Advs Exp. Medicine, Biology - Neuroscience and Respiration (2015) 1: 25–33
DOI 10.1007/5584_2014_8

Published online: 10 October 2014

Green Tea Drinking Improves Erythrocytes and Saliva Oxidative Status in the Elderly

B. Narotzki, A.Z. Reznick, T. Mitki, D. Aizenbud, and Y. Levy

Abstract

We have previously shown that green tea (GT) drinking combined with vitamin E supplementation reduced plasma protein carbonyls and increased erythrocytes catalase activity in exercising healthy elderly. In the present study we set out to investigate the antioxidative effects of GT drinking in an aging population. We performed an interventional, crossover, controlled prospective trial with 35 healthy elderly subjects (mean age 67.3 ± 4.8 years), supplemented with four daily placebo maltodextrin "tea-bags" for 12 weeks, followed by four 1.5 g daily GT bags for another 12 weeks. Data were obtained at baseline, at the end of the placebo period, and at the end of the GT intervention period. We found that GT did not alter erythrocyte catalase activity. However, it provided protection against 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative hemolysis which declined by 10.2 % ($p < 0.001$). No changes were observed in saliva oral peroxidase enzymes. Nonetheless, saliva total antioxidant capacity increased by 42.0 % ($p < 0.01$). Plasma oxidative products, such as protein carbonyls, lipid peroxides and thiobarbituric acid reactive substances (TBARS) were stable throughout the intervention period. We conclude that four daily cups of GT are well tolerated in elderly free living subjects. Our results demonstrate that both erythrocyte resistances to oxidation and saliva antioxidant capacity are improved by GT drinking. The clinical implications of these oxidation modifications require further research.

Keywords

Aging • Erythrocytes • Green tea • Oral peroxidases • Oxidative hemolysis • Protein carbonyls • Saliva antioxidant capacity

B. Narotzki, A.Z. Reznick, and D. Aizenbud
Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel

T. Mitki
Clalit Health Services, Haifa, Israel

Y. Levy (✉)
Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel

Department of Medicine D, Rambam Health Care Campus, POB 31096, Bat Galim, Haifa, Israel
e-mail: ys_levy@rambam.health.gov.il

1 Introduction

Population aging is a global phenomenon. The elderly population has substantially increased over the past 50 years. For instance, in Israel, the elderly population percentage has more than doubled (Mashavh 2012). In some countries, the increase rate is even higher. Japan's aging rate shows more than a fourfold increase in the elderly population (Arai et al. 2012). High incidence of diseases may hamper the elderly life quality. Therefore, strategies for diseases prevention by health promotion in the older population are required.

Oxidative stress (OS) is suggested to be involved in the pathogenesis of various age related disorders, for instance, cancer, diabetes mellitus, neurodegeneration, atherosclerosis and cardiovascular diseases (Lagouge and Larsson 2013). The OS hypothesis suggests that reactive oxygen species (ROS) lead to molecular oxidative damage and senescence associated losses in physiological functions. According to this theory, attenuation of oxidative damage/stress is crucial for delaying the rate of aging (Sohal et al. 2002). Accumulated reactive oxygen species (ROS), on top of inadequate antioxidants function, lead to further OS and oxidative damage to macromolecules and progressive decline in cell functions (Salmon et al. 2010). Hence, much interest has arisen in the role of antioxidants, especially natural food derived antioxidants for maintenance of human health and disease prevention (Niki 2010). Thus, humans can achieve elevated antioxidant potential and alleviate ROS related diseases with increased dietary intake of antioxidant compounds (Jówko et al. 2011).

Tea (*Camellia sinensis*) is a caloric free beverage and constitutes an important source of antioxidants, including carotenoids, tocopherols and vitamin C, as well as polyphenols called catechins. Tea catechins antioxidant properties have been extensively investigated *in vitro*; the main four catechins include epigallocatechin 3 gallate (EGCG), epigallocatechin (EGC), epicatechin 3 gallate (ECG) and epicatechin (EC). Tea catechins reduce oxidation directly and indirectly by chelating prooxidant metals, separation of oxidative enzymes, and induction of antioxidant enzymes. The health potentials of tea, and particularly of green tea (GT) are progressively acknowledged in the western world due to the accumulation of evidence demonstrating favorable health effects in various body compartments, including the oral cavity, heart and vessels, skin, and adipose tissue (Narotzki et al. 2012a; Erba et al. 2005).

