

Nuts and Plasma Lipids: An Almond-Based Diet Lowers LDL-C while Preserving HDL-C

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Objective: To compare lipid-altering effects of an almond-based diet with an olive oil-based diet, against a cheese and butter-based control diet.

Methods: Forty-five free-living hyperlipidemic men (n=12) and women (n=33) with a mean plasma total cholesterol (TC) of 251 ± 30 mg/dL followed one of three diets; almond-based, olive oil-based, or dairy-based for 4 weeks. Total fat in each diet was matched, and the study-provided sources of fat comprised the major portion of fat intake.

Results: Reductions in TC and low-density lipoprotein-cholesterol (LDL-C) between the three groups were significantly different from the almond group (both $p < 0.001$). Within group analysis revealed that the almond-based diet induced significant reductions in TC ($p < 0.05$), LDL-C ($p < 0.001$), and the TC:HDL ratio ($p < 0.001$), while HDL-C levels were preserved. TC and HDL-C in the control diet were significantly increased from baseline (both $p < 0.05$), while the olive oil-based diet resulted in no significant changes over the study period. Weight did not change significantly.

Conclusion: Results suggest that the more favorable lipid-altering effects induced by the almond group may be due to interactive or additive effects of the numerous bioactive constituents found in almonds.

INTRODUCTION

Whole foods, such as nuts, contain numerous beneficial nutritive and bioactive compounds like fatty acids, dietary fibers, micronutrients, and phytochemicals. Many of these compounds, e.g., fatty acids, have been examined as isolated elements either as a formula or as a natural fat, and have shown favorable lipid-altering activity [1,2]. However, in studying the hypocholesterolemic effects of diet, little research has examined the outcome resulting from consuming a diet of whole and unrefined foods that contain naturally occurring salutary elements.

As a commonly available food, tree nuts such as almonds, hazelnuts, and pistachios are rich in several beneficial compounds, such as ω -9 fatty acids, which in the form of olive, canola, and other oils, have demonstrated beneficial effects on blood cholesterol and lipoprotein profiles [1,3–9], and walnuts,

high in ω -6 fatty acids, have also been found to be cardioprotective [10]. Further, protein in nuts have an arginine-rich amino acid profile that is thought to be protective [3]. In addition, nuts are good sources of dietary fiber, ranging from 4% to 11% by weight, and are excellent sources of micronutrients, such as copper and magnesium, and phytochemicals, such as plant sterols, all of which have been documented to contribute to reduced risk of coronary heart disease [4–6]. Additionally, almonds in particular are especially rich in many tocopherols, including α -tocopherol, the most active form of vitamin E, which has also shown potent anti-atherogenic effects [6,7].

We had previously shown [5] that a diet low in saturated fat and high in plant foods which included 100 g/day of raw almonds (*Prunus amygdalus*), a nut rich in ω -9 fatty acids, reduced TC and LDL-C without adversely affecting triglycerides (TG). HDL-C levels, sometimes lowered by other hypocholesterolemic diets, were preserved in that investigation.

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Further, several other clinical studies using diverse research designs and methods with both men and women have demonstrated beneficial changes in lipid profiles feeding either walnuts, almonds, or macadamia nuts [8–12]. Moreover, two population studies, the Loma Linda Adventist Health Study and the Iowa Women's Study, have reported inverse associations between nut consumption and coronary artery disease [13,14].

To confirm the TC-lowering effect we found in our first uncontrolled study [5], we fed a diet where the major portion of fat was supplied by almonds and compared it to a diet with an equivalent amount of fat supplied by olive oil, a highly mono-unsaturated oil. A third group, a control group, consumed a diet high in saturated fatty acids that were supplied by cheese and butter. The balance of the diet was similar in macronutrient content.

