Pistachios Increase Serum Antioxidants and Lower Serum Oxidized-LDL in Hypercholesterolemic Adults

Colin D. Kay, Sarah K. Gebauer, Sheila G. West, and Penny M. Kris-Etherton

Abstract

Pistachios are high in lutein, β-carotene, and γ-tocopherol relative to other nuts; however, studies of the effects of pistachios on oxidative status are lacking. We conducted a randomized, crossover controlled-feeding study to evaluate 2 doses of pistachios on serum antioxidants and biomarkers of oxidative status in 28 hypercholesterolemic adults (LDL-cholesterol ≥2.86 mmol/L). Participants consumed 3 isoenergetic diets for 4 wk each after a 2-wk baseline Western diet. Experimental diets included a lower-fat control diet without pistachios (25% total fat) with 1 serving/d (i.e. 32–63 g/d; energy adjusted) of pistachios (1 PD; 10% energy from pistachios; 30% total fat) or with 2 servings/d (63–126 g/d; energy adjusted) of pistachios (2 PD; 20% energy from pistachios; 34% total fat). When participants consumed the pistachio-enriched diets, they had higher plasma lutein (P < 0.0001), α-carotene, and β-carotene (P < 0.01) concentrations than after the baseline diet. After consuming the pistachio diets, participants had greater plasma lutein (P < 0.001) and γ-tocopherol (P < 0.05; 2 PD only) relative to the lower-fat control diet. After the 2 PD diet period, participants also had lower serum oxidized-LDL concentrations than following the baseline diet period (P < 0.05). After both the 1 PD and 2 PD diet periods, they had lower serum oxidized-LDL concentrations than after the control diet period (P < 0.05). The change in oxidized-LDL from baseline correlated positively with the change in LDL-cholesterol across all treatments (r = 0.42; P < 0.005). After controlling for the change in serum LDL-cholesterol as a covariate, increases in serum lutein and γ-tocopherol following the 2 PD period were still modestly associated with decreases in oxidized-LDL (r = −0.36, P = 0.06 and r = −0.35, P = 0.08, respectively). This suggests that a heart-healthy diet including pistachios contributes to the decrease in the serum oxidized-LDL concentration through cholesterol-lowering and may provide an added benefit as a result of the antioxidants the pistachios contain.

Introduction

Epidemiologic and clinical studies have demonstrated significantly beneficial cardiovascular effects of tree nuts and peanuts (1,2). The cardioprotective effects of tree nuts and peanuts have been associated with their favorable fatty acid profiles in addition to their bioactive constituents/phytochemicals (1,2). We have shown previously that the inclusion of pistachios in a healthy diet beneficially affects lipids and lipoproteins in a dose-dependent manner (3). Relative to other nuts, pistachios are a rich source of antioxidants, including lutein, β-carotene, and γ-tocopherol in addition to containing selenium, flavonoids, and proanthocyanidins (4,5). As would be expected, pistachios have a relatively high in vitro antioxidant capacity (6–8).

Oxidized LDL-cholesterol (9,10) and lipid peroxidation products are found in elevated concentrations in atherosclerotic plaques (11,12) and are thought to play an important role in the development and progression of atherosclerosis (9–11,13). Thus, strategies that reduce in vivo oxidative stress are thought to confer cardioprotective effects. The present study was designed to evaluate a dose-response effect of pistachios on serum antioxidant status and oxidative biomarkers of cardiovascular disease (CVD) (8).

Previous dietary interventions with pistachios conducted in humans have shown improvements in lipoprotein profiles.
(7,14,15) and one reported a beneficial effect on serum antioxidant status (measured using a malondialdehyde assay) in 44 males and females who consumed a diet that provided 20% of their energy as pistachios for 3 wk (7). However, all of these studies were conducted in free-living individuals and none controlled for SFA or antioxidant content of the background diets. Therefore, it is difficult to establish the extent to which pistachio-derived antioxidants contributed to the observed effects on antioxidant capacity over the displacement of saturated fat resulting from nut consumption. The present clinical study of the antioxidant effects of pistachios is unique, because it utilized a controlled-feeding crossover design and 2 doses of pistachios and controlled for saturated fat intake. The aim of our study was to evaluate the effects of supplementing a lower-fat diet with pistachios (10% of energy/d from pistachios (1 PD) and 20% of energy/d from pistachios (2 PD)) on serum oxidative risk factors in a controlled-feeding trial. We evaluated serum oxidative risk factors associated with CVD, including oxidized-LDL and total lipid hydroperoxides, in addition to other markers of serum global redox state, including serum tocopherol, carotenoid, and uric acid and whole blood glutathione concentrations. We hypothesized that a lower-fat diet supplemented with 2 different doses of pistachios would dose-dependently confer greater cardioprotective effects on antioxidant status than the lower-fat control diet.