We have previously shown that GT drinking combined with vitamin E supplementation reduced plasma protein carbonyls, elevated erythrocytes catalase activity, and tended to increase the activity of the antioxidant oral peroxidases (OPO) in exercising healthy elderly (Narotzki et al. 2013b). Moreover, we have demonstrated that GT addition to saliva or mouth rinsing resulted in a sharp rise in the activity of OPO (Narotzki et al. 2013a). Therefore, the purpose of this study was to evaluate the effects of a long term (12 weeks) GT drinking on plasma, erythrocytes, and saliva oxidative stress biomarkers and on antioxidant capacity in healthy aged men and women. We hypothesize that GT drinking would improve antioxidative mechanisms. To examine this hypothesis, plasma oxidation biomarkers such as protein carbonyls, peroxides, thiobarbituric acid reactive substances (TBARS), erythrocyte antioxidant (catalase activity), and resistance to oxidative hemolysis and saliva antioxidants [OPO activity, total antioxidant capacity (TAC)] were investigated.

2 Methods

This study was approved by the institutional Helsinki committee at Rambam Health Care Campus, Haifa, Israel. All subjects provided a written informed consent.

2.1 Green Tea/Placebo Analysis

Subjects received 1.5 g GT bags (provided by Wissotzky Tea Company-Tel Aviv, Israel) and 1.1 g placebo (PB) "tea bags" containing maltodextrin (custom made by Wissotzky Tea Company). Analyses of the GT and PB contents were conducted for total phenols and TAC. The beverages were prepared by brewing PB (5 min) or GT bags (1, 3, 5, 7, or 10 min), in 240 mL of boiling water without stirring. Total phenols content of the drinks was determined by the colorimetric method of Folin-Ciocalteau, according to the modified methodology (Singleton and Rossi 1965). Briefly, 150 μL of GT/placebo drink was added to 500 μL of ethanol (100 %), 2,500 μL of DDW, and 250 μL of Folin-Ciocalteau reagent (50 %). After 5 min, 500 μL of 5 % sodium bicarbonate was added. The mixture was left for 1 h at room temperature and absorbance of the colored product was measured at 765 nm. Gallic acid in ethanol served as standard solution, and the results for total phenols were expressed as μL/mL of gallic acid equivalents.

Tea and placebo hydrophilic and lipophilic antioxidant capacity was measured by TAC kit (ScienCell Research Laboratories, CA, USA) and expressed as mM of Trolox equivalents.

2.2 Subjects

Thirty-five healthy men and women (13 men and 22 women), aged 60–76 years participated in this study. Baseline body mass index (BMI) was 22.4–34.6 kg/m^2. Table 1 displays baseline characteristics. Recruitment took place at Kibbutz Beit HaShita community family clinic in northern Israel. Kibbutz is an Israeli collective settlement; its communities are known for a high level of health, wellbeing, and longevity (Narotzki et al. 2012b). Exclusion criteria included any active disease state or unstable chronic disease (diabetes, vascular, or renal).

Table 1 Baseline characteristics of subjects

Age, year	67 ± 4.6
BMI, kg/m^2	28.6 ± 3.0
Blood pressure systolic/diastolic (mmHg)	136 ± 23/84 ± 15
Fasting glucose (mg/dL)	97.1 ± 14.0
Creatinine (mg/dL)	0.8 ± 0.2

