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Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy nonsmokers

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ABSTRACT

Objective: The aim of this study was to determine whether lutein affected biomarkers related to cardiovascular diseases (CVD) in healthy nonsmokers.
Methods: A randomized, double-blind, placebo-controlled trial of lutein supplementation was conducted in healthy nonsmokers. 117 eligible subjects were randomly assigned to receive 10 or 20 mg/d of lutein or placebo for 12 weeks. Levels of plasma carotenoid concentrations, total antioxidant capacity (TAOC), the lipoprotein profile, and antioxidant enzymes activities were determined at baseline and at 6, and 12 weeks after the initiation of treatment. Biomarkers of oxidative damage to protein and lipids, and C-reactive protein (CRP) concentrations were measured at baseline and after supplementation.
Results: Plasma lutein and TAOC significantly increased in both active treatment groups during 12 weeks. A significant reduction was found in malondialdehyde in the 20 mg lutein group. CRP concentration decreased in a dose-dependent manner for lutein supplementation, and there was a significant between-group difference in CRP between the 20 mg lutein and the placebo group. Serum CRP was directly related to the change in plasma lutein and TAOC for both active treatment groups.
Conclusion: The results support the possibility that lutein supplementation reduce biomarkers of CVD risk via decreased lipid peroxidation and inflammatory response by increasing plasma lutein concentrations and antioxidant capacity.

1. Introduction

Cardiovascular diseases (CVD) are the major health problem and the leading cause of death worldwide, accounting for 32.8% of all deaths in 2008 in the United States [1]. Oxidative damage and inflammation have been implicated in the pathogenesis of CVD [2,3]. If antioxidant defense systems are not sufficient for endogenous or exogenous oxidants and the balance between the production and elimination of reactive oxygen species is disrupted, the redox-active molecules generated during normal aerobic metabolism or pathological biological processes can attack membranes and initiate a chain reaction resulting in lipid peroxidation, leading to cell and tissue damage [2]. Inflammation plays a key role not only in the pathogenesis of all stages of atherosclerosis and plaque rupture but also in the subsequent microcirculatory dysfunction [3]. Both oxidative stress and inflammation are potential therapeutic targets in CVD, and biomarkers could help to identify and monitor the treatment effect.

Lutein is a member of the oxygenated carotenoids abundant in marigold flowers, egg yolks and dark-green leafy vegetables such as spinach and kale. Absorption and metabolism of lutein is assumed to follow a similar route of other lipophilic compounds [4]. After being absorbed via the mucosa of the small intestine and taken up by hepatocytes, lutein is incorporated into lipoproteins, which are then released into the systemic circulation [4]. As one of the major carotenoids found in human plasma and tissues, lutein is hypothesized to have the capacity to modulate defense and repair systems that operate in response to oxidative damage and inflammation [5]. Most of the past studies were designed to focus on the protective roles of lutein in eye health, and had revealed that lutein had beneficial effects on improving visual function and preventing the initiation and progression of age-related macular degeneration, which was also in agreement with findings of our intervention studies [6,7]. Our previous meta-analyses also suggested that high dietary intake of lutein yielded a 32% reduction in risk of choroidal neovascularisation, a disease sharing several inflammatory mechanisms with atherosclerosis [8]. The evidence from in vitro and

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preclinical animal studies had indicated that lutein could inhibit the inflammatory response of monocytes to artery wall cell modification of LDL and reduce atherosclerotic lesion formation; but until recently the human study specifically concerning the efficacy of lutein supplementation is still limited [9]. Moreover, lutein is more effective in preventing lipid peroxidation than other antioxidants, and can retard the cumulative effects of oxidative damage even at high oxygen tension; however, to date, there is little information on the antioxidant activity of lutein in vivo [10]. In addition, although epidemiological studies indicated that lutein consumption above 6–14 mg per day was associated with significant reductions in risk for age-related diseases such as age-related macular degeneration and cataracts, the daily intake of lutein by Americans and Europeans was only approximately 2 mg per day and tended to decline [11,12]. It is worth noting that increasing intakes of lutein should be warranted.

