EFFECTS OF WALNUTS ON SERUM LIPID LEVELS AND BLOOD PRESSURE IN NORMAL MEN

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Abstract *Background.* In a recent six-year follow-up study, we found that frequent consumption of nuts was associated with a reduced risk of ischemic heart disease. To explore possible explanations for this finding, we studied the effects of nut consumption on serum lipids and blood pressure.

Methods. We randomly placed 18 healthy men on two mixed natural diets, each diet to be followed for four weeks. Both diets conformed to the National Cholesterol Education Program Step 1 diet and contained identical foods and macronutrients, except that 20 percent of the calories of one diet (the walnut diet) were derived from walnuts (offset by lesser amounts of fatty foods, meat, and visible fat [oils, margarine, and butter]).

Results. With the reference diet, the mean (±SD) serum values for total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol were, respectively, 182±23, 112±16, and 47±11 mg per deciliter (4.71±0.59, 2.90±0.41, and 1.22±0.28 mmol per liter). With the walnut diet, the mean total cholesterol level was 22.4 mg per deciliter (0.58 mmol per liter) lower than the mean level with the reference diet (95 percent confidence interval, 28 to 17 mg per deciliter [0.72 to 0.44 mmol per liter]); the LDL and HDL cholesterol levels were, respectively, 18.2 mg per deciliter (0.47 mmol per liter) ($P<0.001$) and 2.3 mg per deciliter (0.06 mmol per liter) ($P = 0.01$) lower. These lower values represented reductions of 12.4, 16.3, and 4.9 percent in the levels of total, LDL, and HDL cholesterol, respectively. The ratio of LDL cholesterol to HDL cholesterol was also lowered ($P<0.001$) by the walnut diet. Mean blood-pressure values did not change during either dietary period.

Conclusions. Incorporating moderate quantities of walnuts into the recommended cholesterol-lowering diet while maintaining the intake of total dietary fat and calories decreases serum levels of total cholesterol and favorably modifies the lipoprotein profile in normal men. The long-term effects of walnut consumption and the extension of this finding to other population groups deserve further study. (N Engl J Med 1993;328:603-7.)

MOST previous studies of the effect of diet on risk factors for cardiovascular disease or events related to heart disease have considered the intake of fat, cholesterol, and other nutrients.[1] However, little attention has been paid to the intake of specific foods. It is possible that the effect of diet on ischemic heart disease depends on the combination of nutrients, with the influence of any particular nutrient or food constituent differing according to the presence or absence of other substances. Individual foods should be considered as packages of nutrients and other substances organized in unique proportions.[2]

In a prospective epidemiologic study among California Adventists,[3] we recently reported associations between the intakes of certain foods and the risk of ischemic heart disease. During six years of follow-up, we found that frequent consumption of nuts was associated with a substantial, independent reduction in the risk of myocardial infarction and death from ischemic heart disease.

Little research has been conducted on the effects of nut consumption on the risk of ischemic heart disease or cardiovascular risk factors. Most previous studies have involved animals fed peanut oil.[4] We were thus prompted to investigate the effects of consuming a specific type of nut in a carefully controlled experimental situation. We report here the results of a controlled trial of human diets that compared the effects on serum lipids and blood pressure of a diet rich in walnuts with those of a diet that conformed to the Step 1 recommendations of the National Cholesterol Education Program[5] but did not include nuts.

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METHODS

Subjects

Men who responded to campus and community advertisements were screened by two investigators. They were excluded from the trial if they ate nuts frequently, had known food allergies, smoked cigarettes, had a history of hypertension or atherosclerotic or metabolic disease, were taking any medication on a regular basis, had serum cholesterol values below the 20th percentile or above the 80th percentile for the participants' age range,[6] or were considered unable to comply with the study protocol.

