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Supplementation with lutein or lutein plus green tea extracts does not change oxidative stress in adequately nourished older adults☆

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Abstract

Epigallocatechin gallate, a major component of green tea polyphenols, protects against the oxidation of fat-soluble antioxidants including lutein. The current study determined the effect of a relatively high but a dietary achievable dose of lutein or lutein plus green tea extract on antioxidant status. Healthy subjects (50–70 years) were randomly assigned to one of two groups (n=20 in each group): (1) a lutein (12 mg/day) supplemented group or (2) a lutein (12 mg/day) plus green tea extract (200 mg/day) supplemented group. After 2 weeks of run-in period consuming less than two servings of lightly colored fruits and vegetables in their diet, each group was treated for 112 days while on their customary regular diets. Plasma carotenoids including lutein, tocopherols, flavanols and ascorbic acid were analyzed by HPLC-UVD and HPLC-electrochemical detector systems; total antioxidant capacity by fluorometry; lipid peroxidation by malondialdehyde using a HPLC system with a fluorescent detector and by total hydroxyoctadecadienoic acids using a GC/MS. Plasma lutein, total carotenoids and ascorbic acid concentrations of subjects in either the lutein group or the lutein plus green tea extract group were significantly increased (P<.05) at 4 weeks and throughout the 16-week study period. However, no significant changes from baseline in any biomarker of overall antioxidant activity or lipid peroxidation of the subjects were seen in either group. Our results indicate that an increase of antioxidant concentrations within a range that could readily be achieved in a healthful diet does not affect in vivo antioxidant status in normal healthy subjects when sufficient amounts of antioxidants already exist.

Keywords: Supplementation; Lipid peroxidation; Antioxidant status; Biological system

1. Introduction

Various observational studies suggest a protective effect of lutein against eye diseases such as age-related macular degeneration [1–4] and cataract [5,6]. Lutein may be beneficial to maintain visual performance over time both by absorbing short-wave blue light [7] and by preventing oxidation through antioxidant mechanisms. It has been shown that lutein can quench the triplet state of photosensitizers [8], singlet oxygen [9] and oxygen radicals [10], react with free radicals [11,12] and retard the peroxidation of phospholipids [13,14].

A large US elderly cohort study (American Association of Retired Persons members) [15] indicates that the median fruit and vegetable intake for the first and fifth quintiles, respectively, were 3.1 and 11.6 servings/day for men (n=340,148) and 2.8 and 11.3 servings/day for women (n=227,021). The median fruit and vegetable intakes of the Framingham elderly (67–93 years) men (n=201) and women (n=346) were 4.0 and 4.8 servings/day, respectively [16]. However, the intakes of dark green vegetables, which are the major source of lutein, remain low (0.2 servings/day) [17]. In addition, data from NHANES III indicate that for women 51–70 years, the medians for 50th and 75th percentiles of lutein/zeaxanthin intakes were 1.7 and 2.4 mg/day, respectively [18]. Although lutein is being actively studied for its possible function in the eye, its dietary intake remains low.

Flavonoids are a large group of polyphenolic antioxidants that are present in fruits, vegetables and beverages, such as tea and wine. It has been reported that tea polyphenols protect unsaturated phospholipids from oxidation by directly reacting with the radicals in vitro

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[19], delay oxidation of α-tocopherol and β-carotene in plasma ex vivo [28] and protect against LDL oxidation [29]. It is probable that hydrophilic tea polyphenols interact not only with hydrophilic compounds but also with lipophilic compounds due to their high affinity for lipid bilayers [20]. We also have found [21] that epigallocatechin gallate, a major component of green tea polyphenols, protects the oxidation of fat-soluble antioxidants, including lutein. In addition, a recent cross-sectional study utilizing NHANES 1999–2002 data suggested that a high intake of flavonoid-rich foods may reduce inflammation-mediated chronic diseases [22].