Methods

Participants and study design. Healthy, nonsmoking males and females [n = 10 M, 18 F; ages 35–61 y, BMIs = 26.8 ± 0.7 kg/m²] with moderately elevated LDL-cholesterol (>2.6 mmol/L; 50–95th percentile based on NHANES data and the 3rd Adult Treatment Panel guidelines (16)] completed the study. One participant dropped out of the study due to an inability to comply with study protocol. Participants were required to have triacylglycerol (TG) concentrations < 3.94 mmol/L, blood pressure < 160/90 mm Hg, BMI between 21 and 35 kg/m², and fasting blood glucose ≤ 6.9 mmol/L. The following exclusion criteria were used: use of blood pressure or cholesterol-lowering medication; supplemental use of psyllium, fish oil, soy lecithin, or phytoestrogens; being pregnant or wishing to become pregnant 6 mo before or during the study; having diabetes, liver, kidney, or autoimmune diseases, or previous stroke; and inability to comply with the study protocol. A complete list of baseline characteristics (including blood pressure, glucose, insulin, and other parameters) has been reported previously (3). The study was approved by the Institutional Review Board at The Pennsylvania State University and all participants gave written informed consent prior to enrollment.

A 3-period randomized, crossover controlled-feeding design was implemented. All participants consumed a 2-wk run-in diet, which was a typical Western diet that served as their baseline diet. They were then randomized to each of the 3 experimental diets for 4 wk. Short compliance breaks (average of 2 wk) separated the diet periods.

Diet. The energy and macro- and micronutrient compositions of the test diets (Table 1) were determined by Nutritionist Pro (Axxya Systems) approximations and approximated from the USDA Nutrient Database (5) where necessary. All test diets were isocaloric and matched for saturated fat [≤8% of energy] and cholesterol (<300 mg/d); they varied in the amount of unsaturated fat provided by the pistachios. The diets varied in total fat within the range of 25–35% of energy as recommended by the 3rd Adult Treatment Panel of the National Cholesterol Education Program (16). Diets also were matched for the antioxidants vitamins A, C, and E, tocopherols, lutein, selenium, and folate. The baseline diet and control diet did not contain pistachios. Pistachio intake was calculated as 10 and 20% of energy for the 1 PD and 2 PD, respectively. Pistachio amounts ranged from 32 to 63 g/d for the 1 PD and 63 to 126 g/d for the 2 PD, depending on energy level. All food was prepared and consumed by the participants at the Metabolic Diet Study Center at The Pennsylvania State University. They ate 1 meal per day in the center and had their other meals and snacks packed for offsite consumption. Adherence to the experimental diets was checked daily using compliance questionnaires. In addition, participants were weighed daily to assess compliance and ensure that body weight was maintained. Specific details of the diet protocol have been described elsewhere (3).

Data collection. Blood samples were collected in the morning after a 12-h fast on 2 consecutive days at the end of each diet period by nurses at The Pennsylvania State University General Clinical Research Center (University Park, PA). Serum oxidation markers (oxidized-LDL, lipid hydroperoxides), antioxidant status measures (tocopherols, carotenoids, uric acid, and glutathione), and serum lipid and lipoprotein concentrations were measured at the end of each feeding period. Serum for oxidation and antioxidant analysis was collected on 1 d, whereas plasma for lipid and lipoprotein analysis was collected on 2 consecutive days (and averaged for consistency). Blood was collected into Vacutainer tubes (VWR Scientific Products), allowed to clot at room temperature for 25 min (serum only), and separated by centrifugation for 15 min at 3000 × g at 0°C. Serum was immediately aliquoted into cryovials (VWR Scientific Products), flash-frozen in liquid nitrogen, and stored at −80°C until the completion of the study. Blood samples used for measuring glutathione were first treated with 1-methyl-2-vinylpyridinium tri fluoromethanesulfonate and samples for tocopherol and carotenoid analysis were collected in amber cryovials.

