RESEARCH ARTICLE

Bolus ingestion of white and green tea increases the concentration of several flavan-3-ols in plasma, but does not affect markers of oxidative stress in healthy non-smokers

Nadine Müller[1], Sabine Ellinger[1]*, Birgit Alteheld[1], Gudrun Ulrich-Merzenich[2], Heiner K. Berthold[3], Hans Vetter[2] and Peter Stehle[1]*

[1] Department of Nutrition and Food Science – Nutritional Physiology, University of Bonn, Bonn, Germany
[2] Medical Policlinic, University Hospital Bonn, Bonn, Germany
[3] Lipid Clinic at the Interdisciplinary Metabolism Center, Virchow Clinic Campus, Charité University Medicine Berlin, Berlin, Germany

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White tea (WT) is rich in flavan-3-ols as green tea (GT) and might provide health protective effects due to the strong antioxidant properties of flavan-3-ols. Since intervention studies with WT are lacking, we evaluated the effects of WT consumption on antioxidant status, antioxidant capacity and biomarkers of oxidative stress compared to water and GT. After an overnight fast, 70 healthy non-smokers were randomized to consume 600 mL of WT, GT or water (control). Plasma (epi-)catechin and epi(gallo)catechingallate, antioxidant capacity (Folin assay, trolox equivalent antioxidant capacity test), 8-iso-prostaglandin $F_{2\alpha}$, ascorbic acid and uric acid were determined before and several times within 8 h after consumption. DNA strand breaks were measured *in vivo* and *ex vivo* (H_2O_2 stimulation) in leukocytes. Plasma flavan-3-ols significantly increased after WT and GT ingestion. Trolox equivalent antioxidant capacity was lower after 5 h in controls *versus* WT ($p = 0.031$) and GT ($p = 0.005$). Folin-Ciocalteu reducing capacity, ascorbic and uric acid as well as markers of oxidative stress (8-iso-prostaglandin-$F_{2\alpha}$, DNA strand breaks) were not affected by the beverages. A short-term increase of catechins does not change plasma antioxidant capacity in healthy subjects. Conclusions with respect to health protective effects of WT and GT on the basis of these biomarkers can, thus, not be drawn.

Keywords:
Antioxidant capacity / Bolus ingestion / Green tea / Oxidative stress / White tea

1 Introduction

Epidemiological studies suggest that frequent green tea (GT) consumption lowers the risk of cardiovascular diseases (CVD) [1]. Since oxidative stress is a known risk factor of CVD [2], protective effects of GT may be explained by the strong antioxidant properties of tea catechins (flavan-3-ols) [1]. This hypothesis is supported by several controlled [3–7] and uncontrolled [8] intervention studies, which show that bolus ingestion of GT (300–450 mL) increased plasma antioxidant capacity after 30–120 min, probably due to increased total phenol levels in plasma [7]. The plasma kinetics of individual flavan-3-ols was, however, not investigated in most of these studies [3–7, 9]. Only two studies showed no effects on plasma antioxidant capacity [9, 10].

Correspondence: Dr. Sabine Ellinger, Department of Nutrition and Food Science – Nutritional Physiology, University of Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany
E-mail: ellinger@uni-bonn.de
Fax: +49-228-73-3217

Abbreviations: 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; **C,** catechin; **CVD,** cardiovascular diseases; **EC,** epicatechin; **ECG,** epicatechingallate; **EGCG,** epigallocatechingallate; **FCR,** Folin-Ciocalteu reducing capacity; **GT,** green tea; **SB,** DNA single strand breaks; **TEAC,** trolox equivalent antioxidant capacity; **WT,** white tea

*These authors have contributed equally to this study and could be considered as co-first authors.

White tea (WT) is produced from very young tea leaves or buds of *Camellia sinensis*, which are steamed immediately after harvest to inactivate polyphenol oxidases. Therefore, WT is claimed to provide more flavan-3-ols than GT [11, 12]. Results from studies on flavan-3-ol content are, however, inconsistent. In a German study analyzing 30 different samples of GT and WT, total flavan-3-ol content was on average 12% higher in WT than in GT [12]. In contrast, two studies found up to 2.5 times higher flavan-3-ol concentrations in GT compared to WT [13, 14]. Stronger *in vitro* antimutagenic and cancer-preventive properties for WT extract compared to GT extract [15, 16] strongly support the hypothesis that WT consumption might be even more effective in the prevention of CVD than GT. An increased flavan-3-ol uptake and/or higher plasma concentrations of biomarkers for the antioxidant activity would support this assumption. However, the antioxidant effects of WT consumption have not been investigated so far.

