taking medication or any dietary supplements; alcohol intake of >40g/d; and individuals with a history of major illness including diabetes, heart disease, liver disease, renal disease and/or gastrointestinal disorder (28). The Royal Perth Hospital Ethics Committee approved the study and all subjects gave informed written consent.

Study 1: experimental design. A 3-period randomized crossover study was performed. Green tea, black tea and hot water containing caffeine were consumed for 7 d each in random order. The caffeine concentration in hot water was matched to the green and black tea (50 mg/200 mL). A hot water without caffeine period was not included in this study because of possible carry-over effects associated with caffeine withdrawal. Subjects consumed 1000 mL/d of tea (as 5 cups containing 2 g tea leaves in 200 mL) or hot water containing caffeine in random order. Compliance was measured by using counts of tea bags and caffeine containers. Japanese “Sen-Cha” green tea, blended black tea (infused for 1 min with constant movement) and Japanese “Sen-Cha” green tea, blended black tea (infused for 1 min with constant movement) and...
pure caffeine dissolved in boiled water were consumed without additives, including milk or sugar. During the intervention, subjects ceased intake of caffeine-containing beverages except those assigned. Subjects did not drink tea in the morning before each blood sample to avoid the possibility of acute effects. Apart from these changes, dietary intake, usual level and pattern of alcohol intake, and physical activity levels were unchanged throughout the study. A spot urine sample was collected at the end of each of the intervention periods. Urine samples were frozen at −80°C until assayed.

**Study 2: subjects.** Twenty-two subjects, 16 men and 6 post-menopausal women not taking hormone replacement therapy were recruited from the general population in response to media advertisements. Subjects had total cholesterol ≥ 5.0 mmol/L and/or triacylglycerols ≥ 1.8 mmol/L. Exclusion criteria were the same as for Study 1 (29). The Royal Perth Hospital Ethics Committee approved the study and all subjects gave informed written consent.

**Study 2: experimental design.** A randomized-controlled crossover study with 4-wk intervention periods preceded by a 4-wk baseline period was performed. During baseline, all subjects were instructed to drink 1250 mL/d of hot water (as 5 cups containing 250 mL of hot water). Subjects were then randomized to receive 1250 mL/d of black tea (as 5 cups containing 2 g tea leaves in 250 mL of boiled water) or to continue with 1250 mL/d of hot water for 4 wk, then cross over to the alternate drink for a further 4 wk. Compliance was measured by using total weights of tea leaves used. The blended black tea was infused in 250 mL of boiled water for 1 min with constant movement, then consumed without additives, including milk and sugar. During the intervention, subjects ceased intake of caffeine-containing beverages except those assigned. Subjects did not drink tea in the morning before each blood sample to avoid the possibility of acute effects. Apart from these changes, dietary intake, usual level and pattern of alcohol intake, and physical activity levels were unchanged throughout the study. A 24-h urine sample was collected at the end of baseline and at the end of each of the intervention periods. Urine samples were frozen at −80°C until they were assayed.

**F2-isoprostanes.** F2-isoprostanes were analyzed in spot urine samples (Study 1) and 24-h urine samples (Study 2) by gas chromatography-mass spectrometry using negative chemical ionization. Creatinine-corrected isoprostane concentrations in spot urine samples have been previously shown to be highly correlated with creatinine-corrected concentrations in 12-h urine samples (30). Therefore, creatinine-corrected isoprostane concentrations in spot urine samples and 24-h urine samples are likely to provide a similar indication of isoprostane excretion. The method for measurement of F2-isoprostanes has been previously described in detail (18,31). Briefly, 8-isoprostaglandin (PG)F2α,d4 (5 ng, internal standard) was added to 2 mL of urine and extracted using solid-phase cartridges. After HPLC separation, samples were derivatized, then analyzed on a Hewlett-Packard 6890 Series gas chromatograph coupled to an Agilent 5973 Mass Spectrometer (Agilent, Palo Alto, CA), Chromatography used an HP-5MS column (30 m × 0.25 mm, 0.25 μm, Hewlett-Packard) with helium as the carrier gas. Negative ion chemical ionization used methane as the reagent gas. The mass spectrometer was operated in the selective ion monitoring mode and F2-isoprostanes were detected by monitoring m/z 569 and m/z 573 for 8-isoprostaglandin (PG)F2α,d4. The intra-assay CV was 8%.