Analytical methods. Serum oxidized LDL were measured by a solid phase 2-site ELISA (Mercodia Oxidized LDL ELISA, no. 10–1158–01; Alpco Diagnostics) based on the direct sandwich technique and adapted from previous studies (17) [intraassay CV = 3.2%]. The lipid hydroperoxide assay measures hydroperoxides in the isolated lipid-phase of the serum directly following ferrous ion reduction. This assay involves the Cayman LPO kit (no. 705003) that is based on the FOX assay but has been modified for use with a 96-well plate reader (intraassay CV = 1.4%).
TABLE 2 Circulating concentrations of biomarkers of oxidative stress, antioxidants, and LDL-cholesterol in healthy, moderately hypercholesterolemic men and women before and after consuming 0, 1, and 2 servings of pistachios/d for 4 wk

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Baseline</th>
<th>Control</th>
<th>1 PD</th>
<th>2 PD</th>
<th>Treatment effect&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized-LDL, U/l</td>
<td>48.57 ± 3.02</td>
<td>51.29 ± 3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.57 ± 3.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.43 ± 3.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0002</td>
</tr>
<tr>
<td>Lipid hydroperoxide, μmol/l</td>
<td>0.60 ± 0.21</td>
<td>0.50 ± 0.21</td>
<td>0.54 ± 0.21</td>
<td>0.65 ± 0.21</td>
<td>0.9</td>
</tr>
<tr>
<td>Uric acid, μmol/l</td>
<td>269.04 ± 17.70</td>
<td>245.44 ± 17.70</td>
<td>248.98 ± 17.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>244.85 ± 17.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GSH, μmol/l</td>
<td>934.71 ± 27.33</td>
<td>916.44 ± 27.15</td>
<td>874.26 ± 27.82</td>
<td>847.02 ± 27.33</td>
<td>0.08</td>
</tr>
<tr>
<td>γ-Tocopherol, nmol/l</td>
<td>10.06 ± 0.79</td>
<td>9.72 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.68 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.28 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>α-Tocopherol, nmol/l</td>
<td>31.35 ± 1.51</td>
<td>31.23 ± 1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.97 ± 1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.44 ± 1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td>Lutein, nmol/l</td>
<td>230.72 ± 21.89</td>
<td>239.37 ± 21.89</td>
<td>337.77 ± 22.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>421.89 ± 21.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lycopene, nmol/l</td>
<td>674.37 ± 33.62</td>
<td>616.69 ± 33.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>571.74 ± 33.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>506.25 ± 33.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Zeaxanthin, nmol/l</td>
<td>51.22 ± 3.41</td>
<td>63.84 ± 3.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.23 ± 3.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.81 ± 3.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>α-Carotene, nmol/l</td>
<td>232.18 ± 31.66</td>
<td>304.52 ± 31.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>345.41 ± 32.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>337.53 ± 31.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>β-Carotene, nmol/l</td>
<td>561.52 ± 52.75</td>
<td>642.84 ± 52.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>706.52 ± 54.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>714.87 ± 52.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0007</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>3.43 ± 0.11</td>
<td>3.42 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.08 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are expressed as mean ± SE, n = 28. *Significantly different from baseline, P < 0.05. Means for diet treatments with superscripts without a common letter differ, P ≤ 0.05.

<sup>2</sup> Effect of treatment (raw values).

Serum tocopherol (α and γ) and carotenoid (α and β) analyses were conducted in the laboratory of Dr. Thomas Wilson at the Department of Clinical Laboratory and Nutritional Sciences, University of Massachusetts (Lowell, MA) using HPLC with photodiode array detection for carotenoids and fluorescence detection for tocopherols. Samples were prepared according to the procedures described by Handelman et al. (18). Uric acid was measured commercially by Zeptronix using a Cobas Mira (Roche, no. 25–4668) clinical automated chemical analyzer and standard methods (19,20).