Thus, the aim of this study was to compare the effects of the consumption of equal amounts of WT and GT on flavan-3-ol concentrations, antioxidant status and antioxidant capacity in plasma as well as on biomarkers of oxidative stress (lipid peroxidation, DNA damage) in healthy non-smokers.

2 Materials and methods

2.1 Subjects and study design

For this randomized, controlled, three-arm parallel group intervention study, volunteers were recruited through announcements at the University of Bonn. Inclusion criteria were age between 18 and 65 years, non-smoking and normal weight (BMI 18.5–24.9 kg/m^2). Exclusion criteria were pregnancy, breast feeding, supplementation of vitamins or polyphenols, known gastrointestinal disorders or known metabolic diseases as well as the intake of drugs known to interfere with intestinal absorption. Since the level of physical activity may affect antioxidant status [17] and/or oxidative stress [18], subjects were asked to provide information (questionnaire) on hours of physical activity regularly performed *per* wk.

Participants were stratified by sex and randomly assigned to consume 600 mL WT, GT or water (control) within 15 min after an overnight fast. Blood was collected before and 1, 2, 3, 5 and 8 h after consumption of the study drink. This period was chosen because flavanols [19, 20] as well as their metabolites with di-/trihydroxyphenyl groups [20, 21] were still detectable in plasma 6 and 8 h after GT consumption in bioavailability studies. Thus, antioxidant effects may occur later on as observed by Arendt *et al.* [22] where leukocytes' resistance of DNA against H_2O_2-induced strand breaks increased 6 h after bolus consumption of red wine.

The subjects were instructed to abstain from foods rich in polyphenols 24 h before and until completion of the study to avoid artifacts by flavonoid intake from other sources. Water was allowed to be drunk *ad libitum* after the 2-h blood withdrawal. After the 3-h blood withdrawal, subjects received a standardized breakfast (two rolls with butter and cheese) and after the 5-h blood withdrawal, lunch (two rolls with butter and ham or cheese). The compliance with dietary restrictions at the day before was documented by a self-completed standardized 1-day dietary record.

All participants gave their written informed consent prior to the study. The study was approved by the Ethical Committee of the University of Bonn (No. 031/05) and conducted according to the Declaration of Helsinki.

2.2 Tea preparation

Tea leaves ''Japanese Sencha'' and ''Fancy White Peony'' (both from TeeGschwendner, Meckenheim, Germany) were used for the preparation of the GT and WT, respectively. Eleven grams of leaves were brewed for 5 min with 600 mL boiling water. The concentration of catechin (C), epicatechin (EC), epicatechingallate (ECG), epigallocatechin-3-gallate (EGCG), ascorbic acid as well as the Folin-Ciocalteu reducing capacity (FCR) of both beverages was determined in duplicate (see below). The intake of flavan-3-ols and ascorbic acid by consumption of 600 mL WT or GT as well as the FCR is summarized in Table 1.

2.3 Sample collection and treatment

Blood samples were collected in EDTA-coated vacutainers (Becton Dickinson, Heidelberg, Germany) (analysis of flavan-3-ols, FCR) and in heparinized tubes (trolox equivalent antioxidant capacity, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), ascorbic acid, uric acid). All samples were placed on ice immediately and exposure to light was avoided. Before centrifugation at $3000 \times g$ for 15 min at 4°C, 100 µL of heparinized blood was removed for the Comet Assay. For the determination of ascorbic acid, 500 µL of a cold 6% perchloric acid/2% metaphosphoric acid solution (v/v) was added to 500 µL fresh plasma to stabilize the plasma samples. After centrifugation at $3000 \times g$ for 10 min at 4°C, the supernatant and the remaining plasma were aliquoted and stored at −80°C until analysis.

2.4 Dietary intake of energy and nutrients

The intake of energy, macronutrients, dietary fibers and antioxidant pro-/vitamins on the day before the study was calculated using Ebis Pro 4.0 software based on BLS II.3. The flavonoid intake was estimated by using the USDA databases http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.pdf [23].