Of the 24 subjects selected, 5 withdrew from the study during the run-in period (see below), and 19 thus entered the experimental phase; 1 subject was later excluded because he missed a blood drawing. Fifteen subjects were white, and three were Asian. The 18 men were between 21 and 43 years old (mean, 30), weighed between 60 and 103 kg (mean, 73), and had body-mass indexes (defined as the weight in kilograms divided by the square of the height in meters) ranging from 18.7 to 30.6 (mean, 23.8). Their fasting levels of serum cholesterol before the experiment began ranged from 137 to 250 mg per deciliter (mean, 198) (3.54 to 6.47 mmol per liter [mean, 5.1]). Triglyceride levels averaged 117 mg per deciliter (1.32 mmol per liter); one subject had mild hypertriglyceridemia (plasma triglyceride level, 317 mg per deciliter [3.58 mmol per liter]). Systolic blood pressure averaged 109 mm Hg, and diastolic 72 mm Hg.

Experimental Design

A controlled, single-blind, randomized, crossover design was used. All subjects consumed the reference diet during a five-day run-in period; this phase was succeeded by an eight-week experimental period in which they followed each of two consecutive diets for four weeks. One group followed the walnut diet during the first period and the reference diet during the second period, while the other group followed the diets in reverse order. The subjects were randomly assigned to a particular dietary sequence, with stratification on the basis of age, base-line serum cholesterol level, and body-mass index.[7] Ten subjects followed the walnut diet first, and eight the reference diet. The study personnel performing measurements and analyses were blinded to the subjects' diet sequence.

The study protocol was approved by the institutional review board of Loma Linda University, and all subjects gave informed consent. They were offered an honorarium of $200 for their participation.

Diets

During the 61 days of the study the subjects received all their meals at the nutrition-research kitchen of the university. Breakfast and dinner were eaten on the premises, under the supervision of one of the researchers. Lunches and snacks were packed and distributed at breakfast time. All foods and drinks were weighed and apportioned for each subject. The diets consumed during the study consisted of natural and common food items and were prepared and cooked in customary ways according to a five-day menu cycle. Daily menus were formulated so that there were five levels of energy intake, ranging from 2100 to 2900 kcal per day. The percentage of calories from protein, carbohydrates, total fat, and dietary fiber (Table 1) was held constant during both dietary periods. The two experimental diets were identical except that the walnut diet substituted three servings of walnuts per day (28 g per serving, or 84 g of walnuts per 2500 kcal) for portions of some foods in the reference diet.

A cholesterol-lowering diet was selected as the reference diet. It was designed according to the recommendations of the Expert Panel on the Step 1 diet of the National Cholesterol Education Program.[5] Thus, the total fat content accounted for about 30 percent of calories, with equal proportions of saturated, monounsaturated, and polyunsaturated fat. The reference diet included foods from all major food groups but did not contain nuts, nut butters, or nut oils of any kind. The foods in the walnut diet were identical to those in the reference diet; however, the sizes of the portions of fatty foods, such as potato chips and meat, were reduced, and the amount of visible fat (oils, margarine, and butter) was decreased, to accommodate the percentage of calories derived from walnuts (20 percent). Walnuts were served in several ways: as snacks, mixed in salads and breakfast cereals, or cooked in dinner entrees. Walnuts contributed 55, 14, and 10 percent, respectively, of the total fat, protein, and fiber of the walnut diet.

Complete duplicate samples of the two study diets were collected on 20 randomly selected days during the study period. Mixed samples were analyzed for levels of macronutrients[8] (Michelson Laboratories, Commerce, Calif.) and fatty acids (Agricultural University Department of Human Nutrition Laboratory, Wageningen, the Netherlands). The composition of each diet as determined by chemical analysis conformed closely to the composition planned by computer with the Food Processor II (ESHA Research, Salem, Oreg.) analysis system (Table 1). In addition, the chemical determination of fatty-acid content revealed the expected distribution for each diet (Table 2).