Total cholesterol (TC) and TG were determined by enzymatic procedures with commercially available kits (TC, CHOP/PAP, Boeringer; TG and free glycerol, Abbott Laboratories, Diagnostic Division). HDL-cholesterol was estimated according to the modified heparin-manganese precipitation procedure of Warnick and Albers (21), whereas LDL-cholesterol was calculated by Friedewald’s equation. Intraassay CV ranged from 1.5 to 1.6% for lipids and lipoproteins.

Statistical analyses. All statistical analyses were performed using SAS (version 9.1). Normality was assessed via the Shapiro–Wilk test utilizing the residuals for each variable. Logarithmic or other appropriate transformations were used for variables that did not have normal distributions [lipid hydroperoxides and glutathione (GSH)]. ANOVA was used to determine whether the outcome variables differed among the four dietary conditions (baseline, control, 1 PD, 2 PD). Specifically, the mixed models procedure (PROC MIXED) was used to assess the variance of treatment, period, and their interactions as fixed effects and participant as a random effect. If an effect was significant (P < 0.05); for oxidized-LDL, α-tocopherol, γ-tocopherol, β-carotene, lutein, lycopene, we examined the effect of treatment on the magnitude of change (calculated as treatment − baseline) utilizing the mixed model procedure. In this case, treatment was the fixed effect and participant was the random effect. Degrees of freedom were adjusted for unequal group variance by Satterthwaite’s approximation. Tukey-Kramer adjusted P-values were used to examine the source of significant effects for all mixed models. Values are presented as least squares means ± SEM. P-values were considered significant at P ≤ 0.05. A post hoc analysis of lipid-adjusted carotenoids and tocopherols was conducted where concentrations were divided by the total level of plasma cholesterol and statistically analyzed using the above protocol. For variables that significantly changed with treatment, correlation analysis was used to examine whether individuals exhibiting greater increases in serum concentrations of pistachio-derived antioxidants also had greater changes in biomarkers of oxidation.

Results

Compliance to the study protocol was very good, as indicated by daily compliance questionnaires and maintenance of prestudy body weight. The diets significantly affected serum oxidized-LDL, uric acid, lutein, lycopene, zeaxanthin, α-carotene, β-carotene, γ-tocopherol, and α-tocopherol concentrations (Table 2). However, concentrations of total lipid peroxides, glutathione, and zeaxanthin did not change from baseline. The magnitude of changes in uric acid from baseline after the pistachio and control diet periods did not differ (data not shown). Diet order did not affect any of these variables.

Serum antioxidants. Serum lutein was greater following consumption of the 1 PD and 2 PD diets compared with the baseline and control diets and was greater after the 2 PD than the 1 PD period, indicating a dose-effect (P < 0.0001) (Table 2; Fig. 1). In addition, the change in serum lutein from baseline was inversely correlated with the change in serum lycopene after consumption of the 2 PD diet (r = −0.5; P ≤ 0.01). Serum α-carotene (P < 0.01) and β-carotene (P < 0.01) increased from baseline following the 1 PD and 2 PD periods (Table 2). However, compared with the control diet, the percent increase in serum β-carotene from baseline was significant only following the 2 PD period (P < 0.05) (Fig. 1A). After the 2 PD diet period, participants had a greater serum γ-tocopherol concentration (Table 2) and percent increase (Fig. 1A) compared with the control diet period. The percent decrease from baseline in the serum α-tocopherol concentration after the 2 PD period also was significantly greater than after the control diet or the 1 PD period (Fig. 1A).

Serum lycopene was higher in the participants following consumption of the baseline diet (P < 0.01) relative to all of the dietary treatments and was higher following the control diet relative to the 2 PD treatment (P < 0.01) (Table 2). However, after post hoc analysis of lipid-adjusted carotenoids (ratio of carotenoids:TC; Table 3), the lipid-adjusted lycopene concentration did not differ between the control diet and 2 PD periods (Fig. 1B).

Oxidative biomarkers. After the 2 PD period, participants had lower oxidized-LDL concentrations relative to baseline (P <
without a common letter differ: *P = 0.002). Additional post hoc correlation analyses were conducted to account for the association between LDL-cholesterol and oxidized-LDL by using the change in LDL-cholesterol as a covariate; this slightly reduced the significance of the association between the changes in serum lutein (r = −0.36; P = 0.06) and γ-tocopherol (r = −0.35; P = 0.08) with the change in oxidized-LDL.

