

Including Walnuts in a Low-Fat/Modified-Fat Diet Improves HDL Cholesterol-to-Total Cholesterol Ratios in Patients With Type 2 Diabetes

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OBJECTIVE — The aim of this study was to examine the effect of a moderate-fat diet inclusive of walnuts on blood lipid profiles in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — This was a parallel randomized controlled trial comparing three dietary advice groups each with 30% energy as fat: low fat, modified low fat, and modified low fat inclusive of 30 g of walnuts per day. Fifty-eight men and women, mean age 59.3 ± 8.1 years, started the trial. Dietary advice was given at baseline with monthly follow-up and fortnightly phone calls for support. Body weight, percent body fat, blood lipids, HbA_{1c}, total antioxidant capacity, and erythrocyte fatty acid levels were measured at 0, 3, and 6 months. Data were assessed by repeated-measures ANOVA with an intention-to-treat model.

RESULTS — The walnut group achieved a significantly greater increase in HDL cholesterol-to-total cholesterol ratio ($P = 0.049$) and HDL ($P = 0.046$) than the two other treatment groups. A 10% reduction in LDL cholesterol was also achieved in the walnut group, reflecting a significant effect by group ($P = 0.032$) and time ($P = 0.036$). There were no significant differences between groups for changes in body weight, percent body fat, total antioxidant capacity, or HbA_{1c} levels. The higher dietary polyunsaturated fat-to-saturated fat ratio and intakes of ω -3 fatty acids in the walnut group were confirmed by erythrocyte biomarkers of dietary intake.

CONCLUSIONS — Structured “whole of diet” advice that included 30 g of walnuts/day delivering substantial amounts of polyunsaturated fatty acid improved the lipid profile of patients with type 2 diabetes.

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Walnuts are distinguished from other nuts by virtue of their higher polyunsaturated fat content (and importantly their α -linolenic acid [ALA] content) combined with anti-

oxidants in the form of γ -tocopherol. There are mechanistic explanations for the influence of dietary polyunsaturated fatty acid (PUFA) on insulin action and energy metabolism (1,2), and cohort

studies of women in the U.S. have demonstrated a reduced risk of developing type 2 diabetes with dietary PUFA replacing *trans* or saturated fatty acids (SFAs) (3,4). Intervention trials have demonstrated the benefits of replacing dietary SFAs with monounsaturated fats (MUFAs) (5–7), but the effects of dietary PUFA have been less well studied in people with diabetes (8).

The literature on recommendations varies to a minor degree, and cultural differences may have an influence (9), but a total fat level of $\leq 30\%$ energy (10) and $< 10\%$ saturated fat is reasonably well accepted (11,12). There is some concern that PUFAs are more susceptible to oxidation and therefore may be more atherogenic (11), so a level of $< 10\%$ energy for PUFAs is seen with greater flexibility in the proportions of carbohydrate and MUFA (13). Within the PUFA fraction of the diet, the ratio of ω -6 to ω -3 fatty acids is also considered (9) with daily recommendations for a 2,000-kcal diet of 0.65 g for the long-chain ω -3s (EPA + DHA) and 2.22 g for ALA (14) and the ω -6-to- ω -3 ratio reduced to substantially < 10 (14a).

Fish are the major source of EPA + DHA, but fish oil supplements produce a decreased oxidative stability of plasma LDL (15,16). In contrast, consumption of fish itself may be protective against type 2 diabetes (17). Nuts provide substantial amounts of dietary PUFA, and nut consumption is inversely associated with the risk of type 2 diabetes in women (18). There is no net effect on glucose homeostasis from nut supplementation, but more interestingly, no net weight gain when nuts were used as a replacement food (19). In subjects with dyslipidemia (total cholesterol > 5.17 ; LDL > 3.36 ; triglycerides > 2.26 mmol/l), a low-fat diet supplemented with walnuts was found to reduce total cholesterol compared with a habitual or low-fat diet, and LDL compared with a low-fat diet alone (20), with no differential effects on tri-

