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Journal of Nutritional Biochemistry 16 (2005) 144–149

Journal of Nutritional Biochemistry

Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans

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Received 24 December 2003; received in revised form 5 October 2004; accepted 15 November 2004

Abstract

The antioxidant activity of green tea (GT) has been extensively studied; however, the results obtained from dietary intervention studies are controversial. In the present study we investigated the effect of the addition of two cups of GT (containing approximately 250 mg of total catechins) to a controlled diet in a group of healthy volunteers with respect to a group following the same controlled diet but not consuming GT. Antioxidant status and lipid profile in plasma, the resistance from oxidative damage to lipid and DNA, and the activity of glutathione peroxidase (GPX) in isolated lymphocytes were measured at the beginning and the end of the trial. After 42 days, consumption of GT caused a significant increase in plasma total antioxidant activity [from 1.79 to 1.98 μmol Trolox equivalent (TE)/ml, $P<.001$], significant decreases in plasma peroxides level (from 412 to 288 Carr U, $P<.05$) and induced DNA oxidative damage in lymphocytes (from 14.2% to 10.1% of DNA in tail, $P<.05$), a moderate although significant decrease in LDL cholesterol (from 119.9 to 106.6 mg/dL, $P<.05$) with respect to control. The present study suggests the ability of GT, consumed within a balanced controlled diet, to improve overall the antioxidative status and to protect against oxidative damage in humans.

Keywords: Green tea; Oxidative damage; Antioxidant capacity; Humans; Lipid profile

1. Introduction

Consumption of green tea (GT) has been correlated with low incidence of chronic pathologies in which oxidative stress seems to be involved, such as cancer or cardiovascular diseases [1]; a higher dietary intake of antioxidant compounds might be considered as preventive in the reduction of ROS-related diseases in human by increasing the antioxidant potential of subjects [2].

Green tea beverages are an excellent source of polyphenol antioxidants called catechins, mainly epigallocatechin gallate (EGCg), epigallocatechin (EGC), epicatechin gallate (ECg) and epicatechin (EC), whose antioxidant properties have been extensively investigated in vitro [3–5]. The mechanisms of catechin activity include radical and oxidant scavenging, metal chelation, inhibition of redox-sensitive transcription factors, inhibition of pro-oxidant enzymes and induction of phase II enzymes [6]. The importance of demonstrating the antioxidant effects of tea in human beings is emphasized by the evidence that catechins are extensively metabolised in vivo and that the antioxidant and biological activities of catechin metabolites may differ from those of their parent compounds. Furthermore, catechin concentrations used in in vitro studies are generally higher than dietary concentrations. The health benefits of GT consumption, by means of extract or beverage, have been investigated in human studies, but intervention studies report controversial results probably due to differences in the considered population (i.e., dietary habit and life style) and/or in experimental protocols (i.e., dose and length of treatment) [7–11]. In addition, different markers, such as lipid or DNA-oxidized products or lipid and DNA resistance to oxidative stress, have been selected to evaluate oxidative damage or antioxidant protection in humans [10–13]. Other effects of tea consumption, not related to the

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doi:10.1016/j.jnutbio.2004.11.006

antioxidant activity of catechins, have also been considered, i.e., the reduced cardiovascular mortality associated with tea intake which has also been considered, i.e., the reduced cardiovascular mortality associated with tea intake which has been ascribed to the lowering effect on plasma cholesterol [14].

The positive effects of tea consumption on human health were mainly suggested by epidemiological studies in Eastern Countries, where not only the differences in tea intake but also the differences in the typical diet with respect to Western Countries could affect the biological response.

The aim of the present study was to investigate whether a moderate intake of GT could exert beneficial effects on human health. To this aim several variables were measured in healthy subjects before and after the dietary intervention: the antioxidative status and lipid profile in plasma, the resistance from oxidative damage to lipid and DNA and the activity of antioxidant enzymes in lymphocytes. Results obtained in subjects consuming GT were compared to those obtained in control subjects; during the trial, diet was controlled for fruit and vegetable intake in both groups.

