Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study[1-4]

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ABSTRACT

Background: Increased consumption of $n-3$ (omega-3) fatty acids decreases the incidence of coronary heart disease (CHD).

Objective: The objective was to determine whether walnuts (plant $n-3$ fatty acid) and fatty fish (marine $n-3$ fatty acid) have similar effects on serum lipid markers at intakes recommended for primary prevention of CHD.

Design: In a randomized crossover feeding trial, 25 normal to mildly hyperlipidemic adults consumed 3 isoenergetic diets ($\approx 30\%$ total fat and $<10\%$ saturated fat) for 4 wk each: a control diet (no nuts or fish), a walnut diet (42.5 g walnuts/10.1 mJ), or a fish diet (113 g salmon, twice/wk). Fasting blood was drawn at baseline and at the end of each diet period and analyzed for serum lipids.

Results: Serum total cholesterol and LDL cholesterol concentrations in adults who followed the walnut diet (4.87 ± 0.18 and 2.77 ± 0.15 mmol/L, respectively) were lower than in those who followed the control diet (5.14 ± 0.18 and 3.06 ± 0.15 mmol/L, respectively) and those who followed the fish diet (5.33 ± 0.18 and 3.2 ± 0.15 mmol/L, respectively; $P < 0.0001$). The fish diet resulted in decreased serum triglyceride and increased HDL-cholesterol concentrations (1.0 ± 0.11 and 1.23 ± 0.05 mmol/L, respectively) compared with the control diet (1.12 ± 0.11 and 1.19 ± 0.05 mmol/L, respectively) and the walnut diet (1.11 ± 0.11 mmol/L, $P < 0.05$, and 1.18 ± 0.05 mmol/L, $P < 0.001$, respectively). The ratios of total cholesterol:HDL cholesterol, LDL cholesterol:HDL cholesterol, and apolipoprotein B:apolipoprotein A-I were lower ($P < 0.05$) in those who followed the walnut diet compared with those who followed the control and fish diets.

Conclusion: Including walnuts and fatty fish in a healthy diet lowered serum cholesterol and triglyceride concentrations, respectively, which affects CHD risk favorably. *Am J Clin Nutr* 2009;89(suppl):1657S–63S.

INTRODUCTION

One of the most compelling public health messages to emerge in the past decade for lowering the incidence of coronary heart disease (CHD) has been to increase the amount of $n-3$ fatty acids in the daily diet of Americans (1–3); α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have shown cardiovascular benefits in normolipidemic and hyperlipidemic subjects (2–8). Of the different dietary sources of EPA and DHA, consumption of 2 servings of fatty fish per week is specifically recommended by the American Heart Association for individuals without documented CHD (3). The other $n-3$ fatty acid, ALA, is a precursor to EPA, and DHA and is abundant in walnuts, flaxseed, and rapeseed and soybean oils (9). Of these foods, a qualified health claim issued by the Food and Drug Administration suggests that a daily intake of 1.5 oz (42.5 g) of walnuts as part of a low-fat, low-cholesterol, isoenergetic diet may lower the risk of heart disease (10). Thus, among all the different $n-3$ fatty acid rich foods commonly consumed, fatty fish and walnuts are the 2 that have been recommended at a specific "dose" by recognized organizations (3, 10) for the primary prevention of CHD.

Ideally it is best to include both sources of $n-3$ fatty acids in the diet for optimum cardiovascular protection. However, promoting increased consumption of fatty fish to the public has limitations. For those who follow a vegetarian eating pattern, fish is among the foods they avoid (11). Among nonvegetarians, there are those who do not consume fish because they dislike the taste of fish or are concerned about environmental toxins (1, 12). Even among fish eaters, the primary sources of EPA and DHA are finfish and shellfish (13), which provide only ≈ 88 mg/d when 2 servings per week are consumed. This amount is much lower than the recommended 500 mg/d provided by equivalent servings of other fatty fish, eg, salmon.

Walnuts and fatty fish have been studied individually for their effects on blood lipid markers. Controlled feeding studies (14) have shown that consuming 2–3 servings of walnuts (56–84 g/d) substantially decreases cholesterol in normal and hyperlipidemic individuals. The doses used in these studies are somewhat higher (contributing 12.5–20% of the total energy) than those suggested (10) by the qualified health claim for walnuts (1.5 oz or 42.5 g, $\approx 10\%$ of total energy). Alternatively, studies have shown that fish or fish oil supplements containing 3–4 g/d of EPA and DHA are effective in decreasing plasma triglyceride concentration (2, 15,

16), but the efficacy of a dosage <1 g/d (equivalent of 2 fatty fish servings/wk) in decreasing triglyceride is inconclusive (17, 18).

Thus, for practical applications, a systematic comparison of these 2 foods (walnuts and fatty fish) containing plant compared with marine n−3 fatty acids at "doses" suggested for primary prevention of CHD in a healthy population with normal to mildly increased lipid levels under controlled conditions is warranted.