**Creatinine and 4-O-methylgallic acid.** Urinary creatinine concentrations were measured using a modified Jaffe reaction on a Hitachi 917 automated analyzer (Boehringer Mannheim, Mannheim, Germany). Urinary 4-O-methylgallic acid was measured in the 24-h urine samples by gas chromatography-mass spectrometry according to procedures described previously (7). This compound, a methyl ether derivative of gallic acid, is used as a marker of black tea intake (32).

**Statistics.** Statistical analyses were performed using SPSS software (Chicago, IL). Results are presented as means ± SEM and P < 0.05 was the level of significant difference. The paired-samples t test was used to determine the effects of tea. With 13 subjects in Study 1, there was >80% power to detect a 25% difference in urinary F2-isoprostane excretion. With 22 subjects in the Study 2, there was >80% power to detect a 15% difference in urinary F2-isoprostane excretion.

**RESULTS**

**Study 1: Effects of regular ingestion of green and black tea for 7 d on F2-isoprostanes.** Subjects were between 25 and 72 y old (mean 59.8 ± 2.6 y), and had a body mass index between 20.7 and 32.5 kg/m² (mean 27.0 ± 1.1 kg/m²). Subjects recruited to this study had high normal range systolic blood pressure or mild systolic hypertension. At baseline, mean 24-h ambulatory systolic and diastolic blood pressures were 136.6 ± 0.9 and 76.2 ± 0.7 mm Hg, respectively. Mean urinary F2-isoprostane concentration at baseline was 239 ± 26 pmol/mmol creatinine. The F2-isoprostane concentrations at the end of each 7-d intervention period were as follows: 263 ± 47 pmol/mmol creatinine (hot water), 273 ± 48 pmol/mmol creatinine (green tea) and 274 ± 39 pmol/mmol creatinine (black tea). Relative to hot water, there was no significant effect of green tea or black tea on urinary F2-isoprostane excretion (Fig. 1).

**Study 2: Effects of regular ingestion of black tea for 4 wk on F2-isoprostanes.** Subjects were between 43 and 75 y old (mean 59.1 ± 1.6 y) and had a body mass index between 23.5 and 35.7 kg/m² (mean 27.0 ± 0.6 kg/m²). Subjects recruited had mildly elevated serum total cholesterol or triacylglycerol concentrations. At baseline, mean total serum cholesterol and triacylglycerol concentrations were 5.8 ± 0.2 and 1.1 ± 0.1 mmol/L, respectively. Mean urinary 4-O-methylgallic acid concentrations at baseline were 28 ± 4 nmol/mmol creatinine, and mean urinary F2-isoprostane concentrations at baseline were 265 ± 21 pmol/mmol creatinine. In comparison with hot water, regular ingestion of black tea resulted in a significant increase in urinary excretion of 4-O-methylgallic acid [39 ± 9 (hot water) vs. 547 ± 65 (black tea) nmol/mmol creatinine; P < 0.001]. The F2-isoprostane concentrations at the end of each 4-wk intervention period as follows: 355 ± 75 pmol/mmol creatinine (hot water) and 334 ± 71 pmol/mmol creatinine (black tea). F2-isoprostane concentrations were not altered by black tea, relative to hot water (Fig. 2).

**DISCUSSION**

Promoting the enzymatic oxidation of polyphenols present in the tea leaf produces black teas, whereas these enzymes are inactivated during green tea production (8). Differences between green tea and black tea in polyphenol composition have been suggested to influence antioxidant effects (33). However, we showed that the effects of green tea and black tea on lipoprotein oxidation in vitro (10) and ex vivo (7) were not...
different. We also showed that black tea can inhibit ex vivo lipoprotein oxidation acutely (7), which demonstrates the potential of tea to inhibit oxidation.

In Study 1, we assessed the effects of regular ingestion of green and black tea over 7 d on urinary excretion of F_{2}-isoprostanes in 13 subjects with raised blood pressure. Green tea and black tea in comparison with hot water containing caffeine did not alter F_{2}-isoprostanes. These results suggest that short-term regular ingestion of tea does not influence lipid peroxidation, and that there is no difference in effects between green and black tea, in this population.

