Dose Response of Almonds on Coronary Heart Disease Risk Factors: Blood Lipids, Oxidized Low-Density Lipoproteins, Lipoprotein(a), Homocysteine, and Pulmonary Nitric Oxide

A Randomized, Controlled, Crossover Trial

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Background—Although recent studies have indicated that nut consumption may improve levels of blood lipids, nuts are not generally recommended as snacks for hyperlipidemic subjects because of their high fat content. Furthermore, the effective dose is still unknown.

Methods and Results—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) supplements each for 1 month. Supplements provided 22.2% of energy and consisted of full-dose almonds (73±3 g/d), half-dose almonds plus half-dose muffins, and full-dose muffins. Fasting blood, expired air, blood pressure, and body weight measurements were obtained at weeks 0, 2, and 4. Mean body weights differed <300 g between treatments. The full-dose almonds produced the greatest reduction in levels of blood lipids. Significant reductions from baseline were seen on both half- and full-dose almonds for LDL cholesterol (4.4±1.7%, P=0.018, and 9.4±1.9%, P<0.001, respectively) and LDL:HDL cholesterol (7.8±2.2%, P=0.001, and 12.0±2.1%, P<0.001, respectively) and on full-dose almonds alone for lipoprotein(a) (7.8±3.5%, P=0.034) and oxidized LDL concentrations (14.0±3.8%, P<0.001), with no significant reductions on the control diet. No difference was seen in pulmonary nitric oxide between treatments.

Conclusions—Almonds used as snacks in the diets of hyperlipidemic subjects significantly reduce coronary heart disease risk factors, probably in part because of the nonfat (protein and fiber) and monounsaturated fatty acid components of the nut. (Circulation. 2002;106:1327-1332.)

Key Words: hypercholesterolemia ■ lipids ■ lipoproteins ■ diet ■ antioxidants

Studies over the last decade have demonstrated favorable effects of nuts in modifying lipid risk factors for coronary heart disease (CHD).1–11 However, their use is not yet part of standard advice for patients with hyperlipidemia,12,13 despite recognized health benefits for the general population.13 There is also concern that high-fat foods may cause weight gain.13,14 Furthermore, there are no dose-response data on nuts, and their advantage in a low-saturated fat therapeutic diet is uncertain. Nevertheless, almond consumption fits well with current American Heart Association guidelines13 to replace saturated fats with unsaturated fats and with the National Cholesterol Education Program (NCEP) guidelines to liberalize total fat intake, specifically from monounsaturated fat (MUFA),12 related to its ability to increase HDL cholesterol.15 Although replacing dietary carbohydrate with MUFA does not result in large changes in serum LDL cholesterol, the addition of MUFA to the diet as almonds reduces the LDL:HDL cholesterol ratio.2 Further benefits of almonds may result from their high polysaturated:saturated fatty acid (PUFA:SFA) ratio, nut protein, plant sterols, fiber, and associated phenolic substances.14,16 We therefore assessed the effects of whole unblanched almonds at 2 doses on the blood lipids of hyperlipidemic subjects when provided as supplements to their self-selected therapeutic diets.

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Received April 22, 2002; revision received June 14, 2002; accepted June 17, 2002.
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Authors Jenkins, Kendall, Vidgen, Marchie, Faulkner, and Parker have received funding from the California Almond Board. In addition to research funding, Dr Jenkins has received research presentation subsidization from the California Almond Board.

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Circulation is available at http://www.circulationaha.org DOl: 10.1161/01.CIR.0000028421.91733.20
Methods

Study Protocol

Three 1-month diet phases taken in a randomized crossover design, with each phase separated by a minimum 2-week 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 half-dose almond plus half-dose muffin. During all study phases, subjects followed their own self-selected low-fat therapeutic diets to which they included the supplement. Subjects were counseled on strategies to facilitate weight maintenance.

After overnight fasts (12 to 14 hours), body weight, blood samples, and blood pressure measurements were obtained at the start and at weeks 2 and 4 of each 4-week diet phase. Expired air was also collected through a modified Haldane-Priestley tube for nitric oxide (NO) measurement at weeks 0 and 4 of each phase. Seven-day diet records were collected over weeks 1 and 2 of each phase. All subjects were instructed to weigh all foods consumed with the exception of electrolytes, and supplements provided during the weeks when diets were recorded. After the study, subjects assessed palatability of the supplements on a semantic scale of zero to 10 (0, very distasteful; 5, neutral; and 10, very appetizing).