The subjects were requested to maintain their activities and other lifestyle habits and to record in diaries any signs of illness, medications used, and any deviation from their experimental diets. Inspection of their diaries every two weeks revealed that none deviated from the protocol except one subject, who ate lunch elsewhere four times. Two subjects consumed two cans of beer a week throughout the study; the others drank no alcohol. No subject reported side effects during the walnut diet.

Body weight as measured without shoes or heavy clothing was recorded every day during the run-in period and twice a week thereafter; energy intake was adjusted when necessary to maintain weight. Average (±SD) body weight decreased by 1.4±1.8 kg over the 61 days of the study, but this decrease was not related to a specific diet. The mean difference between the dietary treatments in weight lost was 0.099 kg ($P = 0.97$).

Table 1. Planned and Observed Mean Daily Composition of the Two Study Diets.

Variable	Reference Diet		Walnut Diet	
	Planned	Observed*	Planned	Observed*
Energy (kcal/day)	2523	2583	2536	2620
Fat (% of energy intake)	29.7	29.3	30.8	31.3
Saturated	9.3	9.0	5.5	6.0
Lauric, myristic, palmitic acids		6.3		4.0
Monounsaturated	9.1	8.8	8.6	7.4
Polyunsaturated	8.2	9.5	15.5	16.5
Protein (% of energy intake)	13.8	13.5	13.9	14.4
Carbohydrates (% of energy intake)	56.2	55.5†	55.2	54.2†
Cholesterol (mg/day)	278	237	152	125
Dietary fiber (g/day)	35.6		37.0	

*Values observed during chemical analysis of samples from the study diets.

†The values for carbohydrate intake were calculated by subtracting the values for fat and protein intake from those for total energy intake.

Measurements

Blood was drawn from each subject after an overnight fast once in the run-in period and on two alternate days at the end of each dietary period (day 2, days 30 and 32, and days 58 and 60). Serum and lipoprotein subfractions were analyzed to determine concentrations of cholesterol[9] and triglyceride[10] with the use of enzyme reagent kits (Ciba–Corning Diagnostics, Oberlin, Ohio) and an automated analyzer (Ciba–Corning) at Donner Laboratory (Berkeley, Calif.). High-density lipoprotein (HDL) cholesterol was measured directly after the precipitation of other lipoproteins by dextran sulfate.[11] Low-density lipoprotein (LDL) cholesterol was calculated by subtraction with the Friedewald algorithm.[12] All measurements were standardized according to the Lipid Standardization Program of the Centers for Disease Control and Prevention and the National Heart, Lung, and Blood Institute. For each subject, the fatty-acid composition of serum cholesterol esters was determined in a pool of two serum samples obtained at the end of each diet period and analyzed according to methods previously described.[13]

Blood pressure was measured with a random-zero sphygmomanometer twice during the run-in period (days 3 and 5), weekly during the first two weeks of each diet period, and twice during the last week of each diet period (days 31 and 33 and days 59 and 61). At each session, blood pressure was measured three times at one-minute intervals by two physicians who used training tapes from the London School of Hygiene to limit interobserver variability. The three measurements from each session were averaged. Measurements were obtained during fasting, before breakfast, after urination, and after a five-minute rest in a sitting position.

Statistical Analysis

To reduce variability in individual subjects, the results of all measurements made during the alternate days at the end of each dietary period were averaged; subsequent analyses were based on each subject's average value for that period. Descriptive values are expressed as means ±SD. Statistical analysis included two-tailed t-tests for the comparison of changes in outcome variables in response to dietary treatment and diet period for the two-period crossover design, with methods described by Fleiss.[14] However, we first tested for possible interaction between the dietary treatment and diet period (carry-over effect), also using a two-tailed t-test.[14]

Results

The compositions of the two diets were nearly identical with respect to their total fat, protein, carbohydrate, and fiber (Table 1). The reference diet substantially matched the diet currently recommended

Table 2. Proportions of Major Fatty Acids in the Two Study Diets.