Therefore, the objective of the present study was to examine the effect of supplementation with lutein on biomarkers of oxidative stress and inflammation in healthy nonsmokers in a randomized, double-blind, placebo-controlled trial.

2. Methods

2.1. Subjects

Participants were recruited from local universities and communities through referrals and advertisements. They were eligible for the study if they were aged 20–80 years, had a body mass index between 18 and 30 kg/m^2, and were assessed to be in good health based on medical history, physical examination, and clinical laboratory tests.

Individuals were excluded from the study if they were current smokers, had a history of myocardial infarction or stroke within the previous 1 year, untreated thyroid disorder, cancer, active neoplasms, surgery, or alcohol dependence, had significant gastrointestinal, renal, pulmonary or hepatic disease, had inflammatory diseases or recent infection, were vegetarian, and consumed any nutritional supplements containing vitamin or carotenoid within 6 months of enrollment. Subjects with C-reactive protein (CRP) levels greater than 10 mg/l were also excluded from the study.

The study was carried out in accordance with the instructions of the Declaration of Helsinki. Written informed consent was obtained from each participant prior to participation. The protocol was approved by the institutional review board of the Xi'an Jiaotong University College of Medicine.

2.2. Study design

The study was a randomized, double-blind, placebo-controlled, 12-wk intervention study. During the screening visit, participant information including demographics, clinical history, and physical and laboratory results was collected for eligibility. At the end of the baseline assessment, participants who remained eligible for study entry were randomly assigned in a 1:1:1 ratio to receive 10, or 20 mg/d of lutein or placebo, according to a computer-generated random number sequence in sex-stratified blocks of 6. Lutein (85% pure) was supplied as capsules of marigold flower extract in soybean oil; placebo capsules contained soybean oil. All capsules were manufactured by Beijing Yuguang Bioscience Research Center Co (Beijing, China). The lutein and placebo capsules were identical in appearance, weight and taste. Treatment allocations were blinded to participants, data analysts, and all study and laboratory personal through study completion. The treatment assignment code was produced and maintained by a staff member not directly involved in collection of outcome data.

Participants were counseled to maintain their usual dietary habits and to abstain from taking supplements containing carotenoids during the study. Adherence to the study protocol was evaluated by interviewing the subjects and by returned capsule counts at weekly visits. For accurate estimation of daily food intake during the study, the subjects completed a validated food-frequency questionnaire at baseline and at the last visit. Study assessments were performed at baseline and at 6, and 12 weeks after the initiation of treatment. At each visit, participants were also queried about adverse events.

2.3. Outcome measures

Fasting blood samples were taken at baseline and at 6, and 12 weeks. Plasma was separated within 3 h by centrifugation at 1500 × g for 10 min at 4 °C, and was immediately divided into aliquots for immediate analysis or for storage at −80 °C until analysis. All biochemical measurements were carried out in duplicate or triplicate for individual samples.

Plasma carotenoid concentrations were analyzed by high-performance liquid chromatography (HPLC) according to a method described previously [13]. Proteins were precipitated with ethanol. B-apo-8′-carotenal (Fluka Chemical Corp, Ronkonkoma, NY, USA) was used as internal standard. The carotenoids were extracted twice with hexane and the analysis was performed using an Agilent Technologies 1100 Series HPLC system with a C30 reversed-phase column with detection at 450 nm. Carotenoid concentrations were calculated by means of a mix of standards containing lutein and β-carotene (Sigma Chemical Co, St. Louis, MO, USA). The recovery was between 90% and 100%. The intra-assay coefficients of variation for analysis of lutein and zeaxanthin, and β-carotene averaged 3.6, 7.4, and 8.7%, respectively.