SUBJECTS AND METHODS

Subjects

Normolipidemic to mildly hyperlipidemic but apparently healthy men and women (aged 23–65 y) were recruited through advertisements in and around Loma Linda University. A multistage screening of participants was used that included telephone screening, informational group meeting, personal interview with an investigator, and a preliminary screening blood lipid test. Participants who consumed nuts or ate fish >2 times/wk, drank caffeinated beverages >3 times/d and/or alcohol >2 drinks/wk, had food allergies, smoked, had a history of chronic or metabolic diseases, or regularly used medication or supplements known to affect blood lipids were excluded. Women who had irregular menses or started hormone treatment within the past 5 y also were excluded. Potential participants who were selected after the initial screening had a fasting blood sample drawn for analyses of blood lipids. Participants were excluded if their screening serum total cholesterol concentration was >7.76 mmol/L and if their triglyceride concentration was >3.33 mmol/L. All selected participants ($n = 25$) were asked to maintain their habitual level of physical activity throughout the study. Written informed consent was obtained from participants and the study was approved by the institutional review board of Loma Linda University, Loma Linda, CA.

Study and diet design

This study was a randomized crossover (3 × 3 Latin square) controlled feeding trial with 3 diet periods of 4 wk each with a weekend break in-between diet periods. Before beginning the dietary treatments, participants received an average American diet that contained 34% energy from fat for 1 wk (run-in period); baseline data were collected at the end of this period. Participants then were randomized and stratified on the basis of age, gender, and baseline serum total cholesterol concentration to 1 of 6 possible diet sequences in a crossover fashion for 4 wk each.

The treatment diets were control diet (excluded nuts and fatty fish), walnut diet [included 42.5 g (1.5 oz) walnuts/10 mJ (2400 kcal), 6 d/wk], and fish diet [included 113 g (4 oz raw) salmon, twice/wk]. The diets did not include any n−3 fatty acid rich foods other than those being tested (walnuts or salmon). Added fats were provided by olive oil– or corn oil–based products. The control diet did not include any nuts or seafood. The fish diet was identical to the control diet except that the cooked salmon (113 g) substituted for the same amount of other types of meats twice a week. The dosage of 2 fatty fish meals per week is based on the recommendation of the American Heart Association (3) for individuals without documented CHD. The walnut diet was identical to the control diet except that 42.5 g of walnuts was substituted for meats and dairy foods. The amount of walnuts used in this study is based on the

Food and Drug Administration qualified health claim for walnuts (10). All diets were isoenergetic and were designed to follow the current dietary guidelines for Americans (19), with 25–35% energy from fat, <10% energy from saturated fatty acids (SFAs), and <300 mg cholesterol. The menus were analyzed (**Table 1**) for nutrient composition with Nutrition Data System for Research (NDSR) software, version 4.05 (Nutrition Coordinating Center, Minneapolis, MN).

This study featured a highly controlled feeding protocol (20) in which all meals were prepared in the university's metabolic kitchen. Sunday through Friday of each week, participants ate breakfast and dinner at this facility, whereas daily lunch, snacks, and Saturday meals were packaged for consumption at the participant's discretion. Twelve menus (including 2 Saturday menus) were used in rotation to provide for mealtime variety. Walnuts were consumed during breakfast and/or dinner, alone or on cereals, and in salads or desserts. Salmon was served at dinner twice a week. Menus were designed for 7 energy intake values, ranging from 7.54 to 15.1 mJ/d (1800–3600 kcal/d). Participants were weighed twice weekly and their energy intake adjusted as needed to maintain stable body weights throughout the study.

Quality control and compliance with the dietary protocol were insured among participants by weighing foods to the nearest gram before being served, with at least one investigator present at all meal times, and by requiring participants to maintain a daily diary to record any deviations from the study protocol. In addition, the erythrocyte membrane fatty acid composition was determined at the end of each diet period as a marker of compliance.

Serum sample collection and analyses

Twelve-hour fasting blood samples were obtained on 2 alternate study days at the end of each diet period, including baseline. Within 30 min of collection, the blood samples were centrifuged at 4°C at 1300 × g for 10 min and serum was separated and stored immediately at −80°C until analyzed. The Nutritional Assessment Core of the University of California, Davis (NIH Clinical Research Unit, NIDDK 35747), performed the lipid analyses. Total cholesterol,

TABLE 1
Calculated nutrient composition of the 3 treatment diets[1]

Nutrient	Control diet	Fish diet	Walnut diet
Carbohydrate (% of energy)	57.8	57.8	59.8
Protein (% of energy)	14.6	14.8	14.5
Fat (% of energy)	30	29	31
Saturated	9.4	9	8
Monounsaturated	9.4	9	7.9
Polyunsaturated	4.3	4.5	10.8
trans	1.0	0.96	0.84
Linoleic acid (g/10,048 kJ)	10.1	9.5	24.3
α-linolenic acid (g/10,048 kJ)	1.04	1.09	4.70
EPA + DHA acid (g/10,048 kJ)	0.04	0.78	0.04
Cholesterol acid (g/10,048 kJ)	339	343	282
Fiber acid (g/10,048 kJ)	26.3	26.1	29.3

[1] Analyses were conducted by using Nutrition Data System for Research software, version 4.05 (Nutrition Coordinating Center, Minneapolis, MN). DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. 10,048 kJ = 2400 kcal.