The Ethics Committee of the University of Toronto and St Michael’s Hospital approved the study. All subjects gave informed consent.

Subjects

Healthy hyperlipidemic men and postmenopausal women were recruited by newspaper advertisement and from patients attending the Risk Factor Modification Center, St Michael’s Hospital. Of the 43 subjects who started the study, 16 withdrew during or after completion of 1 to 2 study phases. Three quit for reasons directly related to the study (food allergies, n=2; abdominal discomfort, n=1). The majority (n=13) withdrew for unrelated reasons. Twenty-seven subjects completed all 3 phases: 15 men and 12 postmenopausal women; mean±SD age 64±9 years (range 48 to 86 years; 4 subjects ≥75 years, healthy and interested in the study); body mass index 25.7±3.0 kg/m² (range 20.5 to 31.5 kg/m²); baseline LDL cholesterol 4.3±0.23 mmol/L (range 2.77 to 5.32 mmol/L). All subjects had elevated LDL cholesterol levels on initial assessment at 0.63 mmol/L (range 2.77 to 5.32 mmol/L). All subjects had normal renal function (serum creatinine <147 μmol/L) and liver function (serum transaminase <30 U/L) at baseline. All subjects had normal blood pressure, and fasting blood glucose was ≤5.5 mmol/L. One subject had a history of smoking (n=1) and 2 subjects were taking hormone replacement therapy (n=2). Two subjects were using hormone therapy and were excluded from the study due to potential impact on Lp(a) levels (n=2). Seven subjects completed all 3 phases: 15 men and 12 postmenopausal subjects 75 years, healthy and interested in the study); body mass index 25.7±3.0 kg/m² (range 20.5 to 31.5 kg/m²); baseline LDL cholesterol 4.3±0.23 mmol/L (range 2.77 to 5.32 mmol/L). All subjects had elevated LDL cholesterol levels on initial assessment at 0.63 mmol/L (range 2.77 to 5.32 mmol/L). All subjects had normal renal function (serum creatinine <147 μmol/L) and liver function (serum transaminase <30 U/L) at baseline. All subjects had normal blood pressure, and fasting blood glucose was ≤5.5 mmol/L. One subject had a history of smoking (n=1) and 2 subjects were taking hormone replacement therapy (n=2). Two subjects were using hormone therapy and were excluded from the study due to potential impact on Lp(a) levels (n=2). Seven subjects completed all 3 phases: 15 men and 12 postmenopausal subjects 75 years, healthy and interested in the study); body mass index 25.7±3.0 kg/m² (range 20.5 to 31.5 kg/m²); baseline LDL cholesterol 4.3±0.23 mmol/L (range 2.77 to 5.32 mmol/L). All subjects had elevated LDL cholesterol levels on initial assessment at 0.63 mmol/L (range 2.77 to 5.32 mmol/L).

Dietary supplements, which were weighed and recorded. Subjects recorded supplements consumed, and return of uneaten supplements. To minimize changes in body weight and diet composition, detailed dietary counseling was undertaken before and at weeks 1 and 2 of each phase. During the study, subjects were asked not to consume any additional nuts or nut products or alter consumption of dietary fiber or vegetable protein foods. Compliance was assessed from 7-day diet records, a supplement checklist on which subjects recorded supplements consumed, and return of uneaten supplements, which were weighed and recorded.

Analyses

Serum was analyzed according to the Lipid Research Clinics protocol for total cholesterol, triglyceride, and HDL cholesterol after dextran sulfate-magnesium chloride precipitation. All samples from a given individual were analyzed in the same batch. LDL cholesterol was calculated. Serum apolipoprotein A-I and B were measured by nephelometry, lipoprotein(a) [Lp(a)] by a commercial ELISA (Macra Lp(a) Kit, Trinity Biotech USA), and C-reactive protein by end-point nephelometry (Behring BN-100, N high-sensitivity C-reactive protein reagent, Dade-Behring). Plasma total L-homocysteine was analyzed with a fluorescence polarization immunoassay (IMx homocysteine assay, Axis-Shell).

