Almonds and Almond Oil Have Similar Effects on Plasma Lipids and LDL Oxidation in Healthy Men and Women[1,2]

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ABSTRACT Epidemiologic and clinical studies have shown that nut consumption is associated with favorable plasma lipid profiles and reduced cardiovascular risk. These effects may result from their high monounsaturated fat (MUFA) content but nuts contain constituents other than fatty acids that might be cardioprotective. We conducted a study to compare the effects of whole-almond vs. almond oil consumption on plasma lipids and LDL oxidation in healthy men and women. Using a randomized crossover trial design, 22 normolipemic men and women replaced half of their habitual fat (~14% of ~29% energy) with either whole almonds (WA) or almond oil (AO) for 6-wk periods. Compliance was ascertained by monitoring dietary intake via biweekly 5-d food records, return of empty almond product packages and weekly meetings with a registered dietitian. Fat replacement with either WA and AO resulted in a 54% increase in percentage of energy as MUFA with declines in both saturated fat and cholesterol intake and no significant changes in total energy, total or polyunsaturated fat intake. The effects of WA and AO on plasma lipids did not differ compared with baseline; plasma triglyceride, total and LDL cholesterol significantly decreased, 14, 4 and 6% respectively, whereas HDL cholesterol increased 6%. Neither treatment affected in vitro LDL oxidizability. We conclude that WA and AO do not differ in their beneficial effects on the plasma lipid variables measured and that this suggests that the favorable effect of almonds is mediated by components in the oil fraction of these nuts. J. Nutr. 132: 703–707, 2002.

KEY WORDS: • *lipids* • *monounsaturated fat* • *nuts* • *oxidation* • *humans*

Epidemiologic studies have shown that frequent consumption of nuts is associated with reduced incidence of cardiovascular disease (CVD)[4] in vegetarian and nonvegetarian populations (1–3). This is postulated to be due mainly to the less atherogenic plasma lipid profiles associated with long-term consumption of nuts (4,5). Indeed, a number of clinical studies have demonstrated that the addition of nuts to the habitual diet of both normocholesterolemic and hypercholesterolemic subjects results in a significant reduction in plasma total and LDL cholesterol, whereas HDL is unchanged or increases (6–11).

The cholesterol reduction associated with nut consumption has been attributed to the replacement of saturated fat (SFA) with monounsaturated fatty acid (MUFA) due to the high MUFA content of nuts. However, a review of several feeding trials demonstrated that the magnitude of cholesterol reduction found exceeded that predicted on the basis of inputting the changes in dietary fat composition during nut consumption into equations relating dietary fat composition to plasma lipid levels (5). There are several components in nuts in addition to MUFA that have the potential to affect plasma cholesterol, including dietary fiber, phytochemicals, sterols and possibly arginine (12,13).

In addition, nuts are rich in many nutrients that may reduce risk of CVD by mechanisms that are independent of cholesterol lowering. With the exception of vitamin E and perhaps some phytochemicals, most of these components are present in the nonlipid fraction of the nut. Nuts are a very good source of vitamin E, which may reduce oxidation of LDL cholesterol, a key event in the development of CVD (4,14,15). Moreover, nuts are rich in arginine, a dietary precursor for nitric oxide, which plays an important role in endothelial function and CVD risk (16). Nuts also contain copper and magnesium, each of which may be cardioprotective (17,18). Thus, whole nuts provide a variety of constituents that may be cardioprotective by a number of different mechanisms.

Several studies have specifically reported that consumption of almonds reduces total and LDL cholesterol in plasma (6–8). We conducted a dietary intervention study incorporating equal amounts of almond fat derived from either whole almonds or isolated almond oil to determine whether the consumption of whole almonds would have differential effects on plasma lipids and ex vivo LDL oxidation compared with the purified almond oil component. We hypothesized that the effects of almond oil would differ from those of intact almonds, due to the absence of the additional nonlipid-related constituents in the oil that are present in the whole nut. It was expected that because of these con-

[1] Presented in part in abstract form [Hyson, D. A., Richter, B. D. & Davis, P. A. (1999) Effect of whole almonds versus almond oil intake on serum lipid parameters in healthy men and women. FASEB J. 13: AZ13.

[2] Funding provided by a grant from the Almond Board of California.

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[4] Abbreviations used: AO, almond oil; CVD, cardiovascular disease; MUFA, monounsaturated fat; PUFA, polyunsaturated fat; SFA, saturated fat; TG, triglyceride; WA, almond oil.