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Abbreviations: ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fat; P:S ratio, polyunsaturated fat-to-saturated fat ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—Change in weight, body fat, HbA_{1c}, lipids, and TAOS over time

Variable	Control			Modified fat		
	0 months	3 months	6 months	0 months	3 months	6 months
n (women/men)	8/13			10/10		
Age (years)	60.48 ± 8.16			59.30 ± 7.11		
Weight (kg)*	81.87 ± 11.19	82.07 ± 11.46	82.27 ± 1.67	84.55 ± 4.31	84.70 ± 14.00	84.36 ± 14.07
BMI (kg/m ²)	29.22 ± 2.60	29.34 ± 2.72	29.42 ± 2.80	30.16 ± 4.51	30.12 ± 4.33	30.05 ± 4.23
Percent body fat*	31.23 ± 8.05	32.11 ± 8.41	32.39 ± 8.21	35.13 ± 10.16	35.54 ± 0.09	35.51 ± 9.95
HbA _{1c} (%)	6.56 ± 0.80	6.40 ± 0.85	6.75 ± 0.88	6.82 ± 0.88	6.77 ± 0.86	6.97 ± 0.95
Total cholesterol (mmol/l)	4.79 ± 0.82†	4.71 ± 1.06	4.90 ± 1.08	4.58 ± 0.88	4.58 ± 0.81	4.83 ± 0.99
LDL (mmol/l)	2.70 ± 1.56	2.68 ± 1.76	2.69 ± 1.49	2.58 ± 1.30	2.59 ± 1.29	2.73 ± 1.20
HDL (mmol/l)	1.11 ± 0.22	1.19 ± 0.24	1.25 ± 0.27	1.11 ± 0.24	1.24 ± 0.20	1.34 ± 0.21
HDL cholesterol-to-total cholesterol ratio	0.24 ± 0.06	0.26 ± 0.08	0.26 ± 0.06	0.25 ± 0.07	0.27 ± 0.06	0.29 ± 0.07
Triglycerides (mmol/l)	2.18 ± 0.82	1.85 ± 0.80	2.13 ± 0.71	1.76 ± 0.82	1.65 ± 0.80	1.55 ± 0.73
Total antioxidant status (mmol/l)	1.08 ± 0.25	1.21 ± 0.24	1.14 ± 0.18	1.08 ± 0.14	1.18 ± 0.172	1.13 ± 0.18

Data are means ± SD. *Baseline and repeated measures analysis adjusted for sex; †significantly different at baseline.

glyceride levels. In a summary of walnut studies, Feldman (2002) (21) found that there was evidence of decreased total cholesterol and LDL cholesterol in diets of at-risk subjects supplemented with two to three servings of walnuts per day, with no net gain in body weight. However, most studies considered in that review had been conducted over a limited period of time and were not “real world” in context, and “the effect of walnut ingestion at a practical level, i.e., one serving a day, has not been evaluated.” The aim of the study reported here was to examine the effect on blood lipid profiles of including 30 g of walnuts/day in a modified-fat diet plan compared with the provision of generic low-fat or low-fat/modified-fat dietary advice.

RESEARCH DESIGN AND METHODS

A parallel randomized controlled trial was established to compare the achievement of dietary targets by three different approaches to dietary advice targeting <30% energy as fat: usual practice (low fat, control), low fat/modified fat (using exchange lists inclusive of fatty acid considerations), and walnut inclusive (low-fat/modified-fat approach including 30 g of walnuts supplied per day). All subjects were recruited through advertisements in the local media and e-mail networks (University and Technical College), from a news clip included on local television news, or via letters to general medical practitioners through the Illawarra Division of General Practice in the Wollongong region (a ma-

