Almonds Reduce Biomarkers of Lipid Peroxidation in Older Hyperlipidemic Subjects¹,²

David J. A. Jenkins,³–⁵* Cyril W. C. Kendall,³–⁵ Augustine Marchie,³–⁵ Andrea R. Josse,³–⁵ Tri H. Nguyen,³–⁵ Dorothea A. Faulkner,³–⁵ Karen G. Lapsley,⁶ and Jeffrey Blumberg⁷

¹Clinical Nutrition and Risk Factor Modification Center, and ²Department of Medicine, Division of Endocrinology and Metabolism; St. Michael’s Hospital, Toronto, Ontario M5C 2T2; ³Department of Nutritional Sciences; Faculty of Medicine, University of Toronto, Toronto, Ontario MSS 3E2; ⁴Almond Board of California, Modesto, CA 95354; and ⁵Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111

Abstract

Nut consumption has been associated with reduced coronary heart disease (CHD) risk. In addition to cholesterol-lowering properties, almonds have been shown to lower oxidized LDL concentrations. However, little is known regarding their effects on other markers of oxidative stress. The dose-response effects of whole almonds, taken as snacks, were compared with low-saturated fat (<5% energy) whole-wheat muffins (control) in the therapeutic diets of hyperlipidemic subjects. In a randomized crossover study, 27 hyperlipidemic men and women consumed 3 isoenergetic (mean 423 kcal/d or 1770 kJ/d) supplements each for 1 mo. Supplements consisted of full-dose almonds (73 g), half-dose almonds plus half-dose muffins (half-dose almonds), and full-dose muffins (control). Subjects were assessed at wk 0, 2 and 4. Mean body weights differed ≤300 g between treatments, although the weight loss on the half-dose almond treatment was greater than on the control (P < 0.01). At 4 wk, the full-dose almonds reduced serum concentrations of malondialdehyde (MDA) (P = 0.040) and creatinine-adjusted urinary isoprostane output (P = 0.026) compared with the control. Serum concentrations of α- or γ-tocopherol, adjusted or unadjusted for total cholesterol, were not affected by the treatments. Almond antioxidant activity was demonstrated by their effect on 2 biomarkers of lipid peroxidation, serum MDA and urinary isoprostanes, and supports the previous finding that almonds reduced oxidation of LDL-C. Antioxidant activity provides an additional possible mechanism, in addition to lowering cholesterol, that may account for the reduction in CHD risk with nut consumption. * J. Nutr. 138: 908–913, 2008.

Introduction

The effectiveness of nuts in lowering serum cholesterol has been demonstrated (1–9) and has been used to explain their marked effect in reducing coronary heart disease (CHD) in cohort studies (10–13). On the basis of these data, the FDA has allowed a CHD risk reduction qualified health claim for nuts and nut products or organizations imply their endorsement.

¹ Supported by the Canada Research Chair Endowment of the Federal Government of Canada, USDA Agricultural Research Service under Cooperative Agreement no. 1950-51000-065-02A, and the Almond Board of California. The contents of this publication do not necessarily reflect the views or policies of the U.S. or Canadian governments, nor does mention of trade names, commercial products or organizations imply their endorsement.

² Author disclosures: D. Jenkins and C. Kendall have sat on the Scientific Advisory Board and have been on the speakers panel for the Almond Board of California. D. Jenkins, C. Kendall, and J. Blumberg have received honoraria and grants from the Almond Board of California. K. Lapsley is employed by the Almond Board of California. A. Marchie, A. Josse, T. Nguyen, and D. Faulkner, no conflicts of interest.

³ Abbreviations used: CHD, coronary heart disease; LDL-C, LDL cholesterol; MDA, malondialdehyde; MUFA, monounsaturated fatty acids.

* To whom correspondence should be addressed. E-mail: cyril.kendall@utoronto.ca.

