Substituting Walnuts for Monounsaturated Fat Improves the Serum Lipid Profile of Hypercholesterolemic Men and Women

A Randomized Crossover Trial

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Background: It has been reported that walnuts reduce serum cholesterol levels in normal young men.

Objective: To assess the acceptability of walnuts and their effects on serum lipid levels and low-density lipoprotein (LDL) oxidizability in free-living hypercholesterolemic persons.

Design: Randomized, crossover feeding trial.

Setting: Lipid clinic at a university hospital.

Patients: 55 men and women (mean age, 56 years) with polygenic hypercholesterolemia.

Intervention: A cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content in which walnuts replaced approximately 35% of the energy obtained from monounsaturated fat. Patients followed each diet for 6 weeks.

Measurements: Low-density lipoprotein fatty acids (to assess compliance), serum lipid levels, lipoprotein(a) levels, and LDL resistance to in vitro oxidative stress.

Results: 49 persons completed the trial. The walnut diet was well tolerated. Planned and observed diets were closely matched. Compared with the Mediterranean diet, the walnut diet produced mean changes of −4.1% in total cholesterol level, −5.9% in LDL cholesterol level, and −6.2% in lipoprotein(a) level. The mean differences in the changes in serum lipid levels were −0.28 mmol/L (95% CI, −0.43 to −0.12 mmol/L) (−10.8 mg/dL [−16.8 to −4.8 mg/dL]) ($P < 0.001$) for total cholesterol level, −0.29 mmol/L (CI, −0.41 to −0.15 mmol/L) (−11.2 mg/dL [−16.3 to −6.1 mg/dL]) ($P < 0.001$) for LDL cholesterol level, and −0.021 g/L (CI, −0.042 to −0.001 g/L) ($P = 0.042$) for lipoprotein(a) level. Lipid changes were similar in men and women except for lipoprotein(a) levels, which decreased only in men. Low-density lipoprotein particles were enriched with polyunsaturated fatty acids from walnuts, but their resistance to oxidation was preserved.

Conclusion: Substituting walnuts for part of the monounsaturated fat in a cholesterol-lowering Mediterranean diet further reduced total and LDL cholesterol levels in men and women with hypercholesterolemia.

Ann Intern Med. 2000;132:538-546.

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Lifestyle modification is the cornerstone of population-based strategies for prevention of coronary heart disease and is the first line of therapy in patients with hypercholesterolemia. Diets low in saturated fatty acids and cholesterol have long been recommended to decrease low-density lipoprotein (LDL) cholesterol levels and reduce cardiovascular risk (1). Ample evidence suggests that polyunsaturated fatty acids and monounsaturated fatty acids have a similar cholesterol-lowering effect when substituted for saturated fatty acids (2–4). However, most studies of fatty acids and blood lipids have been done with fats and oils, rarely with whole fatty foods. Because people usually buy and consume whole food products, it is desirable to know the effects of specific foods on risk factors for coronary heart disease.

Recent reports suggest that the regular consumption of nuts might reduce cardiovascular risk (5). Walnuts are particularly rich in polyunsaturated fatty acids (6), and epidemiologic evidence suggests that frequent walnut consumption protects against coronary heart disease (7). In a controlled feeding trial by Sabaté and colleagues (8), a diet in which walnuts represented 55% of the energy from fat reduced blood cholesterol levels in normal young men when compared with a standard low-fat diet. However, the results cannot easily be extrapolated to the population at risk for coronary heart disease because women, older age groups, and hypercholesterolemic persons were not studied (8). In addition, because meals were served at a metabolic kitchen, the study did not address the question of whether free-living persons would incorporate substantial quantities of walnuts into their diets. Because oxidized LDL plays a key role in atherogenesis (9) and oxidative damage involves peroxidation of polyunsaturated fatty acids in LDL lipids (10), there is concern that walnut intake may promote LDL oxidation. Therefore, we designed a dietary intervention study in free-living adult men and women with polygenic hypercholesterolemia to compare the effects of a walnut-rich diet with those of a cholesterol-lowering Mediterranean diet on serum lipid levels, lipoprotein levels, and LDL resistance to oxidation.

Methods

Patients

Adult men and women with polygenic hypercholesterolemia attending the Lipid Clinic of the Hospital Clínic of Barcelona were eligible if they had serum LDL cholesterol concentrations greater than 3.36 mmol/L (130 mg/dL) and triglyceride concentrations less than 2.82 mmol/L (250 mg/dL); no evidence of alcohol, tobacco, or drug abuse; absence of diabetes mellitus and liver, kidney, thyroid, or other endocrine diseases, as assessed by medical history, a complete physical examination, and laboratory tests; no intake in the previous 8 weeks of medications known to affect lipid metabolism, including hypolipidemic agents and estrogen compounds in women; infrequent consumption of nuts and no known history of allergy to them; and no use of multivitamin or vitamin E supplements. Because the target population had common (polygenic) hypercholesterolemia, we excluded persons whose elevated blood cholesterol levels had a strong genetic basis (such as heterozygous familial hypercholesterolemia or familial combined hyperlipidemia), as established by standard criteria. On admission to the Lipid Clinic, all patients were advised to follow a Mediterranean-type hypolipidemic diet (11).