Table 1. Antioxidants and Folin-Ciocalteu reducing capacity of white tea and green tea *per* liter and *per* single dose (600 mL) ingested

	White tea		Green tea	
	Amount *per* liter	Amount ingested	Amount *per* liter	Amount ingested
Flavan-3-ols[a)]				
Catechin (mg)	1.5	0.9	1.7	1.0
Epicatechin (mg)	8.1	4.8	25.5	15.3
Epicatechingallate (mg)	4.9	2.9	11.1	6.7
Epigallocatechingallate (mg)	21.0	12.6	92.3	55.4
Folin-Ciocalteu reducing capacity (mg CE)[b)]	226	136	395	237
Ascorbic acid (mg)[c)]	0.7	0.4	14.8	8.9

All investigations were done in duplicate. Data are mean values. CE, catechin equivalents.
a) Flavan-3-ols were analyzed by reversed phase HPLC with electrochemical detection.
b) The Folin-Ciocalteu reducing capacity was investigated by the Folin assay.
c) Ascorbic acid was determined by HPLC with UV/Vis detection.

2.5 Laboratory analysis

2.5.1 Flavan-3-ols

C, EC, ECG and EGCG were determined in tea samples and in EDTA plasma by HPLC. In plasma, total flavan-3-ols were expressed as sum of unconjugated flavan-3-ols and glucuronidated and sulfated metabolites.

Conjugated metabolites in plasma samples were hydrolyzed by incubation of 0.5 mL plasma for 45 min under nitrogen atmosphere with a purified enzyme preparation of β-glucuronidase (1000 U) and sulfatase ($\geq$3 U, determined according to the manufacturer's protocol, Sigma-Aldrich, Munich, Germany). Afterwards, the samples were prepared according to Kivits *et al.* [24] and stored at −80°C until analysis.

For HPLC separation, a C18 reversed-phased column (Hypersil ODS; 250 × 4 mm, 3 µm, Thermo Electron, Dreieich, Germany) (30°C) was used. The flow rate was 0.6 mL/min for the first 25 min and 0.75 mL/min until the end of the run. The eluent was monitored by an electrochemical detector (signal-to-noise: 3:1) against Ag/AgCl at 0.6 V. The detection limit for C and EC was 2.5 nmol/L and for ECG and EGCG 5.0 nmol/L. Flavan-3-ol concentrations below detection limit were set at 2.5 nmol/L (C, EC) and 5.0 nmol/L (ECG, EGCG), respectively, for data evaluation. The CV (repeated analysis of standard solutions) was <2.5% for all flavan-3-ols.

2.5.2 FCR

In tea and plasma, the FCR (CV 2.0%) (formerly incorrectly called "total phenol content") was measured by the Folin-Ciocalteu Assay [25] with some modifications [22] to exclude interferences with plasma proteins.

2.5.3 TEAC

The antioxidant capacity of plasma was measured according to Miller *et al.* [26] as TEAC (CV 1.2%).

2.5.4 Uric acid and ascorbic acid

Plasma concentrations of uric acid were determined photometrically with a kit (Diaglobal, Berlin, Germany) based on the uricase-PAP-method (CV 2.0%). Ascorbic acid in tea and plasma samples was measured by HPLC-UV/Vis at 243 nm (CV 1.8%) according to Steffan [27].

2.5.5 Lipid peroxidation

Total 8-iso-$PGF_{2\alpha}$ concentration in plasma was determined by a competitive ELISA test kit (Assay Designs, Ann Arbor, Michigan, USA) (CV according to manufacturer 5.7%).

2.5.6 DNA strand breaks

DNA single-strand breaks (SB) were measured in leukocytes *in vivo* and after 20 min incubation at 4°C with 300 µM H_2O_2 *ex vivo* using the single cell gel electrophoresis assay (also called Comet Assay) developed by Ostling and Johanson [28] and modified by Singh *et al.* [29] for the detection of SB. The procedure was done according to Arendt *et al.* [22]. Fifty nuclei *per* slide were evaluated and tail moments were calculated using the Comet Assay III software (Perceptive Instruments, Suffolk, UK). Results from untreated cells (SB *in vivo*) were subtracted from those of treated cells to calculate the DNA damage induced by H_2O_2 challenge (SB *ex vivo*) (CV 22%; [30]).

2.6 Calculation of sample size

Data on FCR in plasma (AUC) determined by the Folin assay in plasma samples from a pilot study obtained after consumption of GT *versus* time compared to the control group (water) were used for the calculation of the sample size. Considering a power of 70 and a standard deviation of 135, a difference of 110 (arbitrary units) between the AUCs could be detected with a sample size of 20 subjects *per* group. To account for drop-outs, 70 participants were included in the study.