LDL cholesterol, HDL cholesterol, and triglyceride (21, 22) were measured by using enzymatic colorimetric assays with the Bayer 550 Express Chemistry Analyzer (Bayer Corporation, Tarrytown, NY). Before analysis, HDL cholesterol was separated from serum with a magnetically enhanced dextran sulfate (molecular weight 50,000) and magnesium chloride reagent by selective precipitation of apolipoprotein (apo) B–containing lipoproteins. All samples were analyzed in duplicate with an internal control and standard curve. Serum concentrations of apo A and B (23) were measured with a nephelometer (Beckman Coulter, Brea, CA) to measure the rate of light scatter resulting from an immunoprecipitation reaction. A commercial calibrator serum was used to set a reference point with which the sample's peak rate of increase of light scatter was compared and then converted into concentration units (mmol/L). The erythrocyte membrane fatty acid composition was measured at the end of each treatment by Lipomics (Sacramento, CA) with methods described previously (20).

Statistical analyses

Power calculations for a crossover design indicated that to detect a mean absolute difference in serum LDL cholesterol concentration of 0.26 mmol/L, 18 participants would need to complete the study ($\alpha = 0.05$, power >0.9). On the basis of this calculation, our study was in excess of 95% power. Statistical analyses were performed with the Statistical Analysis System, version 9.1 (SAS Institute, Cary, NC). Treatment means were estimated with mixed linear models, with fixed terms for diet, treatment period, clinic day, period-by-clinic-day interaction, and a random term for subjects. A significant main effect of diet is indicated when the P value of the F test for the main effect is <0.05. When the main effect of diet is significant, individual treatment effects (differences between pairs of treatment means) are significant when the treatment effect P value (adjusted for multiple comparisons by the Tukey-Kramer method) is <0.05.

Interaction between diet and baseline lipids was investigated by adding fixed terms for baseline lipids and the interaction of baseline lipids plus diet to the above model. Treatment means are presented as least-squares means and SE. Treatment effects are presented as percentage differences between treatment means and SE of the percentage difference.

RESULTS

Subject characteristics

Of the 27 eligible participants who started the study, 2 dropped out because of time conflicts and 25 (14 men and 11 women) completed all 3 treatments. The subjects were aged 23–65 y with a mean body mass index of 24.8 (in kg/m^2; range: 18.7–36.6). The mean body weights at the end of the diet treatments were not significantly different ($P = 0.1658$) from each other (control diet, 71.7 ± 3.1 kg; walnut diet, 71.9 ± 3.1 kg; and fish diet, 71.7 ± 3.1 kg). Subjects ranged from normal to mildly hyperlipidemic as calculated from their baseline values for serum cholesterol and triglyceride concentrations. The mean baseline total cholesterol was 5.41 mmol/L (range: 3.4–7.76 mmol/L), mean baseline LDL cholesterol was 3.53 mmol/L (range: 1.82–5.66 mmol/L), and mean baseline triglyceride was 1.25 mmol/L (range: 0.66–3.33 mmol/L) for the subjects who completed the study ($n = 25$).

Dietary intake and compliance

The calculated nutrient composition of the 3 treatment diets is shown in Table 1. All 3 diets adhered to current dietary guidelines with similar amounts of total fat ($\approx 30\%$ of total energy), including 8–10% energy from SFAs and $<1\%$ from *trans* fat. Although the goal was to keep the dietary cholesterol to <300 mg/d, the fish and control diets had slightly higher amounts of cholesterol but still <350 mg/d. Because walnuts are rich in polyunsaturated fatty acid (PUFA), the percentages of energy from total PUFA, specifically linoleic acid and ALA, were higher in the walnut diet compared with the other 2 diets. The higher PUFA content of the walnut diet resulted in a slight decrease in SFA (1–1.5% energy decrease) and monounsaturated fatty acid (MUFA) content (3–4% energy decrease) compared with the other 2 diets. The fish diet, however, had higher amounts of EPA and DHA, as expected, compared with negligible amounts (<0.05 mg) in the other 2 diets.

Participants' compliance to the diet protocol was verified by the fatty acid composition of the erythrocyte membrane (**Table 2**). Both SFA and MUFA content of the erythrocyte membrane decreased in participants who followed the walnut diet compared with those who followed the other 2 diets. As expected, the total PUFA, specifically ALA, was higher in those who followed the walnut diet compared with those who followed the control and fish diets ($P < 0.0001$) whereas EPA ($P < 0.0001$) and DHA ($P = 0.0002$) were higher in those who followed the fish diet compared with those who followed the other 2 diets. All of the subjects who followed the walnut diet showed an increase in membrane ALA concentration and 71% of the subjects who followed the fish diet showed an increase in membrane DHA concentration. These trends are similar to those observed for the nutrient analyses of the diets and validate compliance to the dietary treatments.