Plasma total antioxidant capacity (TAOC), as an estimation of the total amount of antioxidants present in plasma, was measured using the azinodiethyl-benzthiazoline sulphate (ABTS) method with Trolox as standard. Results were expressed as mmol Trolox equivalent/l of plasma, and intra-assay and inter-assay variation of the TAOC assay were 2.3 and 4.8%, respectively.

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triacylglycerol concentrations were measured using enzymatic colorimetric methods with commercial kits (Boehringer, Mannheim, Germany), with intra- and inter-assay coefficient of variation of 1.2%–3.8%. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated by using the Friedwald formula.

Enzyme activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were analyzed using a Model 450 microtiter plate reader (Bio-Rad Laboratories, Hercules, CA, USA). Plasma GPx activity was evaluated by the subsequent oxidation of NADPH at 340 nm using a coupled reaction system with t-buthyl hydroperoxide (Thermo Fisher Scientific Inc, Chelmsford, MA, USA) as substrate [14]. SOD activity was determined by the inhibition of the rate of cytochrome c reduction mediated by the superoxide anions that were produced by xanthine–xanthine oxidase at 550 nm. CAT activity was determined by measuring the rate of decomposition of hydrogen peroxide at a wavelength of 240 nm. Intra- and inter-assay variations were below 5% for all these assays.

Plasma malondialdehyde (MDA), a marker of lipid peroxidation, was determined as thiobarbituric acid reactive substances (TBARS) using a fluorometric method, with intra- and inter-assay variations of 4.4 and 8.6%, respectively. As a biomarker of protein oxidation, carbonyl content was quantified by the reaction with 2,4-dinitrophenylhydrazine, as previously described by Burcham et al. [15]. The amount of protein was determined by the cyanmethemoglobin method.

The concentration of CRP was measured by polyethylene glycol-enhanced immunoturbidimetric assay on the Hitachi 911 automatic analyzer (Roche Diagnostics, Indianapolis, IN, USA), which has inter- and intra-assay coefficients of variation of <5%.

2.4. Sample size calculations and statistical analysis

It was estimated that 34 subjects per treatment group would be required for 85% power and 5% level of significance to detect an approximate 30%–20% decrease in CRP [16]. To ensure a sufficient sample size, a total of 117 subjects were included in the study.

Data were analyzed according to the intention-to-treat principle. The Kolmogorov–Smirnov test was used to assess whether continuous data were normally distributed. Baseline variables among treatment groups were compared using analysis of variance (ANOVA), Kruskal–Wallis or the chi-squared test, as appropriate. Within-group changes from baseline for each outcome variable were tested by paired *t* tests. Differences in outcomes among treatment groups in terms of change from baseline were analyzed using analysis of covariance (ANCOVA), with covariate adjustment for baseline values; the least significant difference procedure was used for multiple pairwise comparisons. Pearson regression analysis was used to quantify univariate correlations. *P* values less than 0.05 were considered statistically significant. All data analyses were preformed using SPSS statistical software version 11.0 (SPSS Inc, Chicago, IL, USA).

3. Results

A total of 117 participants were randomized into the double-blind treatment phase. The baseline characteristics of the participants are summarized in Table 1. The mean (standard deviation [SD]) age of the study population was 55.2 (11.7) years; the mean (SD) educational achievement was 12.2 (2.6) years; and the mean (SD) body mass index was 23.4 (4.3) kg/m^2. None of subjects had active or prior CVD, but 45 (38.5%) subjects were taking anti-hypertensive, hypolipidemic or antidiabetic medication. There were no significant differences in demographic variables or baseline characteristics among three groups. Of all the subjects randomized, 116 (99.1%) completed the 12-week study and none reported any side effects during the experimental period. One participant assigned to receive 10 mg lutein was terminated from the study because of personal reasons. Mean compliance with study capsule consumption exceeded 95% in all study groups, and there were no significant differences in compliance among groups.