The skins of almonds are rich sources of phenolic antioxidants (20–22) and nuts are also good sources of vitamin E (1). Antioxidants have been proposed to reduce CHD through their ability to decrease oxidative damage to lipids, proteins, and lipoproteins (23). Oxidized LDL is considered to be more atherogenic than native LDL (24). Because almonds have been shown to reduce serum concentrations of oxidized LDL (1), the antioxidant activity of nuts may provide a further mechanism for their cardioprotective effects. In addition to their antioxidant capacity, dietary polyphenols appear also to possess additional

over the high fat content of nuts, because the sources of fat are monounsaturated fatty acids (MUFA) and PUFA. In this respect, there has recently been a liberalization of MUFA intakes in guidelines of the AHA (15), the American Diabetes Association (16), and the National Cholesterol Education Program Adult Treatment Panel III (17).

Nevertheless, it seems unlikely that the ~5% reduction in serum cholesterol with nuts (1) could explain more than part of the average 30% reduction in CHD risk in cohort studies (10–13). In statin trials, a 30% reduction in CHD risk is associated with a 30% reduction in LDL cholesterol (LDL-C) (18,19). Additional mechanisms must therefore be sought to explain the beneficial effect of almonds.

The skins of almonds are rich sources of phenolic antioxidants (20–22) and nuts are also good sources of vitamin E (1). Antioxidants have been proposed to reduce CHD through their ability to decrease oxidative damage to lipids, proteins, and lipoproteins (23). Oxidized LDL is considered to be more atherogenic than native LDL (24). Because almonds have been shown to reduce serum concentrations of oxidized LDL (1), the antioxidant activity of nuts may provide a further mechanism for their cardioprotective effects. In addition to their antioxidant capacity, dietary polyphenols appear also to possess additional
cardioprotective functions, such as altering hepatic cholesterol uptake, triglyceride assembly and secretion, plasma lipoprotein processing, and inflammation (25).

We have therefore assessed the effect of almonds on antioxidant vitamins and lipid markers of oxidative damage, including serum malondialdehyde (MDA) and urinary isoprostane output, to determine whether the antioxidant property of almonds may be a further reason for their cardioprotective effects. The lipid data from this almond dose-response study have been published previously and demonstrated reductions in both LDL-C and oxidized LDL (1).

Methods

Subjects. Healthy hyperlipidemic men and postmenopausal women were recruited by newspaper advertisement and from patients attending the Risk Factor Modification Center of St. Michael’s Hospital, a University of Toronto teaching hospital. Of the 43 subjects who started the study, 16 withdrew during or after completing 1 or 2 study phases. Three quit for reasons directly related to the study (food allergies, n = 2; abdominal discomfort, n = 1). The majority of subjects (n = 13) withdrew for unrelated reasons. Twenty-seven subjects (15 men and 12 postmenopausal women) completed all 3 phases of the study. Four subjects were ≥75 y but were healthy and interested in the study. The 27 subjects who completed the study were (mean ± SD) 64 ± 9 y (range, 48–86 y) and had a BMI of 25.5 ± 4.0 kg/m² (range, 20.5–31.5 kg/m²) and a mean baseline LDL-C of 4.32 ± 0.63 mmol/L (range, 2.77–5.32 mmol/L). The 16 subjects who withdrew from the study had similar characteristics to those who completed: 9 men, 7 postmenopausal women; age 62 ± 8 y (range, 51–74 y); BMI 25.6 ± 4.0 kg/m² (range, 21.3–37.1 kg/m²); and baseline LDL-C 4.24 ± 0.92 mmol/L (range, 3.19–6.40 mmol/L). All subjects had elevated LDL-C levels on initial assessment at recruitment (≥4.1 mmol/L) despite the lower values in some subjects at baseline, and triglyceride concentrations < 4.0 mmol/L. No subjects used tobacco and none had clinical or biochemical evidence of diabetes or liver or renal disease. Of the 27 participants who completed the study, 3 men and 5 women were taking the following medications: a hypolipidemic agent (statin) (n = 2), β-blocking agents (n = 3), angiotensin-converting enzyme inhibitors (n = 3), angiotensin II receptor blockers (n = 1), thiazide diuretics (n = 2), levothyroxine (n = 2), and hormone replacement therapy (n = 2). Medications had been stable for at least 2 wk prior to the study. Medication dosages were kept constant throughout the study.