Serum lipid, lipoprotein, and apolipoprotein concentrations

Mean values for serum lipid, lipoprotein, and apolipoprotein concentrations at the end of each dietary treatment are shown in **Table 3**. In addition, for a few of the variables, the percentage difference for the walnut and fish diets from the control diet is

TABLE 2
Fatty acid composition of erythrocyte membranes at the end of each of the 3 treatment diets[1]

Fatty acid	Control diet	Fish diet	Walnut diet	P value[2]
	mol %	*mol %*	*mol %*	
SFA	38.9 ± 0.39[a]	40.2 ± 0.39[b]	39.9 ± 0.39[a,b]	0.0409
MUFA	15.8 ± 0.19[a]	15.8 ± 0.19[a]	14.5 ± 0.19[b]	<0.0001
PUFA	38.6 ± 0.38[a]	39.4 ± 0.38[a]	41.0 ± 0.38[b]	<0.0001
LA (18:2n−6)	10.5 ± 0.29[a]	9.7 ± 0.29[a]	12.4 ± 0.29[b]	<0.0001
ALA (18:3n−3)	0.13 ± 0.01[a]	0.12 ± 0.01[a]	0.26 ± 0.01[b]	<0.0001
EPA (20:5n−3)	0.51 ± 0.04[a]	1.08 ± 0.04[b]	0.58 ± 0.04[a]	<0.0001
DHA (22:6n−3)	4.52 ± 0.21[a]	5.12 ± 0.21[b]	4.46 ± 0.21[a]	0.0002

[1] All values are means ± SEs; $n = 25$. ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. Values within a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA, with Tukey-Kramer adjustments).

[2] P values for the main effect of diet obtained from a mixed linear model.

TABLE 3
Serum lipid, lipoprotein, and apolipoprotein concentrations at the end of each of the 3 treatment diets[1]

	Control diet	Fish diet	Walnut diet	P value[2]
Cholesterol (mmol/L)				
Total	5.14 ± 0.18^a	5.33 ± 0.18^b	4.87 ± 0.18^c	<0.0001
LDL	3.06 ± 0.15^a	3.20 ± 0.15^b	2.77 ± 0.15^c	<0.0001
HDL	1.19 ± 0.05^a	1.23 ± 0.05^b	1.18 ± 0.05^a	<0.0017
Triglyceride (mmol/L)	1.12 ± 0.11^a	1.00 ± 0.11^b	1.11 ± 0.11^a	0.0419
Apolipoproteins (g/L)				
apo A-I	1.66 ± 0.04	1.66 ± 0.04	1.65 ± 0.04	0.7903
apo B	1.03 ± 0.06^a	1.07 ± 0.06^b	0.94 ± 0.06^c	<0.0001
Total cholesterol:HDL cholesterol	4.49 ± 0.21^a	4.50 ± 0.22^a	4.32 ± 0.21^b	0.0231
LDL cholesterol:HDL cholesterol	2.71 ± 0.18^a	2.74 ± 0.18^a	2.51 ± 0.18^b	0.0005
apo B:apo A-I	0.64 ± 0.04^a	0.66 ± 0.04^a	0.59 ± 0.04^b	<0.0001
LDL cholesterol:apo B	1.17 ± 0.02	1.16 ± 0.02	1.17 ± 0.02	0.5670

[1] All values are means $\pm$ SEs; $n = 25$. To convert cholesterol and triglyceride from mmol/L to mg/dL, multiply by 38.67 and 88.57, respectively. To convert apo A-I and B from g/L to mg/dL, multiply by 100. Values within a row with different superscript letters are significantly different, $P < 0.05$ (with Tukey-Kramer adjustment for multiple comparisons).
[2] P values for the main effect of diet obtained from mixed linear model.

presented in **Figure 1**. When compared with the control and fish diets, the walnut diet decreased total cholesterol significantly, by 0.28 and 0.47 mmol/L, respectively, and decreased LDL cholesterol by 0.28 and 0.43 mmol/L, respectively ($P < 0.0001$). Overall, the walnut diet showed a 5.4% and 9.3% decrease in total cholesterol and LDL cholesterol compared with the control diet.

The fish diet resulted in significantly higher serum total cholesterol and LDL cholesterol concentrations compared with those in the control ($P < 0.02$) and walnut ($P < 0.0001$) diets. This effect of fish on total cholesterol and LDL cholesterol was magnified in subjects with higher baseline values for these variables. The increase in these variables among subjects who followed the fish diet was not significant ($P = 0.24$) for those with normal baseline levels. However, for those with moderate to high baseline total cholesterol (<5.17 mmol/L) and LDL cholesterol (>3.36 mmol/L) values, the increase in these variables among those who followed the fish diet was progressively greater (**Figure 2**).