Table 2 presents the changes in anthropometric measurements from baseline at 12 weeks. No statistically significant differences were found among treatment groups in the changes in any anthropometric variables.

Relative to baseline, plasma lutein concentration increased from 0.30 μmol/l to 0.48 μmol/l in the 10 mg lutein group, and from 0.32 μmol/l to 0.56 μmol/l in the 20 mg lutein group at 12 weeks with no significant change in the placebo group (Table 3). An ANCOVA analysis showed the changes in plasma lutein concentrations for the 20 mg lutein group (0.24 vs −0.04; between-group difference, 0.29; 95% confidence interval [CI], 0.12–0.45; $p = 0.001$) and the 10 mg lutein group (0.18 vs −0.04; between-group difference, 0.23; 95% CI, 0.06–0.39; $p = 0.007$) were significantly greater than those for the placebo group. None of the groups had a significant change in plasma β-carotene level. There was a modest but significant increase in TAOC for both active treatment groups ($p < 0.05$) (Table 3).

Lutein supplementation did not significantly alter serum lipid or lipoprotein levels, or the levels of antioxidant enzyme activities such as GPx, SOD, and CAT (Table 3). Although the LDL/HDL ratio decreased approximately 6.0% in 20 mg lutein group, the reduction did not reach statistical significance ($p = 0.09$).

Level of MDA decreased by 0.03 μmol/l in the 10 mg lutein group and by 0.08 μmol/l in the 20 mg lutein group while it increased by 0.01 μmol/l in the placebo group; and statistically significant change in MDA was noted in the 20 mg lutein group ($p = 0.02$) (Fig. 1A). Carbonyl content remained unchanged throughout the treatment period among groups. Correlations were examined between MDA concentrations and antioxidant status of the subjects; and MDA reductions were only significantly affected by the changes of plasma lutein in the 20 mg lutein group ($r = 0.35$, $p = 0.04$).

Mean 12-week CRP concentration change was 0.09 mg/l (95% CI, −0.02 to 0.19; $p = 0.09$) for the 10 mg lutein group and 0.12 mg/l

Table 1
Baseline participant characteristics.[a]

Characteristics	Placebo group ($n = 39$)	Lutein group		*p*-Value[b]
		10 mg ($n = 38$)	20 mg ($n = 39$)	
Age, y	55.6 (11.9)	54.6 (12.8)	55.2 (11.7)	0.87
Women, *n* (%)	22 (56.4)	22 (56.4)	21 (53.8)	0.97
Education, y	12.4 (2.6)	11.8 (2.7)	12.3 (2.6)	0.58
Family history of premature CVD,[c] *n* (%)	7 (17.9)	7 (17.9)	4 (10.3)	0.55
Blood pressure, mmHg				
Systolic	122.6 (11.4)	123.7 (14.8)	121.8 (15.3)	0.83
Diastolic	72.4 (9.5)	71.0 (8.6)	71.2 (8.0)	0.76
Medication, *n* (%)				
Antidiabetic drugs	6 (15.4)	4 (10.3)	4 (10.3)	0.72
Antihyperlipidemic	7 (17.9)	9 (23.1)	7 (17.9)	0.81
Anti-hypertensive agents	11 (28.2)	10 (25.6)	9 (23.1)	0.87
Dietary intake				
Energy, MJ	9.7 (2.4)	9.6 (2.4)	9.7 (2.3)	0.97
Retinol equivalents, μg/d	681.0 (408.4–972.2)	691.3 (447.7–1050.9)	594.0 (374.9–1087.7)	0.66
Vitamin C, mg/d	90.9 (48.8)	90.9 (59.7)	83.4 (39.0)	0.75
Vitamin E, mg/d	7.0 (6.2–9.1)	8.2 (6.5–10.9)	7.9 (6.3–10.8)	0.39
β-carotene, mg/d	3.0 (2.0–4.2)	2.7 (1.5–5.4)	3.5 (1.7–5.8)	0.68
Lutein, mg/d	2.3 (1.3–3.8)	2.1 (1.5–3.2)	2.2 (1.4–3.4)	0.98
Zinc, mg/d	10.3 (3.3)	9.6 (3.1)	10.0 (4.1)	0.68