MATERIALS AND METHODS

Subjects

Forty-eight hypercholesterolemic adults, all residents of the San Francisco Bay Area and recruited through newspaper advertisements or local physicians, were entered into the study. They were screened for medications known to alter lipids and for heart disease, diabetes, other major diseases, and any known food allergy. The almond and the olive oil groups consisted of 18 subjects each. At the time of randomization, three subjects were lost from the olive oil group. Two of the three withdrew for reasons unrelated to the study, while the third was excluded for noncompliance. Fifteen subjects were assigned to the control group, but three withdrew because they were unwilling to follow a diet expected not to lower TC. The data reported here are based on the 45 subjects who completed the study. There were 12 men and 33 women with a mean age of 53 ± 10 years and a mean body weight of 66 ± 13 kg. They had a mean baseline TC of 251 ± 30 mg/dL, an LDL-C of 166 ± 26 mg/dL, an HDL-C of 59 ± 16 mg/dL, TG of 133 ± 63 mg/dL, and a TC/HDL ratio of 465 ± 1.1 . Subjects were asked not to take any medication proscribed by the investigators and to inform the investigators immediately should any medication become necessary. The study protocol had been approved by an independent review committee and was explained to each subject who then signed an informed consent.

Study Design

A randomized, controlled, parallel design was used to compare the three study diets over a 4-week period after a 1-week baseline period. Sample sizes in the diet groups were based on our experiences with earlier diet studies in which, to reduce TC by approximately 10% and to yield such a reduction in $\geq 90\%$ of subjects on the diet, 15 to 20 subjects per group were sufficient to achieve a power of the test in excess of 0.90 in individuals whose TC was between 200–300 mg/dL. As no

lowering of TC was expected in the control group, a smaller sample size was considered sufficient to validate the results of the other two diet groups. All subjects were ranked by baseline TC from lowest to highest. They were then blocked into groups of three, and within each group assigned randomly to one of the three diet groups. During baseline, 3-day food record data were obtained, an initial blood test was done for routine hematological and biochemical measurements, and body weight and height were measured. Duplicate 12-hour fasting plasma lipid measurements were made on day one and day three of baseline. Two final duplicate lipid measurements were made 2 days apart and 3-day food record data were obtained during the fourth week of the intervention. Following the first baseline blood sample and until the end of the baseline period, subjects were asked not to change their normal diets. They received no specific information about the study diets until the end of this period to discourage independently initiated dietary changes. After 2 weeks subjects met with the investigators to discuss experiences with the diet and to be weighed. In addition, to monitor compliance, random 24-hour dietary recalls and phone interviews were conducted by the study dietitian.

Study Diet

After the baseline week, subjects were given verbal and written detailed diet instruction in a group meeting and individually as needed. During the 4-week study period, all subjects consumed a similar background diet, which consisted primarily of whole and unrefined foods that were matched for carbohydrate, protein, and total fat content. Dietary fiber intake was not matched, since fiber-rich foods, such as fruits, vegetables, and legumes would have confounded findings by their potential to affect lipid values [15]. In each group, approximately 630 calories a day were added to the background diet, about 450 calories of which were supplied by either almonds, olive oil, or butter and cheese (control diet) as the primary sources of fat. The total fat content of each diet was matched, and the study-provided fats comprised the major proportion of fat intake. The fatty acid composition of the three sources of fat is shown in Table 1. The almond group was provided with 100 g/day of raw unblanched almonds, supplied both as whole and ground nuts. The olive oil group was provided with 48 g/day of olive oil and was also given 113 g/day of cottage cheese and 21

Table 1. Fatty Acid Composition of Study-Provided Sources of Fat (g/day)

	nonpareil California almonds (<i>Prunus amygdalus</i>) (100 g)	California virgin olive oil (48 g)	Cheddar cheese (85 g) Butter (28 g)
Total fat	48.0	47.5	55.3
MUFA	30.0	35.7	16.9
PUFA	14.0	3.8	1.7
SFA	4.0	8.0	36.7

g/day of rye crackers to approximate the protein and carbohydrate content of the almond diet, and the control group was provided with 85 g/day of cheddar cheese and 28 g/day of butter, along with 21 g/day of rye crackers also to approximate the protein and carbohydrate content of the almond diet. The macronutrient and dietary fiber content of these foods are given in Table 2. In addition, all subjects were provided with whole grain bread, brown rice, pasta, nonfat yogurt, rice cakes, dry beans, lentils, and couscous and were instructed to eat these foods a set number of times during each week. Subjects rounded out their daily food intake with fruits, vegetables, other whole grains, legumes, lowfat or nonfat milk, egg whites and lean fish. Foods not allowed were commercial or homemade products containing fats other than the study fat and products made with refined flour (e.g., snack foods, chips, crackers, cakes, pastries, pies, candy, ice cream). Whole milk dairy products were not allowed, while lean beef was allowed twice weekly, and poultry and fatty fish were permitted up to four times weekly. Whole eggs were permitted up to four a week, but only if the subject had been consuming eggs prior to the study. Subjects were also instructed to maintain their usual pattern of coffee, tea, alcohol, and soft drink intake, their typical exercise routine, smoking habits, and not to make any special efforts toward changing their weight.