Manuscript received 30 July 2001. Initial review completed 24 August 2001. Revision accepted 2 January 2002.

stituents, whole almonds would have a greater effect on cholesterol lowering and reduced oxidation of LDL cholesterol than almond oil.

SUBJECTS AND METHODS

Subjects. Normocholesterolemic [$n = 24$; plasma cholesterol < 5.2 mmol/L ($\pm$ 10%)] healthy men and women from the university and surrounding community were recruited by newspaper advertisement or local flyers and enrolled in the study. All subjects were required to have normal body weight, be nonsmokers, free of dietary restrictions/food allergies and not using medications known to alter lipid metabolism. They were also screened for diabetes, heart disease and other major illnesses. Study entry cholesterol levels were determined via finger stick samples (Cholestech LDX lipid analyzer, Hayward, CA).

Study design. All subjects were required to record 7 d of baseline dietary intake before the initiation of the study. A registered dietitian provided detailed instructions for food recording and a digital scale was given to each subject for food quantitation. After successful completion of the dietary records, subjects then entered the study. A randomized, crossover design with 6-wk feeding periods was employed to compare the effects of the two diets containing different almond products. Each subject was randomly assigned to either a whole-almond (WA) or almond oil (AO) diet for 6 wk, after which they crossed over to the alternate diet arm for a further 6 wk. Compliance with the study protocol was reinforced and assessed using multiple approaches. The study design required the subjects to return to a central facility each week for body weight measurement, review of the dietary protocol and to obtain weighed allotments of almond products. Subjects were also required to return empty product containers, submit completed 5-d records of weighed food intake on alternate weeks of the study and attend weekly interviews with a registered dietitian. All dietary intake records were analyzed using Nutritionist Four version 2.0 (N-Squared Computing, Silverton, OR); these were reviewed with the subjects at their weekly meetings with the dietitian. The study protocol was approved by the University of California Davis Human Subjects Review Committee and was explained to each subject, who signed an informed consent.

Diet. The goal of the study was to replace 50% of each subject's usual daily intake of dietary fat with fat from almond sources. Habitual consumption was determined from analysis of 7-d food records collected during the baseline period. Dry-roasted nonpareil California almonds were provided in preweighed packages during the 6-wk WA diet period. The mean amount ($\pm$ SEM) of almonds required to replace half of usual daily fat intake was 66 $\pm$ 5 g, providing 35 g of fat. During the AO diet period, subjects were provided a daily average of 35 $\pm$ 2 g of almond oil derived from the same lot of almonds used for the WA diet.

The AO was weighed and blended with a flavored beverage powder and water. The drink powder did not contain any fat or cholesterol and provided an average of 73 $\pm$ 4 kcal (304 $\pm$ 17 kJ)/d. An aliquot of this powder was also provided to each subject for daily consumption during the WA period to control for any potential effects of the beverage powder itself.

A registered dietitian met with each subject to provide individualized strategies for substituting their customary dietary fat sources with almond products to maintain a total fat, energy and macronutrient composition similar to baseline levels. In addition, subjects were asked to eliminate almonds and other tree nuts from their diet other than those provided by the study for the entire 12-wk experimental period. They were further required to maintain their baseline activity patterns as well as body weight.

Lipids. Twelve-hour fasting blood samples were collected by venipuncture into EDTA vacutainers before random assignment into a dietary group (baseline) and after 6 wk of each dietary period. Plasma, separated by centrifugation at 1200 $\times$ *g* for 20 min, was stored under nitrogen for LDL isolation as detailed below. Another aliquot was taken and used for total and HDL cholesterol and triglyceride (TG) determinations using enzyme-based reagents on a Chiron-Bayer 550 Express Chemistry Analyzer (University of California, Davis NIH Clinical Nutrition Research Unit-DK35747, analytical core laboratory). LDL cholesterol concentrations were estimated using the Friedewald equation (19). Apolipoprotein B and apolipoprotein A-1 were measured using an immunoprecipitation reaction on a Beckman-Coulter Array Protein System (Fullterton, CA) as described by the manufacturer.