Study protocol. Three 1-mo diet phases taken in a randomized cross-over design with each phase separated by a minimum 2-wk washout period were completed by 27 subjects. The 3 phases consisted of a muffin phase (control) and 2 almond phases: 1 full-dose almond and the other 0.5-dose almond. During all study phases, the background diet that the subjects followed was their own self-selected low-fat therapeutic diet. Subjects were counseled on strategies to facilitate weight maintenance, including holding exercise constant throughout the study, and were questioned by the dietitian at each clinic visit to ensure they had not deviated from their usual exercise routine during the previous 2 wk.

After overnight fasts (12–14 h), body weight, blood samples, and blood pressure were obtained at the start and at wk 2 and 4 of each 4-wk diet phase. A 24-h urine sample was also collected at the end of wk 4. Seven-day weighed diet records were obtained prior to baseline (Table 1) and at wk 4 (Table 2) of each phase. Subjects were instructed to weigh all foods consumed with the self-taring electronic food scales provided during the week when diets were recorded.

The Ethics Committee of the University of Toronto and St. Michael’s Hospital approved the study. All subjects gave informed consent. The clinical trial registration number is: NCT00507520.

Diets. Before the study, all subjects had been instructed to follow a therapeutic National Cholesterol Education Program Step 2 diet (<7% energy from saturated fat and <200 mg/d dietary cholesterol) for at least 2 mo prior to the study. Achievement of the dietary goals at baseline was assessed by 7-d food records (Table 1). Subjects added 1 of 3 supplements to their diet: whole raw unblanched almonds (73 ± 3 g/d); muffins (147 ± 6 g/d); and half portions of almonds (37 ± 2 g/d) plus muffins (75 ± 3 g/d) as described previously (1). All subjects took each supplement and the intake level was based on subjects’ estimated daily energy requirement (26). The muffins were made from whole-wheat flour with corn oil sufficient to provide the same amount of SFA, PUFA, and fiber as the almonds, and with skim-milk powder and egg white to provide a similar level of protein, although the muffin protein was 46% of animal origin. MUFAs from almonds balanced the starch from muffins. The macronutrient composition of the muffins as a percentage of energy was 14.7% protein, 53.3% available carbohydrate, 32.1% fat, 4.3% SFA, 7.6% MUFA, and 18.9% PUFA with 18 g/1000 kcal (4.3 g/1000 kJ) dietary fiber and 6 mg/1000 kcal (1.4 mg/1000 kJ) cholesterol. Nonhydrogenated corn oil was the only fat used in muffin preparation to avoid the addition of trans fatty acids to the diet. Muffin supplements were provided at biweekly intervals and were stored in the freezer until the day before use. Subjects were instructed to reduce total food intake, especially starchy foods (breads, bagels, nonstudy muffins, and breakfast cereals), to allow supplements to be taken as snacks without increasing total energy intake and to keep the background diet constant across all 3 phases. Detailed dietary counseling was undertaken prior to and at wk 1 and 2 of each phase. During the study, we asked subjects to not consume any additional nuts or nut products or alter consumption of dietary fiber or vegetable protein foods. Compliance was assessed from 7-d diet records (Table 2), a supplement checklist on which subjects recorded supplements consumed and return of un eaten supplements, which were weighed and recorded.

Analyses. Samples stored at −70°C were used for determination of serum vitamin A (retinol), α- and γ-tocopherol, MDA, and urinary isoprostanes. Following extraction with hexane, α- and γ-tocopherol and retinol were determined via reverse phase HPLC at UV 292 nm for tocopherols and 540 nm for retinol according to Bieri et al. (27). MDA was determined by reverse phase HPLC according to Volpi and Tarugi (28), in which a thiobarbituric acid-MDA conjugate derivative was injected onto a C18 column and fluorometrically quantified at excitation 515 nm and emission 533 nm. MDA concentration was calculated from calibration curves of authentic standard, with a linear relationship of $R^2 > 0.995$. F₂,₂-isoprostanes were measured in duplicate by GC/MS after isolation using HPLC according to the method described by Sacheck et al. (29) and modified from that of Walter et al. (30). Briefly, samples were thawed and deuterated, and prostaglandin F₂ was added as an internal standard. Pentfluorobenzyl esters of isoprostanes were prepared and purified by HPLC, silylated, and analyzed by GC/MS with a mass selective detector operated in negative chemical ionization mode. Results were obtained as μg/L and converted to pmol/L using a mean molecular weight for isoprostanes of 354 atomic mass units. We determined urinary