Exhaled NO samples were collected by an offline method with fixed-flow expired air (6 L/min), stored at −20°C in plastic syringes with 3-way valves, and measured by a rapid-response chemiluminescent NO analyzer (Sievers, model 280). Oxidized LDL was measured as conjugated dienes in the LDL fraction after isolation of LDL particles by precipitation with buffered heparin at their isoelectric point. The results were expressed as total serum conjugated dienes (oxidized lipids) in the LDL fraction. Study supplements were analyzed by Association of Official Analytical Chemists methods for fat, protein, and fiber, with available carbohydrate calculated by difference. Fatty acid composition was determined by gas chromatography. Dietary macronutrient intake was calculated by computerized nutrient analysis software (Table 1). The results were expressed as total serum conjugated dienes (oxidized lipids) in the LDL fraction.

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<table>
<thead>
<tr>
<th>TABLE 1. Calculated Macronutrient Intakes on the Control, Half-Almond, and Full-Almond Treatment Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
</tr>
<tr>
<td>Total protein, %</td>
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<tr>
<td>Vegetable protein</td>
</tr>
<tr>
<td>Available carbohydrate, %</td>
</tr>
<tr>
<td>Total dietary fiber, g/1000 kcal</td>
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<tr>
<td>Total fat, %</td>
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<tr>
<td>SFA</td>
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<tr>
<td>MUFA</td>
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<tr>
<td>PUFA</td>
</tr>
<tr>
<td>Dietary cholesterol, mg/1000 kcal</td>
</tr>
<tr>
<td>Alcohol, %</td>
</tr>
</tbody>
</table>

n=27. Values are mean±SEM.
TABLE 2. Body Weight, Blood Lipid, and Blood Pressure Data on the Control, Half-Dose, and Full-Dose Almond Diet Periods

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Half-Dose Almond</th>
<th>Full-Dose Almond</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Treatment*</td>
<td>Week 0</td>
</tr>
<tr>
<td>Body weight, kg†</td>
<td>71.0±2.4</td>
<td>71.2±2.5</td>
<td>71.1±2.4</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.54±0.16</td>
<td>6.44±0.15</td>
<td>6.47±0.15</td>
</tr>
<tr>
<td>LDL</td>
<td>4.34±0.14</td>
<td>4.22±0.13</td>
<td>4.30±0.12</td>
</tr>
<tr>
<td>HDL</td>
<td>1.43±0.09</td>
<td>1.41±0.08</td>
<td>1.38±0.08</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.69±0.13</td>
<td>1.80±0.12</td>
<td>1.75±0.16</td>
</tr>
<tr>
<td>Apolipoproteins, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.74±0.07</td>
<td>1.73±0.06</td>
<td>1.71±0.06</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.30±0.03</td>
<td>1.27±0.03</td>
<td>1.27±0.03</td>
</tr>
<tr>
<td>Ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total:HDL cholesterol</td>
<td>4.95±0.28</td>
<td>4.89±0.24</td>
<td>5.07±0.28</td>
</tr>
<tr>
<td>LDL:HDL cholesterol</td>
<td>3.32±0.22</td>
<td>3.23±0.18</td>
<td>3.40±0.22</td>
</tr>
<tr>
<td>ApoB:ApoA-1</td>
<td>0.78±0.04</td>
<td>0.76±0.03</td>
<td>0.77±0.04</td>
</tr>
<tr>
<td>Oxidized LDL†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL conjugated dienes, μmol</td>
<td>64±3</td>
<td>60±2</td>
<td>65±3</td>
</tr>
<tr>
<td>Homocysteine, μmol/L†</td>
<td>8.9±0.5</td>
<td>8.6±0.4</td>
<td>8.9±0.5</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>15.3±3.0</td>
<td>15.5±3.2</td>
<td>15.4±3.3</td>
</tr>
<tr>
<td>C-reactive protein, mg/L†</td>
<td>3.21±1.39</td>
<td>2.37±0.45</td>
<td>1.81±0.27</td>
</tr>
<tr>
<td>Pulmonary nitric oxide, ppm†</td>
<td>25.4±3.2</td>
<td>18.2±1.3</td>
<td>15.3±1.8</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>119±3</td>
<td>121±2</td>
<td>121±3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75±2</td>
<td>76±2</td>
<td>75±2</td>
</tr>
<tr>
<td>Estimated CHD risk, 10 y, %‡</td>
<td>10.7±1.2</td>
<td>11.0±1.1</td>
<td>11.2±1.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=27.

To convert cholesterol and triglycerides and mg/dL multiply by 38.67 and 88.57, respectively. To convert apolipoprotein A-I and B values to mg/dL multiply by 100.