For a crossover design, statistical power calculations indicated that to detect mean differences of 0.39 mmol/L (15 mg/dL), 34 patients would need to complete the two treatment periods (α statistic, 0.05; power > 0.8). From a computerized register of clinical records, 75 hypercholesterolemic patients (35 women and 40 men) who initially met the eligibility criteria were selected for screening and were asked to participate in the study. They were offered free walnuts but no monetary compensation.

Study Design

A crossover design was used. Patients were randomly assigned to the two diet sequences by using a computer-generated random-number table, with stratification by sex. Because patients followed each diet for 6 weeks and lipoprotein changes due to dietary intervention stabilize in less than 4 weeks (12), we did not incorporate a washout period between diets. In their crossover feeding study with walnuts, Sabaté and colleagues (8) did not observe a carryover effect. In the week before the trial began, patients received expert dietary counseling individually and in a group class. Twice during the pretrial week and on weeks 5 and 6 of each one of the two dietary periods, patients came to the clinic for a medical visit, an interview with the dietitian, anthropometric measurements, and blood extraction. The main outcomes of the study were changes in serum levels of total and LDL cholesterol from the control diet period to the walnut diet period. Secondary outcomes were changes in other lipid variables and oxidizability of LDL particles. The study protocol was approved by the institutional review board of the Hospital Clínic of Barcelona, and all patients gave informed consent.

Diets

The experimental diets were individually prescribed and were based on estimated energy requirements. Because participants ate on their own, detailed dietary information was provided to them and, if appropriate, to their partners. Diets were calculated in increments of 200 kcal to cover the range from 1600 to 2200 kcal. The control diet was Mediterranean and was composed of natural foodstuffs. Red meat and eggs were limited, vegetable products and fish were emphasized, olive oil was indicated for culinary use, and no nuts were allowed. The walnut diet was similar to the control diet, but walnuts partially replaced olive oil and other fatty foods. Prepackaged daily allowances of raw, shelled walnuts were provided daily in amounts varying from 41 g to 56 g (the equivalent of 8 to 11 walnuts), according to the participants' total energy intake. Walnuts were consumed as snacks or with meals in desserts or salads. In the walnut diet, walnuts contributed approximately 18% of the total energy and 35% of the total fat. To improve compliance, each family unit was given 1000-g packs of walnuts at the beginning of the walnut diet period.

Adherence to the study diets was carefully monitored. Unannounced 24-hour diet recalls were performed weekly by telephone during the two dietary periods, for a total of 12 recalls per patient. This method allows reliable estimations of food intake (13). The nutrient composition of the diets was calculated with Food Processor Plus software, version 5.0 (ESHA Research, Salem, Oregon), which was adapted to nutrient databases of specific Mediterranean foods when appropriate. We defined *noncompliance* as at least 20% deviation from dietary instructions regarding walnut or nutrient intake. Compliance during the walnut diet was also assessed at each clinic visit by a count of the empty walnut packages. The fatty acid content of LDL lipids was analyzed as a biological measure of adherence to the prescribed diets.

Laboratory Measurements

Blood samples were obtained after an overnight fast, and serum and EDTA plasma were collected and processed immediately. Serum lipid and apolipoprotein levels were determined as described else-

where (11). In brief, serum cholesterol and triglyceride levels were measured by using enzymatic reagents (Trinder, Bayer Diagnostics, Tarrytown, New York) adapted to a Cobas Mira automated analyzer (Hoffmann-LaRoche, Basel, Switzerland). High-density lipoprotein (HDL) cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. Levels of apolipoprotein A-I and apolipoprotein B were determined by an immunoturbidimetric method (Unimate 3, Roche Diagnostic Systems, Basel, Switzerland). Measurements of the cholesterol content of very-low-density lipoprotein particles and LDL particles were based on preparative ultracentrifugation (11). Lipoprotein(a) levels were measured by using an immunoturbidimetric method (Lipoprotein(a) SPQ II Test Kit, DiaSorin, Stillwater, Minnesota).