A cohort study of 35,369 postmenopausal women in Iowa indicated that about 58% of women reported tea consumption as "never" or "once per month," 24% at least once a week, 8.7% daily, while 8.6% drank two or more cups of tea a day [23]. A recent report on dietary intakes from NHANES 1999–2002 indicated that only 21.3% of US adults reported drinking tea daily and their daily total flavonoid intake was over 20 times that of tea nonconsumers (698 vs. 33 mg/day) [24].

The current study focuses on whether supplementation of lutein and/or green tea, which have been shown to exert antioxidant actions and interact with each other yet whose intakes remain low in the US population, can alter overall antioxidant status/oxidative stress in an older population using doses which are high and can readily be obtained in a healthful diet.

2. Materials and methods

2.1. Chemicals and reagents

All-*trans*-β-carotene (type II), α-carotene, cryptoxanthin, lycopene, α-tocopherol, catechin, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate (ECG), L-ascorbic acid, uric acid, human serum albumin and phosphatidylcholine (type XVI-E) were purchased from Sigma Chemical Co. (St. Louis, MO). Lutein was purchased from Kemin Industries (Des Moines, IA). The fatty acid analogue 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY 581/591) was purchased from Molecular Probes (Eugene, OR). The lipophilic radical initiator, 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) were purchased from Wako Chemicals (Richmond, VA, USA). All HPLC solvents were obtained from JT Baker Chemical and were filtered through a 0.45-μm membrane filter before use.

2.2. Subjects

Forty nonsmoking men and postmenopausal women aged 50–70 years (males, 15; females, 25) successfully completed the current study. For this study, 1097 potential subjects were recruited, 566 subjects were prescreened based on a prescreening questionnaire, 132 subjects were screened with a physical examination and blood test, 47 subjects were admitted and 7 subjects dropped out due to personal reasons (n=2) or due to developing an eye condition falling under the study's exclusion criteria (n=5).

All study subjects who participated in the current study were in good health, as determined by a medical history questionnaire, physical examination and normal results of complete blood count. All study subjects did not have any history of cardiovascular, hepatic, gastrointestinal, or renal diseases, were not alcoholic, were nonsmokers and were not exogenous hormone users. Subjects were not taking any supplemental vitamin or carotenoid for more than 6 weeks before the start of the study and were not heavy tea drinkers (>2 cups/day). In addition, subjects who had plasma lutein concentrations that were more than 200% of median of the normal population (as previously reported in NHANES III for subjects of same age group) were excluded from the study. In addition, subjects who had cataract, glaucoma or age-related macular degeneration were excluded from the study except for those with age-appropriate progression of eye status (e.g., mild lens clouding). The study protocol was approved by the institutional review board of the Tufts Medical Center and Tufts University Health Sciences, and written informed consent was obtained from each study subject.

Subjects underwent a 2-week run-in period to limit fruit and vegetable intake to two servings/day of lightly colored fruits and vegetables and to avoid green tea before starting the study in order to maximize the blood response to the lutein supplementation. Subjects were randomly assigned to one of two groups (n=20 in each group): (1) a lutein (12 mg/day) supplemented group or (2) a lutein (12 mg/day) plus green tea extract (200 mg/day) supplemented group.

On sampling Days 1, 29, 57 and 85, the subjects were provided with a 4-week supply of supplements. The subjects were instructed to take the supplement with their first meal of the day, which contained more than 10 g of fat to facilitate absorption of the lutein supplement. There was no recommendation to alter dietary habits of the study subjects during the intervention period in order to make the application of data obtained from this study relevant to the overall population. Lutein (5% beadlets) was supplied by Kemin Foods, L.C. (Des Moines, IA), and the green tea extract was provided by Indena USA, Inc (Seattle, WA). Lutein (12 mg) or lutein (12 mg) plus green tea extract (200 mg) was encapsulated in a Vcap capsules (Capsugel, Greenwood, SC) by Thorne Res Inc (Dover, ID). The lutein and flavanols in the green tea extract were stable throughout the study as determined by HPLC systems with a photodiode array detector and an electrochemical detector (ECD), respectively. Flavanols in the green tea extract were determined by HPLC-ECD after a 24-h sequential acidified methanol extraction [25]. Our HPLC-ECD analysis indicated that there were 63.1 mg EGCG, 28.3 mg ECG, 19.9 mg EGC, 8.42 mg EC and 1.5 mg catechin in 200 mg of green tea extract.