Plasma Cholesterol and Lipoprotein Measurements

Duplicate plasma lipid measurements were made at baseline and at 4 weeks. Two 10-ml blood samples were drawn into vacutainer tubes containing 15 mg sodium ethylenediaminetetraacetic acid (EDTA) from the antecubital vein after a 12-hour fast. Each vacutainer tube was then centrifuged for 10 to 15 minutes, and the specimens shipped by overnight air carrier under refrigeration. All samples were analyzed the following day. HDL-C was separated from the plasma by a precipitation procedure using dextran sulfate (50,000 daltons) and magnesium chloride [16]. TC in the remaining plasma and in the separated HDL-C fraction was measured by an enzymatic procedure on the Spectrum Analyzer (Abbott Laboratories, North

Chicago, IL). TG corrected for the glycerol blank were analyzed by an enzymatic UV procedure (Agent—a TG reagent, Abbott Laboratories, North Chicago, IL) on the same Spectrum Analyzer [17]. These analytical procedures were standardized and met the performance requirements of the Lipoprotein Standardization Program of the Centers for Disease Control (Atlanta, GA) and are traceable to the National Reference System for Cholesterol. LDL-C was estimated according to the Friedewald algorithm [18] using a value for very low-density lipoprotein obtained by dividing TG in mmol/L by 2.22. The long-term interassay correlation coefficient (CV) during the study was between 1–2% for TC and less than 2.5% for HDL-C at all concentrations measured. Intra-assay CV was less than 1.5% for both TC and HDL-C chloride [16–18].

Statistical Methodology

The effects of the study diets on plasma lipids were estimated by analysis of variance for repeated measures (ANOVA). Student's paired t-test (two tailed) from baseline to four weeks for each diet group was computed. A p-value of <0.05 was taken to be statistically significant. All results are expressed as mean ± SD. Three-day diet records were computer analyzed by the Nutritionist III nutrition analysis program with an expanded database (N-Squared Computing, Inc., Salem, OR).

RESULTS

Changes in Diet and Weight

Compliance with the protocol and acceptance of the three study diets were assessed by 3-day diet records, 24-hour dietary recalls conducted on random days, and verbal reports by subjects at the study group meetings, and was found to be excellent. Table 3 shows the composition of each study diet at baseline and at 4 weeks. Caloric intake was significantly higher ($p < 0.05$) in the olive oil group compared with the almond or control groups. Total fat intake increased significantly

Table 2. Composition of Study-Provided Foods

	Almond-based diet	Olive oil-based diet	Control diet
	Whole and ground almonds (100 g)	Olive oil (48 g) Cottage cheese (113 g) Rye crackers (21 g/day)	Cheddar cheese (85 g) Butter (28 g) Rye crackers (21 g)
Total energy (kcal)	636	627	621
Protein (g)	21	19	24
Carbohydrate (g)	9	20	19
Dietary fiber (g)	12	3	3
Total fat (g)	48	49	52
MUFA (g)	30	35	18
PUFA (g)	14	4	2
SFA (g)	4	10	32

Table 3. Changes in Body Weight and Food Intake (Mean±SD)