Abbreviation: CVD, cardiovascular diseases.
[a] Value expressed as mean (standard deviation) or as median (interquartile range) unless otherwise noted.
[b] *p*-Values for any difference in groups derived from analysis of variance or Kruskal–Wallis for continuous variables or the χ^2 test for categorical variables.
[c] The CVD event was considered premature if it occurred before the age of 55 years in male and before 65 year of age in female.

Table 2
Change in anthropometric measurements from baseline at 12 weeks[a].

Characteristics	Placebo group ($n = 39$)			10 mg lutein group ($n = 38$)			20 mg lutein group ($n = 39$)			p-Value[b]
	Baseline, mean (SD)	Mean (SD), change	p-Value (vs Baseline)	Baseline, mean (SD)	Mean (SD), change	p-Value (vs baseline)	Baseline, mean (SD)	Mean (SD), change	p-Value (vs baseline)	
Weight, kg	64.1 (11.7)	−0.14 (0.98)	0.14	61.2 (11.5)	0.02 (0.77)	0.87	63.2 (12.9)	−0.04 (0.68)	0.69	0.47
Body mass index,[c] kg/m^2	23.8 (3.6)	0.06 (0.30)	0.23	23.0 (3.5)	0.05 (0.29)	0.27	23.4 (4.3)	−0.01 (0.22)	0.82	0.85
Waist circumference, cm	87.2 (11.0)	0.08 (0.65)	0.47	86.2 (10.3)	0.12 (1.31)	0.56	88.1 (10.5)	0.08 (0.92)	0.58	0.64
Body fat, %	26.2 (6.4)	0.03 (0.40)	0.63	25.8 (6.3)	−0.04 (0.61)	0.66	25.8 (6.7)	−0.03 (0.39)	0.57	0.30

[a] Value expressed as mean (standard deviation).
[b] p-Values for between-group difference in change from baseline derived from analysis of covariance analysis adjusting for baseline value.
[c] Body mass index calculated as weight in kilograms divided by height in meters squared.

(95% CI, 0.03–0.22; $p = 0.01$) for the 20 mg lutein group, and there was a significant between-group difference between the 20 mg lutein and the placebo group (−0.12 vs 0.01; between-group difference, −0.14; 95% CI, 0.02–0.25; $p < 0.02$) (Fig. 1C). Repeated-measure analyses of variance found significant time effects for CRP concentration ($p = 0.007$). In addition, CRP concentration decreased in a dose-dependent manner for lutein supplementation ($p = 0.02$). In contrast with the slight association between antioxidant status and MDA reduction, the changes in CRP concentrations were significantly associated with the changes in plasma lutein ($p < 0.005$) and TAOC ($p < 0.05$) for both active treatment groups (Fig. 2).

4. Discussion

In the present study, lutein supplementation resulted in a significant reduction of C-reactive protein that correlated with the improvement of plasma lutein and antioxidant capacity in healthy nonsmokers. Moreover, we also found that lutein induced significant decreases in lipid peroxidation.

As a defense mechanism, the body is equipped with strong antioxidant defenses to maintain an optimal balance between the prooxidant forces and the antioxidant defense systems, and thereby it is essential for normal cellular function and health [17]. The antioxidant pathways that form the major line of defense against oxidative damage can be categorically divided into nonenzymatic and enzymatic systems. Consistent with previous studies, we found plasma lutein concentrations were significantly increased after 12 weeks of supplementation with lutein [18]. In addition, our results suggested that there seemed to be an increasing tendency for TAOC, and were indicative of a slight improvement in antioxidant status of the subjects as a result of the supplementation with lutein. This increase in antioxidant capacity could be explained by the increase in plasma lutein levels; and the small magnitude of increase might have been due to our subjects' good baseline antioxidant status. Total antioxidant status has been reported to be reduced in smokers, and the nonsmokers generally exhibit a significantly lower toxin burden than smokers [19]. As the cells also protect themselves against oxidative damage by enzymatic antioxidant systems, we further examined the effects of lutein supplementation on antioxidant defense enzymes like SOD, GPx and CAT. In the present study the levels of these enzyme activities remained unchanged, which was in accordance with studies conducted by others [20].