Subject compliance was determined by counting any remaining pills and checking the compliance calendar on each sampling day. In addition, the research dietitian at the Jean Mayer US Department of Agriculture-Human Nutrition Research Center on Aging at Tufts University interviewed each study subject on each sampling day.

Blood samples were collected at 0 h (14-h fasting) and 2 h after ingesting supplements in vacutainers containing EDTA at baseline and at Days 29, 57, 85 and 113. Aliquots of plasma samples were stored at −80°C for subsequent biochemical analyses.

2.3. Measurement of fat-soluble antioxidants

Plasma carotenoid concentrations were measured by an HPLC system as previously described with minor modification [26]. Plasma samples (200 μl) were extracted with 2 ml of chloroform/methanol (2:1) followed by 3 ml of hexane. Samples were dried under nitrogen and resuspended in 75 μl ethanol/methyl *tert*-butyl ether (2:1) of which 25 μl was injected onto the HPLC. The HPLC system consisted of a Waters 2695 Separation Module, 2996 Photodiode Array Detector, a Waters 2475 Multi λ Fluorescence Detector, a C30 carotenoid column (3 μm, 150×3.0 mm, YMC, Wilmington, NC) and a Waters Millenium 32 data station. The mobile phase was methanol/methyl *tert*-butyl ether/water (85:12:3 by volume with 1.5% ammonium acetate in water; Solvent A) and methanol/methyl *tert*-butyl ether/water (8:90:2 by volume with 1% ammonium acetate in water; Solvent B). The gradient procedure has been reported earlier [26]. Results were adjusted by an internal standard containing echinenone. The interassay coefficient of variation (CV) (n=25) was 4% and intraassay CV was 4% (n=9). Recovery of the internal standard averaged 97%.

2.4. Measurement of water-soluble antioxidants

Ascorbic acid was measured by an HPLC system using an ECD (Bioanalytical System, N. Lafayette, IN), as described by Behren et al. [27] with minor modification.

Uric acid was measured by an Olympus analyzer (Olympus America Inc., Melville, NY), as reported by Fossati et al. [28] with minor modification.

2.5. Determination of plasma flavanols

Concentrations of free plus Phase II enzyme-conjugated flavanols in plasma including EGCG, ECG, EGC, EC and catechin were determined by the HPLC method of Chen et al. [25]. Briefly, 20 μl vitamin C–EDTA (200 mg ascorbic acid plus 1 mg EDTA in 1.0 ml 0.4 mol/L NaH_2PO_4, pH 3.6), 20 μl of a 5 mg/L internal standard (2′,3′,4′-trihydroxyacetophenone) and 20 μl β-glucuronidase/sulfatase (98 U/L β-glucuronidase and 2.4 U/L sulfatase) were added to 200 μl plasma and the mixture incubated at 37°C for 45 min. After enzyme digestion, flavanols were extracted with 500 μl acetonitrile, then the 500 μl supernatant was removed, dried under purified nitrogen and reconstituted in 200 μl of an aqueous HPLC mobile phase. Flavanols were determined using an HPLC system equipped with a Zorbax ODS C_{18} column (4.6×150 mm, 3.5 μm) and a Coularray 5600 A detector (ESA, Inc. Chelmsford, MA). The quantification of individual flavanols was calculated according to calibration curves constructed with commercially available standards (Sigma), with linear relationships of R^2>.999. Analyses were performed in duplicate. The quantitation limit on the column for flavan-3-ols was 0.357 pmol. The CVs of the intra- and interday assays were 3.0 and 9.0%, respectively.