To obtain the LDL fraction for fatty acid and oxidizability studies, lipoproteins were fractionated by sequential centrifugation adjusting with NaBr to separate very-low-density lipoprotein particles (d = 1.006 g/mL) and to obtain LDL particles (d = 1.063 g/mL), as described elsewhere (14). Low-density lipoprotein fatty acids were measured by capillary gas chromatography in the cholesteryl ester, phospholipid, and triglyceride lipid fractions (14). Low-density lipoprotein susceptibility to oxidation was determined by measuring the α-tocopherol content of LDL particles and conjugated diene kinetics after incubation of 50 μg of LDL protein with 5 μmol of copper sulfate at 37 °C (15, 16).

Statistical Analysis

The two measurements obtained at baseline and at the end of each dietary period were averaged. Means and SDs are presented for each measurement. With methods described by Fleiss (17), two-tailed *t*-tests were used to compare changes in outcome variables in response to dietary treatment and diet period and carryover effects for the two-period crossover design. Differences between the walnut and control diets were also tested by analysis of covariance using general linear models; baseline values or sex were used as covariates. Analyses were

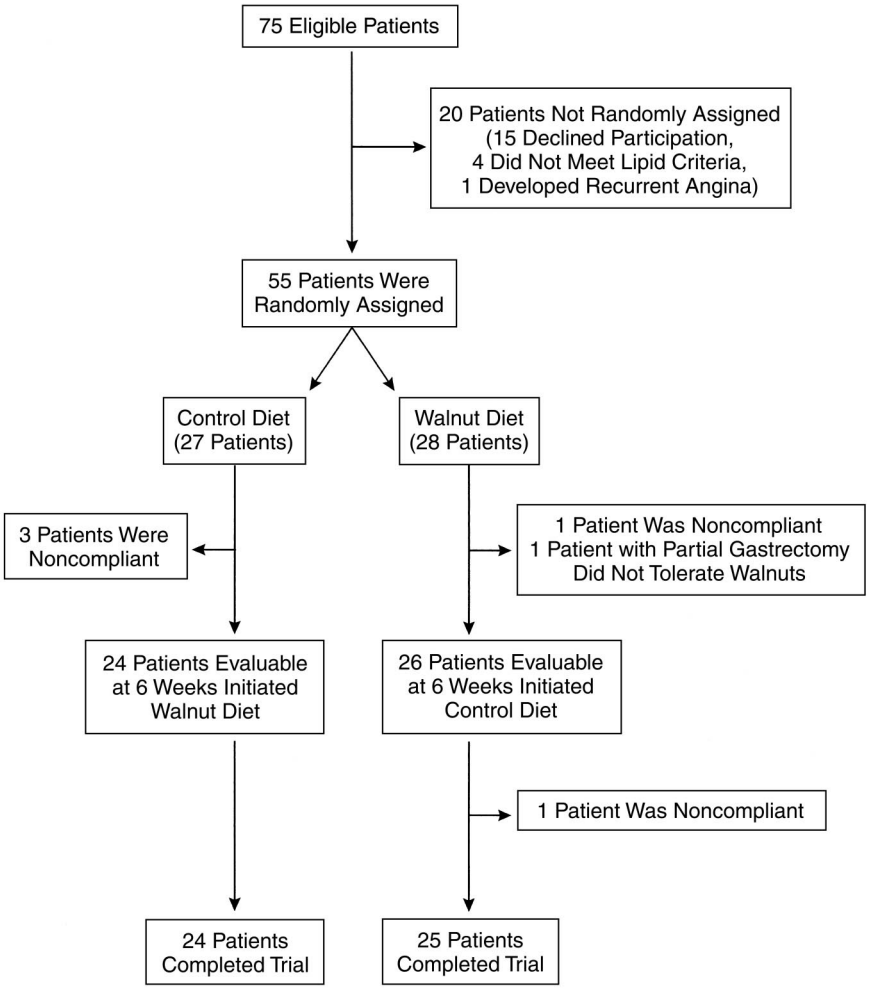

Figure 1. Flow of patients.