Fatty Acid	Reference Diet	Walnut Diet
	grams per 100 g of fatty acid	
Lauric (C12:0)	1.0	0.3
Myristic (C14:0)	2.8	0.7
Palmitic (C16:0)	18.8	12.0
Palmitoleic (C16:1)	1.2	0.6
Stearic (C18:0)	7.5	5.1
Oleic (C18:1)	29.3	22.9
Linoleic (C18:2)	30.3	45.0
α-Linolenic (C18:3)	3.3	9.2

for the prevention of ischemic heart disease; the fatty-acid composition of the walnut diet was closer to that of walnut fat,[15,16] with a larger proportion of polyunsaturated fatty acids, especially α-linolenic acid (Table 2). The significant differences in the fatty-acid composition of the subjects' serum cholesterol esters during the two dietary periods confirmed that they had adhered closely to the diets (Table 3), since the changes occurred in the same direction as the differences in the fatty-acid composition of the study diets (Table 2).

The changes in lipid levels during the study are shown in Figure 1. A clear crossover pattern was observed in the values for total and LDL cholesterol. Since there was no evidence of a carry-over effect between the periods, the values for serum lipids and lipoproteins are presented in Table 4 for all study subjects during each diet, irrespective of the order of the diets. The mean serum total cholesterol level during the walnut diet was 22.4 mg per deciliter (0.58 mmol per liter) lower than the level during the reference diet, representing a reduction of 12.4 percent. The serum total cholesterol level decreased in all subjects during the walnut diet (range of reduction, 2 to 41 mg per deciliter [0.05 to 1.06 mmol per liter]), and the subjects' responses were similar regardless of whether their values for base-line serum cholesterol and body-mass index were in the lower or the upper half of the distribution of these values. The LDL cholesterol level during the walnut diet was 18.2 mg per deciliter (0.47 mmol per liter) lower than the level during the reference diet, representing a reduction of 16.3 percent; the HDL cholesterol level during the walnut diet was 2.3 mg per deciliter (0.06 mmol per liter) lower than that during the reference diet. The mean ratio of LDL cholesterol to HDL cholesterol was 2.5±0.6 during the reference diet and decreased to 2.2±0.7 during the walnut diet ($P<0.001$). The ratio of total cholesterol to HDL cholesterol also decreased, from 4.0±1.0 during the reference diet to 3.7±1.0 during the walnut diet ($P<0.001$). The level of the serum triglycerides during the walnut diet was 9.5 mg per deciliter (0.11 mmol per liter) lower than the level during the reference diet.

The mean blood pressure was 108/71 mm Hg during the reference diet and 110/71 mm Hg during the walnut diet. No significant changes were observed in the systolic ($P = 0.2$) or diastolic ($P = 0.6$) blood pressure at the end of each diet period when these values were compared with the values measured at base line or any time throughout the study.

Discussion

This randomized, crossover trial was conducted to determine the effect of eating walnuts on serum lipid levels and blood pressure in free-living healthy men. The walnut diet was a modified version of the recommended diet of the National Cholesterol Education Program that incorporated walnuts, a fatty food, without increasing the percentage of calories from total dietary fat. The results of the study suggest that replacing a portion of the fat in a cholesterol-lowering diet with walnuts further lowers serum cholesterol levels by more than 10 percent in normal men. The effect of the walnut diet on the lipoprotein risk profile was more favorable than that of the recommended reference diet. It is noteworthy that these effects were observed despite the relatively low base-line cholesterol levels of the study subjects.