Oxidative damage is present throughout life and thought to be involved in the pathogenic processes of various chronic diseases including CVD and cancers [2]. Reactive oxygen species (ROS) are responsible for the cumulative oxidative damage of biological molecules such as lipid and protein. It has been suggested that carotenoids played a part in protecting cells from oxidative damage [21]. In our study, there was a modest and significant decline in the levels of MDA for high-dose lutein group during the supplementation. The underlying mechanism of action includes trapping chain-carrying peroxyl radical and retarding the chain propagation

Table 3
Change in plasma carotenoids, total antioxidant capacity, defense enzymes activities, and lipoprotein profile from baseline at 12 weeks.[a]

Characteristics	Placebo group ($n = 39$)			10 mg lutein group ($n = 38$)			20 mg lutein group ($n = 39$)			p-Value[b]
	Baseline, mean (SD)	Mean (SD), change	p-Value (vs Baseline)	Baseline, mean (SD)	Mean (SD), change	p-Value (vs Baseline)	Baseline, mean (SD)	Mean (SD), change	p-Value (vs baseline)	
Plasma lutein (μmol/l)	0.30 (0.29)	−0.04 (0.33)	0.42	0.30 (0.21)	0.18 (0.34)[b]	0.002	0.32 (0.20)	0.24 (0.41)[c]	0.001	0.002
Plasma β-carotene (μmol/l)	0.20 (0.18)	−0.04 (0.14)	0.11	0.21 (0.22)	0.03 (0.19)	0.36	0.21 (0.18)	−0.04 (0.14)	0.09	0.95
TAOC (mmol Trolox equivalent/l)	0.48 (0.28)	0.04 (0.24)	0.31	0.42 (0.31)	0.10 (0.29)	0.03	0.41 (0.26)	0.08 (0.23)	0.04	0.50
GPx (U/ml)	0.41 (0.20)	−0.00 (0.09)	0.99	0.38 (0.12)	0.02 (0.10)	0.13	0.43 (0.16)	−0.02 (0.09)	0.16	0.11
SOD (U/l)	34.9 (10.2)	0.9 (12.6)	0.66	34.9 (8.0)	1.6 (12.4)	0.43	33.9 (12.8)	1.6 (8.9)	0.59	0.97
CAT (U/l)	12.4 (3.0)	−0.1 (0.8)	0.28	12.6 (2.1)	−0.1 (0.9)	0.25	12.4 (2.6)	−0.1 (0.4)	0.15	0.41
Total cholesterol (mmol/l)	4.74 (1.58)	−0.08 (1.21)	0.70	4.81 (0.94)	−0.13 (0.56)	0.16	4.70 (0.96)	0.05 (0.47)	0.51	0.62
HDL-C (mmol/l)	1.39 (0.40)	−0.08 (0.35)	0.15	1.43 (0.29)	−0.05 (0.34)	0.33	1.34 (0.24)	0.04 (0.21)	0.21	0.78
LDL-C (mmol/l)	2.88 (0.69)	−0.09 (0.66)	0.37	3.06 (0.71)	−0.14 (0.64)	0.20	2.97 (0.75)	−0.11 (0.57)	0.23	0.96
Triacylglycerol (mmol/l)	1.32 (1.38)	0.01 (0.74)	0.98	1.33 (0.65)	0.02 (0.34)	0.76	1.35 (0.75)	0.12 (0.45)	0.09	0.56

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SOD, superoxide dismutase; TAOC, total antioxidant capacity.
[a] Value expressed as mean (standard deviation).
[b] p-Values for between-group difference in change from baseline derived from analysis of covariance analysis adjusting for baseline value.
[c] Significantly ($p < 0.05$) different from the placebo group.