2. Materials and methods

2.1. Reagents

Reagents were purchased from Sigma (St Louis, MO, USA) and Merck (Darmstad, Germany); the GT extract, Greenselect, was purchased from Indena (Milan, Italy). Green tea catechin composition was EGCg 67%, ECg 7.2%, EGC 1.1%, EC 1.9%, catechin (C) 0.6%, gallic acid 0.8%, as previously reported [4].

2.2. Subjects

Twenty-four healthy female subjects were recruited. Average age was 26 (range 20–39) years and average BMI was 19.1 (range 17.2–23.0) kg/m^2. Potential volunteers were selected following a dietary questionnaire on their dietary habit, in order to enroll people with similar eating behaviour, in particular for fruit and vegetable consumption. Subjects with low fruit and vegetable intake and on hypocaloric, vegetarian or vegan diet were excluded. Subjects reporting the use of any medication or dietary supplement, history of major illness and current smoking were excluded as well. Volunteers received oral and written information about the study and gave their written consent.

2.3. Experimental protocol

Volunteers were randomly divided into two groups: 12 acting as control (C) and 12 receiving the GT supplementation (GT). The latter consumed a total of two cups of GT per day at breakfast and dinner. Every cup was prepared solving 160 mg of GT extract in 200 ml of warm water; the total daily intake of catechins was approximately 250 mg. Supplementation lasted for 42 days, and during this period subjects of both groups followed a controlled diet, developed considering dietary habit and season, in order to conform with the antioxidant intake without significantly affecting their dietary habits. Particularly, all subjects received a detailed list of allowed foods, together with a list of foods with fixed frequencies of consumption (Table 1), in order to reach the frequency of four portions per day of fruits and vegetables. Furthermore, they received a list of not-allowed foods representing the main dietary source of polyphenols among food normally consumed by our volunteers. As reported above, volunteers were selected on the basis of their eating behaviour, in order to minimally affect their dietary habits during the study. The subjects did not report any difficulty in following the suggestions as confirmed by the dietary diaries collected at the end of the study. Blood samples were collected at the beginning (T=0) and at the end (T=42) of the experimental trial and immediately centrifuged to obtain plasma or serum for further analyses or used to isolate lymphocytes. Blood sample at T=42 was collected 12 h after the last GT ingestion.

2.4. Analysis of total polyphenols

Analysis was performed by the method of Scalbert et al. [15] on deproteinated plasma samples. Calibration was achieved with gallic acid aqueous solution (0–50 μg/ml); results are expressed as gallic acid equivalents per millilitre of plasma.

2.5. Analysis of total antioxidant activity

Total antioxidant activity (TAA) of plasma samples was measured using the method of Re et al. [16], based on the ability of the antioxidant molecules in the sample to reduce the radical cation of ABTS, determined by the decolorization of ABTS$^{\circ+}$ and measured as quenching of absorbance at 740 nm. Values obtained for each sample were compared

Table 1
List of "not allowed" and "allowed" food with frequencies of consumption (based on 2 weeks)

Food not allowed	beer, chocolate and cocoa, fruit juices, grape, pesto sauce for pasta (based on basil), tea and wine
Food with fixed frequencies of intake (number of portion per 2 weeks)	apple without peel (2), asparagus (1), aubergine (2), banana (6), bean (2), beet (1), broccoli (1), carrot (1), cauliflower (1), decaffeinated coffee (14), fennel (2), figs (4), French bean (2), green salad (2), kaki (2), kiwi (2), maize (1), orange (4), pear without peel (6), peas (2), pineapple (2), pumpkin (1), spinach (2), Swiss chards (white) (1), tomato (2), tomato sauce (2), zucchini (2)

