The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia[1–3]

Rogelio U Almario, Veraphon Vonghavaravat, Rodney Wong, and Sidika E Kasim-Karakas

ABSTRACT

Background: Epidemiologic studies show an inverse relation between nut consumption and coronary heart disease.

Objective: We determined the effects of walnut intake on plasma fatty acids, lipoproteins, and lipoprotein subclasses in patients with combined hyperlipidemia.

Design: Participants sequentially adhered to the following diets: *1*) a habitual diet (HD), *2*) a habitual diet plus walnuts (HD+W), *3*) a low-fat diet (LFD), and *4*) a low-fat diet plus walnuts (LFD+W).

Results: In 13 postmenopausal women and 5 men ($\bar{x} \pm$ SD age 60 ± 8 y), walnut supplementation did not increase body weight despite increased energy intake and the LFD caused weight loss (1.3 ± 0.5 kg; $P < 0.01$). When comparing the HD with the HD+W, linoleic acid concentrations increased from 29.94 ± 1.14% to 36.85 ± 1.13% and α-linolenic acid concentrations increased from 0.78 ± 0.04% to 1.56 ± 0.11%. During the LFD+W, plasma total cholesterol concentrations decreased by 0.58 ± 0.16 mmol/L when compared with the HD and by 0.46 ± 0.14 mmol/L when compared with the LFD. LDL-cholesterol concentrations decreased by 0.46 ± 0.15 mmol/L when compared with the LFD. Measurements of lipoprotein subclasses and particle size suggested that walnut supplementation lowered cholesterol preferentially in small LDL (46.1 ± 1.9% compared with 33.4 ± 4.3%, HD compared with HD+W, respectively; $P < 0.01$). HDL-cholesterol concentrations decreased from 1.27 ± 0.07 mmol/L during the HD to 1.14 ± 0.07 mmol/L during the HD+W and to 1.11 ± 0.08 mmol/L during the LFD. The decrease was seen primarily in the large HDL particles.

Conclusions: Walnut supplementation may beneficially alter lipid distribution among various lipoprotein subclasses even when total plasma lipids do not change. This may be an additional mechanism underlying the antiatherogenic properties of nut intake. *Am J Clin Nutr* 2001;74:72–9.

KEY WORDS Walnuts, cholesterol, lipoprotein, plasma fatty acid, habitual diet, low-fat diet, coronary heart disease, combined hyperlipidemia

INTRODUCTION

Epidemiologic studies consistently show that consumption of 142 g (5 oz) nuts/wk is associated with a 30–50% decrease in coronary heart disease (1–5). This inverse relation remains significant after adjustment for age; body mass index; exercise; smoking; dietary intake of fats, fiber, vegetables, fruit, and alcohol; and supplementation with multivitamins or vitamin E. The protective effects of nuts are mediated through several mechanisms. Nuts are a rich source of unsaturated fatty acids, vitamin E (which may function as an anitoxidant), fiber, magnesium, potassium, and arginine (1). Unsaturated fatty acids and fiber may improve plasma lipids (by decreasing triacylglycerol and cholesterol concentrations) (6–8), decrease platelet aggregation (9–11), and prevent arrhythmias (12). Cations, such as magnesium and potassium, may improve blood pressure (13), and arginine may lower blood pressure by promoting the production of nitrous oxide and causing vasodilation (14).

Although epidemiologic studies do not differentiate between the intakes of various nuts, the fatty acid composition of different nuts varies considerably. Although almonds, hazelnuts, macadamia nuts, and peanuts are rich in monounsaturated fatty acids (MUFAs), walnuts are rich in polyunsaturated fatty acids (PUFAs) (1, 15, 16). A unique aspect of walnuts is that they are a rich source of both n−6 and n−3 PUFAs (16). Replacement of dietary saturated fats with either MUFAs or PUFAs decreases plasma total and LDL-cholesterol concentrations (17–20), and n−3 PUFAs in fish oils lower triacylglycerol concentrations (21–24). The n−3 PUFA in walnuts, ie, α-linolenic acid (ALA; 18:3n−3), is the essential precursor of n−3 PUFAs in fish oils [eicosapentaenoic acid (EPA; 20:5 n−3), and docosahexaenoic acid (DHA; 22:6n−3)] and can be elongated and desaturated in the human body (15, 25). Therefore, it is conceivable that the consumption of walnuts can lower both plasma triacylglycerol and cholesterol concentrations.

Studies in normolipidemic individuals showed that the replacement of other dietary fats with walnuts lowers plasma total and LDL-cholesterol concentrations without changing

[1] From the Department of Internal Medicine, Division of Endocrinology and Metabolism, School of Medicine, the Department of Statistics, University of California, Davis.

[2] Supported by grants from The California Walnut Commission, Sacramento, and the ALSAM Foundation, Los Angeles (to SEKK).

[3] Reprints not available. Address correspondence to SE Kasim-Karakas, University of California, 4150 V Street, PSSB, Suite G400, Sacramento, CA 95817. E-mail: sekarakas@ucdavis.edu.

Received March 16, 2000.

Accepted for publication December 7, 2000.

either triacylglycerol or HDL-cholesterol concentrations (6–8). We investigated the effects of walnut consumption in subjects with high plasma total cholesterol and triacylglycerol concentrations. We focused on hyperlipidemic patients because fish oil supplementation lowers plasma triacylglycerol concentrations only when the baseline concentration is high (22, 26). Therefore, just because there is no decrease in plasma triacylglycerol concentrations in normolipidemic subjects does not necessarily exclude the possibility of a beneficial effect in hyperlipidemic patients.