Treatment values represent the mean of: *Weeks 2 and 4 or †Week 4 alone.

‡P<0.01; §P<0.050; or ||P<0.001, significance of the difference of half- and full-almond dose vs control treatment using the least squares method with Tukey adjustment in SAS. No significant differences were seen between the half- and full-dose almonds.

¶CHD risk was estimated using the Framingham cardiovascular risk equation (21).

Results

Compliance was good. Percentages of the prescribed supplements consumed during the 3 phases were as follows: control muffins, 98.1±0.8%; half-dose almonds plus half-dose muffins, 99.5±0.6%; and full-dose almonds, 97.8±0.7%, with no significant differences between treatments. The full-dose almonds were perceived as more palatable than the control muffin (control 6.5±0.4 on a scale of 0 to 10; almond 8.4±0.3 on a scale of 0 to 10; P<0.001). There was virtually no effect of any of the supplements on body weight change across the treatment periods (muffins, 0.1±0.1 kg; half-dose almonds, −0.3±0.2 kg; and full-dose almonds, −0.2±0.1 kg; Table 2).

Baseline blood lipid values did not differ among the 3 treatments (Table 2). On the muffin control diet, the only significant change from baseline was the increase in triglycerides (10.8±4.7%, P=0.031). Almonds tended to reduce blood lipids in a dose-dependent manner (Figure 1). Compared with baseline values, half- and full-dose almond sup-

Statistical Analysis

Results are expressed as mean±SEM. The response variable was the mean of the week 2 and 4 values for each dietary treatment. Absolute differences between treatments were assessed by the method of least squares means within the mixed model procedure (PROC MIXED/SAS) with a Tukey adjustment for multiple means comparisons. The statistical model included diet, the interaction term of diet by sex and sequence, a random term that represented the individual (nested within sex by sequence), and baseline as a covariate. To test for the overall effect of the diet-sex interaction, ANCOVA (PROC GLM/SAS) was used with the same model specification. Student’s t test for paired data (2-tailed) was used to assess the significance of percentage changes across diets. CHD risk was calculated with the total: HDL cholesterol ratio and systolic blood pressure in the Framingham cardiovascular disease risk assessment equation (21). SAS software was used throughout (SAS/STAT version 8, 1997; SAS Institute).
control values (Table 2) confirmed the significantly lower blood lipid concentrations for total cholesterol ($P=0.006$), LDL cholesterol ($P=0.002$), and apolipoprotein B ($P=0.002$); the ratios of total:HDL cholesterol ($P<0.001$), LDL:HDL cholesterol ($P<0.001$), and apolipoprotein B:A-1 ($P<0.001$); and higher HDL cholesterol levels ($P=0.041$; Figure 2). Lp(a) values were lower on the full-dose almond phase than for control ($P=0.026$). The 2 subjects taking statins responded similarly to the rest of the group, and the reduction in lipoprotein ratios in the 4 subjects aged ≥75 years was similar to that for the younger subjects.

Conjugated dienes in the LDL fraction as a marker of LDL oxidation were also significantly reduced on almonds compared with baseline values (control, 3.1±4.2%; $P=0.466$; half-dose almonds, 15.0±4.0%; $P<0.001$; full-dose almonds, 14.0±3.8%; $P<0.001$). The difference between almond and control treatments was confirmed with absolute values (half-dose almonds, $P=0.007$; full-dose almonds, $P=0.001$). No treatment differences were seen in homocysteine, C-reactive protein, blood pressure, or pulmonary NO (Table 2). Calculated CHD risk for the full dose of almonds was significantly reduced compared with baseline (9.2±3.5%; $P=0.015$) and compared with the control value ($P=0.029$; Table 2).

Discussion
Consumption of 73 g of almonds daily as a supplement in the diets of hyperlipidemic subjects reduced LDL cholesterol from baseline by 9.4%, whereas 37 g/d almonds (a “hand full”) reduced LDL cholesterol by 4.4%. These data suggest a dose response in which ∼7 g of almonds per day reduces LDL cholesterol by ∼1%, which translates into a 2% risk reduction for CHD.22 The full-dose of almonds was also associated with a significant reduction in calculated CHD risk based on the total:HDL cholesterol and blood pressure data.21 The Adventist23 and Nurses’ Health Study24 data have demonstrated that nut consumption is associated with reduced CHD risk. The nut consumption in those studies (<20 g/d) was below the half dose of almonds in the present study.