The fish diet resulted in significantly higher HDL cholesterol ($\approx$4%; $P < 0.002$) and decreased triglyceride ($\approx$11%; $P =$

0.04) compared with those in the control and walnut diets. The ratios of total cholesterol:HDL cholesterol ($P = 0.02$), LDL cholesterol:HDL cholesterol ($P < 0.0005$), and apo B:apo A-I ($P < 0.0001$) all were significantly lower in those who followed the walnut diet compared with those who followed the control and fish diets.

DISCUSSION

This study compared the effect of 2 foods (walnuts and salmon) rich in plant compared with marine n−3 fatty acids on blood lipid markers in a healthy population at "doses" suggested for primary prevention of CHD. Incorporating 1.5 oz (42.5 g) of walnuts in a diet based on the current dietary guidelines markedly decreased total cholesterol and LDL cholesterol by 5.4% and
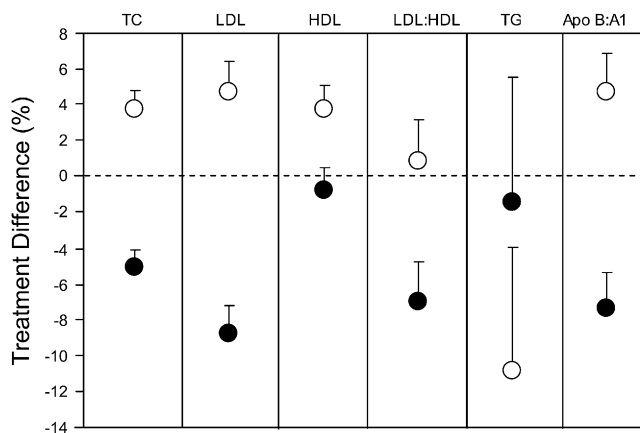

FIGURE 1. The absolute mean ($\pm$SE) percentage difference in total cholesterol (TC), LDL cholesterol, HDL cholesterol, triglyceride (TG), and apolipoprotein (apo) B:apo A-I ratio in walnut and fish diets compared with the control diet. Results obtained from mixed linear model (with Tukey-Kramer adjustment for multiple comparisons). ●: Walnut diet values – control diet values; ○: fish diet values – control diet values ($n = 25$).
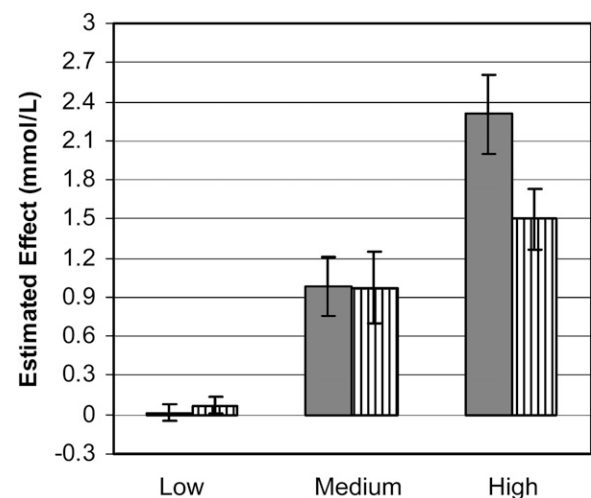

FIGURE 2. The magnitude of change in total cholesterol (■) and LDL cholesterol (▥) after the fish diet in subjects stratified on the basis of their baseline values as low, medium, and high. Values are expressed as means $\pm$ SEs. For total cholesterol, low is <5.18 mmol/L ($n = 9$), medium is 5.18–6.21 mmol/L ($n = 11$), and high is >6.21 mmol/L ($n = 5$). For LDL cholesterol, low is <3.36 mmol/L ($n = 5$), medium is 3.36–4.14 mmol/L ($n = 10$), and high is >4.14 mmol/L ($n = 10$). Results estimated by using a mixed linear model. The model contained fixed terms for diet, period, baseline lipids, and the interaction between baseline lipids and diet and random term for subjects.

9.3%, respectively. Every 1% decrease in LDL cholesterol results in 2% decrease in risk of CHD (24), which means there is an ≈18.6% decrease in the risk of CHD for subjects on the walnut diet. Alternatively, 2 servings (226 g) of fatty fish (salmon) per week resulted in decreasing triglyceride by ≈11% and increasing HDL cholesterol by ≈4%. The "dose" of walnuts and salmon used in our study was based on suggested amounts for the primary prevention of CHD (3, 10).

Our findings from the walnut diet are in accord with previous feeding studies in which a decrease of LDL cholesterol in the range of 8–16% was observed at intakes of 56–84 g (2–3 oz/d) of walnuts in normal and hypercholesterolemic patients (7, 14). Walnuts are rich in linoleic acid and ALA, which are known to lower cholesterol when they replace SFA or MUFA in the diet, possibly by increasing the receptor-mediated uptake of LDL cholesterol (25). The cholesterol-lowering effect of walnuts is, however, not entirely due to the fatty acid makeup of this food. Consistent with what we and others have found (7, 14), the predicted decrease in LDL cholesterol in this study was smaller than the observed decrease, suggesting that other nonlipid components of walnuts may have mediated some of the cholesterol-lowering effects.