In all the published reports, walnut supplementation was accompanied by simultaneous decreases in dietary fat intake from other sources (6–8). Therefore, it was impossible to determine conclusively whether supplementation of walnuts or the restriction of other dietary fats was responsible for the improvement in plasma lipids. To address this question directly, we investigated the effects of walnut consumption along with 2 different background diets, a habitual diet and a low-fat diet.

SUBJECTS AND METHODS

Subjects

Seven men and 16 postmenopausal women with fasting plasma triacylglycerol concentrations >2.26 mmol/L, total cholesterol concentrations >5.17 mmol/L, and LDL-cholesterol concentrations >3.36 mmol/L while consuming their habitual diets and who had not been taking any antihyperlipidemic medicine for ≥2 mo were recruited after they signed informed consent forms approved by the Institutional Human Investigation Committee of the University of California, Davis. All participants were examined by the principal investigator and chemistry-20 panels and lipid profiles were obtained after subjects had fasted overnight for 12 h. Individuals with any systemic illness (eg, diabetes mellitus, liver or kidney disease, or hypertension) that required medication were excluded. Smokers and individuals who consumed >3 servings of alcohol/wk (1 serving = 120 mL wine, 240 mL beer, or 30 mL hard liquor) were also excluded. Hypothyroid patients who were euthyroid with a stable dose of thyroid replacement therapy were accepted. Menopause was defined as ≥12 mo of amenorrhea or surgical removal of both ovaries. Only women receiving no hormone therapy or continuous hormone therapy were included. Women receiving cyclical hormone replacement were excluded. Doses of hormones and all other medications or supplements remained unchanged throughout the study. Each subject's exercise level was kept constant and monitored by physical activity questionnaires.

Five subjects discontinued the study for the following reasons: 1 was intolerant to walnuts (canker sores in the mouth), 2 had unrelated medical conditions, 1 relocated, and 1 could not afford the time required for the study. The remaining 5 men and 13 women (aged 60 ± 8 y) completed all 4 dietary periods.

Study design

Subjects adhered to the following 4 different diets sequentially in free-living conditions: *1*) a habitual diet (HD), *2*) a habitual diet plus walnuts (HD+W), *3*) a low-fat diet (20% fat; LFD), and *4*) a low-fat diet plus walnuts (LFD+W). Although lack of randomization limits the ability to control for seasonal variations in plasma lipids, these diets were not administered in a random order for many reasons. First, in our experience, individuals who are trained to consume a low-fat diet do not fully resume a high-fat intake. Second, dietary intervention periods of ≥5 wk are adequate to achieve stable plasma lipid profiles (27). Third, randomization would have approximately quadrupled the number of group sessions and increased the time and resources required.

The total duration of the study was 5.5 mo. The HD lasted 4 wk, whereas the other diet periods lasted 6 wk each. During the HD and HD+W periods, the participants received no nutrition education to avoid influencing their usual dietary habits. At the start of the LFD period, the participants received intensive group education and training on the amount of fat in food items, types of fats, calculating energy obtained from fat, the basic principles of low-fat cooking, shopping for low-fat food items, and behavior-modification techniques to change dietary habits. In addition, each participant attended individual counseling sessions with a dietitian (≈3 h) to review general nutrition principles and to address specific questions (eg, ethnic foods and what to do when traveling). During the 3-mo period that involved consuming the low-fat diets, 1-h group sessions were held every 2–3 wk to review and reinforce the principles of a low fat intake and to answer any new questions.

During the second and fourth intervention periods, the diets were supplemented with 48 g walnuts/8460 kJ energy intake. This particular amount of walnut supplementation was selected because 48 g walnuts contains 3.3 g n−3 fatty acids (ALA). Previously we showed that 3.3 g n−3 PUFAs in fish oils lowered plasma triacylglycerol concentrations by 37% (22). In that study the average energy intake was 8106 ± 456 kJ/d. Therefore, similar ratios of n−3 PUFAs per energy intake were provided with walnuts. Furthermore, Abbey et al (28) reported that a diet containing 68 g walnuts and 10951 kJ/d (or 52 g walnuts/8460 kJ) reduced total cholesterol by 5% and LDL-cholesterol concentrations by 9% in healthy subjects. Forty-eight grams of walnuts contains 1570 kJ: 1269 kJ from 30 g of fat, 132 kJ from 7 g of protein, and 169 kJ from 9 g of carbohydrates. The daily supplementation of walnuts was provided in preweighed packages to each study participant. Although the participants were told that they could use the walnuts for cooking and baking, most subjects ate the walnuts as they were provided.

Data collection

At the middle and end of each dietary period, subjects were seen in the morning after they had fasted for 12 h overnight. Subjects were weighed and blood samples were collected in tubes containing EDTA. The blood tests obtained at the end of each period were used for the statistical analysis and reporting.

Determination of fatty acid composition

Plasma fatty acid composition was determined at the Clinical Nutrition Research Unit–Metabolism Core Laboratory at the University of California, Davis. The total plasma lipids were extracted and the fatty acids were esterified with 5% HCl in methanol. Methyl esters of fatty acids were extracted in petroleum ether (boiling point: 30–60 °C), dried under nitrogen, and submitted to analysis by capillary gas chromatography. A Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 50 m × 0.25 mm bonded 007-

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

FFAP fused silica capillary column (Quadrex Corp, New Haven, CT) was used to separate the methyl esters from the fatty acids. The column was programmed to raise the temperature from 190 to 220 °C at 2 °C/min with a final hold, separating 12:0–22:6n−3 fatty acids. The detector temperature was 270 °C and the injector temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.4 mL/min and a split ratio of 1:65. Fatty acid methyl esters were identified by comparison with authentic standards and peak areas were integrated as relative weight with the use of a microprocessor.