LDL isolation. LDL was isolated from frozen EDTA-containing plasma aliquots using stepwise density gradient centrifugation (total 3.5 h at 649,826 $\times$ *g* using S120-AT2 rotor) in a Sorvall RC-M120GX Microultracentrifuge (Sorvall Products L.P., Newton, CT). The LDL was obtained by aspiration of the LDL band and dialyzed overnight at 4°C in 2 L of PBS containing a chelating ion exchange resin (Bio-Rad Laboratories, Hercules, CA) in the dark. Cholesterol content of LDL was measured with a cholesterol oxidase based kit (Boehringer Mannheim, Indianapolis, IN).

LDL oxidation. LDL oxidation was measured essentially as described by Esterbauer et al. (20). An aliquot of LDL was added to PBS in a 1-cm quartz cuvette to obtain a final concentration of 250 mg cholesterol/L. The oxidation procedure was conducted at 37°C and was initiated by the addition of $CuCl_2$ to give a final concentration of 1.66 μmol/L. The kinetics of conjugated diene formation were monitored spectrophotometrically (Shimadzu-UV-160; Columbia, MD) by measuring absorbance at 234 nm every 5 min until a plateau occurred. Calculations were used to determine lag time, propagation rate and maximum diene formation during LDL oxidation as described previously (20).

Statistics. One way repeated-measures ANOVA was used to assess dietary effects on nutrient intake, plasma lipid parameters and LDL oxidation indices using Sigmastat version 2.0 for Windows 95 (Jandel Scientific Software, San Rafael, CA). Because fasting TG concentrations were not normally distributed, the log of the TG values was used in the analysis (21). The LDL oxidation variables did not meet the criteria for normality set by the statistical package; thus, nonparametric analyses were done using the Friedman repeated-measures analysis on ranks. Tukey's or Dunnet's multiple comparison procedures were used for the post-hoc analysis. Differences with a $P < 0.05$ were deemed significant.

RESULTS

Subject characteristics. The mean age for the group was 43.5 $\pm$ 8 y. The mean body mass index for the group at baseline was 23.7 $\pm$ 1.2 kg/m^2 and remained stable throughout the 12-wk study period. The data reported here describes the 22 subjects (12 women and 10 men) who completed the entire study protocol. One subject withdrew after 6 wk due to pregnancy and one subject withdrew after 4 wk due to relocation.

Dietary intake. Food records were analyzed for daily energy, total fat, SFA, polyunsaturated fat (PUFA), MUFA, cholesterol, and dietary fiber. These parameters, except for dietary fiber, did not differ between the WA and AO periods and are therefore presented in **Table 1** as means for the entire 12-wk study. The intake of dietary fiber was, as expected, greater (36%) during the WA period compared with baseline and the AO period.

Compared with baseline, intake of MUFA increased ~10 g/d during the WA and AO periods, accompanied by a modest but significant reduction in SFA intake (~5 g/d). MUFA provided 8.1% of energy at baseline and 12.4% during the study; SFA provided 9.8% of total energy at baseline and 8.1% during the 12-wk period. Cholesterol intake was reduced ~60 mg/d during the WA and AO periods compared with baseline.

Fasting lipids. There were significant reductions in plasma total (4%) and LDL (6%) cholesterol compared with baseline but there were no differences between the WA and AO diet periods (**Table 2**). Similarly, TG concentration was significantly decreased (14%) after 6 wk of almond product consumption; however, there was no difference between the

TABLE 1

Energy and lipid intake of men and women consuming almond products for 12 wk[1,2]

Item	Baseline intake	Study intake
Energy, *kcal/d* (*kJ/d*)	2121 ± 106 (8840 ± 442)	2103 ± 103 (8763 ± 429)
Total fat, *g/d*	68 ± 5	69 ± 4
SFA,[3] *g/d*	23 ± 2	19 ± 1*
MUFA, *g/d*	19 ± 7	29 ± 2*
PUFA, *g/d*	10 ± 0.8	13 ± 0.8
Cholesterol, *mg/d*	250 ± 22	189 ± 14*

[1] Values are means ± SEM, $n = 22$; * different from baseline, $P < 0.05$.
[2] Combined whole-almond and almond oil periods.
[3] SFA, saturated fat; MUFA, monounsaturated fat; PUFA, polyunsaturated fat.

two almond product diet periods. Plasma VLDL cholesterol also tended to be reduced by the almond products ($P = 0.27$). Plasma HDL cholesterol was significantly higher than baseline after each of the treatment periods but the WA and OA intake periods did not differ.