Given the known effects of dietary fats, the composition of the fatty acids found in walnuts should lower serum cholesterol levels.[17-22] About 81 percent of the total calories of walnuts are derived from fat, which accounts for 58 percent of their weight. Walnut fat is qualitatively similar to that of some commonly used oils extracted from grains and seeds. The ratio of polyunsaturated to saturated fat in walnuts is 7.1, one of the highest among naturally occurring foods. Walnuts contain relatively large amounts of the n−3 linolenic acid, about 7 g per 100 g of edible portion, or 12 percent of their total fat content.[15,16] Thus, they can be considered an alternative food source of n−3 fatty acids that does not add cholesterol to the diet.[23] However, recent studies have not demonstrated that linolenic acid has any greater cholesterol-lowering effect than linoleic acid.[24,25] We used the equation of Keys

Table 3. Mean ±SD Changes in Fatty-Acid Composition of Serum Cholesterol Esters.

Fatty Acid	Reference Diet	Walnut Diet	Change*	P Value†
	percent of total fatty acids by weight			
Myristic (C14:0)	1.0±0.1	1.2±0.1	0.1±0.1	<0.001
Palmitic (C16:0)	10.1±0.5	9.5±0.6	−0.6±0.5	<0.001
Palmitoleic (C16:1n−7)	1.8±0.6	1.6±0.6	−0.3±0.5	0.024
Stearic (C18:0)	0.8±0.1	0.7±0.1	−0.2±0.1	<0.001
Oleic (C18:1n−9)	13.7±1.0	11.8±1.4	−1.8±0.9	<0.001
Linoleic (C18:2n−6)	60.1±2.6	63.8±3.4	3.7±2.1	<0.001
α-Linolenic (C18:3n−3)	0.5±0.1	1.1±0.2	0.6±0.2	<0.001
Arachidonic (C20:4n−6)	7.5±1.2	6.5±1.1	−1.0±0.5	<0.001
Others	4.5±1.0	3.9±0.9	−0.6±1.0	0.075

*Reference-diet values were subtracted from walnut-diet values. Values have been rounded off.

†By two-tailed parity test.

et al.[19-22] to determine the extent to which the difference between the two experimental diets in the content of fats and cholesterol could explain the decrease in serum cholesterol levels during the walnut diet. This equation predicted a decrease of 17.8 mg per deciliter (0.46 mmol per liter) during the walnut diet, which is approximately 5 mg per deciliter (0.13 mmol per liter) less than the decrease actually observed in our study. Although walnuts and other foods with similar fat composition may lower the serum cholesterol level through the actions of their fatty acids, the type of dietary fiber and the very low ratio of lysine to arginine[26] that are characteristic of walnuts may also have some effect in this regard.

Table 4. Serum Lipid and Lipoprotein Levels at the End of Each Diet Period.

Variable*	Reference Diet†	Walnut Diet†	Estimated Difference in Diet Effects‡	Percent Change
Total cholesterol (mg/dl)	182±23	160±23	−22.4 (−28.2 to −16.6; P<0.001)	−12.4
LDL cholesterol (mg/dl)	112±16	94±17	−18.2 (−23.2 to −13.2; P<0.001)	−16.3
HDL cholesterol (mg/dl)	47±11	45±10	−2.3 (−3.9 to −0.7; P = 0.009)	−4.9
LDL cholesterol:HDL cholesterol	2.5±0.6	2.2±0.7	−0.3 (−0.4 to −0.2; P<0.001)	−12.0
Triglycerides (mg/dl)	114±59	103±45	−9.5 (−20.5 to 1.5; P = 0.101)	−8.3

*To convert values for total, HDL, and LDL cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129.

†Values are means ±SD.

‡Reference-diet values were subtracted from walnut-diet values according to methods described by Fleiss.[14] Because of minor adjustments for the diet-period effect, the estimated differences do not exactly equal the difference between the values for each diet. Values in parentheses are 95 percent confidence intervals.

Several cross-sectional studies suggested that low-fat diets or the consumption of unsaturated fat decreases blood pressure.[27,28] However, controlled dietary trials have not provided clear support for this hypothesis.[29] Our study also does not support the notion that dietary fat has a role in the control of blood pressure, since the blood pressure of our normotensive subjects did not change during the two study diets or from base line; the base-line values reflected the effects of the subjects' habitual diets, which were probably higher in fat. Our findings may or may not apply to hypertensive subjects.