Lipid and apolipoprotein measurements

Triacylglycerol and cholesterol concentrations were measured enzymatically. Each assay included appropriate standards and calibrators. The interassay CV was 3.6% for triacylglycerol and 1.9% for cholesterol. HDL cholesterol was separated from plasma by the precipitation method of Warnick et al (29) with use of dextran sulfate and magnesium chloride. The CV for HDL cholesterol was 2% for the normal values and 5% for the extremes. Apolipoproteins B and A-I were measured by immunonephelometry; the CVs for these measurements were 3.6% and 3.3%, respectively.

Determination of lipoprotein particle size

Particle sizes of VLDL, LDL, and HDL were determined by nuclear magnetic resonance as previously described (30). The LDL and HDL subclass distributions determined by gradient-gel electrophoresis and nuclear magnetic resonance correlate closely (31). The following lipoprotein subclass categories were used: $VLDL_{large}$ (>40 nm), $VLDL_{intermediate}$ (31–40 nm), and $VLDL_{small}$ (27–31 nm); intermediate-density lipoprotein (IDL; 23–27 nm); LDL_{large} (21.3–23 nm), $LDL_{intermediate}$ (19.8–21.2 nm), and LDL_{small} (18.3–19.7 nm); and HDL_{large} (8.8–13 nm), $HDL_{intermediate}$ (7.8–8.8 nm), and HDL_{small} (7.3–7.7 nm). Each VLDL subclass was expressed as a percentage of triacylglycerol in the total VLDL fraction. Concentrations of IDL and LDL subclasses were expressed as a percentage of cholesterol in the total LDL fraction, and concentrations of HDL subclasses were expressed as a percentage of cholesterol present in the total HDL fraction.

Nutrition assessment

During each intervention period, 7-d food records were obtained and analyzed using updated version of Nutrition Data System (NDS 93; University of Minnesota, Minneapolis).

Statistical analysis

Analyses were conducted with use of SAS for WINDOWS, release 6.12 (32, 33). Results are reported as means ± SEMs unless otherwise noted. Changes over time were examined with a repeated-measures analysis with an unstructured covariance matrix (mixed procedure). Significance (P values) for testing the null hypothesis of no difference among the 4 dietary regimens was computed on the basis of approximate F statistics. Significance for testing the null hypothesis between 4 prespecified pair wise dietary comparisons (HD+W compared with HD, LFD compared with HD, LFD+W compared with LFD, and LFD+W compared with HD) was also computed. For each variable, an overall significance level of $\alpha = 0.05$ was maintained by adjusting $\alpha^* = \alpha/4 = 0.0125$ to account for the multiple comparisons by use of the Bonferroni procedure.

RESULTS

Changes in dietary energy and fat intakes and weight

Diet records indicated that during the HD period, participants consumed 8106 ± 456 kJ/d, providing 31.4 ± 1.9% of daily energy from fat (**Table 1**). The dietary ratio of polyunsaturated to monounsaturated to saturated fat (P:M:S) was 1:1.8:1.7; the HD provided 11.2 ± 1.4 g linoleic acid (LA; 18:2n−6)/d and 1.3 ± 0.2 g ALA/d. The addition of the walnuts increased the energy intake by 1661 ± 466 kJ. The P:M:S became 1:0.8:0.8 and the intakes of LA and ALA increased by 19.1 ± 4.1 g/d and 4.1 ± 0.2 g/d, respectively. During the LFD, daily energy intake decreased by 1451 ± 395 kJ and fat intake decreased by 11.8% when compared with the HD period. The P:M:S ratio became 1:1.8:1.6 and the intakes of LA and ALA decreased. When walnuts were added back to the LFD (LFD+W), energy and total fat intake increased by 1514 ± 338 kJ and 14%, respectively, compared with the LFD. The P:M:S became 1:0.7:0.5 and LA and ALA intakes increased by 19.7 ± 1.1 and 4.2 ± 0.2 g/d, respectively. During the HD and LFD+W periods, energy and fat intakes did not differ; however, the composition of dietary fat (P:M:S) was significantly different.

Despite the significant increase in energy intake during the HD+W, there were no increases in body weight among subjects (78.7 ± 3.3 kg compared with 78.8 ± 3.4 kg, HD+W compared with the HD, respectively). During the LFD, significant weight loss occurred (1.3 ± 0.5 kg; $P < 0.01$ compared with the HD), and even when walnuts were added back to the LFD (LFD+W), there was no significant weight gain (76.9 ± 3.3 kg compared with 77.5 ± 3.2 kg, LFD+W compared with LFD, respectively) despite the significant increases in energy (1514 ± 338 kJ/d) and fat intake (30.3 ± 3.4 g/d).

Changes in plasma fatty acid composition

The addition of walnuts to the HD increased the LA and ALA concentrations in the plasma. There were compensatory decreases in palmitic and oleic acid concentrations, as shown in **Table 2**. Interestingly, concentrations of EPA and DHA did not change, and concentrations of arachidonic acid (AA, 20:4n−6) decreased. These changes were completely reversed during the LFD. The addition of walnuts to the LFD increased the LA and ALA concentrations and decreased plasma palmitic, oleic, and AA concentrations, yet did not change either EPA or DHA concentrations.