Table 2
Serum lipid profile of healthy subjects consuming or not two cups of GT daily

Variable	C group		GT group	
	$T=0$	$T=42$	$T=0$	$T=42$
Triacylglycerol (mg/dl)	66.1±5.4	65.5±6.0	84.9±14.0	84.4±15.3
Total cholesterol (mg/dl)	170.9±8.7	172.5±10.0	174.6±10.2	173.9±9.6
HDL cholesterol (mg/dl)	56.8±2.6	56.3±2.4	55.8±3.9	56.3±3.6
LDL cholesterol (mg/dl)	115.5±9.0	107.5±9.5	119.9±9.1	106.6±8.3*

Blood samples were obtained at the beginning ($T=0$) ant the end ($T=42$) of the experimental trials, and the different analyses were performed on plasma as reported in Materials and methods. Result are mean±SE ($n=10$). Statistical analysis was by ANOVA as specified in the text.

* Significantly different from $T=0$ value within the same variable and experimental group ($P<.05$).

to the concentration–response curve of standard Trolox solutions and expressed as μmol Trolox equivalent (TE)/ml.

2.6. Peroxide levels determination

The d-ROMs test (Diacron, Grosseto, Italy) was applied on serum samples immediately after collection. This test is based on the ability of transition metals to react with peroxides by Fenton reaction. The reaction produces free radicals that, trapped by an alchilamine, form a coloured compound detectable at 505 nm. Results are expressed in Carr units where 1 Carr U corresponds to 0.08 mg hydrogen peroxide/100 g; normal range is 260–340 Carr U. [17].

2.7. Analysis of plasma lipid profile

Triacylglycerol, total cholesterol, LDL cholesterol and HDL cholesterol were determined by colorimetric methods [18–21].

2.8. Isolation of lymphocytes

Depending on the number of lymphocytes necessary, different aliquots of whole blood were added to equal volumes of Histopaque 1077 (Sigma) for separation. Then samples were centrifuged at 300×*g* for 30 min and lymphocyte fractions harvested and washed with PBS. The cell number was quantified by haemocytometer count.

2.9. Analysis of malondialdehyde

Lymphocytes isolated from 3 ml of whole blood were divided in control (C) and oxidized cells (OX). The latter were subjected to oxidative treatment with 100 μmol/L Fe^{2+} (as $FeSO_4$) for 15 min at 37 °C. At the end of the incubation, cells were washed twice with PBS, centrifuged, suspended in 0.5 ml PBS and finally stored at −80 °C. Malondialdehyde was measured by the reaction with thiobarbituric acid (TBA), then the adduct (MDA-TBA2) was separated by HPLC as previously reported [22]. Results are expressed in nanograms MDA/10^6 cells.

2.10. Analysis of glutathione peroxidase

Lymphocytes isolated from 1 ml of whole blood were suspended in 0.5 ml of 0.25 mol/L phosphate buffer pH 7 and stored at −80 °C. After thawing, samples were sonicated for 20 s, five cycles, 40% power (Sonopuls model UW 2070, Bandelin, Berlin, Germany) and centrifuged at 5000×*g* for 1 h at 4 °C. The enzyme activity was evaluated on supernatants according to the method of Belsten and Wright [23]. Briefly, the enzyme activity was quantified by the rate of loss of NADPH when a peroxide compound (*t*-butyl hydroperoxide) was added to the reaction solution. The kinetic was followed for 2 min at 340 nm in a thermostated spectrophotometer (37 °C) (Cary 3E Varian, Mulgarve, Victoria, Australia). Results were expressed in units (nmol NADPH oxidized×min^{-1})/10^6 cells.