Data are means ± SD
BMI body mass index

2.3 Experimental Design

The study was a prospective, crossover trial, in which every individual served as its own control. The experimental design is displayed in Fig. 1. All subjects underwent a 4 weeks washout period, during which they were briefed to avoid tea drinking and antioxidant supplements until the end of the study. At the end of the washout period, blood and saliva samples were collected after 12 h of night fast. Subsequently, all subjects received the maltodextrin containing PB "tea bags". The subjects were instructed to brew the PB sachets for 5 min in 240 mL boiling water without stirring and to drink 4 cups per day for 12 weeks period (PB period). After the PB period, fasting samples were collected once more and the subjects received the GT bags. Preparation and drinking instructions were the same as those for the PB drink. At the end of the 12 weeks, GT drinking, fasting blood, and saliva samples were drawn for the third time. During the study, every 4 weeks the subjects were given additional PB or GT bags. For compliance purposes, leftover tea bags were counted and actual consumption was assessed. Nutritional consumption of macronutrients and antioxidants was assessed using a food diary at the beginning, middle, and end of the study. The mean intakes were analyzed using Tzameret (version 2) dietary analysis program (Department of Nutrition, Ministry of Health, Jerusalem, Israel).

2.4 Blood Analysis

Blood was collected into disposable vials containing EDTA and separated for plasma

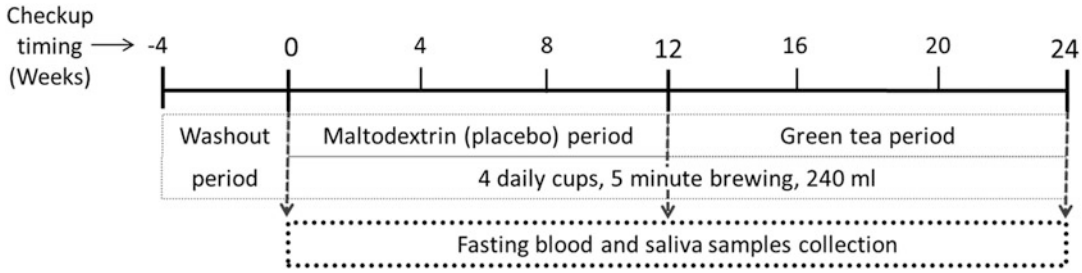

Fig. 1 Time plot of experimental design

and erythrocytes. Plasma was stored at −80 °C for subsequent analyses of oxidized proteins and lipids. Plasma protein carbonyls assay was performed according to the Reznick and Packer (1994) procedure and is presented in nmol per mg of plasma proteins. Plasma lipid peroxidation TBARS assays and plasma peroxides are described elsewhere and expressed as nmol per ml (Lavie et al. 2004). Oxidized lipid results was also adjusted for total plasma lipids [total lipids = 1.28(total cholesterol + triglycerides) + 96 (mg/dL)] (Thuresson et al. 2005).

Erythrocytes were washed 3 times with isotonic saline, kept for 6 days in 4 °C for catalase assay and analyzed immediately for 2,2′-azo-bis (2-amidinopropane) dihydrochloride (AAPH)-induced hemolysis test (HT). The test was based on previously reported protocols (Costa et al. 2009; Verma et al. 2006) and modified as follows: 0.4 ml washed erythrocytes was added to 9.5 ml saline. Two ml of diluted erythrocytes was added to DDW, 100 mM AAPH or saline to a final tube volume of 4 ml. All tubes were incubated at 37 °C for 2.5 h with intermittent shaking. After centrifuging the incubated tubes at 4,000 rpm for 10 min, absorbance was read spectrophotometrically at 540 nm. The HT percentage was calculated by dividing absorbance of AAPH-induced hemolysis from DDW-induced hemolysis. Saline tubes served as a negative control. Catalase activity was determined in erythrocytes hemolysates according to Aebi's method (1984) and expressed as mU per mg of hemoglobin protein.

2.5 Saliva Analysis

Non-stimulated whole saliva was collected between 7:00 and 8:00 a.m. to avoid circadian variations and stored in 4 °C until the formation of solid sediment containing squamous cells and cell debris. The supernatant was used for the following assays. The activity of OPO was measured according to the 5,5′-dithiobis, 2-nitrobenzoic acid thiocyanate (NBS-SCN) assay as described previously (Narotzki et al. 2013a) and expressed as mU per mg of saliva supernatant protein. Saliva was diluted 1:2 with DDW and used for TAC analysis as described above. Saliva TAC expressed as mM Trolox equivalents per mg of saliva supernatant protein.