2.6. Measurement of total antioxidant performance

The fluorescent probe BODIPY 581/591 was incorporated into the lipid compartment of plasma at a final concentration of 2 μmol/L, as reported previously [29,30]. Samples were then diluted 1:10 (v/v) with PBS and incubated at 37°C with MeO-AMVN (2 mM). Lipid oxidation kinetics were monitored by measuring the green fluorescence (λex=500, λem=520 nm) of the oxidation product of BODIPY 581/591 using a multiwell plate reader (Wallack Victor 2, PerkinElmer, Boston, USA). Results are expressed as a Q_{value} calculated as follows: $Q=T_{sample}-T_{control}/T_{control}$, where T_{sample} and $T_{control}$ represent the time required to reach half of the maximum fluorescence unit. Control samples were prepared using liposomes (phosphatidyl choline 2.5 mg/ml) in PBS.

Table 1
Characteristics of the study participants at baseline (Day 1)

Characteristics	Lutein	Lutein+green tea
Age (years)	61.8±6.26	59.0±6.52
Weight (kg)	79.3±12.2	68.7±11.01
Height (cm)	170.0±6.80	168.6±8.87
BMI (kg/m^2)	27.8±3.73	24.2±3.54
Waist/hip ratio	0.87±0.12	0.88±0.28
Albumin (g/dL)	4.28±0.05	4.25±0.16
Total cholesterol (mmol/L)	5.18±0.81	4.67±0.73
LDL cholesterol (mmol/L)	3.21±0.65	2.82±0.58
HDL cholesterol (mmol/L)	1.43±0.41	1.48±0.36

All values are means±S.D.

2.7. Measurements of lipid peroxidation

2.7.1. Malondialdehyde analysis

Lipid peroxidation was assessed by the measurement of malondialdehyde (MDA) using an HPLC system, as reported previously [31]. Briefly, plasma or plasma incubated with 5 mM AMVN at 42°C for 2h was treated with BHT (5% in EtOH), followed by protein precipitation using TCA (10% w/v). The mixture was reacted with TBA (0.4% w/v, in an acetate buffer, pH 3.5) and analyzed for MDA-TBA adducts by an HPLC system equipped with a Pecosphere-3 C18 column (83×4.6 mm) and a fluorescence detector (Waters 2475 multi λ), which was set Ex 515 nm and Em 553 nm. The HPLC mobile phase was 20 mM potassium phosphate buffer/acetonitrile (80:20, v/v) and the flow rate was set at 0.8 ml/min. The lower limit of detection on the column is 0.2 pmol for the MDA-TBA adduct. Endogenous MDA and AMVN-induced MDA were determined to evaluate the lipid peroxidation and susceptibility of plasma against oxidation, respectively.

2.7.2. Total HODE and 8-iso-PGF$_{2\alpha}$ analyses

Total hydroxyoctadecadienoic acid (t-HODE) and 8-iso-PGF$_{2\alpha}$ were measured by GC/MS as previously reported [21] with slight modification. Briefly, plasma was reduced with an excessive amount of sodium borohydride followed by saponification with potassium hydroxide. The identification and quantification of total t-HODE and 8-iso-PGF$_{2\alpha}$ were determined by their retention times and mass patterns (m/z=440, 369, 225 for HODE and 571, 481 for 8-iso-PGF$_{2\alpha}$). Ions at 440 and 481 were selected for quantification for HODE and 8-iso-PGF$_{2\alpha}$, respectively, using the internal standard 8-iso-PGF$_{2\alpha}$ −d$_4$ (m/z=485). The isomers of 9-(*Z*,*E*)- and 13-(*Z*,*E*)-HODE, 9-(*E*,*E*)-HODE and 13-(*E*,*E*)-HODE were adequately separated using this method.

2.8. Dietary assessment

Fruits and vegetable intakes were assessed with Fred Hutchinson Food Frequency Questionnaire [32,33] and calculated using Nutrition Data System for Research software version 4.04 32, developed by the Nutrition Coordinating Center, University of Minnesota, MN. The questionnaires were self-administered and reviewed for completeness by a research dietician.