2.11. Evaluation of DNA damage by COMET assay

The assay was applied as previously described [24]. Briefly, lymphocytes isolated from 100 μl of whole blood were suspended in 50 μl PBS. The suspension was embedded in agarose gel on slides and dipped in oxidative solution (Fe^{2+} 500 μmol/L in 10 mmol/L HEPES solution) for 15 min; control cells were dipped in HEPES solution for the same time. Cells on slides were then subjected to electrophoresis, neutralized and stained with ethidium bromide. Cell images were electronically captured and analysed for fluorescence intensity. The DNA damage was expressed as percentage of DNA in tail.

2.12. Statistical analysis

Data are means±SE. Statistical analysis was by the one-way analysis of variance (ANOVA) followed by Tukey's test for honestly significant difference (HSD) using the Statistica Software (Stat Soft, Tulsa, OK). Statistical significance was defined at $P<.05$.

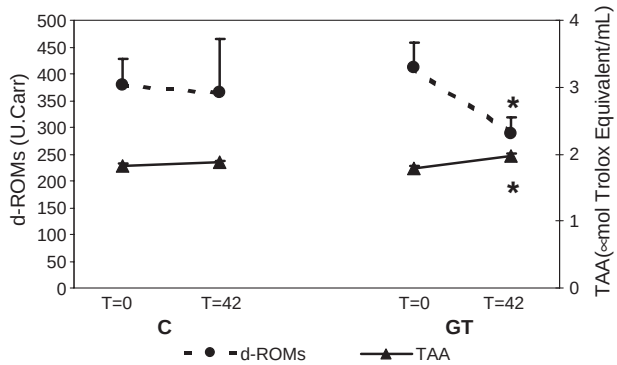

Fig 1. Data of TAA and peroxide level (d-ROMs) in plasma of healthy subjects consuming or not 2 cups of GT daily. Blood samples were obtained as reported in Table 1, and analysis performed as reported in Materials and methods. Data are mean±SE ($n=10$). Statistical analysis was by ANOVA as specified in the text. * Significantly different from $T=0$ value within the same variable and experimental group ($P<.05$).

3. Results

3.1. Plasma variables

In Table 2 the serum lipid profile of control and GT-consuming subjects, at the beginning and the end of the trial, is reported. At $T=0$, no differences were detected between the two groups, apart from triacylglycerol level which was significantly higher in the GT group ($P<.05$). However, in both control and experimental group the triacylglycerol level, as well as total cholesterol and HDL cholesterol levels, was not modified by GT consumption. On the contrary, compared to basal value, at $T=42$, LDL cholesterol was significantly decreased ($P<.05$) in the GT group.

The level of plasma total polyphenols was similar in both groups at the beginning of the trial (C: 4026±160 μg gallic acid/ml; GT: 3928±228 μg gallic acid/ml), and GT consumption for 42 days caused a slight but not significant increase (C: 3950±154 μg gallic acid/ml; GT: 4199±250 μg gallic acid/ml).

As reported in Fig. 1, at $T=0$, TAA and peroxide plasma level were similar between the two groups, but moderate intake of GT for 42 days significantly increased TAA (from 1.79 to 1.98 μmol TE/ml; $P<.001$) and significantly decreased peroxide plasma level (from 412 to 288 Carr. Units; $P<.05$); conversely, no significant differences were found in the control group.

3.2. Variables of oxidative stress in lymphocytes

Data on indicators of oxidative stress and glutathione peroxidase (GPX) activity in lymphocytes of control and GT-supplemented subjects at the beginning and end of the experimental trial are reported in Table 3. Lymphocyte MDA production represents the differences between MDA production in lymphocytes not subjected and subjected to oxidative treatment for each volunteer and experimental time. At $T=0$, similar values were detected for all variables comparing control and GT group. In the GT group, MDA level showed a trend towards a decrease at $T=42$, although not significant. Lymphocyte resistance to DNA damage induced ex vivo was significantly increased after GT intake. Glutathione peroxidase activity was significantly decreased at $T=42$ in both C group (about 50% decrease) and GT group (about 70% decrease).