Table 1. Baseline Characteristics of Study Patients

Variable	Women (n = 27)		Men (n = 28)		All Patients (n = 55)
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Age, *y*	59 ± 8	42–68	53 ± 12	28–72	56 ± 11
Weight, *kg*	65.0 ± 8.9	47–79	75.2 ± 12.7	55–101	70.6 ± 12.1
Body mass index, *kg/m²*	26.3 ± 3.4	19–34	27.5 ± 2.9	20–33	27.0 ± 3.1
Waist-to-hip ratio	0.88 ± 0.09	0.75–1.05	0.95 ± 0.04	0.85–1.01	0.91 ± 0.07
Blood pressure, *mm Hg*					
Systolic	124 ± 20	95–160	123 ± 16	90–160	123 ± 18
Diastolic	81 ± 12	60–100	77 ± 9.2	55–95	79 ± 11
Serum lipids, *mmol/L (mg/dL)*					
Total cholesterol level	7.42 ± 0.75 (287 ± 29)	6.05–8.79 (234–340)	6.98 ± 0.88 (270 ± 34)	5.74–8.61 (222–333)	7.21 ± 0.83 (279 ± 32)
Low-density lipoprotein cholesterol level	5.22 ± 0.70 (202 ± 27)	4.09–6.47 (158–250)	4.78 ± 0.85 (185 ± 33)	3.36–6.52 (130–252)	5.05 ± 0.75 (195 ± 29)
High-density lipoprotein cholesterol level	1.60 ± 0.34 (62 ± 13)	1.01–2.22 (39–86)	1.32 ± 0.28 (51 ± 11)	0.67–1.91 (26–74)	1.44 ± 0.31 (56 ± 12)
Triglyceride level	1.42 ± 0.51 (126 ± 45)	0.73–2.66 (65–236)	1.56 ± 0.41 (138 ± 36)	0.89–2.81 (79–249)	1.51 ± 0.43 (134 ± 38)

performed by using SAS software (SAS, Inc., Cary, North Carolina) (18).

Role of the Funding Sources

The California Walnut Commission provided funding and walnuts. Research grants were also obtained from national and local nonprofit agencies. The funding sources were not involved in the design of the study and had no role in the collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication. None of the authors has any financial interest in the nut food industry.

Results

Patient Characteristics

Of the 75 eligible patients, 20 left the study before randomization for various reasons (**Figure 1**). **Table 1** shows the baseline characteristics of the 55 patients (27 women and 28 men) who met all of the eligibility requirements, entered the study, and were randomly assigned to one of two dietary intervention sequences. Twenty-two women were postmenopausal. Nine patients (6 men and 3 women) had high blood pressure that was controlled with antihypertensive medication. Seven patients (5 men and 2 women) had coronary heart disease.

As shown in **Figure 1**, 6 patients withdrew before completing the two phases of the study. The baseline serum lipid profiles of the patients who were excluded for noncompliance were similar to the mean values of the overall group. Forty-nine participants (23 women and 26 men) completed both phases of the study. Subsequent data refer only to this group.

Dietary Compliance and Body Weight

The nutrient content of the self-reported diets was close to that of the planned diets (**Table 2**). The fatty acid composition of the control diet reflected the high monounsaturated fatty acid content of olive oil, and the fatty acid composition of the walnut diet mirrored the constitution of walnut fat, which is particularly rich in polyunsaturated fatty acids. Other nutrients had differences that were small but statistically significant. The significance can be explained by the high statistical power of the study, the use of calorie-adjusted nutrient values, and the participants' close adherence to the prescribed diets; all of these factors resulted in small SDs. According to participants' reports and to recounts of empty packages, compliance with walnut ingestion was 100%. The analysis of LDL cholesteryl ester fatty acids during the two dietary periods confirmed that participants had closely adhered to the prescribed diets. When the treatment effect of the walnut diet was compared with that of the control diet, molar percentages of oleic acid decreased 3.9 (CI, −4.6 to −3.2), molar percentages of linoleic acid increased 6.6 (CI, 8.0 to 5.0), and molar percentages of α-linolenic acid increased 0.34 (CI, 0.44 to 0.25) ($P <$ 0.001 for all comparisons). The respective mean changes of molar percentages of oleic acid, linoleic acid, and α-linolenic acid were −20%, 14%, and 83%. Similar changes occurred in LDL phospholipids and triglycerides.

Body weight was stable throughout the two intervention diet periods (**Table 3**). Daily walnut consumption was well tolerated by most patients. Twenty-five patients reported softening of the stools associated with walnut consumption. After walnut ingestion, 3 patient described mild symptoms of postprandial heaviness and bloating; however, these symptoms did not lead to withdrawal.