2.7 Statistics

Nominal variables between the groups were compared with Chi-square test. Metric data were checked for normal distribution and data were log-transformed if necessary. ANOVA or Kruskal–Wallis test was used for comparisons at baseline between the groups.

The effect of time and beverage on plasma antioxidant status, antioxidant capacity, and markers of oxidative stress separately and in combination were investigated with the repeated measures analysis of variance. In case of significant differences, *t*-test was performed subsequently.

Flavan-3-ols concentrations were not normally distributed. Therefore, Mann–Whitney *U*-test was used for the comparison of baseline values between the groups. Wilcoxon signed rank test was performed to analyze changes within each group.

Results are shown as mean and standard deviation or as median and interquartile range. All tests were performed with SPSS 17.0 (SPSS, Chicago, IL, USA).

3 Results

Seventy participants were enrolled in this study. Two subjects dropped out due to vomiting and/or circulatory problems. Thus, 68 volunteers completed the study *per* protocol and their data were included in the statistical evaluation.

Age, BMI and physical activity (Table 2) as well as the intake of energy, macronutrients and antioxidant pro-/vitamins at the day before the study were not different between the groups (mean $\pm$ SD for all subjects: energy: 1717 $\pm$ 699 kcal, protein: 79 $\pm$ 39 g, fat: 78 $\pm$ 41 g; β-carotene: 0.2 $\pm$ 0.2 g; ascorbic acid: 11 $\pm$ 16 g; tocopherol equivalents: 4.4 $\pm$ 3.2 g). Flavonoid-rich foods were not ingested by any participant, indicating 100% compliance with dietary restrictions.

C content of WT and GT were similar, whereas WT provided only 2–4 times lower amounts of ECG, EC and EGCG than GT (Table 1).

C, EC, ECG and EGCG were not detectable in subjects of the control group consuming water. Thus, statistical analysis of flavan-3-ols concentration in plasma was limited to the tea groups, without differences between the WT and GT group at baseline.

At baseline, C was only detectable in four subjects of the WT group (median: 18 nmol/L) and in four subjects of the GT group (median: 20 nmol/L). After consumption of WT and GT, median plasma levels of 19–24 and 16–22 nmol/L were reached, respectively (Figs. 1A and B). The question if the concentrations after tea consumption were higher than baseline remains open as the Wilcoxon signed rank test could be done with the small sample size. EC did not increase after consumption of WT (Fig. 1C), but was higher 1, 2, 3 and 8 h after consumption of GT (Fig. 1D) ($p \leq 0.05$). An increase of ECG and EGCG *versus* baseline occurred after consumption of WT and GT ($p \leq 0.05$), which was still significant after 5 h in group GT ($p = 0.05$ for ECG, $p = 0.008$ for EGCG) (Figs. 1E–H).

At baseline, FCR, TEAC, uric acid, ascorbic acid, 8-iso-$PGF_{2\alpha}$ and SB *in vivo* and *ex vivo* were not different between the groups (Table 3). The beverage *per se* did not affect any of these parameters, whereas effects by time on ascorbic acid ($p = 0.004$), uric acid ($p < 0.001$), 8-iso-$PGF_{2\alpha}$ ($p = 0.049$) and SB *in vivo* ($p < 0.001$) could be observed (Table 3). Interactions between beverage and time had only an impact on TEAC ($p = 0.007$) (Table 3). Even though *t*-test is not the test of choice because individual changes in TEAC depend on baseline level (results of regression analysis, data not shown), differences after 5 h could be observed after

Table 2. Characteristics of the subjects

	White tea ($n = 23$)	Green tea ($n = 22$)	Water ($n = 23$)
Sex, females/males[a)]	15/8	16/6	16/7
Age (years)[b)]	25 $\pm$ 5	24 $\pm$ 5	24 $\pm$ 4
Weight (kg)[b)]	67 $\pm$ 13	64 $\pm$ 9	67 $\pm$ 11
Height (m)[c)]	1.73 $\pm$ 0.09	1.73 $\pm$ 0.08	1.73 $\pm$ 0.12
BMI (kg/m^2)[c)]	22.1 $\pm$ 2.5	21.4 $\pm$ 2.1	22.4 $\pm$ 2.0
Physical activity (h/wk)[c)]	3.2 $\pm$ 2.7	3.8 $\pm$ 4.5	4.2 $\pm$ 3.9

a) No differences between groups according to Chi-square test.
b) Data: mean $\pm$ SD. No differences between groups according to Kruskal–Wallis test.
c) Data: mean $\pm$ SD. No differences between groups according to ANOVA.