2.6 Statistical Analysis

Results are expressed as means ± SD. SPSS Statistics 17 (SPSS Software, Chicago, IL, USA) was used for GT and placebo drinks total phenols and TAC analysis (one way ANOVA followed by post hoc Tukey's test).

SAS software (version 9.2, SAS Institute Inc, Cary, NC, USA) was used for The MIXED model analyzes of the time changes. Time was specified as categorical. The covariance structure was unspecified and was estimated on the basis of the data. In the comparisons of the means that correspond to different time points, Tukey's procedure was used to allow for the multiple comparisons. All statistical tests were 2-tailed with a significance level set at $P < 0.05$.

3 Results

Total phenols content of PB bags brewing in boiling water, was close to zero. GT phenols content rose substantially from 1 to 5 min brewing in boiling water, and marginally increased from 5 to 10 min (Fig. 2). Similar GT different brewing time trends were observed with TAC analysis, while PB capacity was lower than the TAC assay buffer (Fig. 3).

Compliance with PB and GT drinking was 94.0 ± 5.9 % and 95.9 ± 3.4 %, respectively. According to the nutritional food diary, the consumption of total calories, fat, carbohydrates, dietary fibers, vitamin C, vitamin E, and carotenes remained stable during the study.

Plasma, erythrocytes, and saliva oxidative stress biomarkers as well as antioxidant capacity are summarized in Table 2. Plasma oxidative stress biomarkers: protein carbonyls, TBARS, and peroxides remained unchanged by GT drinking. However, peroxides concentrations slightly increased after PB drinking (4.4 % increase at Week 12, compared with baseline, $p = 0.006$). Oxidized lipids, adjusted for the total plasma lipid, did not make any difference compared with crude data.

Erythrocytes catalase activity was not different at any time point. Nonetheless, the extent of AAPH-induced hemolysis was significantly lower after GT drinking (10.2 % decline at Week 24, compared with Week 12, $p = 0.001$), but not after PB drinking. Saliva OPO activity was not affected by either drink. However, saliva TAC measurements revealed that GT, but not PB drinking, increased its antioxidant capacity (41.7 % increase at Week 24, compared with Week 12, $p = 0.0063$). The effects of GT *vs.* PB drinking in relation to baseline oxidative status are illustrated in Fig. 4.

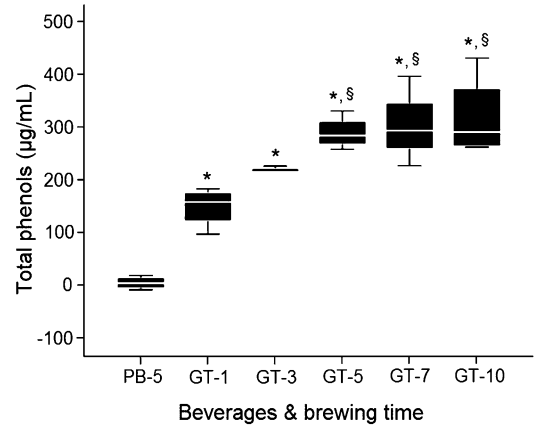

Fig. 2 Brewing time effects on placebo (PB)/green tea (GT) total phenols content. PB or GT bags were added to 240 ml of boiling DDW for different brewing time points (min). Changes in total phenols expressed as µg/mL of gallic acid equivalents. *Difference from placebo, $p < 0.05$; §difference from 1 min brewing, $p < 0.05$. Data are means ± SD (n = 4)