	Almond-based diet (n=18)		Olive oil-based diet (n=15)		Control diet (n=12)	
	Baseline	4 weeks	Baseline	4 weeks	Baseline	4 weeks
Weight (kg)	65±13	65±13	69±15	67±14	64±11	63±11
Energy (kcal)	1668±362	1703±283	2013±362	2183±362 ⁴	1852±321	1917±257
% calories						
Protein	17±3	16±2	8±5	17±4	15±2	17±2
Carbohydrate	47±8	44±5	52±6	47±6	48±6	45±7
Total fat	34±8	39±5 ¹	28±8	35±4 ¹	33±5	35±4
(g/day)						
Protein	73±18	70±16	89±19	93±26	73±15	81±13
Carbohydrate	199±52	197±46	264±53	260±53	228±50	224±58
Total fat	65±21	76±12 ¹	65±27	87±17 ¹	68±16	75±7
MUFA	36±9	53±2 ^{3,5}	39±10	65±12 ³	39±5	31±2 ¹
PUFA	11±6	13±1 ⁵	9±6	8±2	9±4	6±2 ²
SFA	18±7	10±3 ^{3,5}	18±11	14±3	20±7	37±3 ³
Dietary fiber	15±6	25±4 ³	22±9	29±7 ¹	18±6	26±6 ²
(mg/day)						
Cholesterol	205±92	72±41 ³	216±91	119±68 ²	213±71	269±75 ²

Significant within group from baseline—¹p<0.05, ²p<0.01, ³p<0.001.

Significant between groups at 4 weeks—⁴p<0.05, ⁵p<0.001.

(p<0.05) in the almond and olive oil groups, but not in the control group. Monounsaturated fatty acid (MUFA) intake increased significantly in both the almond and olive oil groups (p<0.001), while it decreased significantly (p<0.05) in the control group. Reduction in polyunsaturated fatty acid (PUFA) intake was significant (p<0.01) only in the control group, while reductions in saturated fatty acid (SFA) intake in the almond group and the increase in SFA in the control group were highly significant (both p<0.001). Dietary fiber increased significantly in all three groups (almonds—p<0.001; olive oil—p<0.05; control—p<0.01). Dietary cholesterol intake decreased significantly in the almond and olive oil groups (p<0.001 and p<0.01, respectively), while the control group significantly increased their intake (p<0.01). Also, there were

no significant changes in body weight between groups at 4 weeks (Table 3).

Changes in Plasma Cholesterol and Lipoproteins

Fig. 1 illustrates changes in plasma cholesterol and lipids for each group at baseline and 4 weeks. At 4 weeks there were highly significant differences between the three groups for TC (almond group: 222±28 mg/dL, olive oil group: 240±27 mg/dL, control group: 263±42 mg/dL; p<0.001) and LDL-C (almond group: 141±25 mg/dL, olive oil group: 157±21 mg/dL, control group: 174±39 mg/dL; p<0.001), but no significant changes in HDL-C, TG, or TC/HDL levels. Within group analysis revealed that the almond-based diet induced

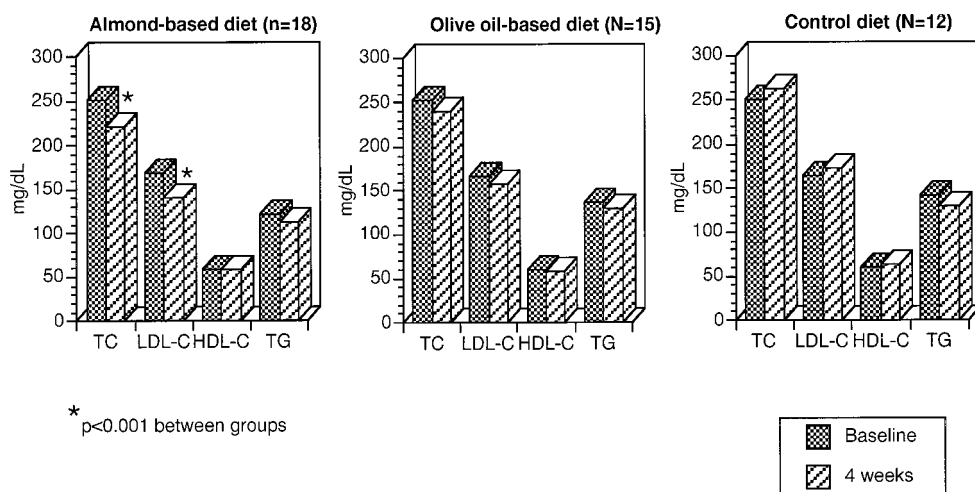


Fig. 1. Changes in total cholesterol and lipoproteins between diet groups.

significant reductions in TC ($p < 0.05$), LDL-C ($p < 0.001$), and the TC:HDL ratio ($p < 0.001$), while HDL-C levels were preserved. TC and HDL-C in the control diet were significantly increased from baseline (both $p < 0.05$), while the olive oil-based diet resulted in no significant changes over the study period. Weight did not change significantly.