We recently reported that eating nuts appeared to have a protective effect against both fatal and nonfatal coronary heart disease in a cohort study of California Adventists.[3] This population had a wide range of nut-consumption patterns. As compared with persons who almost never ate nuts, those who ate them one to four times per week had a relative risk of myocardial infarction of 78 percent, whereas those who ate them five or more times per week had a relative risk of 49 percent. Similar findings were also made for the risk of death from ischemic heart disease. The protective effect of nuts appeared to be independent of other risk factors, including a number of other foods, and was consistent across several population subgroups. Moreover, our data did not suggest any increase in the risk of cancer among nut consumers, but did indicate a clear decrease in the risk of death from all causes.[30]

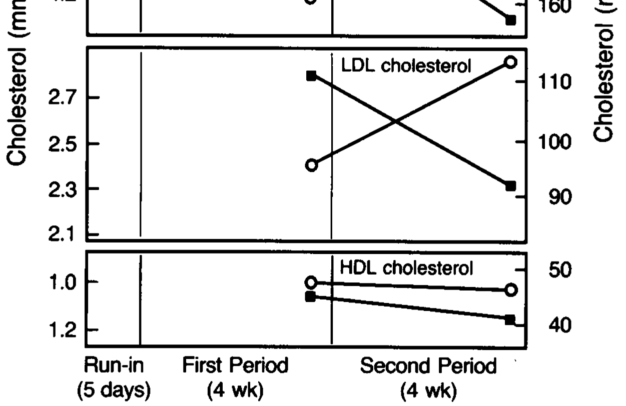

Figure 1. Mean Serum Concentrations of Total, LDL, and HDL Cholesterol during Each Diet Period.

All 18 subjects followed each diet, but 10 followed the walnut diet first (○) and 8 followed the reference diet first (■).

There are many nutritional similarities among types of nuts. The total fat content is high (accounting for 73 to 90 percent of calories) but consists largely of monounsaturated and polyunsaturated fat, with small amounts of saturated fat.[15] The fiber content is high, ranging from 5.2 to 14.3 g per 100 g of edible nut. Thus, nuts other than walnuts may also have cholesterol-lowering properties. Preliminary studies of almond[31] and hazelnut (Margalef J: personal communication) supplements have revealed a decrease in total serum cholesterol levels and a beneficial effect on the lipoprotein profile.[31] The present study suggests that the apparent protective effect of nut consumption against cardiovascular disease that was found in the study of California Adventists[3] may be mediated at least in part through blood lipids. The effects of walnuts and other nuts on platelet function, prostaglandin metabolism, and antioxidant potential can also be postulated, but direct evidence is lacking.

Although some studies in animals suggest that a very high intake of polyunsaturated fats may increase the risk of cancer,[32] we note that walnut consumption could be substantially increased before it exceeded the currently recommended 10 percent maximum of calories from polyunsaturated fat.[33] If the effect of walnuts on serum cholesterol is assumed to be proportional to intake, an increase in the current per capita consumption of 4 g per week[34] to 28 g per day would be expected to decrease serum levels of total and LDL cholesterol by 4 and 6 percent, respectively.

Whether our results can be extended to women, subjects with hypercholesterolemia, or subjects followed longer than ours is unknown. The effects of oil-roasted nuts may differ from those seen in this study. Furthermore, our results may not apply if walnuts are eaten as a dietary supplement and thus increase caloric intake and possibly body weight.

In conclusion, the results of this study indicate that a diet that includes moderate quantities of walnuts without an overall increase in total dietary fat and calories decreases serum cholesterol levels and favorably modifies the lipoprotein profile in normal men, an effect beyond that of the currently recommended diet for lowering cholesterol. The versatility of walnuts, which allows their ready use in the diet as snacks or components of desserts, breads, or entrees, suggests that their consumption would be acceptable to most as part of a cholesterol-lowering diet.

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