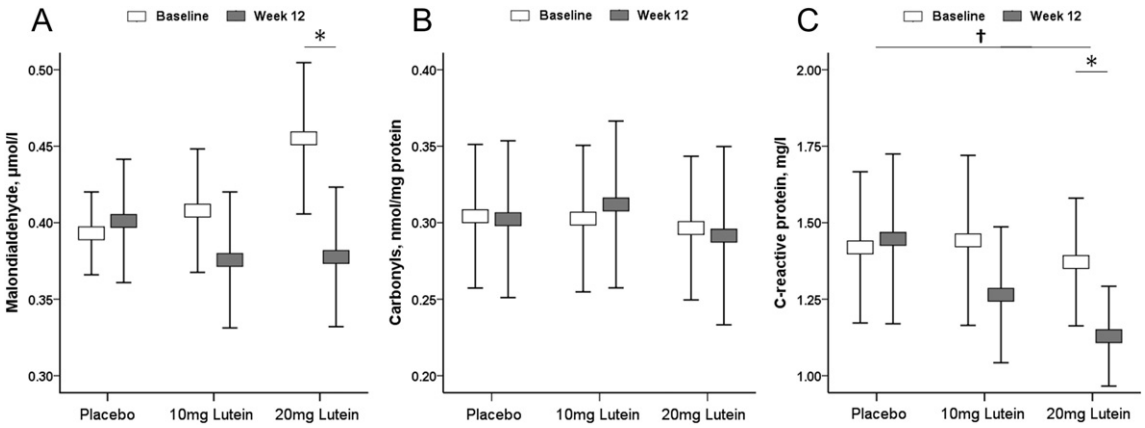

Fig. 1. Change in malondialdehyde (panel A), carbonyls (panel B), and C-reactive protein (panel C) from baseline at 12 weeks for three treatment groups after lutein supplementation. The horizontal bars indicate mean change and error bars indicate 95% confidence intervals. *Significantly ($p < 0.05$) different from baseline to 12 weeks. †Significantly ($p < 0.05$) different from the placebo group.

reaction. In contrast to the present study, some intervention trials that involved increased other antioxidant nutrients intakes have shown negative results regarding lipid damage [22]. This discrepancy might be explained by the special chemical structure and orientation in the membrane of lutein. Because of the presence of two hydroxyl groups, lutein is more hydrophilic and polar in blood and tissues relative to hydrocarbon carotenoids. Lutein can be oriented perpendicular to the lipid membrane [23]. It spans cell membranes with the lipophilic polyene chain inside the lipid bilayer with its polar hydrophilic hydroxyl groups close to the hydrophilic head groups of the phospholipids. This localization optimizes contact with the highly oxidizable cell membrane lipids, and enhances the effectiveness of its ability to protect the lipid matrix against oxidative damage in vivo [23,24].

Most of evidence supports the notion that inflammation plays the pivotal role in all stages of atherosclerosis, and high concentrations of CRP have been shown to predict increased cardiovascular events in several prospective studies [25]. Results from cell culture studies indicated that lutein might have the capacity to maintain the normal vascular function, and might effectively inhibit the inflammatory response of monocytes in the artery wall via signal transduction with cellular receptors; however, few human clinical trials that specifically focused on the effect of lutein on CRP concentrations have been performed [26,27]. The present study showed that lutein supplementation effectively lowered CRP concentrations. Furthermore, this CRP-lowering action might be dose-dependent because a greater reduction of CRP to a high dose of lutein supplementation had been found in our study. It should be noted that there were significant correlations between the reduction of CRP and the increase in both plasma lutein concentration and TAOC, indicating that CRP concentration can be modulated by the antioxidant status. Taken together, these findings supported the hypothesis that increased antioxidant capacity by supplementation with lutein might attenuate inflammatory responses, and ultimately lower cardiovascular risk.