Table 3
Markers of antioxidant status in lymphocytes of healthy subjects consuming or not two cups of GT daily

Variable	C group		GT group	
	$T=0$	$T=42$	$T=0$	$T=42$
MDA (ng/10^6 cells)	102±30	106±21	110±24	82±10
DNA (percentage of DNA in tail)	13.4±0.9	15.3±1.4	14.2±1.4	10.1±0.5*
GPX (U/10^6 cells)	28±7	14±3*	24±5	7±2*

Blood samples were obtained as reported in Table 1. Lymphocytes were obtained and different analyses were performed on lymphocytes as reported in Materials and methods. Results are mean±SE ($n=10$). Statistical analysis was by ANOVA as specified in the text.

* Significantly different from $T=0$ value within the same variable and experimental group ($P<.05$).

4. Discussion

Despite the general agreement on the antioxidant activity of catechins in vitro and on their bioavailability after GT beverage ingestion in humans, conflicting results are present with regard to catechin effect on lipid plasma profile or lipid oxidability [8,25,26]. Several epidemiological studies show a lowering effect on plasma cholesterol and triacylglycerol levels due to GT consumption [14,27], but a recent intervention study reported no significant effects on plasma lipids of six cups per day intake of GT for 4 weeks [8]. In the present study we found a significant decrease in LDL cholesterol, but a similar trend, although not significant, was also observed in control group. Taking into consideration the complexity of the relations between food intake and modifications of body variables, the lowering effect on LDL cholesterol could be ascribed to the synergic action of GT consumption and diet. Although further evidences are needed to confirm GT effect on plasma LDL cholesterol, this synergy of action must be taken into account.

It has been extensively demonstrated that plasma TAA increases after a few hours from the intake of single doses of GT [7], but controversial data are reported about the effect of regular consumption of tea. Recently, Sung et al. [28] reported that a 7-day intake of GT beverages significantly increased plasma TAA; conversely, no changes in plasma TAA, evaluated by FRAP value, were found in the study of Kimura et al. [29] after 7 days of tea polyphenols extract ingestion three times a day, despite the increase of plasma-conjugated EGCg. Our results support the hypothesis that a dietary intervention with moderate amount of GT (two cups a day for 42 days) in human subjects can improve plasma antioxidant status, as demonstrated by the increase in TAA levels and the decrease in peroxide levels. These results are in agreement with those of van het Hof et al [8], which found a slight but significant increase in plasma TAA following 4 weeks of repeated intake of GT. It must be underlined that, in the present study, blood samples were obtained about 12 h after the last GT consumption, indicating that the effect on the antioxidative status of plasma persists at least for that time.

When tea consumption is regular, the mean plasma concentration of catechins reaches a steady state that is significantly higher than baseline level and remains for at least 12 h after the last ingestion [12,30]. However, in our study plasma total polyphenols concentration was not significantly increased in the GT group at $T=42$. This result, which could be ascribed to the lower intake of GT

with respect to other studies (2 cups per day vs. 8–12 cups per day), is not conflicting with the improvement in the antioxidative status of plasma considering that catechin supplementation could have an effect on oxidative stress not only by a direct action but also by affecting the levels of other antioxidant compounds normally present in the human body. Pietta and Simonetti [31] suggested that catechins and their metabolites might exert their antioxidant protection in vivo through a cascade involving ROS and hydrophilic antioxidant, resulting in an overall protection of liposoluble vitamin E and β-carotene. Therefore the improvement in the antioxidant status observed after GT consumption could reflect mainly modifications of the whole antioxidant system rather than of single compounds.