Unlike walnuts, fatty fish did not lower cholesterol but, as shown by others (15, 26, 27), had a cholesterol-raising effect. Of the 2 long-chain n−3 PUFA found in fish, DHA is reported to be more potent in raising cholesterol and this may be due to greater conversion of DHA-enriched VLDL to LDL cholesterol or downregulation of LDL cholesterol receptor (26, 28). We found that the hypercholesterolemic effect of DHA was magnified in those subjects who had increased baseline cholesterol levels. The increase in LDL cholesterol from the fish diet may not suggest an increased risk of CVD as this may be counteracted by a shift of LDL cholesterol toward a larger, less atherogenic LDL cholesterol particle (29). Furthermore, combining walnuts and fish may help to blunt the cholesterol-raising effect of the latter and provide a more comprehensive way of managing mild to moderate hyperlipidemia. This needs to be tested in subsequent studies. A similar approach that used a combination of fish oil and garlic was successfully able to prevent the cholesterol-raising effect of fish oil in hypercholesterolemic adults (30).

The hypotriglyceridemic effect of fish oil is well documented (1, 4, 31). The most effective dosage of EPA and DHA is ≈4 g/d, and this produces a 25–30% decrease in triglyceride. For lower dosages (<1 g/d), the triglyceride-lowering effect has not been consistently shown (17, 18), although in our study we observed an ≈11% decrease in triglyceride at intakes of EPA and DHA (780 mg/d) that were equivalent to 2 servings of fatty fish per week. An important determinant of triglyceride decrease is the saturation of erythrocyte membrane with EPA and DHA, which is known to increase the clearance of circulating triglyceride through the activity of endothelial lipases (31). In previous studies that used <1 g/d of EPA and DHA, researchers observed no change in these fatty acids in serum after the intervention (17) or an increase of <2% of these fatty acids in plasma phospholipids (18). In our study we observed an ≈23% increase in EPA and DHA concentrations in erythrocyte membrane phospholipids, which explains the decrease we observed in triglyceride despite consumption of a low dosage (<1 g/d) of long-chain n−3 PUFA.

The walnut diet did not result in a higher DHA concentration in erythrocyte membrane and resulted in only slightly higher EPA concentration. This explains the lack of hypotriglyceridemic effect

of walnuts at the current intake (42.5 g walnuts is equivalent to 3.3 g ALA) in the study. The conversion of ALA to EPA and DHA in the body is inefficient (9) and it is estimated that ≈7 g ALA is needed to increase membrane phospholipid EPA and DHA concentrations. However, even at higher intakes (9.5 g/d) of ALA, only EPA concentration has been shown to increase (18). This lack of increase in DHA may be due to the retroconversion of DHA to EPA or poor conversion of EPA to DHA due to an inhibition of the $\delta6$ desaturase enzyme by EPA (9).

Paralleling the decrease in triglyceride was the expected increase in HDL cholesterol with the fish diet, an observation consistent with other study results (2, 15). It is suggested that this increase in HDL cholesterol may be mostly the HDL_2, its more protective subfraction. This could be due to the inhibition of the transfer protein that allows the cholesterol ester to remain in the HDL cholesterol for a longer time (32). Although the walnut diet did not influence HDL cholesterol, the overall ratios of total cholesterol:HDL cholesterol and LDL cholesterol:HDL cholesterol decreased substantially in those who followed the walnut diet. These ratios are considered more important predictors of CHD (24) and clinically are more meaningful.

One of the consistent public health messages that has emerged for the prevention of heart disease is increasing n−3 fatty acids in the diet of Americans (1–3). Specific foods, eg, walnuts and fatty fish, that are n−3 fatty acid–rich sources have been recommended at specific "doses" for the primary prevention of CHD by recognized organizations (3, 10). Our findings suggest that individuals who have mild to moderate hyperlipidemia and who need to lower cholesterol may consider adding 1.5 oz per day of walnuts to a heart healthy diet, whereas those who need to lower triglyceride may benefit from eating at least 2 servings of fatty fish per week.

Although this study does not address the question of whether ALA and EPA/DHA are equivalent, it does provide important information about whole foods that are rich in these n−3 fatty acids. One of the dietary strategies to increase a specific nutrient in the diet is to promote the inclusion of specific foods that contain these bioactive nutrients. Individuals who choose to exclude fish from their diet because they are vegetarians or for other reasons (11, 12) may need to consider alternate sources of EPA and DHA, eg, microalgae oils and DHA-enriched eggs. Alternatively, because walnuts seem to influence different blood lipid fraction compared with fatty fish, it would be prudent for those who do eat fatty fish regularly to consider including plant foods rich in n−3 fatty acids. Recently a portfolio diet that combined several cholesterol-lowering foods, including nuts, showed a decreased plasma cholesterol concentration that was equivalent to that accomplished by statin drugs (33). The overall effect on blood lipids when fatty fish and walnuts or other foods rich in EPA/DHA and ALA are combined remains to be determined in future studies and would provide valuable information for use in clinical settings. (Other articles in this supplement to the Journal include references 34–60.)