2.9. Statistical analysis

A repeated-measures analysis of variance with a Holm–Sidak multiple comparison test was used to measure the effect of supplementation on plasma carotenoid concentrations and to measure biomarkers for antioxidant activity for each day with respect to Day 1. When an equal variance test failed, Friedman repeated-measures analysis of variance on ranks with a Tukey multiple comparison test was used. Data analysis was carried out with SigmaStat (Ver 3.1, Systat Software Inc, Point Richmond, CA).

3. Results

Characteristics of the study subjects are presented in Table 1. Although the difference was not significant at baseline (Day 1), body mass index (BMI) tended to be higher in the lutein group as compared to that in the lutein+green tea extract group. The mean baseline (Day 1) albumin and lipid profiles were within normal ranges, with no significant difference between the two groups.

The median fruit and vegetable intakes of the lutein plus green tea group were reduced by 0.58 servings/day after the 2-week run-in phase of eating less than two servings of light-colored fruits and vegetables per day. This was reflected by a significant decrease in plasma total carotenoids in the lutein plus green tea extract group, as shown in Table 2. During the run-in phase, vegetable intake was reduced by 0.59 and 0.5 servings/day in the lutein and lutein plus green tea extract groups, respectively. Plasma lutein concentrations also tended to be decreased in both groups, although the differences were not significant.

Fig. 1 shows the plasma lutein, total carotenoid and α-tocopherol concentrations after supplementing the lutein or lutein plus green tea extract for 16 weeks. Plasma lutein concentrations were significantly increased from baseline on Day 29 and throughout the study period (P<.01) by supplementing 12 mg/day of lutein, so that the values were 177% and 190% of baseline (Day 1) at Days 29 and 113, respectively. The plasma lutein concentrations of subjects in the lutein plus green tea group were also significantly increased from baseline throughout the subsequent study period (P<.05). There were no significant differences in plasma lutein concentrations between the lutein vs. lutein plus green tea extract groups throughout the study period. Plasma total carotenoid concentrations were significantly increased (P<.05) by 154% of baseline value at Day 57 and by 150% at Day 113 by supplementing lutein. For the subjects supplemented with lutein plus green tea extract, plasma total carotenoid concentrations were significantly increased from baseline to 176% at Day 57 and to 189% at Day 113 (P<.05). There were no significant differences in plasma α-tocopherol concentrations throughout the study period.

There were no significant changes in 0-h (14-h fasting) and 2-h (after ingesting supplement) plasma concentrations of five flavanols in 20 subjects supplemented with the lutein plus green tea extract

Table 2
Changes in plasma antioxidant concentrations and fruit and vegetable intakes of study participants after 14 days of low fruit and vegetable diets[a]

	Lutein (n=20)						Lutein+green tea extract (n=20)					
	Before			After			Before			After		
Plasma antioxidant concentrations, means±S.D. (μmol/L)												
Lutein	0.33±0.15			0.23±0.11			0.34±0.14			0.22±0.10		
Total carotenoids[b]	2.81±1.28			1.82±0.86			3.00±1.39			1.94±0.73 *		
α-Tocopherol	34.36±12.67			29.49±8.23			31.40±7.73			27.15±5.42		
Ascorbic acid	61.52±23.57			50.59±21.24			68.76±17.94			55.96±20.89		
Uric acid	326.6±93.9			328.3±101.7			279.7±74.7			286.7±75.0		
Fruits and vegetable intakes (servings/day)[c]												
Intakes (percentile)	25th	50th	75th	25th	50th	75th	25th	50th	75th	25th	50th	75th
Fruits (F)	0.20	0.64	1.25	0.21	0.79	1.25	0.50	1.00	1.50	0.15	0.50	1.00
Vegetables (V)	0.21	0.79	1.25	0.14	0.20	0.79	0.50	1.00	1.63	0.15	0.50	1.00
F and V	0.64	1.14	2.27	0.68	1.21	2.18	1.14	2.00	3.37	0.64	1.42	2.11

[a] Limited to two servings/day of light-colored fruit and vegetables.
[b] Lutein+β-carotene+lycopene.
[c] A Mann–Whitney rank sum test was performed when an equal variance test failed.
* P<.05, significantly different from Day −14 (paired t test).