Table 2. Composition of the Prescribed and Observed Study Diets

Variable	Control Diet		Walnut Diet		*P* Value for Comparisons between Actual Diets
	Prescribed	Actual*	Prescribed	Actual*	
Energy, *kcal/d*	1600–2200	1771 ± 152	1600–2200	1824 ± 178	0.116
Energy derived from fat, %	30.2	31.2 ± 1.2	32.7	33.2 ± 1.3	<0.001
Saturated fatty acids	5.8	6.9 ± 0.7	5.0	6.0 ± 0.7	>0.2
Monounsaturated fatty acids	17.6	17.5 ± 1.1	13.2	13.5 ± 0.6	<0.001
Polyunsaturated fatty acids	4.2	4.8 ± 0.5	11.8	11.7 ± 0.8	<0.001
Linoleic acid (C18:2n-6)	3.3	3.8 ± 0.4	9.6	9.5 ± 0.6	<0.001
α-Linolenic acid (C18:3n-3)	0.4	0.4 ± 0.1	1.9	1.8 ± 0.1	<0.001
Energy derived from protein, %	18.1	19.0 ± 1.1	16.6	17.9 ± 1.1	<0.001
Energy derived from carbohydrates, %	51.7	49.8 ± 2.1	50.7	48.0 ± 1.9	<0.001
Cholesterol, *mg/1000 kcal*	103.9	124.8 ± 24.2	77.8	90.8 ± 17.0	<0.001
Soluble fiber, *g/1000 kcal*	4.8	4.7 ± 0.8	5.0	4.7 ± 0.8	>0.2
Vitamin E (total), *mg/1000 kcal*	8.2	7.1 ± 0.8	8.9	9.9 ± 0.8	<0.001
α-Tocopherol, *mg/1000 kcal*	5.8	5.8 ± 0.6	5.1	4.7 ± 0.6	<0.001
Vitamin C, *mg/1000 kcal*	76.2	97.8 ± 28.8	76.3	83.0 ± 23.9	0.007

* Values are the mean ± SD and were estimated from one 3-day food record and six 24-hour diet recalls during each diet period.

Effects on Serum Lipids and Lipoproteins

Figure 2 shows the changes from baseline values in serum lipids, lipoproteins, and apolipoproteins A-I and B. The actual values at baseline and at the end of each dietary period as well as the differences of effect between dietary interventions are presented in **Table 3**. No carryover effect was seen between the periods. The mean total cholesterol level decreased by 9.0% (0.65 mmol/L [25 mg/dL]) during the walnut diet and by 5.0% (0.36 mmol/L [14 mg/dL]) during the control diet. Similarly, the mean LDL cholesterol level decreased by 11.2% (0.57 mmol/L [22 mg/dL]) during the walnut diet and by 5.6% (0.28 mmol/L [11 mg/dL]) during the control diet. The two diets did not differ with respect to their effects on levels of HDL cholesterol, very-low-density lipoprotein cholesterol, triglycerides, or apolipoprotein A-I. Apolipoprotein B levels decreased after the two diets in parallel with LDL cholesterol levels (**Table 3**). The mean ratio of LDL cholesterol to HDL cholesterol did not change during the control diet and decreased by 8% during the walnut diet. The differences between the effects of the diets on the lipid profile did not change materially when they were adjusted for baseline values or sex by analysis of covariance.

Table 3 and **Figure 2** show the effects of the two diets on lipoprotein(a) levels. Lipoprotein(a) levels decreased by 9.1% (0.033 g/L) during the walnut diet and 3.4% (0.012 g/L) during the control diet. The difference in lipoprotein(a) reduction between the two diets was statistically significant in men ($P = 0.041$) but not in women ($P > 0.2$). In addition, this difference was statistically significant in patients with baseline lipoprotein(a) levels less than or equal to 0.3 g/L ($P = 0.042$) but not in those with baseline levels greater than 0.3 g/L ($P > 0.2$).

Low-Density Lipoprotein Oxidation

The α-tocopherol content of the LDL particles and the lag time of conjugate diene formation during copper-induced LDL oxidation were similar during the control and walnut diets (**Table 3**).

Table 3. Serum Lipid and Lipoprotein Levels, Analytes Related to Low-Density Lipoprotein Oxidation, and Body Weight at the End of Each Diet Period*

Variable	Mean Baseline Measurements ± SD	Mean Measurements during Control Diet ± SD	Mean Measurements during Walnut Diet ± SD
Total cholesterol level, *mmol/L (mg/dL)*	7.16 ± 0.85 (278 ± 33)	6.81 ± 0.79 (2.64 ± 31)	6.52 ± 0.90 (253 ± 35)
LDL cholesterol level, *mmol/L (mg/dL)*	5.05 ± 0.77 (196 ± 30)	4.77 ± 0.64 (185 ± 25)	4.48 ± 0.77 (174 ± 30)
HDL cholesterol level, *mmol/L (mg/dL)*	1.44 ± 0.33 (56 ± 13)	1.37 ± 0.31 (53 ± 12)	1.42 ± 0.36 (55 ± 14)
VLDL cholesterol level, *mmol/L (mg/dL)*	0.67 ± 0.33 (26 ± 13)	0.64 ± 0.36 (25 ± 14)	0.59 ± 0.33 (23 ± 13)
Triglyceride level, *mmol/L (mg/dL)*	1.54 ± 0.48 (136 ± 43)	1.51 ± 0.50 (134 ± 44)	1.42 ± 0.50 (126 ± 44)
Apolipoprotein A-I level, *g/L*	1.70 ± 0.24	1.61 ± 0.21	1.62 ± 0.23
Apolipoprotein B level, *g/L*	1.65 ± 0.23	1.52 ± 0.21	1.44 ± 0.22
LDL:HDL ratio	3.6 ± 1.1	3.7 ± 1.0	3.4 ± 1.0
Lipoprotein(a) level, *g/L*	0.35 ± 0.24	0.34 ± 0.24	0.32 ± 0.22
α-Tocopherol level, *μg/mg of LDL protein*	9.2 ± 1.5	7.2 ± 2.2	7.5 ± 2.4
Lag time of conjugated diene production, *min*	41.9 ± 6.5	42.0 ± 6.8	40.6 ± 6.0
Body weight, *kg*	70.6 ± 12.1	70.1 ± 12.3	69.9 ± 12.5

* HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.
† Average differences between control and walnut diets were calculated by the method described by Fleiss (17).
‡ Walnut diet compared with control diet.

Discussion

In this 12-week crossover dietary intervention trial in 49 free-living, hypercholesterolemic men and women, we found that substituting walnuts for approximately 35% of the energy from fat without changing saturated fatty acid intake in a cholesterol-lowering Mediterranean diet further decreased total cholesterol and LDL cholesterol levels. Use of walnuts did not affect HDL cholesterol levels and thereby improved the ratio of LDL cholesterol to HDL cholesterol. Our findings are consistent with those of an earlier study in normal young men (8) and extend the results of that study to older persons of either sex with elevated blood cholesterol levels. In addition, apolipoprotein B levels decreased after the walnut diet in parallel with LDL cholesterol levels, and lipoprotein(a) levels decreased in men and in patients whose baseline levels were greater than 0.3 g/L. Furthermore, LDL particles were enriched with polyunsaturated fatty acids from walnuts but preserved their resistance to oxidation.

The design of the study—an outpatient crossover feeding trial—presented difficulties in ensuring compliance. However, these difficulties were partially offset by detailed dietary instructions, regular reinforcement, and frequent 24-hour diet recalls performed weekly by telephone throughout the trial. Manageable daily allowances of walnuts and their distribution into different recipes also facilitated compliance during the walnut phase of the study. In fact, compliance was very good and the actual diets consumed closely matched the prescribed diets. The observed changes in the fatty acid composition of LDL lipids are congruent with this assertion. Besides the predictable differences in monounsaturated fatty acid and polyunsaturated fatty acid content, the caloric value and nutrient composition of the two diets were similar (**Table 2**). The patients consumed slightly less cholesterol during the walnut diet than during the control diet, a small difference that only partially explains the extent of the walnut diet's cholesterol-lowering effect (19). Because body weight was not modified, our findings cannot readily be attributed to changes in body weight or caloric intake. In a recent 8-week intervention study with a lower degree of dietary control, a diet containing substantial quantities of walnuts had no hypolipidemic effect when compared with a standard low-fat diet in 21 hypercholesterolemic men (20).

Substantial epidemiologic and experimental evidence indicate that a diet high in saturated fatty acids from animal fat is associated with elevated levels of blood cholesterol, which in turn are related to high incidence of coronary heart disease (1). It is also widely acknowledged that cholesterol levels can be reduced if saturated fat in the diet is replaced by unsaturated fatty acids from vegetable oils and fats; this effect can be predicted by the amounts of fatty acid classes exchanged (2–4). Recent meta-analyses of studies comparing dietary monounsaturated and polyunsaturated fatty acids suggest that their effects on serum lipids are similar when 4% to 10% of the energy of each fatty acid class is substituted for the other (2–4). To ascertain the extent to which the different fat content of the two diets could explain the observed reduction in LDL cholesterol during the walnut diet, we applied the recently developed predictive models that include the regression coefficients for percentage energy changes in dietary saturated and polyunsaturated fatty acids, monounsaturated fatty acids (2, 3), and dietary cholesterol (4). These equations predicted decreases ranging from 0.13 to 0.18 mmol/L (5 to 7 mg/dL), which are smaller than the average observed decrease of 0.29 mmol/L (11 mg/dL) but close to the lower 95% CI of the actual change (**Table 3**). Therefore, the hypolipidemic effect of the walnut diet can be explained in part by its fat content. Most comparative studies of the effects of unsaturated fats on serum lipid levels have used plant and vegetable oils as sources of monounsaturated fatty acids (oleic acid) and polyunsaturated fatty acids (mainly linoleic acid). However, walnuts are solid, complex foods that may influence blood lipids by the nonfat components of their matrix; this could be investigated in a feeding trial that compares the lipid effects of whole walnuts with those of walnut oil.