REFERENCES

1. Gebauer SK, Lsota TL, Harris WS, Kris-Etherton PM. n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 2006;83:1526S–35S.
2. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2006;106: 2747–57.
3. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: A scientific statement from the American Heart Association Nutrition Committee. Circulation 2006;114:82–96.
4. Hu FB, Stampfer MJ, Manson JAE, et al. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr 1999;69:890–7.
5. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. Am J Clin Nutr 2000;71:208S–12S.
6. Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am J Clin Nutr 2006;84:5–17.
7. Sabaté J, Fraser GE, Burke K, Knutsen SF, Bennett H, Lindsted KD. Effect of walnuts on serum lipid levels and blood pressure in normal men. N Engl J Med 1993;238:603–7.
8. Djousse L, Pankow JS, Eckfeldt JH, et al. Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr 2001;74: 612–9.
9. Davis BC, Kris-Etherton PM. Achieving optimal essential fatty acid status in vegetarians: current knowledge and practical implications. Am J Clin Nutr 2003;78:640S–6S.
10. Tarantino LM. Qualified health claims: letter of enforcement discretion –walnuts and coronary heart disease. Docket No 02P-0292, March 9, 2004. Available from: http://www.cfsan.fda.gov/%7Edms/qhcnuts3.html (cited 9 October 2007).
11. Rosell MS, Llyod-Wright Z, Appleby PN, Sanders TA, Allen NE, Key TJ. Long chain n-3 polyunsaturated fatty acids in plasma in British meat eating, vegetarians, vegan men. Am J Clin Nutr 2005;82:327–34.
12. Guallar E, Sanz-Gallardo MI, van't Veer P, et al. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 2002;347:1747–54.
13. Environmental Protection Agency. Estimated per capita fish consumption in the United States Washington, DC: EPA, 2002.
14. Griel AE, Kris-Etherton PM. Tree nuts and the lipid profile: a review of clinical studies. Br J Nutr 2006;96:68–78.
15. Harris WS. N-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65:1645S–54S.
16. Wilkinson P, Leach C, Ah-Sing EE, et al. Influence of α-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an atherogenic lipoprotein phenotype. Atherosclerosis 2005;181:115–24.
17. Seierstad SL, Seljeflot I, Johansen O, et al. Dietary intake of differently fed salmon; the influence on markers on human atherosclerosis. Eur J Clin Invest 2005;5:52–9.
18. Finnegan YE, Minihane AM, Leigh-Firbank EC, et al. Plant- and marine-derived n-3 fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL-C to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr 2003;77:783–95.
19. Departments of Health and Human Services and the Department of Agriculture. Dietary Guidelines Advisory Committee report 2005, September 5, 2005. Available from: http://www.health.gov/dietaryguidelines/dga2005/report/ (cited 9 October 2007).
20. Sabaté J, Haddad E, Tanzman JS, Jambazian P, Rajaram S. Serum lipid response to the graduated enrichment of a Step I diet with almonds: a randomized feeding trial. Am J Clin Nutr 2003;77:1379–84.
21. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.
22. Nagele U, Hagele EO, Sauer G, et al. Reagent for the enzymatic determination of serum total triglycerides with improved lipolytic efficiency. J Clin Chem Clin Biochem 1984;22:165–74.
23. Sternberg JC. A rate nephelometer for measuring specific proteins by immunoprecipitation reactions. Clin Chem 1977;23:1456–64.
24. Fletcher B, Berra K, Ades P, et al. Managing abnormal blood lipids: a collaborative approach. Circulation 2005;112:3184–209.
25. Mori TA, Burke V, Puddey IB, et al. Purified eicosapentaenoic and docosapentaenoic acids have differential effects on serum lipids and lipoproteins, LDL-C particle size, glucose, and insulin in mildly hyperlipidemic men. Am J Clin Nutr 2000;71:1085–94.
26. Leigh-Firbank EC, Minihane AM, Leake DS, et al. Eicosapentaenoic acid and docosahexaenoic acid from fish oils: differential associations with lipid responses. Br J Nutr 2002;87:435–45.
27. Laidlaw M, Holub BJ. Effects of supplementation with fish oil-derived n-3 fatty acids and γ-linolenic acid on circulating plasma lipids and fatty acid profiles in women. Am J Clin Nutr 2003;77:37–42.
28. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk factors: a systematic review. Atherosclerosis 2006;189:19–30.
29. Theobald HE, Chowienczyk PJ, Wittall R, Humphries SE, Sanders TAB. LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women. Am J Clin Nutr 2004;79:558–63.
30. Adler AJ, Holub BJ. Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic men. Am J Clin Nutr 1997;5:445–50.
31. Harris WS, Bulchandan D. Why do omega-3 fatty acids lower serum triglycerides? Curr Opin Lipidol 2006;17:387–93.
32. Abbey M, Clifton P, Kestin M, Belling B, Nestel P. Effect of fish oil on lipoproteins, lecithin:cholesterol acyltransferase, and lipid transfer protein activity in humans. Arteriosclerosis 1990;10:85–94.
33. Jenkins DJA, Kendall CWC, Marchie A, et al. Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. Am J Clin Nutr 2005;81:380–7.
34. Rajaram S, Sabaté J. Preface. Am J Clin Nutr 2009;89(suppl):1541S–2S.
35. Jacobs DR Jr, Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. Am J Clin Nutr 2009;89(suppl): 1543S–8S.
36. Jacobs DR Jr, Haddad EH, Lanou AJ, Messina MJ. Food, plant food, and vegetarian diets in the US dietary guidelines: conclusions of an expert panel. Am J Clin Nutr 2009;89(suppl):1549S–52S.
37. Lampe JW. Interindividual differences in response to plant-based diets: implications for cancer risk. Am J Clin Nutr 2009;89(suppl):1553S–7S.
38. Simon JA, Chen Y-H, Bent S. The relation of α-linolenic acid to the risk of prostate cancer: a systematic review and meta-analysis. Am J Clin Nutr 2009;89(suppl):1558S–64S.
39. Pierce JP, Natarajan L, Caan BJ, et al. Dietary change and reduced breast cancer events among women without hot flashes after treatment of early-stage breast cancer: subgroup analysis of the Women's Healthy Eating and Living Study. Am J Clin Nutr 2009;89(suppl):1565S–71S.
40. Newby PK. Plant foods and plant-based diets: protective against childhood obesity? Am J Clin Nutr 2009;89(suppl):1572S–87S.
41. Barnard ND, Cohen J, Jenkins DJA, et al. A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. Am J Clin Nutr 2009; 89(suppl):1588S–96S.
42. Mangat I. Do vegetarians have to eat fish for optimal cardiovascular protection? Am J Clin Nutr 2009;89(suppl):1597S–601S.
43. Willis LM, Shukitt-Hale B, Joseph JA. Modulation of cognition and behavior in aged animals: role for antioxidant- and essential fatty acid–rich plant foods. Am J Clin Nutr 2009;89(suppl):1602S–6S.
44. Fraser GE. Vegetarian diets: what do we know of their effects on common chronic diseases? Am J Clin Nutr 2009;89(suppl):1607S–12S.
45. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1613S–9S.
46. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Cancer incidence in vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1620S–6S.
47. Craig WJ. Health effects of vegan diets. Am J Clin Nutr 2009;89(suppl): 1627S–33S.
48. Weaver CM. Should dairy be recommended as part of a healthy vegetarian diet? Point. Am J Clin Nutr 2009;89(suppl):1634S–7S.
49. Lanou AJ. Should dairy be recommended as part of a healthy vegetarian diet? Counterpoint. Am J Clin Nutr. 2009;89(suppl):1638S–42S.
50. Sabaté J, Ang Y. Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr 2009;89(suppl):1643S–8S.
51. Ros E. Nuts and novel biomarkers of cardiovascular disease. Am J Clin Nutr 2009;89(suppl):1649S–56S.
52. Lampe JW. Is equol the key to the efficacy of soy foods? Am J Clin Nutr 2009;89(suppl):1664S–7S.