Changes in plasma lipids and apoproteins

Plasma triacylglycerol concentrations did not change significantly during any of the diets (**Table 3**). Total and LDL-cholesterol concentrations did not change during either the HD+W or LFD. Compared with both the HD and LFD, the LFD+W decreased total cholesterol concentrations significantly. LDL-cholesterol concentrations also decreased during the LFD+W compared with the LFD. Plasma apolipoprotein B was not affected by walnut supplementation, but increased during the LFD compared with the HD. The addition of walnuts to the HD decreased the HDL-cholesterol concentrations, but increased apolipoprotein A-I. During the LFD, HDL-cholesterol concentrations remained low. Apolipoprotein A-I returned to the baseline during the LFD and remained stable after the LFD+W.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

TABLE 1
Anthropometric and dietary variables during the habitual diet (HD), habitual diet plus walnuts (HD+W), low-fat diet (LFD), and low-fat diet plus walnuts (LFD+W)[1]

	HD	HD+W	LFD	LFD+W	*P*
Weight (kg)	78.8 ± 3.4	78.7 ± 3.3	77.5 ± 3.2[2]	76.9 ± 3.3[2]	0.0076
BMI (kg/m²)	29.0 ± 1.2	29.0 ± 1.2	28.7 ± 1.1	27.7 ± 1.2	0.0279
Energy (kJ)	8106 ± 456	9767 ± 541[2]	6655 ± 371[2]	8169 ± 404[3]	0.0001
Carbohydrate					
(g)	249 ± 15	267 ± 17	232 ± 14	214 ± 14	0.0499
(%)	50.6 ± 2.0	47.0 ± 1.5	61.2 ± 1.4[2]	50.2 ± 1.9[3]	0.0001
Fiber (g)	19.0 ± 1.6	21.4 ± 1.4	18.8 ± 1.5	20.9 ± 2.3	0.1462
Protein					
(g)	82.7 ± 6.7	88.9 ± 6.6	69.9 ± 4.5	67.3 ± 4.4	0.0045
(%)	17.0 ± 1.5	15.5 ± 0.7	18.6 ± 1.0	15.6 ± 0.7	0.0354
Total fat					
(g)	71.9 ± 7.6	95.4 ± 7.4	32.8 ± 2.1[2]	63.1 ± 3.8[3]	0.0001
(%)	31.4 ± 1.9	37.2 ± 1.4	19.7 ± 1.0[2]	33.7 ± 1.6[3]	0.0001
Saturated fat					
(g)	21.4 ± 2.1	23.2 ± 2.4	11.7 ± 3.8[2]	15.3 ± 5.8[2]	0.0001
(%)	11.0 ± 0.9	9.8 ± 0.6	7.5 ± 0.4[2]	8.2 ± 0.6	0.0001
MUFA					
(g)	23.8 ± 2.6	30.2 ± 2.7	13.1 ± 1.0[2]	22.5 ± 1.5[3]	0.0001
(%)	12.0 ± 0.9	12.8 ± 0.6	8.3 ± 0.4[2]	11.6 ± 0.5[3]	0.0001
PUFA					
(g)	12.9 ± 1.6	36.5 ± 2.1[2]	7.4 ± 0.5[2]	31.5 ± 1.4[2,3]	0.0001
(%)	6.4 ± 0.5	16.0 ± 0.6[2]	4.7 ± 0.3[2]	16.5 ± 0.4[2,3]	0.0001
Linoleic acid (g)	11.2 ± 1.4	30.3 ± 1.8[2]	6.2 ± 0.5[2]	25.8 ± 1.2[2,3]	0.0001
α-Linolenic acid (g)	1.3 ± 0.2	5.4 ± 0.3[2]	0.7 ± 0.1[2]	4.9 ± 0.2[2,3]	0.0001

[1] $\bar{x}$ ± SEM; *n* = 18. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] Significantly different from HD, $P < 0.0125$ (Bonferroni adjusted).
[3] Significantly different from LFD, $P < 0.0125$.

Changes in the particle size and subclass distribution of lipoproteins

Overall particle sizes of VLDL, LDL, or HDL did not change significantly during the different diets (Table 3). However, distribution of cholesterol in the IDL and LDL subclasses did change. When the cholesterol in IDL and LDL was considered as 100% and percentage distribution of cholesterol in each subclass was calculated, IDL cholesterol was lower during the LFD+W (9.7 ± 1.8%) than during the LFD (14.2 ± 1.8%). There was a decrease in small-density LDL, from 46.1 ± 4.9% to 33.4 ± 4.3%, in the HD+W. The LFD reversed this change. Changes in HDL–cholesterol subclass distribution indicated that during the HD+W and LFD, the large HDL subfraction decreased.

DISCUSSION

Walnut intake did not cause weight gain despite an increased energy intake. The reasons for this are not clear. In experimental animals, PUFAs caused less weight gain than did saturated fats (34). Another explanation may be noncompliance with walnut intake. However, during both walnut periods, plasma LA and ALA increased. Another consideration is that this study was not blind or randomized; therefore, intake of other dietary fats may have decreased. Self-reported data can underestimate energy and fat intakes (35, 36); however, we found that even when underreporting does occur, there is consistency within the data obtained from an individual subject over time (35). Furthermore, low-fat intake was associated with a 58 040 kJ energy deficit and a 1.3 kg weight loss, suggesting relative reliability of the self-reported data. Therefore, the absence of weight gain seems to be an accurate finding and requires further investigation.