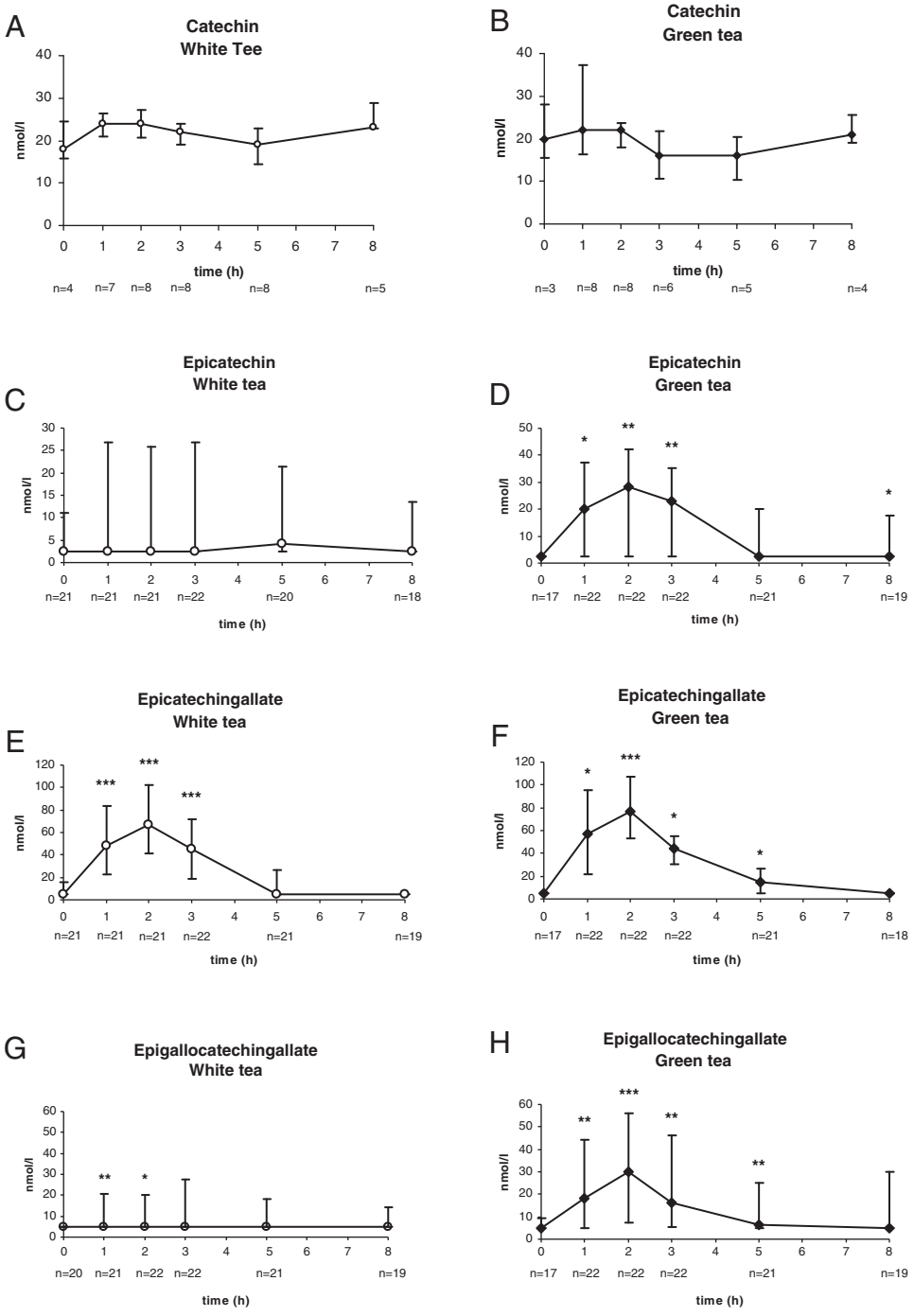

Figure 1. Plasma concentration of total catechin (A, B), epicatechin (C, D), epicatechingallate (E, F) and epigallocatechin-3-gallate (G, H) *versus* time profiles after bolus ingestion of white tea (open circles) or green tea (filled rhombi). Data: medians (interquartile range). Asterisks indicate significant differences *versus* baseline within each group: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (Wilcoxon signed rank test).

consumption of WT and water ($p = 0.031$) as well as between GT and water ($p = 0.005$) (Table 3).