**Discussion**

The present study demonstrates beneficial effects of pistachios on multiple biomarkers of oxidative state. We found significant decreases in serum oxidized-LDL in participants following the pistachio-enriched treatment diets relative to the control diet. This is important, because oxidized-LDL are recognized as a contributing factor for the initiation and progression of CVD (9,10). The decrease in oxidized-LDL was accompanied by a significant increase in serum concentrations of antioxidants (relative to the control diet), including γ-tocopherol (2 PD), lutein (1 PD and 2 PD), and β-carotene (2 PD) (Fig. 1A), thus indicating a beneficial effect of pistachios on concentrations of serum antioxidants. Furthermore, changes in serum lutein and γ-tocopherol were correlated with changes in oxidized-LDL during the 2 PD period, suggesting that pistachio’s antioxidants confer cardiovascular benefits that are associated with their favorable effects on oxidized-LDL.

Significant increases in γ-tocopherol were associated with decreases in α-tocopherol following the pistachio interventions, resulting in significantly lower α-tocopherol concentrations in participants following the 2 PD diet compared with the control diet period. This is a known effect of tocopherol supplementation, as one tocopherol species can inversely affect the levels of the other, although this response is generally observed at much higher intakes (22,23).

Serum lycopene was significantly lower following both the pistachio diet periods relative to the control diet period. The diets were initially matched for vitamins A, C, and E, tocopherols, and lutein, but were not initially matched for lycopene specifically. Therefore, slight differences in the amount of tomato products between the treatments could have resulted in the diets having different quantities of lycopene (24–26). In fact, postintervention HPLC analysis of representative diet treatments indicated that the lycopene concentration of the control diet (1.7 µg/g) was higher than the 1 PD (1.2 µg/g) and 2 PD (1.0 µg/g) diets, thus explaining the lower serum concentrations of lycopene following the pistachio treatments. However, when serum carotenoids were expressed relative to TC (i.e. lipid-adjusted ratios; Fig. 1B), the differences in lipid-adjusted lycopene concentrations between pistachio treatments and the control diet period were no longer significant.

Despite the decreases in lycopene, α-tocopherol, and uric acid following the diet treatments, participants still had significantly lower circulating oxidized-LDL concentrations following consumption of the pistachio diets. This suggests that foods that have multiple antioxidants, like pistachios, may be critically important in reducing a biomarker of chronic disease such as oxidized-LDL (9,10).

In the present investigation, there were significant decreases in uric acid in participants following consumption of the 1 PD and 2 PD, compared with baseline (P < 0.05). However, the magnitude of change in uric acid from baseline did not differ after consumption of the pistachio and control diets. Therefore, differences in uric acid do not appear to confound the observed

![FIGURE 1](http://example.com/figure1.png)

**FIGURE 1** Percent change from baseline in absolute (A) and lipid-adjusted (B) serum biomarkers of oxidative stress, antioxidants, and oxidized-LDL in healthy, moderately hypercholesterolemic men and women before and after consuming 0, 1, and 2 servings of pistachios/d for 4 wk. The absolute change (difference between baseline and treatment; change score) was calculated only for variables significantly altered from baseline and having significant treatment effects in the mixed model analysis of raw data. The lipid-adjusted serum carotenoids and tocopherols were established relative to TC, while oxidized-LDL were adjusted to LDL-cholesterol concentrations. Values are expressed as mean ± SEM, n = 28. Means for diet treatments without a common letter differ: *P = 0.05, **P = 0.01, ***P = 0.001, ****P = 0.0001. Abbreviations: Car, carotene; LDL-C, LDL-cholesterol; Lycop, lycopene; Ox-LDL, oxidized-LDL; Toc, tocopherol.

0.05) whereas both the 1 PD and 2 PD periods resulted in lower post-treatment concentrations compared with the control diet (P < 0.05) (Table 2). In addition, the percent decrease in oxidized-LDL from baseline after the 2 PD period was greater than following the control diet (P < 0.0001) (Fig. 1A). Correlation analysis revealed that increases in lutein and γ-tocopherol following the 2 PD period were correlated with the decreases in oxidized-LDL (r = −0.37 and r = −0.38, respectively; P = 0.05).