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal (kJ)</td>
<td>1789 ± 92 (7489 ± 385)</td>
</tr>
<tr>
<td>Total protein, %</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Vegetable protein, %</td>
<td>7 ± 0.4</td>
</tr>
<tr>
<td>Available carbohydrate, %</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>Total dietary fiber, g/1000 kcal (g/1000 kJ)</td>
<td>16 ± 1 (3.8 ± 0.2)</td>
</tr>
<tr>
<td>Total fat, %</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>SFA, %</td>
<td>8 ± 0.4</td>
</tr>
<tr>
<td>MUFA, %</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>PUFA, %</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>Dietary cholesterol, mg/1000 kcal (mg/1000 kJ)</td>
<td>100 ± 8 (23.9 ± 1.9)</td>
</tr>
<tr>
<td>Alcohol, %</td>
<td>2 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 27; all % values reported as a percentage of energy.
The results are expressed as means ± SEM. Statistical analyses. The results are expressed as means ± SEM. The data were analyzed using SAS software (SAS Institute, 2004, SAS 9.1 for Windows). Mixed models were constructed (SAS: Proc Mixed) to assess the effect of diet on serum and urinary measures at wk 4. All models contained a subject effect to control for variance in the outcome by accounting for the crossover nature of the study. All models initially included diet, total cholesterol, subject sex, diet sequence, and the interactions between sex and diet and sex and cholesterol. Nonsignificant (>0.10) effects were removed sequentially until the model contained significant interactions or significant main effects. The exception was that the main effects of interest (sex and diet) were retained in all reduced models. The final model included subject as a random factor, sex, diet sequence, interactions between sex and diet and sex and sequence, and baseline serum measures as a covariate. Diet sequence and interactions were removed from the final models if they were not significant. When diet was significant, post hoc contrasts were used to compare outcomes between the 3 diets. A Tukey adjustment was used for multiple means comparisons.

Results

Compliance with supplement consumption was good at 97.8 ± 0.7% to 99.5 ± 0.6% for all 3 treatments. Baseline dietary data are shown in Table 1 and treatment dietary data are shown in Table 2. Body weight did not differ at baseline and weight loss did not differ between the full-dose almonds and the control, although the half-dose almond treatment resulted in a weight loss that was significantly greater than the control (Table 3; previously published data) (1). Blood lipid data have also been published previously (Table 3) (1). The serum total cholesterol concentration was significantly lower in subjects while taking both the half- and full-dose almond treatments compared with the control treatments. Subjects taking the full-dose almond treatment also had significantly lower serum LDL-C and higher serum HDL-C concentrations compared with the control treatment (Table 3). The serum triglyceride concentration was not affected by the treatments (Table 3).

Measures of oxidative damage. Serum MDA, a biomarker of lipid peroxidation, decreased during the full-dose almond intervention and this differed from the change during the control period (P = 0.040). The changes during the treatments were: control, from 1.6 ± 0.1 to 1.7 ± 0.2 μmol/L; half-dose almonds, from 1.4 ± 0.1 to 1.6 ± 0.3 μmol/L; and full-dose almonds, from 1.6 ± 0.1 to 1.3 ± 0.1 μmol/L. The baseline MDA concentration was a significant predictor of the treatment effect (P = 0.002). The overall diet effect approached significance (P = 0.051). Subjects’ 24-h urine volumes did not differ at the ends of the 3 treatments. In 24-h urine collections, the isoprostane concentrations during the control, half-dose, and full-dose almond treatments were 63.0 ± 15.0, 48.6 ± 10.2, and 53.7 ± 11.3 nmol/L, respectively. After creatinine correction, urinary isoprostane outputs during the half- (0.7 ± 0.2 nmol/mmol) and the full-dose almond (0.8 ± 0.2 nmol/mmol) treatments were comparable to those in the control treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Half-almond</th>
<th>Full-almond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>71.0 ± 2.4</td>
<td>71.1 ± 2.4</td>
<td>71.2 ± 2.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.54 ± 0.16</td>
<td>6.47 ± 0.15</td>
<td>6.60 ± 0.16</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>4.02 ± 0.12</td>
<td>4.10 ± 0.12</td>
<td>4.01 ± 0.12</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.40 ± 0.08</td>
<td>1.43 ± 0.08</td>
<td>1.45 ± 0.09</td>
</tr>
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</table>