LDL oxidation. Consumption of WA or AO for 6 wk did not affect lag time, propagation rate or total conjugated diene formation (**Table 3**).

DISCUSSION

Replacing half of the habitual fat intake with fat from almonds or almond oil had a favorable effect on plasma lipids in normolipemic men and women. A number of studies have examined lipid profile responses to dietary incorporation of whole almonds but to our knowledge, this is the first report of a direct comparison between equal amounts of high MUFA consumed as whole almonds and almond oil.

TABLE 2

Fasting plasma lipids of men and women consuming whole almonds (WA) or almond oil (AO) for 6 wk[1]

Variable	Baseline	WA	AO
	mmol/L (mg/dL)		
Total cholesterol	5.35 ± 0.13 (206.5 ± 5.0)	5.12 ± 0.14* (197.6 ± 5.4)*	5.11 ± 0.14* (197.2 ± 5.4)*
LDL cholesterol	3.47 ± 0.11 (133.9 ± 4.2)	3.26 ± 0.12* (125.8 ± 4.6)*	3.24 ± 0.13* (125.1 ± 5.0)*
HDL cholesterol	1.16 ± 0.07 (44.8 ± 2.7)	1.21 ± 0.06* (46.7 ± 2.3)*	1.24 ± 0.06* (47.9 ± 2.3)*
Triglyceride	3.52 ± 0.38 (135.8 ± 14.7)	3.01 ± 0.32* (116.2 ± 12.4)*	2.98 ± 0.28* (115.0 ± 10.8)*
VLDL cholesterol	0.71 ± 0.08 (27.4 ± 3.1)	0.60 ± 0.06 (23.2 ± 2.3)	0.60 ± 0.06 (23.2 ± 2.3)
	μmol/L (g/L)		
Apolipoprotein A-1	50.8 ± 5.7 (1.44 ± 0.16)	47.7 ± 6.6 (1.35 ± 0.19)	48.6 ± 6.4 (1.38 ± 0.18)
Apolipoprotein B	1.87 ± 0.25 (0.96 ± 0.13)	1.89 ± 0.32 (0.97 ± 0.16)	1.97 ± 0.45 (1.00 ± 0.23)

[1] Values are means ± SEM, $n = 22$; * different from baseline, $P < 0.05$.

TABLE 3

Indices of in vitro plasma LDL oxidation in men and women consuming whole almonds (WA) or almond oil (AO) for 6 wk[1]

LDL oxidation indices	Baseline	WA	AO
Lag time, *min*	50 ± 3	56 ± 3	55 ± 4
Propagation rate, Δ*OD/min*	1.31 ± 0.06	1.24 ± 0.06	1.31 ± 0.03
Conjugated diene formation,[2] *units*	2.57 ± 0.08	2.63 ± 0.10	2.73 ± 0.07

[1] Values are means ± SEM, $n = 22$.
[2] $\times 10^{-5}$ mol conjugated dienes/75 μg cholesterol.

We observed reductions of 4 and 6% in total cholesterol and LDL cholesterol, respectively, regardless of whether the subjects consumed almonds or almond oil. Abbey et al. (7) provided 84 g of raw almonds to the diet of healthy men as a replacement for half of their habitual fat intake. They observed a reduction in total and LDL cholesterol of 7 and 10%, respectively. The subjects in our study had a slightly higher baseline cholesterol level and consumed fewer almonds than in the above study [66 g/d vs. 84 g/d in (7)]. It is possible that these factors contributed to a more modest cholesterol reduction in our group.

Declines in total and LDL cholesterol in this and other almond feeding trials occurred despite unchanged total dietary fat intake. At baseline, our subjects were consuming 29% of daily energy as fat and maintained this level throughout the almond and almond oil periods. Spiller et al. (6) reported that adding 100 g of almonds to the diets of hypercholesterolemic volunteers reduced total and LDL cholesterol from 9 to 12% compared with baseline values. In a separate study, they used a parallel arm design to study the effects of daily consumption of 100 g of almonds compared with a high SFA diet and reported reductions of 16% for total and 19% for LDL cholesterol (8). In these studies, total fat intake was actually increased by the addition of almonds. Collectively, these reports add to the growing body of evidence suggesting that, within limits, the type of dietary fat consumed is more important than the total amount of fat in reducing plasma cholesterol levels.