53. Badger TM, Gilchrist JM, Pivik RT, et al. The health implications of soy infant formula. Am J Clin Nutr 2009;89(suppl):1668S–72S.

54. Messina M, Wu AH. Perspectives on the soy–breast cancer relationship. Am J Clin Nutr 2009;89(suppl):1673S–9S.

55. Lönnerdal B. Soybean ferritin: implications for iron status of vegetarians. Am J Clin Nutr 2009;89(suppl):1680S–5S.

56. Chan J, Jaceldo-Siegl K, Fraser GE. Serum 25-hydroxyvitamin D status of vegetarians, partial vegetarians, and nonvegetarians: the Adventist Health Study-2. Am J Clin Nutr 2009;89(suppl):1686S–92S.

57. Elmadfa I, Singer I. Vitamin B-12 and homocysteine status among vegetarians: a global perspective. Am J Clin Nutr 2009;89(suppl):1693S–8S.

58. Marlow HJ, Hayes WK, Soret S, Carter RL, Schwab ER, Sabaté J. Diet and the environment: does what you eat matter? Am J Clin Nutr 2009;89(suppl):1699S–703S.

59. Carlsson-Kanyama A, González AD. Potential contributions of food consumption patterns to climate change. Am J Clin Nutr 2009;89(suppl):1704S–9S.

60. Eshel G, Martin PA. Geophysics and nutritional science: toward a novel, unified paradigm. Am J Clin Nutr 2009;89(suppl):1710S–6S.