Our findings are in line with previous studies of almonds and other types of nuts. For almonds, reductions of 15% have been noted in LDL cholesterol per 100 g/d (1% reduction for 8 g/d).26 One-percent reductions in LDL cholesterol for walnuts, pecans, peanuts (an oil seed legume), macadamias, and pistachios would be achieved with 7 g of almonds per day reduces LDL cholesterol by 4.4%. These data suggest a dose response in which ∼7 g of almonds per day reduces LDL cholesterol by ∼1%, which translates into a 2% risk reduction for CHD.22 The full-dose of almonds was also associated with a significant reduction in calculated CHD risk based on the total:HDL cholesterol and blood pressure data.21

In the present study, the main macronutrient difference between the almond and muffin diets was the higher MUFA and lower carbohydrate intake with almonds. The SFA and PUFA contents of the almonds and muffins were the same. In general, exchanging carbohydrate for MUFA does not appear to result in large changes in LDL cholesterol levels.15,27,28 The meta-analysis by Mensink and Katan29 indicated a coefficient for LDL cholesterol reduction by MUFA that was less than half that for PUFA. Application of the equation29 to the present data indicated that the MUFA exchange for carbohydrate accounted for 29% of the reduction in LDL cholesterol seen with almonds. The difference in LDL cholesterol between the control and full-dose almonds remained significant
after adjustment for MUFA (**P=0.011**). MUFA has, however, been associated with higher HDL cholesterol concentrations, reflected in lower total:HDL cholesterol and LDL:HDL cholesterol ratios, as potentially important predictors of cardiovascular risk.\(^{21}\) Higher SFA intakes in exchange for carbohydrate in the DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) study were associated with a lower Lp(a) level,\(^{21}\) an effect associated in the present study with higher MUFA intakes. This lipid risk factor for CHD is not altered by most dietary and pharmacological treatments, and the effect of almonds is therefore notable.

Another study with almonds also assessed the dose response in metabolically controlled conditions. In that study, 10\% and 20\% of energy of a background NCEP Step 1 diet was isonenergetically replaced with almonds for 4 weeks. A 1\% reduction in LDL cholesterol was noted for every 10 g of almonds consumed (Joan Sabaté, MD, DrPH, unpublished data, 2002).

The present study addressed a somewhat different question of the effect of nuts plus their constituent monounsaturated fats in diets that were low in saturated fats. The data indicated an advantage of the higher-fat diet in the context of increased monounsaturated fat from nuts.

Almonds also reduced oxidized LDL. Similar reductions and other evidence of antioxidant activity, including lower urinary isoprostane outputs, have been reported after feeding soy and other vegetable protein diets.\(^{20,22}\) The proteins and other components of nuts may therefore share properties with legumes, especially soy, that contribute additionally to the cardioprotective effect of nuts.\(^{23,24}\) In addition, the full-dose almonds add 18 mg of the antioxidant vitamin E to the diet per day. Finally, an increased intake of monounsaturated fat in the diet has been associated with reduced susceptibility of LDL to oxidation.\(^{25}\) There are therefore a number of components of almonds that may reduce LDL oxidation.

We conclude that almonds substituted for whole-wheat flour muffins of similar calories and SFA, PUFA, and protein content reduce lipid risk factors for CHD even in diets already low in saturated fat. The macronutrient profile of the muffins in the present study is likely to have reduced the contrast that would have been seen had nuts been compared with commercial muffins high in saturated and trans fatty acids. The dose response to almonds appears linear in the acceptable range of intake, with a 1\% reduction in LDL cholesterol resulting from each 7-g portion of almonds. These data support epidemiological studies that suggest that nut consumption may reduce the risk of CHD and the suggestion that nuts should be considered for inclusion in lipid-lowering diets.\(^{13}\)

Acknowledgments

This study was supported by the Almond Board of California, Modesto, Calif. Dr Jenkins is funded by the Federal Government of Canada as a Canada Research Chair in Nutrition and Metabolism. The authors sincerely thank Yu-Min Li, George Koumbris, and Lydia Yung, who provided excellent technical assistance.

References


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*Circulation.* 2002;106:1327-1332; originally published online August 19, 2002; doi: 10.1161/01.CIR.0000028421.91733.20

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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