4 Discussion

To the best of our knowledge, this is the first human intervention study that investigated the antioxidant effects of WT in comparison with equal volumes of GT. The groups were comparable with regard to sex, age, weight, height, BMI and physical activity (Table 2). Energy and nutrient intake at the day before the study were similar between the groups. In line with the dietary restrictions, the mean intake of micronutrients present in fruit and vegetables were lower in our participants than in the 19- to 25-year-old general population in Germany [31], *e.g.*, β-carotene (0.2 *versus* 2.4 mg), ascorbic acid (11 *versus* 111 mg), vitamin E (4.1 *versus* 14.0 mg TE) and dietary fibers (8 *versus* 22 mg). Even if

Table 3. Impact of beverage, time and beverage × time on antioxidant status, antioxidant capacity and oxidative stress markers

	White tea ($n = 23$)			Green tea ($n = 22$)			Water ($n = 23$)			Rm ANOVA[a)]		
	0 h	2 h	5 h	0 h	2 h	5 h	0 h	2 h	5 h	Drink	Time	Drink × time
FCR (mg CE/L)	22.5 ± 3.5	22.9 ± 3.6	22.4 ± 3.8	22.5 ± 3.0	22.9 ± 2.9	22.7 ± 2.9	22.2 ± 3.4	22.2 ± 2.4	21.8 ± 3.1	ns	ns	ns
TEAC (mmol TE/L)	1.67 ± 0.07	1.70 ± 0.06	1.68 ± 0.07[a]	1.67 ± 0.09	1.67 ± 0.08	1.69 ± 0.10[b]	1.69 ± 0.07	1.70 ± 0.12	1.64 ± 0.05[ab]	ns	ns	**
Uric acid (μmol/L)	191 ± 67	186 ± 60	193 ± 63	180 ± 55	176 ± 58	196 ± 61	220 ± 54	214 ± 53	216 ± 40	ns	***	ns
Ascorbic acid (μmol/L)	54.3 ± 13.4	55.8 ± 16.0	54.6 ± 14.5	58.1 ± 15.5	63.3 ± 16.3	58.0 ± 18.8	57.7 ± 15.3	62.0 ± 18.0	58.4 ± 16.2	ns	**	ns
8-Iso-$PGF_{2\alpha}$ (ng/mL)	9.1 ± 3.6	9.3 ± 3.8	9.4 ± 3.5	8.8 ± 3.1	9.0 ± 3.4	9.3 ± 2.9	8.5 ± 3.0	9.3 ± 4.1	8.3 ± 2.6	ns	*	ns
SB *in vivo* (TM)	2.05 ± 1.33	3.65 ± 1.53	1.85 ± 1.36	2.67 ± 1.41	4.70 ± 2.02	2.17 ± 1.72	2.59 ± 1.51	3.84 ± 1.88	1.46 ± 1.36	ns	***	ns
SB *ex vivo* (TM)	0.75 ± 1.55	0.58 ± 1.65	0.58 ± 1.41	0.78 ± 1.43	0.34 ± 1.09	0.83 ± 1.21	1.09 ± 1.41	0.56 ± 1.98	0.50 ± 1.36	ns	ns	ns

CE: catechin equivalents; FCR: Folin-Ciocalteu reducing capacity; 8-Iso-$PGF_{2\alpha}$: 8-iso-prostaglandin $F_{2\alpha}$; ns: not significant; rm ANOVA: repeated measures ANOVA; SB: DNA strand breaks; TE: trolox equivalents; TEAC: trolox equivalent antioxidant capacity; TM: tail moment.
Data: mean ± SD. Identical letters indicate significant differences between the groups (*t*-test): [a] $p \leq 0.05$, [b] $p \leq 0.01$.
a) Asterisks indicate significant effects of repeated measures ANOVA: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

underreporting cannot be excluded, this observation confirms the excellent compliance with dietary restrictions before the study. This interpretation is supported by the fact that at baseline, most flavan-3-ols were not detectable in plasma or could only be found in traces (Fig. 1). Thus, any short-term antioxidant effects observed can be explained by tea consumption.