In Study 2, we assessed the effects of regular ingestion of black tea over 4 wk on urinary excretion of F_{2}-isoprostanes in 22 subjects with mildly elevated serum total cholesterol concentrations. In this study, we examined the effects of black tea only. Results of our previous studies suggest no substantial difference in effects of green and black tea on lipid peroxidation in vitro and ex vivo (7,10), and results of prospective studies are suggestive of benefits of black tea on cardiovascular risk (4–6). Urinary excretion of 4-O-methylgallic acid was measured as a marker of intake of polyphenols derived from black tea. 4-O-Methylgallic acid concentrations were significantly increased during regular ingestion of black tea, but F_{2}-isoprostanes were not changed. These results are consistent with those of Study 1, and suggest that regular ingestion of tea does not inhibit lipid peroxidation in this population.

Several studies have shown improvement in isoprostanes with dietary interventions in humans (15,16,22–24,26,27,34–36). These improvements have been between ~10 and 30%, which is similar to the difference that our studies were powered to detect. In uncontrolled studies, decreases in isoprostane concentrations have been observed with olive oil phenols in subjects with mild elevations in serum cholesterol and/or triacylglycerol concentrations (18,20). Isoprostanes have been measured in plasma of dietary antioxidants on isoprostanes. In the present study, we used a gas chromatography-mass spectrometry method to measure F_{2}-isoprostanes, predominantly 8-epi-PGF_{2α} (31). Other methods have included gas chromatography-mass spectrometry to measure specific F_{2}-isoprostane isomers and enzyme-linked immunoassay to measure 8-epi-PGF_{2α}. Previous comparisons of an enzyme-linked immunoassay method and a gas chromatography-mass spectrometry method for the measurement of F_{2}-isoprostanes have demonstrated proportional bias, with the enzyme-linked immunoassay method overestimating lower concentrations and underestimating higher concentrations of F_{2}-isoprostanes relative to gas chromatography-mass spectrometry (37).

In conclusion, regular ingestion of tea did not alter urinary excretion of F_{2}-isoprostanes in subjects with mildly elevated blood pressure or serum cholesterol concentrations. The use of different methods to measure isoprostanes, as well as differences in study designs and populations studied, may account in part for inconsistent effects of dietary antioxidants on isoprostanes. In the present study, we used a gas chromatography-mass spectrometry method to measure F_{2}-isoprostanes, predominantly 8-epi-PGF_{2α} (31). Other methods have included gas chromatography-mass spectrometry to measure specific F_{2}-isoprostane isomers and enzyme-linked immunoassay to measure 8-epi-PGF_{2α}. Previous comparisons of an enzyme-linked immunoassay method and a gas chromatography-mass spectrometry method for the measurement of F_{2}-isoprostanes have demonstrated proportional bias, with the enzyme-linked immunoassay method overestimating lower concentrations and underestimating higher concentrations of F_{2}-isoprostanes relative to gas chromatography-mass spectrometry (37).

In addition, isoprostanes have been measured in plasma (15,19,27,33) and urine (15–18,20–24,26). Although plasma and urinary isoprostanes are both markers of in vivo lipid peroxidation, each represents a different pool. Plasma isoprostanes are predominantly phospholipid bound, whereas urinary isoprostanes are nonesterified and may represent both systemic and renal production and renal excretion. Which pool best reflects lipid peroxidation in the arterial wall is not known. Another possible reason for the inconsistent effects of dietary antioxidants on isoprostanes is that elevated oxidative stress may be required to detect improvements in oxidative stress (20). However, hypertension (38) and dyslipidemia (14) are thought to be conditions of increased oxidative stress. The subjects involved in the present studies had mild-to-moderate elevations in blood pressure or serum cholesterol concentrations and isoprostane excretion similar to that of several groups we studied previously (15,18,25,36). In smokers (15), hyperlipidemics (36) and diabetics (35), we found improvements in isoprostanes with dietary interventions, and in a group with mild elevations in blood pressure (18), we found that isoprostanes were not altered. Therefore, although falls in isoprostanes may be more likely in subjects with elevated oxidative stress, decreases have been observed in healthy populations (26,27) and no benefit found in populations likely to have raised oxidative stress (19,21).

In conclusion, regular ingestion of tea did not alter urinary excretion of F_{2}-isoprostanes in subjects with mildly elevated blood pressure or serum cholesterol concentrations. These results are not consistent with the suggestion that polyphenolic antioxidants derived from tea inhibit in vivo lipid peroxidation.

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LITERATURE CITED