The cholesterol-lowering effect of increased intake of MUFA is not unexpected. A number of early and recent studies have provided evidence that MUFA intake lowers total and LDL cholesterol when substituted for saturated fat energy or when added to the total fat content of the diet (22–24). However, an important and somewhat unexpected finding from our study was that almond consumption, either as the whole nut or the oil, reduced fasting plasma TG levels by 14% compared with baseline ($P = 0.04$). These findings differ from the results of a recent meta-analysis of seven studies addressing the effects of MUFA intake, in which the authors concluded that substitution of MUFA for SFA did not alter TG or HDL cholesterol levels (25). However, a recent investigation of the effects of peanuts and peanut oil intake, both rich in MUFA, also found a significant reduction in plasma TG levels (13%) after either peanut oil or peanut/peanut butter consumption (9). Although replacement of dietary carbohydrate with MUFA has been associated with reductions in plasma TG (22–24),

in both our study and the above peanut study, subjects maintained their baseline level of carbohydrate intake. It seems unlikely that the modest reduction in SFA intake (<5 g) noted in our study would account for the relatively large change in plasma TG levels noted. Support for this comes from the fact that the modest decline in SFA intake (~1.7%) noted in our study is about the same as the difference in SFA intake between peanut oil and the peanut butter group (~1%) both of which lowered plasma TG to a similar extent. Thus, the reason(s) for this decline in TG levels after increased MUFA consumption in either the peanut oil report or the current study remain to be clarified.

The TG-lowering effect associated with MUFA consumption assumes considerable importance because the absence of a TG increase and the lack of an HDL cholesterol–lowering effect are considered major advantages of consuming a MUFA-rich diet compared with a low fat, high carbohydrate diet (22–24). In addition to the decline in plasma TG, we in fact observed no decline but an actual increase in HDL cholesterol after consumption of almonds or almond oil for 6 wk. This finding may be mediated by the 14% decrease in TG in our subjects and related to the well-established inverse relationship between plasma TG and HDL cholesterol levels (26).

The addition of whole almonds to the diet provides constituents beyond those present in the fat found in the almond oil. A major goal of this study was to compare the lipid-lowering effects of intact whole-almond consumption relative to consumption of an equivalent amount of almond fat supplied as almond oil. We found that the magnitude and direction of the lipid changes associated with intake of whole almonds did not differ from those of almond oil. In fact, the lipid subfraction responses were remarkably consistent; both diets reduced total and LDL cholesterol and TG to the same extent and resulted in comparable increases in HDL cholesterol. During each of these diet periods, daily intake of cholesterol and fatty acid classes remained consistent. One difference noted was that whole almond consumption was associated with a modestly greater intake of dietary fiber. However, our results suggest that, in this setting, the additional fiber in whole almonds did not confer any further benefits in terms of plasma lipid outcomes.

Our study design sought to increase MUFA intake while minimizing changes in SFA, PUFA and cholesterol consumption during the whole-almond and almond oil periods. These variables showed only modest, if any changes, i.e., PUFA intake remained unchanged throughout and there was a small decline in SFA intake (~1.7% of energy) in parallel with a modest decrease in daily cholesterol intake (~60 mg/d). Several model equations have been developed to predict changes in plasma cholesterol based upon dietary changes in the percentage of daily energy provided by SFA, MUFA, PUFA and cholesterol (27,28), and it was of interest to see the variable extent to which the changes observed in our study matched the predictions of the different equations.

In conclusion, we hypothesized that nonfat-associated constituents in whole nuts are responsible in part for the lipid-lowering associated with almond consumption and that feeding whole almonds compared with almond oil would demonstrate differential effects on plasma lipids and LDL oxidation. However, the effects of whole almonds and almond oil on our selected end points were not different from one another. Thus, we conclude that the lipid-lowering effect of almonds is mediated primarily by the almond oil fraction of these nuts. However, the favorable changes in lipid profiles found in the current study suggest that in healthy subjects, consumption of almonds or their oil can be an effective strategy to lower total and LDL cholesterol while preventing the HDL cholesterol-lowering and potential TG elevation associated with low fat, high carbohydrate diets (29). Furthermore, the amount of almonds consumed by these subjects was much less than most reported studies and was easily incorporated into the daily diet of our study subjects. Finally, nuts contain a host of other nutrients and components that have the potential to reduce risk for CVD, beyond the limited number of risk factors monitored in this study.

ACKNOWLEDGMENT

The authors thank Diane Richter for her expertise in performing LDL oxidation assays.

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