Lipoprotein(a) is an apolipoprotein B–containing lipoprotein that has been found to be a determinant of the risk for coronary heart disease in clinical and epidemiologic studies (21–24). Evidence suggests that it is also an independent predictor of nonfatal myocardial infarction and coronary death in hypercholesterolemic men (25). Almost all cross-sectional and retrospective studies have shown an increase in

Table 3—*Continued*

Treatment Effect (95% CI)†	Percentage Change‡	*P* Value for Comparisons between Diets
−0.28 (−0.43 to −0.12) (−10.8 [−16.8 to −4.8])	−4.1	<0.001
−0.29 (−0.41 to −0.15) (−11.2 [−16.3 to −6.1])	−5.9	<0.001
0.04 (0.10 to −0.01) (1.6 [3.9 to −0.5])	3.2	0.134
−0.05 (−0.12 to 0.03) (−1.8 [−4.6 to 1.0])	−7.2	>0.2
−0.09 (−0.20 to 0.02) (−8 [−18 to −2])	−6.1	0.103
0.01 (0.05 to −0.03)	0.6	0.103
−0.07 (−0.11 to −0.03)	−4.7	<0.001
−0.3 (−0.5 to −0.1)	−8.1	<0.001
−0.02 (−0.042 to −0.001)	−6.2	0.042
0.3 (−1.2 to 1.8)	4.0	>0.2
−1.54 (−3.40 to 0.32)	−3.3	0.101
−0.2 (−0.4 to 0.0)	−0.3	0.07