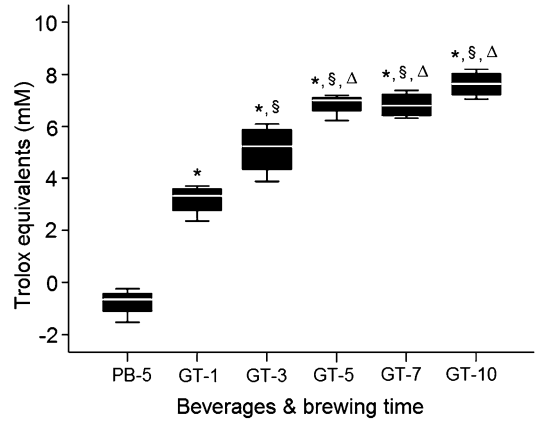

Fig. 3 Brewing time effects on placebo (PB)/green tea (GT) total antioxidant capacity (TAC). PB or GT bags were added to 240 ml of boiling DDW for different brewing time points (min). Changes in TAC expressed as mM of Trolox equivalents. *Difference from placebo, $p < 0.05$, §difference from 1 min brewing, $p < 0.05$; Δdifference from 3 min brewing, $p < 0.05$. Data are means ± SD (n = 4)

4 Discussion

The results of this study show that regular consumption of four daily cups of GT for 12 weeks may improve erythrocytes and saliva antioxidant capacities and contribute to the antioxidative mechanisms in healthy elderly men and women. On the basis of the obtained concentration curves regarding the content of total phenols and the

Table 2 Plasma, erythrocytes, and saliva oxidative stress biomarkers as well as antioxidant capacities

	Baseline	Week 12	Week 24	% change (Week 24 *vs.* Week 12)
Plasma				
Protein carbonyls, nmol/mg	0.65 ± 0.27	0.62 ± 0.20	0.66 ± 0.17	+6.5
TBARS, nmol/mL	11.27 ± 1.53	11.51 ± 1.80	11.50 ± 1.87	−0.1
Peroxides, nmol/mL	487.43 ± 41.38	508.83 ± 42.81[a]	519.77 ± 48.22	+2.2
Erythrocytes				
Catalase activity, mU/mg	32.95 ± 3.34	31.42 ± 3.72	32.99 ± 4.18	+5.0
HT, percentage	69.55 ± 15.39	71.27 ± 9.89	64.01 ± 12.48[b]	−10.2
Saliva				
OPO activity, mU/mg	347.51 ± 286.14	313.18 ± 299.85	328.20 ± 189.44	+4.8
TAC, mM/mg	7.17 ± 5.53	9.08 ± 4.04	12.87 ± 5.13[b]	+41.7

Data are least squares means ± SD

TBARS Thiobarbituric acid reactive substance, *HT* erythrocytes AAPH-induced hemolysis test, *OPO* oral peroxidases, *TAC* total antioxidant capacity

[a]Significant difference of Week 12 *vs.* Week 0 (placebo effect), $p < 0.01$

[b]Significant difference of Week 24 *vs.* Week 12 (green tea effect), $p < 0.001$

level of TAC, a protocol of 5 min' brewing time was selected.

In vitro antioxidant activity of GT's catechins and their bioavailability after tea drinking are generally agreed upon; yet there are conflicting results there regarding the *in vivo* antioxidant activity (Ellinger et al. 2011; Erba et al. 2005). Markers of protein oxidation are decreased after GT consumption (Ellinger et al. 2011). In an animal study of GT extract administration (500 mg/kg) to the aged rats, the percentage of cardiac, hepatic, and renal protein carbonyls was reduced by 30–40 %, compared with the age-matched control group (Wang 2013). In a human study we have shown that GT drinking combined with vitamin E supplementation reduced plasma protein carbonyls in exercising healthy elderly (Narotzki et al. 2013b). However, in our current study the administration of GT alone did not attenuate plasma protein carbonyls. It is possible that a reduction of protein carbonyls has occurred in tissues other than plasma, which was not monitored. Secondly, the combination of daily exercise with aging might have enhanced oxidative stress in our previous protocol (Narotzki et al. 2013b). We suggest that a lesser extent of ROS production was expected in our current study, in which the subjects were not exposed to exercise-induced OS. Consequently, GT administration alone was not advantageous, compared with the combination of GT, exercise, and vitamin E regarding the protection against plasma protein carbonyl production.