Although Mantzioris et al (37) showed that flaxseed oil, a rich source of ALA, increases plasma EPA and DHA concentrations (37), walnut intake did not increase either EPA or DHA, and plasma AA concentrations decreased. This may be due to the competition between LA (n−6 fatty acid) and ALA (n−3 fatty acid) for the rate-limiting Δ^6-desaturase (15).

We expected that increased ALA intake would lower plasma triacylglycerol concentrations because ALA would be desaturated and then elongated to EPA, the major fatty acid found in fish oil that has potent triacylglycerol-lowering activity. However, because walnut intake did not raise plasma EPA, triacylglycerol concentrations did not change either. A recent study by Kris-Etherton et al (38) compared the American Heart Association's Step II Diet with 3 different MUFA-rich diets. Restriction of total fat intake during the American Heart Association's Step II diet caused an increase in plasma triacylglycerol concentrations, whereas all 3 MUFA-rich diets lowered triacylglycerol concentrations. We did not find any increases in plasma triacylglycerol concentrations during the LFD. This was probably due to the decreased energy intake during the LFD period. We previously showed that a eucenergetic low-fat diet increases plasma triacylglycerol concentrations (35, 39). On the other hand, ad libitum low-fat diet results in decreased energy intake and does not raise plasma triacylglycerol concentrations (35). There may be several reasons for the lack of a decrease in plasma triacylglycerol concentrations during walnut intake, eg, our patients had combined hyperlipidemia, our patients were older, or the walnut-rich diet contained more PUFAs than MUFAs.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

TABLE 2
Plasma fatty acid composition as measured by gas-liquid chromatography during the habitual diet (HD), habitual diet plus walnuts (HD+W), low-fat diet (LFD), and low-fat diet plus walnuts (LFD+W)[1]

Fatty acid	HD	HD+W	LFD	LFD+W	*P*
			% by wt		
Myristic	1.50 ± 0.14	1.22 ± 0.09	1.34 ± 0.11	1.24 ± 0.09	0.1706
Palmitic	26.80 ± 0.69	24.47 ± 0.77[2]	26.59 ± 0.7	23.96 ± 0.64[2,3]	0.0001
Stearic	7.62 ± 0.17	7.76 ± 0.18	7.49 ± 0.16	7.51 ± 0.18	0.1985
Oleic	22.93 ± 0.77	18.91 ± 0.83[2]	22.84 ± 0.67	18.73 ± 0.81[2,3]	0.0001
Linoleic	29.94 ± 1.14	36.85 ± 1.13[2]	29.76 ± 1.08	37.11 ± 0.97[2,3]	0.0001
α-Linolenic	0.78 ± 0.04	1.56 ± 0.11[2]	0.86 ± 0.21	1.85 ± 0.17[2,3]	0.0001
Arachidonic	7.26 ± 0.42	6.33 ± 0.39[2]	7.55 ± 0.41	6.52 ± 0.46[3]	0.0025
Eicosapentaenoic	0.89 ± 0.22	0.86 ± 0.13	0.99 ± 0.26	0.83 ± 0.17	0.5574
Docosahexaenoic	2.28 ± 0.30	2.04 ± 0.18	2.59 ± 0.34	2.25 ± 0.27	0.0516

[1] $\bar{x}$ ± SEM; $n = 18$.
[2] Significantly different from HD, $P < 0.0125$.
[3] Significantly different from LFD, $P < 0.0125$.

The HD+W did not raise plasma total cholesterol, LDL-cholesterol, and apolipoprotein B concentrations possibly because saturated fat intake did not increase in the diet (19, 20). In contrast with previous reports (35, 38–42), the LFD did not decrease plasma total cholesterol and LDL-cholesterol concentrations in our subjects. Our participants had genetic hyperlipidemia and habitually consumed less total fat and saturated fat and more MUFAs than the general population. This may have blunted the response to further dietary fat restriction. However, the LFD+W lowered total cholesterol concentrations compared with both the HD and LFD, and lowered LDL-cholesterol concentrations compared with the LFD. It is assumed that PUFAs or MUFAs lower plasma lipids by replacing saturated fats. However, the improvement observed after the addition of PUFAs to the LFD suggests possible independent effects. In support of this, Zambon et al (43) recently reported that substituting walnuts for MUFAs improves plasma lipoprotein concentrations in hyperlipidemic patients.

A novel finding was that the subfraction distribution of lipoproteins changed, even when plasma lipids did not. The most atherogenic lipoproteins are IDL and small-density LDL. Although it is generally believed that normal fasting plasma does not contain IDL, this may be inaccurate because standard laboratory assays do not measure IDL. In a large Framingham Offspring study, which analyzed lipoproteins by nuclear mag-

TABLE 3
Plasma lipids, apolipoproteins, lipoprotein particle sizes, and distribution of triacylglycerol (TG) in VLDL and of cholesterol (C) in intermediate density lipoproteins (IDL), LDLs, and HDLs during the habitual diet (HD), habitual diet plus walnuts (HD+W), low-fat diet (LFD), and low-fat diet plus walnuts (LFD+W)[1]