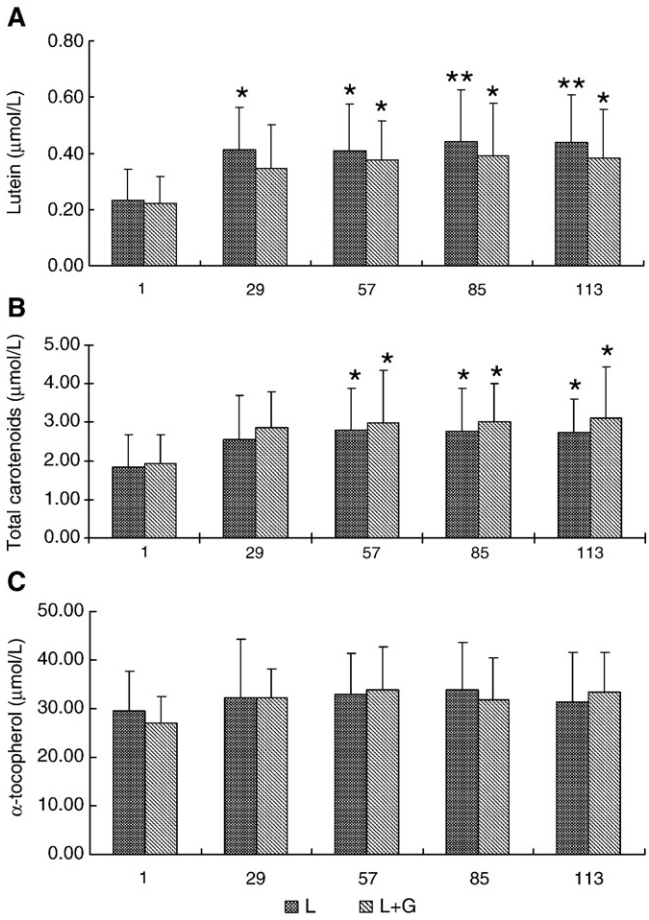

Fig. 1. Plasma fat-soluble antioxidant concentrations over time in older adults (aged 50–70 years) supplemented with either lutein (n=20) or lutein+green tea extract (n=20) for 112 days. The lutein group (L) supplemented with 12 mg/day lutein and the lutein +green tea extract group (L+G) supplemented with 12 mg/day lutein and 200 mg/day green tea extract. (A) Lutein. (B) Total carotenoid (lutein+β-carotene+lycopene). (C) α-Tocopherol. The data are expressed as mean±S.D. One-way repeated-measures ANOVA with Tukey multiple comparison was performed. When an equal variance test failed, Friedman repeated-measures analysis of variance on ranks with a Tukey multiple comparison test was used. *P<.05, **P<.01, significantly different as compared with the baseline. Samples were analyzed by HPLC with UV detection for carotenoids and tocopherol.