Other studies which investigated GT have not demonstrated any affects on lipid peroxidation. Ellinger et al. (2011) suggested that regular GT drinking may be effective when the antioxidative/oxidative balance is impaired due to harmful environmental exposure conditions and non-healthy life style. Such interventional studies, which failed to show GT effects on lipid peroxidation, included only nonsmokers. In agreement with this, our current study included non-smoking elderly and, no GT effects on plasma TBARS and peroxides were demonstrated. Adjusting oxidative biomarkers to total plasma lipids may provide additional information. Higher plasma lipids concentrations can result in higher lipid peroxides. Nonetheless, such adjustment did not make any difference compared with the crude data in our study (data not shown).

Erythrocytes can be used to study oxidative defense systems at the cellular level. Animal studies have shown that green tea was able to increase the activity of catalase in different tissues (Lin et al. 1998). GT and vitamin E supplementation with exercise resulted in a 10 % increase in erythrocytes catalase activity (Narotzki et al. 2013b). However, this increase was not followed in the current study.

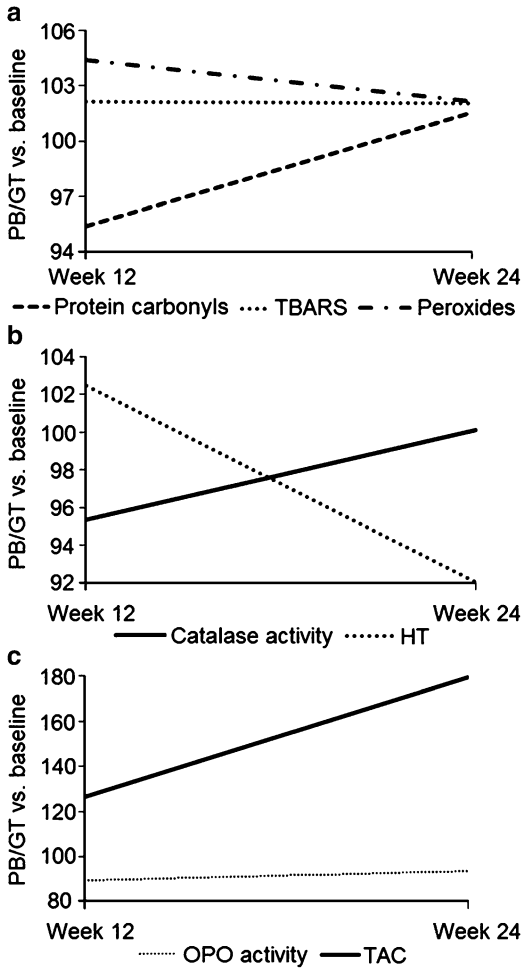

Fig. 4 Oxidation biomarkers and antioxidative mechanisms. The change from baseline levels is presented as Week 12 (Placebo (PB) effect) and Week 24 (green tea (GT) effect). Baseline levels are expressed as 100 %. Full line represents antioxidants activity, broken line represents oxidation. (**a**) **Plasma oxidation biomarkers**. The effects of PB/GT drinking on protein carbonyls, thiobarbituric acid reactive substances (TBARS) and peroxides; (**b**) **Erythrocyte antioxidative mechanisms**. The effects of PB/GT drinking on catalase activity and AAPH-induced hemolysis test (HT); (**c**) **Saliva antioxidative mechanisms.** The effects of PB/GT drinking on oral peroxidases (OPO) activity and total antioxidant capacity (TAC)

By adding AAPH to the erythrocytes, an initial lag time due to erythrocyte endogenous antioxidants, such as glutathione, tocopherols, and catalase is followed by hemolysis due to peroxyl radicals. Polyphenols are known for their ability to reduce membrane lipid peroxidation. They may interact with the membrane bilayer; decrease its fluidity and the diffusion of free radicals. Addition of GT and GT extracts to human and rat erythrocytes *ex vivo*, before performing oxidant induced HT, decreased the extent of hemolysis (Costa et al. 2009; Zhang et al. 1997). Moreover, jasmine GT, given 20 min before blood collection in rats, resulted in hemolysis amelioration (Zhang et al. 1997). Our results demonstrate that regular GT consumption can considerably enhance erythrocytes resistance to oxidative hemolysis. GT affected HT results 12 h after drinking the last GT, which may suggest an interaction of GT polyphenols with erythrocyte membranes. However, this was not investigated in the current study and verification of this hypothesis requires further research.