	HD	HD+W	LFD	LFD+W	*P*
Lipids (mmol/L)					
TG	2.47 ± 0.22	2.22 ± 0.22	2.31 ± 0.18	2.28 ± 0.18	0.3625
Total C	5.97 ± 0.29	5.79 ± 0.31	5.84 ± 0.31	5.39 ± 0.31a[2,3]	0.0098
LDL-C	3.57 ± 0.28	3.63 ± 0.31	3.67 ± 0.31	3.22 ± 0.27[3]	0.0148
HDL-C	1.27 ± 0.07	1.14 ± 0.07[2]	1.11 ± 0.08[2]	1.13 ± 0.04	0.0003
Apolipoprotein (mmol/L)					
B	2.15 ± 0.13	2.22 ± 0.13	2.31 ± 0.13[2]	2.22 ± 0.17	0.0061
A-I	5.25 ± 0.29	5.71 ± 0.32[2]	5.32 ± 0.25	5.14 ± 0.25	0.0100
Particle size (nm)					
VLDL	48.1 ± 1.1	46.2 ± 1.3	49.7 ± 1.2	47.9 ± 1.2	0.0154
LDL	20.4 ± 0.2	20.9 ± 0.1	20.5 ± 0.2	20.7 ± 0.2	0.0342
HDL	8.6 ± 0.1	8.6 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	0.2278
Lipid distribution (%)					
$VLDL\text{-}TG_{large}$	26.7 ± 2.4	18.5 ± 2.7	27.9 ± 3.4	22.5 ± 2.5	0.0147
$VLDL\text{-}TG_{inter}$	37.2 ± 1.5	40.0 ± 1.7	34.9 ± 1.6	41.4 ± 1.8[3]	0.0280
$VLDL\text{-}TG_{small}$	36.4 ± 1.9	41.0 ± 2.6	36.0 ± 2.7	34.3 ± 2.9	0.0536
IDL-C	12.9 ± 1.4	8.6 ± 1.7	14.2 ± 1.8	9.7 ± 1.8[3]	0.0112
$LDL\text{-}C_{large}$	40.1 ± 5.4	53.5 ± 5.5	43.7 ± 5.6	48.8 ± 5.3[2]	0.0602
$LDL\text{-}C_{small}$	46.1 ± 4.9	33.4 ± 4.3[2]	42.1 ± 4.7	39.1 ± 5.0	0.0425
$HDL\text{-}C_{large}$	33.6 ± 4.6	22.8 ± 2.6[2]	23.0 ± 3.3[2]	24.7 ± 2.3	0.0517
$HDL\text{-}C_{small}$	63.4 ± 4.4	73.7 ± 3.1	73.7 ± 4.0	71.4 ± 3.3	0.1005

[1] $\bar{x}$ ± SEM; $n = 18$. Inter, intermediate. To convert from SI units, divide by 0.01129 for TG, 0.02586 for cholesterol, 0.0185 for apolipoprotein B, and 0.0357 for apolipoprotein A-I.
[2] Significantly different from HD, $P < 0.0125$.
[3] Significantly different from LFD, $P < 0.0125$.

The American Journal of Clinical Nutrition
Downloaded from ajcn.nutrition.org by guest on June 13, 2016

netic resonance, did not report IDL concentrations (44). In our patients, 12.9% of the LDL cholesterol was transported in the IDL at times of fasting during the HD. The reason for the IDL presence in the fasting plasma may be that our subject had combined hyperlipidemia. Plasma IDL-cholesterol concentrations were lower during the LFD+W than during the LFD. Walnut intake also affected LDL-cholesterol concentrations favorably. The addition of walnuts to the HD decreased the amount of cholesterol in the small LDL fraction. Low-fat intake reversed this beneficial change. To our knowledge, these findings are the first to suggest that dietary fat composition may affect lipoprotein subclass distribution, even without a change in plasma lipids.

Another important finding was that, during the HD+W, HDL-cholesterol concentrations decreased, but apolipoprotein A-I concentrations increased. Dietary fat affects plasma HDL cholesterol (35, 45, 46). Kinetic studies show that a high fat intake increases HDL-cholesterol concentrations both by increasing the transport and decreasing the fractional catabolic rate of apolipoprotein A-I, whereas low-fat intake reduces the transport rate (45, 46). Our results suggest that both the amount and composition of dietary fat are important. Apolipoprotein A-I may be regulated primarily by the amount, whereas HDL cholesterol may be regulated more by the composition of dietary fat. In agreement with this, walnut intake decreased the cholesterol in the large, but not in the small, HDL particles. The LFD was also associated with a decrease in large HDL-cholesterol subfractions. Although low plasma HDL-cholesterol concentrations are associated with increased coronary artery disease risk, the etiology may also be very important (47). Dietary fat restriction lowers HDL-cholesterol concentrations (35, 45, 46, 48, 49). This diet-induced decrease probably has different implications than would genetically low HDL-cholesterol concentrations. Our finding is novel in that increased PUFA intake reduced HDL-cholesterol concentrations without a decrease in either saturated fat or MUFA intake, suggesting that PUFAs may compete with saturated fat and MUFAs, thus mimicking the effects of dietary fat restriction.

Plasma lipoproteins are metabolized by several receptors, enzymes, and lipid exchange proteins. Plasma LDL-cholesterol concentrations are regulated by the hepatic LDL receptors, which are reduced by saturated fats. Replacement of saturated fats by MUFAs or PUFAs restores LDL-receptor affinity and decreases LDL-cholesterol concentraitons (38). Lipoprotein lipase hydrolyzes VLDL triacylglycerol and contributes to the production of HDL (50). Dietary fat restriction decreases and n−3 fish oils increase postheparin plasma lipoprotein lipase activity (26, 50, 51). There is no information about the effects of LA and ALA on lipoprotein lipase. Hepatic lipase facilitates the uptake of IDL and HDL cholesterol and contributes to the production of small-density LDL. We did not see any change in hepatic lipase activity during either dietary fat restriction or n−3 fish oil supplementation (26, 52). Effects of other PUFAs on hepatic lipase activity are not known.