Major strengths of this study included strict eligibility criteria, recruitment of individuals living in the community, low dropout rate, and high adherence to treatment. In addition, none of the participants reported supplemental use of vitamins, zinc or carotenoids, and no significant variation in these nutrient intake was found during intervention; thus, the treatment effects found in the present study can be attributed to lutein supplements rather than the use of other dietary antioxidants, which may also be related to prevention of CVD. Our study also has several limitations. First, our

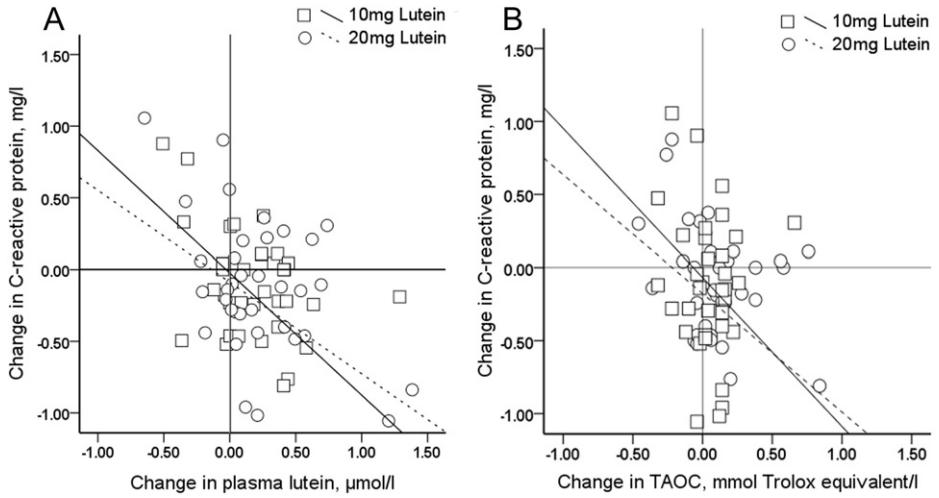

Fig. 2. Relationships between change in C-reactive protein and change in plasma lutein (panel A) and plasma total antioxidant capacity (panel B) for both active treatment groups after 12-week lutein supplementation. Abbreviations: TAOC, total antioxidant capacity. The curves in both plots indicate the linear regression functions for each group. Correlation coefficients r and nominal p-values are calculated by the Pearson test. Panel A, $r_{10\ mg\ Lutein} = -0.45$, $p = 0.004$; $r_{20\ mg\ Lutein} = -0.44$, $p = 0.005$; panel B, $r_{10\ mg\ Lutein} = -0.46$, $p = 0.003$; $r_{20\ mg\ Lutein} = -0.32$, $p < 0.05$.

sample overrepresented well-educated persons, in comparison with the whole eligible population. This might have affected the lifestyle factors, dietary habits or the distribution of antioxidants [28]. Second, the study was conducted at a single center, which potentially may limit the generalizability of study results. Third, although the duration of the study was relatively longer than most previous studies in this population, it was still short and the magnitude of benefit was smaller than was anticipated, because the clinical benefit was often delayed. Finally, the present study was not adequately powered to detect a reduction in CVD incidence; and further larger-scale and longer-term studies are needed to evaluate the effect of lutein on prevention of CVD.

5. Conclusion

In conclusion, the results of the present study showed that lutein supplementation improved plasma lutein and antioxidant capacity in healthy nonsmokers without adverse biological effects. In addition, it concomitantly inhibited the inflammatory response and reduced lipid peroxidation, thus offering an effective strategy to decrease the risk of the development of diseases related to oxidative stress and inflammation.

Conflict of interest

All authors state that they have no conflict of interest.

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