1 Values are means ± SEM, n = 27. Means in a row with superscripts without a common letter differ, P < 0.05.

2 Mean of wk 2 and wk 4 measurements.
lower than during the control treatment (1.1 ± 0.3 nmol/mmol) (P < 0.03). The 24-h urinary isoprostane concentrations were higher in women (83.9 ± 15.0 nmol/L) than in men (31.4 ± 13.6 nmol/L; P = 0.016) as were the creatinine-corrected outputs (1.5 ± 0.2 vs. 0.4 ± 0.2 nmol/mmol; P = 0.001). The diet × sex interaction was not significant for urinary isoprostane output (P = 0.415).

Also, urinary creatinine outputs during the half-dose almond (8.9 ± 0.7 mmol/d) and full-dose almond (8.6 ± 0.7 mmol/d) periods were higher than during the control period (7.1 ± 0.5 mmol/d; P ≤ 0.03).

**Vitamin E and A.** The serum concentrations of α- or γ-tocopherol as well as those of other vitamins, adjusted or unadjusted for total cholesterol, were not affected by the treatments.

**Discussion**

This study provides support for the antioxidant property of nuts as an additional mechanism of action which, together with lowering cholesterol, may account for the cardioprotective property of nut consumption. Reactive aldehydes formed as products of free radical-mediated lipid peroxidation can modify apolipoprotein B. These more negatively charged particles are avidly bound to scavenger receptors and thus are more likely to be taken up by the arterial wall (33). Consequently, arteriosclerotic plaques contain increased levels of oxidized lipids (34). In this study, the full dose of almonds decreased serum MDA and both the full and half dose of almonds decreased urinary isoprostanes as biomarkers of lipid peroxidation. Previously, almond consumption was shown to reduce concentrations of oxidized LDL, the more atherogenic form of the particle (24,35). The present data suggest that this may be a part of an overall lipid antioxidant effect of almonds.

Nuts, unsalted, raw, or dry roasted, possess cardioprotective properties (1–9,11,14,36,37). The focus has been on serum cholesterol reduction as the primary mechanism, but this is modest by comparison with the often dramatic benefits for CHD risk reduction demonstrated in cohort studies (10–13). Nuts are also rich sources of antioxidants. This property has been demonstrated clearly for almonds (20–22). For example, almonds are rich sources of antioxidants. This property has been demonstrated in cohort studies (10–13). Nuts are also rich sources of antioxidants. This property has been demonstrated clearly for almonds (20–22).

The components of nuts such as flavanols, flavonols, flavanones, anthocyanins, procyanins, and phenolic acids all found in almonds may be analogous to the isoflavones in legumes, especially soy, and the lignans in flax and whole-grain cereals that have also attracted attention for their antioxidant properties. These substances with antioxidant activity could contribute to the cardioprotective effect currently ascribed to nuts (10–13).

Increased intake of MUFA in the diet has also been associated with reduced susceptibility of LDL to oxidation (41). Thus, there is no evidence that nut consumption increases protein turnover. Increased creatinine clearance on almonds is a further possibility. Unfortunately, we do not have the corresponding serum measurements for this assessment and there is no preexisting literature on this specific topic.