Phosphatidylcholine–sterol *o*-acyltransferase (otherwise known as lecithin–cholesterol acyltransferase) facilitates the uptake and incorporation of cholesterol by HDL. In general, PUFAs are poor substrates for phosphatidylcholine–sterol *o*-acyltransferase (53). This may partially explain the drop in HDL cholesterol during walnut intake. Finally, cholesterol ester transfer protein exchanges core lipids between triacylglycerol-rich lipoproteins and HDL. Although n−3 fish oils increase cholesterol ester transfer protein activity, effects of other PUFAs are not known (54).

Despite the favorable plasma lipoprotein changes, there may also be potential concerns about increasing the dietary intake of nuts, particularly walnuts. Although not observed in this study, nuts may promote weight gain because of their high fat contents. Second, increased n−3 fatty acid intake may adversely affect glucose homeostasis (55), although Sirtori et al (56) and Uusitipa et al (57) did not show a hyperglycemic effect. Third, LA, which is an n−6 fatty acid, may be proinflammatory and carcinogenic (25). The culprit is believed to be AA, a significant component of phospholipids and the precursor for various prostaglandins (25). In this study, walnut intake did not increase, yet instead decreased plasma AA. The simultaneous increase in the intake of ALA, which is an n−3 fatty acid, may have interfered with the production of AA by competition.

In conclusion, although nut intake may affect human health significantly, mechanisms underlying these effects are not known. The present study suggests that the metabolic effects of walnuts, a food rich both in n−6 and n−3 fatty acids, are very different from the individual effects of oils rich in either n−6 or n−3 fatty acids. The potential antiatherogenic effects of nuts and their possible adverse effects necessitate further research.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

REFERENCES

1. Dreher ML, Maher CV, Kearney P. The traditional and emerging role of nuts in healthful diets. Nutr Rev 1996;54:241–5.
2. Hu FB, Stampfer MJ, Manson JE, et al. Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. BMJ 1998;317:1341–5.
3. Fraser GE, Shavlik DJ. Risk factors for all-cause and coronary heart disease mortality in the oldest-old. The Adventist Health Study. Arch Intern Med 1997;157:2249–58.
4. Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. Am J Epidemiol 1992;136:192–200.
5. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary artery disease: the Adventist Health Study. Arch Intern Med 1992;152:1416–24.
6. Berry EM, Eisenberg S, Harahtz D, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins—the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. Am J Clin Nutr 1991;53:899–907.
7. Chan JK, Bruce VM, McDonald BE. Dietary α-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. Am J Clin Nutr 1991;53:1230–4.
8. Sabate J, Fraser GE, Burke K, Knetsen SF, Bennett H, Linsted KD. Effects of walnuts on serum lipid levels and blood pressure in normal men. N Engl J Med 1993;328:603–7.
9. Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ, Holub BJ. Effect of dietary acid and its ratio to LA on platelet and plasma fatty acids and thrombogenesis. Lipids 1993;28:811–7.
10. Mest HJ, Beitz J, Heinroth I, Block HU, Forster W. The influence of linseed oil diet on fatty acid pattern in phospholipids and thromboxane formation in platelets in man. Klin Wochenschr 1983;61:187–91.
11. Kinsella JE. Effects of polyunsaturated fatty acids on factors related to cardiovascular disease. Am J Cardiol 1987;60:23G–32G.
12. Hu FB, Stampfer MJ, Manson JE, et al. Dietary intake of α-linoleic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr 1999;69:890–7.
13. Elin RJ. Is the magnesium content of nuts a factor for coronary heart disease? Arch Intern Med 1993;153:779–80.