Lymphocyte resistance from lipid oxidation, evaluated as MDA production, was not significantly changed by GT consumption. The protocol used in this study (ex vivo oxidation of cells with Fe^{2+} ions) was defined in a previous study on a lymphoid cell line, in which the protective effect of 15 μmol/L EGCg on lipid damage was tested, confirming the ability of this catechin to decrease MDA production [22]. Malondialdehyde production was also reduced in the same cellular model following 10 mg/L GT extract supplementation, corresponding to 15 μmol/L EGCg [4]. Notably, in those studies the concentration of catechins was very high, about eight times higher than that reachable in vivo following an intake of 400 mg EGCg as a tablet [9]. Considering that the total daily intake of catechins with tea beverages in our volunteers was approximately 250 mg, it is conceivable that the consequent catechin plasma concentration was not sufficient to reduce lipid peroxidation products in lymphocytes. Moreover, Chen et al. [32] demonstrated a different susceptibility to oxidative stress of cancer cells with respect to their normal counterparts. We cannot exclude that an effect of antioxidant supplementation on lipid peroxidation could be detectable in subjects normally exposed to oxidative stress, such as smokers, and not in healthy nonsmoker subjects.

Regarding protection against DNA oxidative damage, in previous studies we demonstrated that Jurkat cells supplemented with 10 mg/L GT extract were protected from DNA damage induced by iron ions [4], while no effect was detected when DNA damage is induced by H_2O_2 [13]. For this reason, in the present study we used iron ions as oxidative stimulus, even though they led to a lower DNA damage with respect to H_2O_2 [4,13]. The latter is probably due to the fact that, differently from H_2O_2, iron ions cannot pass through the cell membrane, so they cause DNA damage indirectly, via the production of other reactive species (e.g., MDA) [33]. In the literature the positive effect of GT catechins on DNA oxidative damage is widely reported in different cellular and animal models [34], but only very few data are available in vivo. Several authors demonstrated a positive effect of tea consumption on markers of DNA damage [10,35], while no effect was found by others [11]. The present results indicate that an increased cell protection from Fe^{2+}-induced DNA damage is achievable in vivo after a moderate and regular intake of GT beverage.

At $T=42$, independently of GT consumption, a decrease in lymphocyte GPX activity was detected. In a previous study, we showed a significant decrease in GPX activity and a prevention of enzyme induction due to Fe^{2+} ions in Jurkat T cells supplemented with 15 μmol/L EGCg for 24 h [36]. Similarly, GPX activation, due to differently induced oxidative stress, was prevented in mice by GT extract supplementation and in HepG2 cells by authentic catechin supplementation [37]. Recently, it has been demonstrated that ROS may affect the activation of nuclear factors, like NF-kB or AP-1, involved in the regulation of cellular gene expression [38]. Therefore, since consensus site for those nuclear factors has been identified in the regulatory regions of antioxidant enzyme (AE) genes [39], we can hypothesize that catechins, by decreasing the oxidative stress in cells, modulate the expression of AE genes. This hypothesis is also consistent with the feedback mechanism of antioxidant compounds on AE activities suggested by Breinholt et al. [40]. Several studies have investigated the effect of polyphenols supplementation on GPX activity in cell cultures and animal models, while, to our knowledge, few studies on the effect of GT or flavonoids intake in humans are available. Moreover, these studies are controversial; some reporting no effect [8,11], others a slight [41] or a significant increase [42] in erythrocyte GPX activity following supplementation with GTE or polyphenols. Even dietary intervention studies with other antioxidants report controversial results [43]. Further studies are needed to clarify whether, and by which mechanism, antioxidant supplementation could influence GPX activity in human. Anyway, our results indicate a control on GPX activity by dietary factors possibly antioxidant; since this effect was present not only in the GT but also in the C group, although to a lesser extent, it has to be ascribed to the controlled diet and not to GT alone.

In conclusion, the present study suggests that, in healthy subjects, a moderate intake of GT ameliorates antioxidant defences in plasma and protects lymphocytes from DNA oxidative damage. Since the effects obtained in GT-consuming subjects have been compared to those obtained in controls consuming the same diet controlled for vegetable and fruit intake, our data support the beneficial effect of GT in humans and underline its particular relevance for human health.

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