The majority of studies that have followed bolus ingestion of GT have demonstrated an *ex vivo* increase in plasma antioxidant capacity (assays include the ferric reducing ability of plasma (FRAP) and total radical antioxidant power (TRAP)) (Ellinger et al. 2011). However, regular consumption was less conclusive. Unlike plasma, information on saliva antioxidant capacity is limited. We have observed a substantial and significant increase in saliva TAC after GT drinking, which occurred 12 h after last tea drink. The effects of GT on saliva TAC were described in another study on chemical laboratory workers. In that study, a single daily cup of 300 mL of GT for a month, was followed by an elevation of TAC. Nonetheless, contrary to our study, TAC was measured short time after GT drinking (Tavakol et al. 2013).

Oral defense mechanisms against free radicals are particularly dependent on the antioxidant enzymes OPO (Narotzki et al. 2013b). The addition of black tea, EGCG, and particularly GT to saliva or mouth rinsing resulted in an increase in the activity of OPO (Narotzki et al. 2013a). Nevertheless, no changes in OPO activity were seen after drinking, despite the sharp elevation in the saliva TAC in our current study. Our former study suggested that GT catechins may be responsible for the increase in OPO activity.

Some of GT components may be present in saliva, even after 12 h fast, as evidenced by TAC modification. However, it is possible that saliva concentration is too low for a change in OPO activity. Since GT catechins undergo methylation, glucuronidation, and sulfation in the human body (Feng 2006), it is also conceivable that GT metabolites have different effects on OPO activity.

Limitations of the study include the selection of subject from a community with unique healthy life style, not representative of similar age groups in Israel. Increased study size would be favorable for some of our marginal results. Quantification of blood and saliva polyphenols would have shown availability of GT catechins. Consumption of more than 4 daily GT cups might have achieved stronger biological effects. However, regarding Israeli GT drinking habits, compliance is suspected to be low. Advantages of this study include the crossover-controlled experimental design with high compliance. Administration of maltodextrin placebo "tea bags" enabled a placebo controlled study.

In conclusion, drinking four daily cups for 12 weeks increased erythrocytes and saliva antioxidative mechanisms in healthy elderly, as demonstrated by a reduction in AAPH-induced hemolysis and elevation in saliva TAC. Despite our subjects' age, they were generally healthy with minimal oxidative stress exposure. Future studies in this age-group should investigate GT antioxidative effects in individuals prone to oxidative damage such as smoking, diseases states (diabetes), and exposure to environmental pollution.

Acknowledgements We thank Kibbutz Beit HaShita medical staff, Yoke Roded, Smadar Lustgarten and Hannah Shulami, for excellent assistance. This study was supported by the Krol foundation of Barnegat NJ, USA, Research and Scholarships Fund in Food and Nutrition Fields with Public Health Implication. Rappaport Institute for Research and Myers-JDC-Brookdale Institute of Gerontology and Human Development and Eshel-the Association for the Planning and Development of Services for the Aged in Israel and by Wissotzky Tea Company-Tel Aviv, Israel.