14. Fraser GE. Nut consumption, lipids, and risk of a coronary event. Clin Cardiol 1999;22(suppl):III11–5.
15. Connor WE. α-Linolenic acid in health and disease. Am J Clin Nutr 1999;69:827–8.
16. Greve LC, McGranahan G, Hasey J, et al. Variation in polyunsaturated fatty acids composition of Persian walnuts. J Am Soc Hort Sci 1992;117:518–22.
17. Willett WC, Sacks F, Trichopolou A, et al. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 1995; 61(suppl):1402S–6S.
18. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985;26:194–202.
19. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel II). JAMA 1993;269:3015–23.
20. Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. Am J Clin Nutr 1992; 56:320–8.
21. Kasim-Karakas SE. Impact of n−3 fatty acids on lipoprotein metabolism. Curr Opin Lipidol 1995;6:167–71.
22. Kasim-Karakas SE, Herrmann R, Almario R. Effects of omega−3 fatty acids on intravascular lipolysis of very-low density lipoproteins in humans. Metabolism 1995;44:1223–30.
23. Harris WS. n−3 Fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65(suppl):1645S–54S.
24. Connor SL, Connor WE. Are fish oils beneficial in the prevention and treatment of coronary artery disease? Am J Clin Nutr 1997; 66:1020S–31S.
25. Simopoulos AP. Health effects of ω−3 polyunsaturated fatty acids in seafoods. In: Simopoulos AP, Kifer RR, Martin RE, Barlow SM, eds. World review of nutrition and dietetics. Vol 66. Basel, Switzerland: Karger, 1991:1–592.
26. Kasim SE, Stern B, Khilnani S, McLin P, Baciorowski S, Jen KL. Effects of omega−3 fish oils on lipid metabolism, glycemic control, and blood pressure in type II diabetic patients. J Clin Endocrinol Metab 1988;67:1–5.
27. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, et al. Body weight and low-density lipoprotein cholesterol changes after consumption of a low-fat ad libitum diet. JAMA 1995;274:1450–5.
28. Abbey M, Noakes M, Nestel PJ. Dietary supplementation with orange and carrot juice in cigarette smokers lowers oxidation products in copper-oxidized low-density lipoproteins. J Am Diet Assoc 1995;95:671–5.
29. Warnick GR, Benderson JM, Albers JJ. Quantitation of high density lipoprotein subclasses after separation by dextransulfate and Mg^{2+} precipitation. Clin Chem 1972;18:499–502.
30. Otvos JD. Measurement of lipoprotein subclass profile by nuclear magnetic resonance. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington, DC: AACC Press, 1997:497–508.
31. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 1992;9:1632–8.
32. SAS Institute Inc. SAS/STAT user's guide, version 6, 4 edition. Cary, NC: SAS Institute, Inc, 1989.
33. Neter J, Wasserman M, Kutner MH. Applied linear statistical models. 2nd ed. Homewood, IL: Richard D Irwin Inc, 1985.
34. Hill JO, Peters JC, Lin D, Yakubu F, Greene H, Swift L. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. Int J Obes Relat Metab Disord 1993;17:223–6.
35. Kasim-Karakas SE, Almario RU, Mueller WM, Peerson J. Changes in plasma lipoproteins during low-fat, high-carbohydrate diets: effects of energy intake. Am J Clin Nutr 2000;71:1439–47.
36. Mertz W, Tsui JC, Judd JT, et al. What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. Am J Clin Nutr 1991;54:291–5.
37. Mantzioris E, James MJ, Gibson RA, Cleland LG. Dietary substitution with an α-linolenic acid–rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. Am J Clin Nutr 1994; 59:1304–9.
38. Kris-Etherton PM, Pearson TA, Wan Y, et al. High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr 1999;70:1009–15.
39. Kasim-Karakas SE, Lane E, Almario R, Mueller W, Walzem R. Effects of dietary fat restriction on particle sizes of plasma lipoproteins in postmenopausal women. Metabolism 1997;46:431–5.
40. Lichtenstein AH, Ausman LM, Carrasco W, Jenner JL, Ordovas JM, Schaefer EJ. Short-term consumption of a low-fat diet beneficially affects plasma lipid concentrations only when accompanied by weight loss. Arterioscler Thromb 1994;14:1751–60.
41. Anderson JW, Ward K. Hypolipidemic effects of high-carbohydrate, high-fiber diets. Metabolism 1980;29:551–8.
42. Ullmann D, Connor WE, Hatcher LF, et al. Will a high-carbohydrate, low-fat diet lower plasma lipids and lipoproteins without producing hypertriglyceridemia? Arterioscler Thromb 1991;11:1059–67.
43. Zambon D, Sabate J, Munoz S, et al. Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women. A randomized crossover trial. Ann Intern Med 2000;132:538–46.
44. Couture P, Otvos JD, Cupples LA, Wilson PWF, Schaefer EJ, Ordovas JM. Association of the A-204C polymorphism in the cholesterol 7α-hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study. J Lipid Res 1999;40:1883–8.
45. Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J Clin Invest 1990;85:144–51.
46. Hayek T, Ito Y, Azrolan N, et al. Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein A1. J Clin Invest 1993;91:1665–71.
47. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14: 173–94.
48. Berglund L, Oliver EH, Fontanez N, et al. HDL-subpopulation patterns in response to reductions in dietary total and saturated fat intakes in healthy subjects. Am J Clin Nutr 1999;70:992–1000.
49. Williams PT, Krauss RM. Low-fat diets, lipoprotein subclasses, and heart disease risk. Am J Clin Nutr 1999;70:949–50.
50. Eckel RH. Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic disease. N Engl J Med 1989;320:1060–8.
51. Harris WS, Lu G, Rambjor GS, et al. Influence of n−3 fatty acid supplementation on the endogenous activities of plasma lipases. Am J Clin Nutr 1997;66:254–60.
52. Kasim SE, Martino S, Kim PN, et al. Dietary and anthropometric determinants of plasma lipoproteins during a long-term low-fat diet in healthy women. Am J Clin Nutr 1993;57:146–53.
53. Parks J, Thuren T, Schmitt J. Inhibition of lecithin:cholesterol acyltransferase activity by synthetic phosphatidylcholine species containing eicosapentaenoic acid or docosahexaenoic acid in the *sn*-2 position. J Lipid Res 1992;33:879–87.
54. Bagdade J, Ritter M, Davidson M, Subbaiah P. Effect of marine lipids on cholesterol ester transfer and lipoprotein composition in patients with hypercholesterolemia. Arterioscler Thromb 1992;12:1146–52.
55. Kasim-Karakas SE. Omega-3 fish oils and insulin resistance. In: Wildman REC, ed. Handbook of nutraceuticals and functional foods. Boca Raton, FL: CRC Press, 2000:345–52.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016

56. Sirtori CR, Paoletti R, Mancini M, et al. n−3 Fatty acids do not lead to an increased diabetic risk in patients with hyperlipidemia and abnormal glucose tolerance. Italian Fish Oil Multicenter Study. Am J Clin Nutr 1997;65:1874–81.

57. Uusitupa M, Schwab U, Makimattila S, et al. Effects of two high-fat diets with different fatty acid compositions on glucose and lipid metabolism in healthy young women. Am J Clin Nutr 1994;59: 1310–6.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 13, 2016