According to our knowledge, our study is the first investigating plasma concentrations of various individual flavan-3-ols (C, EC, ECG, EGCG) in addition to parameters of antioxidant status, antioxidant capacity and oxidative stress after bolus consumption of GT or WT. In general, flavan-3-ols concentrations in plasma reflect the dietary intake.

In contrast to the assumptions of Song and Chun [11], WT provided about 2–4 times lower amounts of ECG, EC and EGCG (Table 1), which corresponds with recent results from other investigations [13, 14]. This observation might be explained by slower flavanol extraction from WT as the lipophilic cuticle of leaves or buds could impair migration kinetic of hydrophilic flavan-3-ols [14].

It is not surprising that C was only detectable before and after tea consumption in a few individuals (Figs. 1A and B). C-rich foods were not consumed due to the dietary restrictions and C was only a minor component of GT and WT (Table 1). EC increased in plasma after ingestion of GT (Fig. 1D) as previously shown in pharmacokinetic studies [19, 20, 32, 33]. This was not the case after ingestion of WT (Fig. 1C), probably due to the 70% lower intake (Table 1). Despite the low ECG content in WT and GT (Table 1), a transient increase in plasma occurred in both groups (Figs. 1E and F). In pharmacokinetic studies, ECG was shown to be bioavailable from GT, but with higher doses of ECG ingested compared to our study (22 mg [19], 33/66/99 mg [32], 154 mg [20], 62 mg [33] *versus* 6.7 mg). EGCG was bioavailable from both teas (Figs. 1G and H) as already shown in pharmacokinetic studies where slightly higher doses were ingested with GT (105 mg [19], 110/219/329 mg [32], 195 mg [20], 161 mg [33] *versus* 55 mg).

Although single flavan-3-ols increased, this uptake of tea flavan-3-ols did not affect the FCR (Table 3). This is not surprising as the Folin-Ciocalteu method measures the FCR of an antioxidant by electron transfer of phenolic and non-phenolic compounds (*e.g.*, ascorbic acid, aromatic amino acids), thus lacking the specificity for phenolic compounds [34, 35]. It is not a marker for total phenolic contents [34, 35]. Obviously, the sum of flavan-3-ols ingested is negligible compared to the high concentrations of other reducing compounds in plasma. In addition, glucuronidated flavan-3-ols, which account to the main metabolites in plasma after ingestion of GT [19], cannot form stable phenoxyl radicals and thus cannot be detected using this method [36].

GT and WT consumption *per se* did not affect the plasma antioxidant capacity measured by TEAC (Table 3). This is in contrast to most intervention studies with either controlled [3–7] or uncontrolled design [37] using GT prepared with

comparable doses of tea leaves (0.7 [7], 1.6 [37], 2.0 [3–5], 5.0 g/100 mL [6] *versus* 1.8 g/100 mL). Even consuming lower volumes of GT (300–450 mL) than in our study increased the plasma antioxidant capacity within 2 h [3–7, 38], irrespective of the analytical method applied (FRAP [6, 7], TEAC [37] or TRAP [3–5]). Unfortunately, only in the study of Leenen *et al.* [7], data on the flavan-3-ol content and composition are given. Since the flavanol content of tea leaves varies largely [39], it can be assumed that the ''true'' flavanol intake varied widely in the different studies. The interaction ''time × beverage'' was significant for TEAC (Table 3), indicating that the pattern of changes of TEAC over time differed under the influence of different beverages. This suggests a decrease of TEAC after consumption of water probably due to the lack of intake of nutritive antioxidants. Effects from the flavonoid-free diet on the day before the study on TEAC cannot be excluded as TEAC was shown to decline in another study 24 h after a diet low in flavonoids was given [40]. However, information on diet is lacking in most studies [3–6], which makes an evaluation of the role and/or extent of dietary restrictions for TEAC in our study difficult. Moreover, the decline of TEAC in our control group may also result from postprandial oxidative stress [41, 42] induced by the breakfast. Polyphenol-rich foods like WT and GT may compensate for these changes, as similar findings have been observed after the consumption of juices and fruit [41].

The known diurnal rhythm of uric acid in plasma [43–45] may explain the changes of plasma uric acid over the 5-h study period (Table 3). As uric acid accounts for 19% of the TEAC in plasma [46], diurnal variations and the postprandial consumption of uric acid [41, 47, 48] might have contributed to the decline of TEAC in our controls.