There was a dose-dependent decrease in the atherogenic lipoprotein profile (LDL-cholesterol:HDL-cholesterol ratio) after the pistachio diet periods, as reported elsewhere (3). The change in LDL-cholesterol from baseline was positively correlated with the change in oxidized-LDL across all treatments, indicating that the absolute concentration of LDL was the greatest determinant of oxidized LDL concentrations (r = 0.42;
effects of the pistachio diets on oxidized-LDL. It is difficult to identify the cause of the decrease in serum uric acid following the pistachio diet periods; however, it may be the result of differences in the amount of purines (27–29) in the baseline and experimental diets as a result of displacing 10 and 20% of energy with pistachios. In the present investigation, all uric acid concentrations were within the normal clinical range (29,30).

Previous studies have found that acute consumption of walnuts or almonds (at 75% energy intake) improves postprandial serum antioxidant status in humans, as measured by the total radical absorption potential, ferric reducing antioxidant potential, and oxygen radical absorption capacity assays (31). In contrast, chronic (8 wk) consumption of walnuts or cashews (20% energy intake) did not alter antioxidant status, as measured using the oxygen radical absorption capacity and prooxidant burden assays and serum GSH, in another study (32). The inconsistency in findings from studies of nut consumption has been described in several recent reviews (1,2,33). Much of this inconsistency has been attributed to a general lack of control for dietary saturated fat and/or antioxidant intakes between the control and treatment diets within studies.

One dietary intervention with pistachios in humans has shown beneficial effects on serum antioxidant status as measured by the malondialdehyde assay (7). However, this study was conducted in free-living individuals and, as suggested above, dietary SFA and antioxidant intakes were not matched among the groups, making it difficult to distinguish the contribution of antioxidants within the nuts to any change in antioxidant capacity compared with the effects of the displacement of saturated fat on serum lipoproteins and oxidation. The novelty of our study is that it was a controlled-feeding study designed not only to control for SFA intake but also levels of antioxidants in the background diets.

There was no effect of the pistachio treatments on serum lipid hydroperoxides or glutathione in the present study. Because we corrected for many of the shortfalls of previous nut interventions, including controlling for dietary SFA and background diet antioxidant content, we attribute the differential effects on the various antioxidant activity measures (i.e. lipid hydroperoxides and glutathione) to complexities in the measurement of redox state in human studies. The lack of effect of the pistachio treatments on these serum biomarkers of antioxidant status may be the result of differential rates of oxidation of proteins (i.e. lipoproteins) compared with lipids, differential activities of the various serum antioxidants, a lower sensitivity of the assays relative to the oxidized-LDL ELISA, or a basic lack of responsiveness of basal redox state in relatively healthy individuals. These are common limitations in studies investigating antioxidant status/antioxidant activity (34,35).

In summary, oxidized-LDL were correlated with LDL-cholesterol across all treatments (within-participant variation) and serum lutein and y-tocopherol were associated with reductions in oxidized-LDL following the 2 PD relative to the control diet period. When controlling for changes in LDL-cholesterol as a covariate in correlation analysis, the associations between lutein, y-tocopherol, and oxidized-LDL lost some statistical power but still suggested an added benefit of the 2 PD. In addition, the consumption of the pistachio diets resulted in significant lowering of LDL-cholesterol as well as a dose-dependent lowering of the atherogenic lipoprotein profile (LDL-cholesterol:HDL-cholesterol ratio) (3). Overall, our study suggests that consumption of pistachios in the context of a heart-healthy diet confers cardioprotective benefits beyond established lipid-lowering effects, including a decrease in oxidized-LDL, which we think is the result of both a decrease in LDL-cholesterol concentrations and an increase in serum antioxidants, such as lutein and y-tocopherol.

In conclusion, the consumption of the pistachio-enriched diets resulted in increases in serum antioxidants and decreases in oxidized-LDL relative to the control diet. These data suggest that a heart-healthy diet rich in pistachios has a beneficial effect on serum antioxidants, as well as oxidized-LDL. Beneficial effects on multiple CVD risk factors would be expected to reduce overall CVD risk beyond that achieved by decreases in LDL-cholesterol alone.

Acknowledgments
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Literature Cited