Conflicts of Interest Statement Wissotzky Tea Company supported the study and provided placebo and GT bags. The company was not involved in any phase of the study including design and data analysis. The authors declare no other potential conflicts of interest in relation to this article.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Arai H, Ouchi Y, Yokode M, Ito H, Uematsu H, Eto F, Oshima S, Ota K, Saito Y, Sasaki H, Tsubota K, Fukuyama H, Honda Y, Iguchi A, Toba K, Hosoi T, Kita T (2012) Toward the realization of a better aged society: messages from gerontology and geriatrics. Geriatr Gerontol Int 12:16–22

Costa RM, Magalhães AS, Pereira JA, Andrade PB, Valentão P, Carvalho M, Silva BM (2009) Evaluation of free radical-scavenging and antihemolytic activities of quince (Cydonia oblonga) leaf: a comparative study with green tea (Camellia sinensis). Food Chem Toxicol 47:860–865

Ellinger S, Müller N, Stehle P, Ulrich-Merzenich G (2011) Consumption of green tea or green tea products: is there an evidence for antioxidant effects from controlled interventional studies? Phytomedicine 18:903–915

Erba D, Riso P, Bordoni A, Foti P, Biagi PL, Testolin G (2005) Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J Nutr Biochem 16:144–149

Feng WY (2006) Metabolism of green tea catechins: an overview. Curr Drug Metab 7:755–809

Jówko E, Sacharuk J, Balasińska B, Ostaszewski P, Charmas M, Charmas R (2011) Green tea extract supplementation gives protection against exercise-induced oxidative damage in healthy men. Nutr Res 31:813–821

Lagouge M, Larsson NG (2013) The role of mitochondrial DNA mutations and free radicals in disease and aging. J Intern Med 273:529–543

Lavie L, Vishnevsky A, Lavie P (2004) Evidence for lipid peroxidation in obstructive sleep apnea. Sleep 27:123–128

Lin YL, Cheng CY, Lin YP, Lau YW, Juan IM, Lin JK (1998) Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione s-transferase in rats. J Agric Food Chem 46:1893–1899

Mashavh- national database for aging planning. Elderly demographic characteristics, Israeli elderly yearbook (2012) Myers-JDC-Brookdale institute of gerontology and human development and Eshel-the association for the planning and development of services for the aged in Israel. Available from: http://igdc.huji.ac.il/mashavh.aspx. Accessed on 16 Sept 2013

Narotzki B, Reznick AZ, Levy Y (2012a) Green tea: a promising natural product in oral health. Arch Oral Biol 57:429–435

Narotzki B, Reznick AZ, Navot-Mintze D, Dagan B, Levy V (2012b) Six minute walk test as a valuable assessment tool for exercise capacity in healthy kibbutz elderly. J Aging Res Clin Pract 1:61–63

Narotzki B, Levy Y, Aizenbud D, Reznick AZ (2013a) Green tea and its major polyphenol EGCG increase the activity of oral peroxidases. Adv Exp Med Biol 756:99–104

Narotzki B, Reznick AZ, Navot-Mintze D, Dagan B, Levy Y (2013b) Green tea and vitamin E enhance exercise-induced benefits in body composition, glucose homeostasis, and antioxidant status in elderly men and women. J Am Coll Nutr 32:31–40

Niki E (2010) Assessment of antioxidant capacity in vitro and in vivo. Free Radic Biol Med 49:503–515

Reznick AZ, Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233:357–363

Salmon AB, Richardson A, Perez VI (2010) Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? Free Radic Biol Med 48:642–655

Singleton VLJ, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

Sohal RS, Mockett RJ, Orr WC (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free Radic Biol Med 33:575–586

Tavakol HS, Akram R, Azam S, Nahid Z (2013) Protective effects of green tea on antioxidative biomarkers in chemical laboratory workers. Toxicol Ind Health April 10 (Epub ahead of print)

Thuresson K, Bergman A, Jakobsson K (2005) Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame-retarded rubber. Environ Sci Technol 3:1980–1986

Verma RJ, Trivedi MH, Chinoy NJ (2006) Amelioration by black tea extract of sodium fluoride induced hemolysis of human red blood cell corpuscles. Fluoride 39:261–265

Wang YC (2013) Supplementation of green tea attenuates protein carbonyls formation in aged mice. Life Sci J 10:1034–1037

Zhang A, Zhu QY, Luk YS, Ho KY, Fung KP, Chen ZY (1997) Inhibitory effects of jasmine green tea epicatechin isomers on free radical-induced lysis of red blood cells. Life Sci 61:383–394