DISCUSSION

The results of this study showed that the almond-based diet induced significant favorable changes in TC and LDL-C relative to the olive oil-based and control diets at a fat intake higher than that recommended by the National Cholesterol Education Program for cholesterol lowering, i.e., 39% calories vs. 30% calories [19]. In addition, there were no significant changes in body weight over the study period. The primary food sources of fat in each group, almonds, olive oil, and cheese/butter, were reflected in plasma lipid changes. The decreases in TC and LDL-C in the almond group were similar to the decreases previously achieved in 27 subjects who were placed on an almond-based diet [20]. In that investigation TC decreased from 235 ± 4 to 216 ± 4 mg/dl after 3 weeks and remained at approximately that level until the end of the 9-week study. Also in that study, as in the present almond-based diet, HDL-C levels were unchanged, with the reduction in TC being attributed to changes in LDL-C. However, the lack of a control group in our previous investigation precluded us from concluding whether the effect on lipids was due to the almonds or to other factors such as the influence of other substances contained in the diet. Both the olive oil and control groups in this study had lower total fat intakes (both 35% calories/fat) at 4 weeks than the almond group (39% calories/fat) suggesting that the effects were independent of total fat intake.

This present investigation also showed that the almond-based diet resulted in lower plasma TC and LDL-C by comparison with the other two study diets. An explanation for the enhanced lipid-altering activity of the almond-based diet may be the contribution made by an increased intake of plant foods and a decreased intake of animal foods and refined products. This is suggested by both the significant increases in dietary fiber (almond diet— $p < 0.001$; olive oil diet— $p < 0.05$) and the significant decreases in dietary cholesterol (almond diet— $p < 0.001$; olive oil diet— $p < 0.01$). The differences in effectiveness between the almond and olive oil groups may be due to several variables, considering that other investigators have demonstrated greater reductions in TC with olive oil or other oils high in monounsaturated fatty acids than we did [21–26]. The first possibility may be the source of protein that was added to the olive oil diet. We used 113 g of cottage cheese which supplied about 16 g of milk protein (mostly as casein) to replace the majority of the protein provided by almonds. There is significant literature indicating that amino acid profiles,

including the favorable arginine:lysine ratio in nuts, have beneficial effects on blood lipids when compared with animal proteins [6,23,27]. A second possible explanation may be the contribution of the dietary fibers supplied by the almonds (about 11 g/day). Numerous studies have demonstrated lipid-lowering effects of fibers such as those found in nuts [2]. Even though the dietary fiber content (Table 3) of the three diets was similar, the type of fiber in the almonds might have hypolipemic effects. Further study of this fiber and nut fibers in general are needed to separate their effect from that of the protein and lipid pattern. A third possible explanation is the presence of lipid-altering phytochemicals such as plant sterols and saponins that are found in almonds [4,28]. Finally, the significantly higher calorie intake in the olive oil group may have influenced the outcome.

CONCLUSIONS

This study supports the benefits of a diet supplying a reasonable amount of fat as monounsaturated fat, while low in saturated fat, for control of plasma cholesterol. The results suggest that almonds and other tree nuts represent an appropriate food choice for individuals following a hypocholesterolemic diet, and that including them as a replacement for some of the saturated fat in hypolipidemic diets, such as the NCEP Step 1 or 2 diet [24] can contribute to anti-atherogenic activity [6,28,29]. Moreover, since almonds are a ready-to-eat snack food and could be incorporated easily into whole grain products or entrees they can be a convenient addition to a cholesterol-lowering diet. Some of the problems found in feeding very low fat diets in sedentary populations (e.g., reduction in HDL-C or elevation of plasma TG) may be prevented by nuts such as almonds. Further research on the beneficial properties of whole and unrefined foods high in unsaturated fats, such as nuts, on cardiovascular risk factors is needed, as are more comparisons of very low fat diets to diets supplying reasonable amounts of nuts or seeds high in oils.

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