Our data did not show a dose response for antioxidant activity, because both the half-dose and full-dose almonds reduced urinary isoprostanes equally, suggesting the possibility of a threshold effect for this biomarker.

The reduced creatinine-corrected isoprostane output on almonds may have resulted from altered creatinine metabolism or renal function on almonds. It is possible that increased urinary creatinine output on almonds may have been due to increased meat intake or increased muscle turnover. However, animal protein intake was lower during the almond phases and there is no evidence that nut consumption increases protein turnover. Increased creatinine clearance on almonds is a further possibility. Unfortunately, we do not have the corresponding serum measurements for this assessment and there is no preexisting literature on this specific topic.

Reducions in oxidized LDL and lower urinary isoprostane outputs have been reported after feeding soy and other vegetable protein diets (42,43). In the present study, baseline urinary isoprostanes were not measured. We assumed that after 4 wk of the diet, a steady state would be reached that would allow the almond treatments to be compared with the 100% muffin as the control.

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Vitamin E did not appear to influence blood levels despite an increased intake on the full-dose almond diet by 18 mg/d. Our results contrast with those of Jambazian et al. (2) who found that, relative to baseline, feeding almonds for 4 wk at 28 and 56 g/d significantly increased plasma α-tocopherol from 26.3 to 29.9 μmol/L. However, our baseline α-tocopherol levels were >40% higher than those reported by Jambazian et al. (2), i.e. ~44 compared with 31.2 μmol/L. In NHANES III, serum vitamin E concentrations for the 50th and 90th percentiles in men were 25.9 and 43.6 μmol/L, respectively, and were 29.7 and 48.3 μmol/L, respectively, for women (44). Thus, our subjects started the study in the top decile of α-tocopherol status of the population. Significantly higher concentrations of α-tocopherol are difficult to achieve by diet alone. The reason for our subjects’ higher prestudy vitamin E levels is not clear.

It is also possible that the antioxidant benefit of almonds demonstrated as a reduced concentration of oxidized LDL may have been associated with other antioxidant systems not measured in the present study. Acute test meal studies have shown that almond supplementation of a bread meal reduced postprandial destruction of serum protein thiols groups (45). Serum protein thiols were not measured in the present study. Nevertheless, both serum MDA and urinary isoprostanes are consis-
ered to be reliable biomarkers of oxidative stress and would be expected to be favorably affected, because oxidative stress is reduced by the provision of exogenous antioxidants (46,47).

The issue of antioxidants and CHD has not been a simple one (48). Early cohort studies demonstrated the protective effect of vitamin E in preventing CHD (49,50). The theoretical basis for oxidative damage to lipids, lipoproteins, and DNA was developed (35,46,51) with relevance to CHD and diabetes (52–54). However, antioxidants, including vitamin E, or carotenoids given singly or in combination as supplements have not demonstrated effectiveness in reducing the risk of cardiovascular disease in randomized controlled trials (35–57).

At the same time, these trials did not undertake any assessment of biomarkers of oxidative stress to determine the effectiveness of the dose or duration of the supplements as antioxidants (58). It therefore still remains possible that antioxidants may have a cardioprotective role if a meaningful reduction in oxidative damage can be achieved by dietary or pharmacologic interventions.

We conclude that the antioxidant potential of almonds was demonstrated in this study as reductions in serum MDA and urinary isoprostanes as markers of lipid peroxidation. However, serum vitamin E concentrations did not increase despite an additional 18 mg/d for the full-dose almonds. It is likely that the antioxidant property of almonds we obtained was related to the effect of flavonoids and other phenolic antioxidants and the high MUFA content of almonds (50% of calories). Flavonoids and other phenolic antioxidants in almonds may reduce oxidative damage directly as well as indirectly via synergy with vitamin E and other components of the antioxidant defense network. The antioxidant ingredients in nuts, combined with their favorable effects on the blood lipid profile, may help to explain their success in cohort studies in protecting from CHD (10–13).

Acknowledgments

We acknowledge the statistical expertise of Mr. Edward Viden, Toronto, and Dr. Laurel Duquette, the Department of Statistics, University of Toronto.

Literature Cited