Plasma concentrations of ascorbic acid were not affected in our study (Table 3) probably due to the relatively low intake (Table 1). Similar results were reported after bolus ingestion of GT and GT extract, respectively [7, 49]. Since circadian rhythms exist for plasma concentrations of ascorbic acid, reaching maximal concentrations around noon [50], the effect of time on the plasma ascorbic acid level in our study may reflect circadian fluctuations which become evident within 6 h of investigation, but not within 2 h in the studies mentioned above [7, 49].

The beverages did not affect the plasma concentration of 8-iso-$PGF_{2\alpha}$ (Table 3). This is in line with the results of previous studies where effects of 400–450 mL GT on lipid peroxides [9], malondialdehyde [51] and on LDL oxidation *ex vivo* [10] did not occur. It could be speculated that the majority of plasma flavan-3-ols was glucuronidated or sulfated. These metabolites exhibit hydrophilic properties and are, thus, not incorporated into the LDL *in vivo* [52]. Consequently, the 8-iso-$PGF_{2\alpha}$ concentration remains unchanged. Whether there is an impact of circadian rhythms [45, 53] and postprandial changes [42, 54] on lipid peroxidation is still unclear.

SB *in vivo* were only affected by time (Table 3). Previous studies exhibited circadian rhythms for the urinary excretion of 8-OHdG (min 6 am, max 6 pm) [45], an accepted biomarker for the total rate of the organisms' DNA damage. Similarly, circadian rhythms for SB *in vivo* – an indicator for the balance between development and repair of DNA damage – cannot be ruled out and might explain the time effect observed.

SB in leukocytes challenged by H_2O_2 *ex vivo* were not affected by time and beverage (Table 3). Perhaps, bolus consumption of GT and WT did not modulate the cellular redox state and thus did not affect SB *ex vivo*.

Negative values calculated for SB *ex vivo* (Table 3) are not plausible. They may be explained by either the different sensitivity of leukocyte subsets (polymorphonuclear *versus* mononuclear cells) against H_2O_2 challenge (25–1000 μM, 5 min, 4°C) *ex vivo*, and/or the relatively low increase of SB in leukocytes from whole blood compared to basal damage, which has shown to be much higher if isolated cells were used instead of non-isolated cells [55].

The study has some limitations: the concentration of selected biomarkers shows high variability. This may be explained by individual differences in flavanols' bioavailability, a well-known phenomenon [32, 56, 57] that may be explained by nutrigenetic effects (*e.g.* polymorphisms in genes decoding transport proteins) as recently verified for carotenoids [58]. However, recruiting of genetically homogeneous subjects would afford a test system, which was not available when planning the study. For the analysis of 8-iso-$PGF_{2\alpha}$, a commercially available ELISA kit was used instead of GC-MS technique because these methods of first choice with excellent specificity and selectivity were not available. Even if cross-reactivity with some of the 64 F_2-isoprostanes cannot be excluded in case of using an ELISA kit [59, 60] (according to the manufacturer cross-reactivity with PGF1α = 4.6%, PGF2α 1.85% and three other PGF2-isomers <0.008%), the proportional bias allows a comparison of data within this study, but not with data obtained by GC-MS [59, 60].

In conclusion, Bolus ingestion of either WT or GT initially increased plasma C concentrations but did not improve overall plasma antioxidant capacity in young healthy non-smokers. Consequently, selected markers of oxidative stress were neither influenced by WT or GT. By definition, ''healthy'' subjects are in a pro-/antioxidant balance with no signs of oxidative stress. In this situation, it seems difficult or even impossible to show any effects of dietary intervention with antioxidants. Conclusions with respect to health protective effects of antioxidant-containing foods like WT and GT can, thus, not be drawn.

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together with H. K. B. who was the medical advisor and estimated the sample size. The study was implemented by N. M., G. U. M. and H.V. who was responsible for blood sampling. Analysis of flavan-3-ols and vitamin C was implemented by N. M. with support from B. A. Furthermore, S. E. and B. A. were responsible for laboratory analysis. Statistical analysis was done by S. E. and N. M. with help from H. K. B. S. E., P. S. and H. K. B. strongly contributed to the interpretation of results. S. E. and P. S. wrote a major portion of the article. All authors contributed to the final version.

The authors have declared no conflict of interest.

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