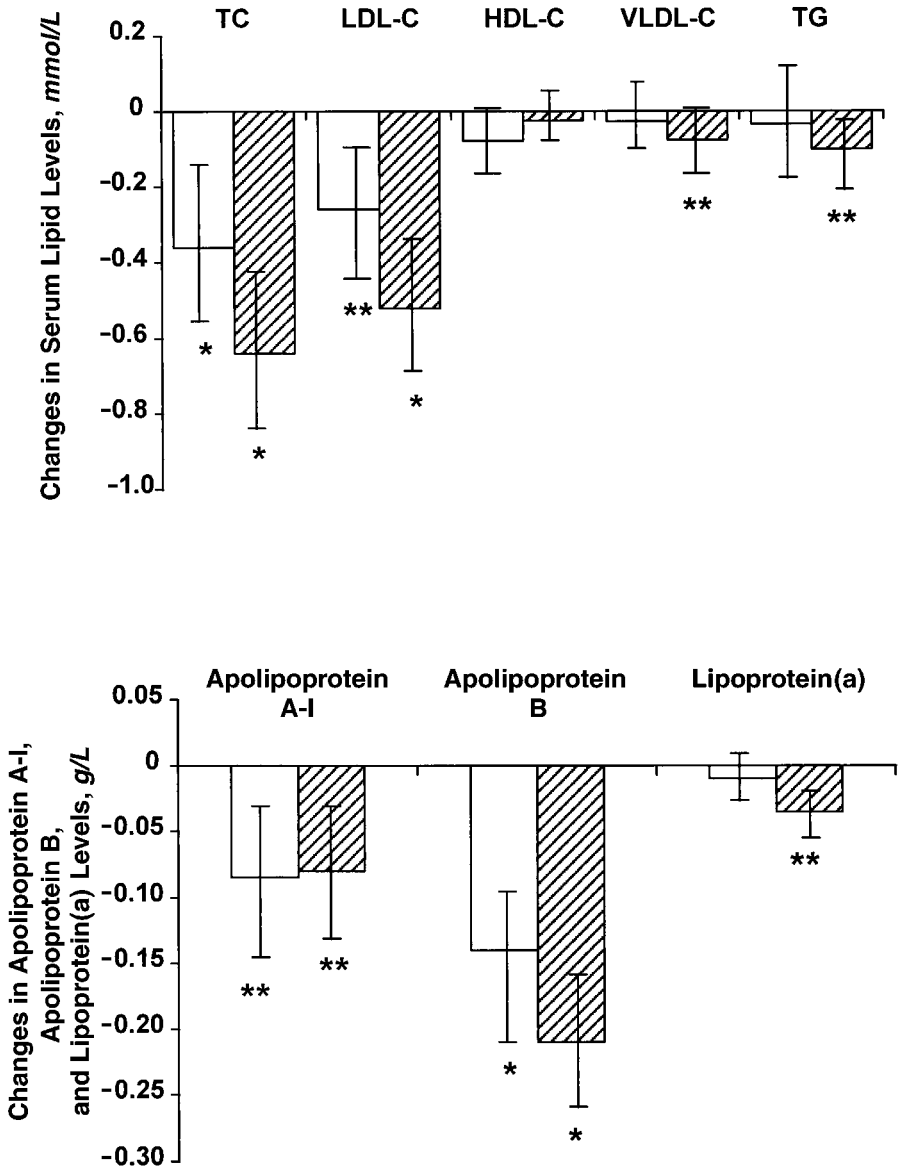

Figure 2. Changes from baseline values in levels of serum lipids, lipoproteins, and apolipoproteins in all patients who completed the study. **Top.** Mean changes from baseline in serum lipid levels. **Bottom.** Mean changes from baseline in levels of apolipoprotein A-I, apolipoprotein B, and lipoprotein(a). White bars indicate the control diet; striped bars indicate the walnut diet. HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglyceride; VLDL-C = very-low-density lipoprotein cholesterol. Error bars represent 95% CIs. To convert triglyceride values to mg/dL, divide by 0.01129; to convert high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, and very-low-density lipoprotein cholesterol values to mg/dL, divide by 0.02586. Asterisks indicate significant differences from baseline by the two-tailed *t*-test. $*P < 0.001$; $**P < 0.05$.

cardiovascular risk associated with plasma levels of lipoprotein(a) greater than the 80th percentile (>0.3 g/L) (21). Blood concentrations are primarily controlled by genetics, and it is generally believed that dietary factors have a negligible effect. The walnut diet modestly but significantly reduced lipoprotein(a) levels in men (but not in women as a group) and in patients with baseline serum levels greater than 0.3 g/L. This is interesting in view of the observation that pharmacologic doses of n-3 polyunsaturated fatty acids from fish oils might have a beneficial effect on lipoprotein(a) levels (26–28). The effect of n-3 fatty acids from plant sources on lipoprotein(a) requires further study.

Dietary fatty acid composition largely determines the fatty acid composition of serum lipoproteins, which in turn influences the rate and extent of their oxidation (10). Unlike saturated and monounsaturated fatty acids, polyunsaturated fatty acids are susceptible to oxidation (10). Nevertheless, enrichment of LDL particles with polyunsaturated fatty acids during the walnut diet did not alter their resistance to oxidative damage, as assessed by α-tocopherol content (the principal protection of LDL particles against oxidation) and the lag time of conjugated diene formation (the only measure of LDL oxidation that has been associated with coronary heart disease in clinical studies [29, 30]). Other components of

walnuts that have antioxidant potential, such as α-tocopherol and other phytochemicals, may be responsible for the lack of change in LDL oxidizability. Nonetheless, the fact that walnut intake is associated with a reduced risk for coronary heart disease (7) argues against an atherogenic effect caused by altered susceptibility of lipoproteins to oxidative damage.

Besides improving the serum lipid profile, walnut consumption may offer additional cardiovascular protection. Among naturally occurring foods, walnuts are one of the highest sources of the n-3 fatty acid α-linolenic acid; they contain approximately 7 g of α-linolenic acid per 100 g of edible matter (6). In recent reports from large prospective studies, dietary intake of α-linolenic acid has been inversely associated with risk for fatal coronary heart disease (31–33). In a secondary prevention trial, a Mediterranean diet enriched with α-linolenic acid had a striking beneficial effect on coronary heart disease morbidity and mortality when compared with a prudent western-type diet (34, 35). The low ratio of linoleic acid to α-linolenic acid in that study (4.5:1) was similar to that seen during the walnut diet in our study (5:1). Dietary α-linolenic acid may reduce the risk for death from coronary heart disease because of its antiarrhythmic properties (36) and other antiatherogenic effects (37, 38).

Our investigation adds further weight to the accumulating evidence that regular intake of nuts has a cholesterol-lowering effect (5). Since the first epidemiologic evidence that frequent nut consumption reduces the risk for coronary heart disease was reported (7), two large prospective studies have confirmed the observation (39, 40). It is reasonable to assume that cardiovascular protection is due, at least in part, to improvement of the lipid profile. The proportion of saturated fat in western diets is generally higher than that in Mediterranean diets. If the lipid profile is improved by partially substituting walnuts for typical Mediterranean foods and oils, which are rich in monounsaturated fatty acids and low in saturated fat, greater benefits might be obtained by partially substituting walnuts for traditional western dietary fats.

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Acknowledgments: The authors thank Nahyr Schinca, RD, and Ana Pérez-Heras, RD, for dietary counseling of the participants in the study. Catherine Bouchet, Antonia Codinach, and Ana Asensio provided skillful technical assistance with the laboratory measurements.

Grant Support: By the California Walnut Commission, Fundació Privada Catalana de Nutrició i Lípids, Fondo de Investigaciónes Sanitarias de la Seguridad Social (FIS 94/0077), and Comisión Interministerial de Ciencia y Tecnología (CICYT, SAF 97/0215, and OLI 96/2132).

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