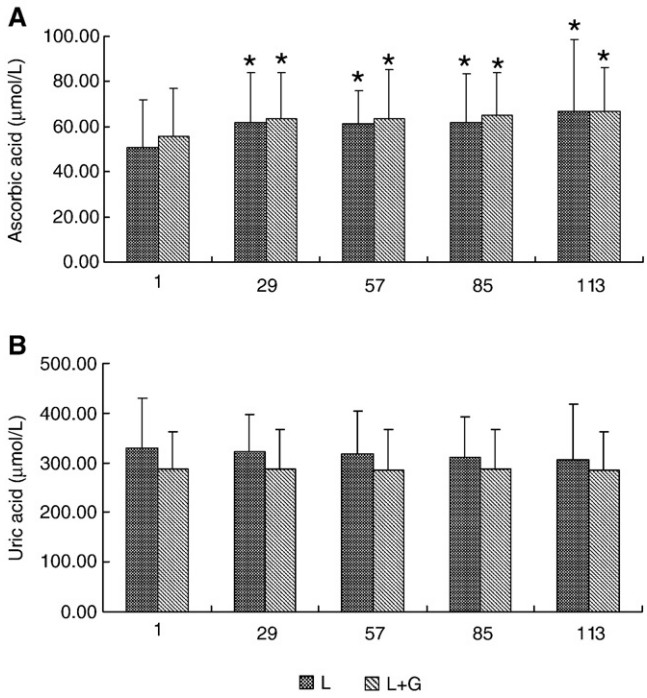

Fig. 2. Plasma water-soluble antioxidant concentrations over time in older adults (aged 50–70 years) supplemented with either lutein (n=20) or lutein+green tea extract (n=20) for 112 days. The lutein group (L) supplemented with 12 mg/day lutein and the lutein+green tea extract group (L+G) supplemented with 12 mg/day lutein and 200 mg/day green tea extract. The data are expressed as mean±S.D. One-way repeated-measures ANOVA with Tukey multiple comparison was performed. When an equal variance test failed, Friedman repeated-measures analysis of variance on ranks with a Tukey multiple comparison test was used. *P<.05, significantly different as compared with the baseline. Samples were analyzed by HPLC with electrochemical detection for ascorbic acid.

throughout the study period. Plasma water-soluble antioxidant concentrations of ascorbic acid and uric acid are presented in Fig. 2. The plasma ascorbic acid concentration was significantly increased to 126% and 135% of the baseline value on Days 29 and 113, respectively, in the lutein-supplemented group (P<.05), and 13% and 20% increase at Days 29 and 113, respectively (P<.05), in the lutein plus green tea extract-supplemented group (P<.05). No significant increase in plasma uric acid concentration was observed in subjects supplemented with either lutein or lutein plus green tea extract throughout the study period.

There were no significant changes in plasma total, LDL- and HDL-cholesterol concentrations after supplementing lutein or lutein plus green tea extracts (data are not shown). The effects of lutein or lutein plus green tea extract supplementation on plasma antioxidant capacity and lipid peroxidation are shown in Table 3. There was no significant change in any biomarker for antioxidant activity as well as in lipid peroxidation throughout the study period.

Table 3
Antioxidant capacity and lipid peroxidation of subjects with lutein or lutein plus green tea extract

	Lutein (n=20)			Lutein+green tea extract (n=20)		
	Day 0	Day 29	Day 113	Day 0	Day 29	Day 113
TAP (Q value)	2.41±0.43	2.22±0.65	2.51±0.22	2.56±0.37	2.47±0.58	2.65±0.20
MDA (μM)	0.50±0.08	0.52±0.11	0.52±0.09	0.54±0.13	0.52±0.08	0.52±0.10
MDA ratio[a]	8.16±4.27	8.51±3.55	6.95±1.47	8.99±6.01	9.11±4.85	9.24±4.57
8-isoprostane (nM)[b]	1.89±1.92	1.34±1.32	1.42±0.75	2.51±2.41	2.59±2.28	2.87±2.70
t-HODE (μM)	0.37±0.09	0.37±0.11	0.38±0.09	0.31±0.09	0.36±0.11	0.37±0.09
ZE/EE[c]	1.46±0.39	1.55±0.40	1.46±0.41	1.51±0.29	1.59±0.40	1.53±0.50

All values are means±S.D. No significant difference among groups (one-way repeated-measures ANOVA with Tukey multiple comparison was performed; when an equal variance test failed, Friedman repeated-measures analysis of variance on ranks with a Tukey multiple comparison test was used). TAP, total antioxidant performance.

[a] MDA-AMVN/MDA: 2,2′-azobis(2,4-dimethylvalenomitrile)-induced MDA/MDA.

[b] t8iso-P: total 8-iso-prostaglandin $F_{2\alpha}$.

[c] The ratio of stereoisomers, 9,13-(*Z*/*E*)-HODE/9,13-(*E*,*E*)-HODE; 9,13 (*Z*/*E*)-HODE=9-(Z/E)-HODE and 13-(*Z*/*E*)-HODE, 9,13-(*E*/*E*)-HODE=9-(*E*/*E*)-HODE and 13-(*E*/*E*)-HODE.

4. Discussion

The current study shows that daily supplementation of either 12 mg lutein or 12 mg lutein plus 200 mg green tea extract for 16 weeks to older (50–70 years) subjects increased the plasma concentrations of lutein but did not affect overall antioxidant status or oxidative stress in vivo. Lutein concentration showed an obviously significant increase at the 4 weeks of supplementation and then throughout the study period in both groups in accordance with our previous study [34]. However, there were no significant changes in the fasting plasma flavanol concentrations after supplementing 200 mg of green tea extract along with 12 mg of lutein/day probably due to the rapid clearance of flavanol from the blood stream. In addition, the reason of no significant change in flavanol at 2-h plasma could be explained by the low dose (121.22 mg of total catechins/day), form of administration (green tea extract powder) and the nature of green tea extract supplement used in the current study (free catechins). A phospholipid complex of green tea has been reported to be more efficiently absorbed, as compared to that of free catechins [35]. Further, intraindividual [36] and interindividual [37] variations in absorption, metabolism, excretion, and distribution probably contribute to the variation of the blood response.

There was a significant increase (0.39 servings/day) in fruit and vegetable intakes at Day 29 ($P<.05$) in the lutein plus green tea extract group only. In addition, vegetable intakes were significantly increased at Days 85 (0.29 servings/day increase) and 113 (0.29 servings/day increase) in the lutein group and at Day 113 (0.5 servings/day increase) in the lutein plus green tea extract group, as compared to intakes at baseline, Day 1. These minor changes in fruits and vegetable intakes may contribute to the significant increase in plasma total carotenoids and ascorbic acid in addition to lutein during the intervention period.

Measurement of prostaglandin F_2-like compounds (F_2-isoprostanes), which are produced in vivo by nonenzymic free radical-mediated peroxidation arachidonic acid, has emerged as one of the most reliable approaches assessing oxidative stress status [38,39]. In addition, t-HODE, an oxidation product of linoleic acid, is also believed to be one of the sensitive and specific indices of lipid peroxidation and a more reliable biomarker for assessing oxidative stress status in vivo [40]. In the current study, no significant changes were found in the plasma t-HODE as well as isoprostane concentrations, indicating that oxidative stress status was not significantly affected in our study subjects. Another crude marker of lipid peroxidation [41,42], MDA, also showed no significant change throughout the study period. The current study results are in accordance with the recent report by Valtuena et al. [43] showing that the food selection based on total antioxidant capacity can modify antioxidant intake without altering markers of oxidative stress or total antioxidant activity in plasma. However, it should not be overlooked that the antioxidant supplementation may alter other markers of biological function such as systemic inflammation and liver function without any changes in various markers of antioxidant capacity and lipid peroxidation as shown in the previous study by Valtuena et al [43]. It is interesting to note that several studies have pointed out that the increase in plasma antioxidant capacity observed after the consumption of flavonoid-rich foods such as wine was due to a plasma uric acid increase and not caused by the flavonoids [44–46].

Several attempts to alter overall antioxidant activity by supplementing antioxidant nutrients or implementing dietary modification in healthy subjects [47–49] have not been successful. Considering that the biological antioxidant network in healthy subjects already contains adequate amounts of water- and fat-soluble antioxidants working in an interactive manner, further increases of single or combinations of antioxidants within a physiologic range might not affect the overall in vivo antioxidant network.

Even though various combinations of “two” antioxidants in physiologic concentrations showed additive/synergistic interactions within and between the hydrophilic and lipophilic compartments in vitro [50], the much more complex in vivo system, where many different antioxidants such as uric acid and protein already exist as part of biological antioxidant network, is generally maintained in homeostasis. Thus, the potency of the entire antioxidant network is not subject to swift modifications through supplementation of single antioxidant or their combinations when given in physiologic doses to healthy people.

The data from the current study indicate that although supplementation either with lutein or lutein plus green tea extract can increase the plasma antioxidant concentrations, it does not significantly affect body antioxidant status or oxidative stress in well-nourished adults aged 50–70 years.

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