jeor coastal city 50 miles south of Sydney, Australia). To be included, subjects had to be aged 35–75 years, diagnosed with type 2 diabetes for at least 1 year, and generally well. Exclusion criteria were insulin therapy (or with HbA_{1c} >9%), BMI >35 kg/m² with major debilitating illness, known food allergies or food habits inhibiting the study, illiteracy, and inadequate conversational English. After an introductory session at the university, baseline measurements of clinical and dietary data were undertaken, and subjects were randomized into one of the three treatment groups. Subjects then were advised to attend one of two sites, the University Clinic or the Diabetes Centre, to avoid possible interaction between groups. Two research dietitians undertook all dietary assessments, all at the University Clinic at 0, 3, and 6 months. Another three experienced dietitians provided advice only; two were randomly allocated to see subjects on a monthly basis at the University Clinic (walnut-inclusive group) and the Diabetes Centre (low-modified-fat group), whereas the third dietitian, who was blinded to the intervention diets, provided standard clinical practice with follow-up advice based on clinical judgment at the Diabetes Centre (control group). One of the dietitians providing advice on the intervention diets contacted these subjects by phone at the 2-week interval between appointments to provide additional support. Each group was given advice on the number of servings of carbohydrate-rich foods, the type of protein-rich foods (low fat) and oils/spreads

(MUFA or PUFA rich), and to include two fish meals per week. In addition to this advice, the modified low-fat and walnut groups were advised on the number of servings of these protein-rich foods and oils/spreads, using an exchange list, and the walnut group was given 30 g of walnuts per day (included as one exchange). Ethics approval to conduct the study was received from the University of Wollongong Human Research Ethics Committee.

Clinical assessments

Dietary data were assessed by a validated diet history method (22,23) and a 3-day food record at each time point (0, 3, and 6 months). The meal-based diet history interview noted the types, amounts, and frequency of consumption of all foods consumed routinely within a 3-month reference time. Dietary data were entered into the Foodworks nutrient analysis software program (version 3, 2002; Xyris Software, Brisbane, Australia) using the Australian nutrient database AUSNUT and the fatty acid database Australian Fatty Acids Rev six (2002; RMIT, Melbourne, Australia). Nutrient intake data were assessed according to achievement of dietary targets, which were defined by authoritative guidelines (8). A number of approaches to dietary validation and modeling were used in this study and are described in detail elsewhere (22 and L.J.G., L.C.T., C.S.P., A.O., M.B., unpublished observations). As changes in fatty acid intakes were the primary outcomes of the study, changes in biomarkers of

Table 1—Continued

Walnut			P		
0 months	3 months	6 months	Time	Group	Time × group
6/11					
57.71 ± 8.97					
87.61 ± 12.83	87.05 ± 13.06	86.33 ± 13.07	0.570	0.493	0.248
30.72 ± 3.85	30.51 ± 4.33	30.26 ± 3.84	0.264	0.599	0.229
34.48 ± 9.12	33.72 ± 8.56	34.00 ± 8.97	0.374	0.379	0.057
6.94 ± 1.22	6.58 ± 0.92	6.89 ± 0.82	0.000	0.489	0.380
4.11 ± 0.81†	3.94 ± 0.70	4.02 ± 0.77	0.037	0.021	0.434
2.17 ± 1.31	2.01 ± 1.06	1.95 ± 0.75	0.634	0.032	0.316
1.10 ± 0.24	1.16 ± 0.24	1.30 ± 0.62	0.000	0.766	0.046
0.27 ± 0.08	0.30 ± 0.08	0.33 ± 0.10	0.000	0.109	0.049
1.90 ± 0.74	1.72 ± 0.60	1.70 ± 0.68	0.006	0.208	0.174
1.14 ± 0.17	1.23 ± 0.23	1.21 ± 0.14	0.000	0.341	0.975

PUFA intake (erythrocyte fatty acids) were assessed (25,26).

Body weight and percent body fat were assessed using bioelectrical impedance scales (Tanita TBF-622 foot-to-foot analyzers), applicable to standard clinical practice and found to compare reasonably well with dual X-ray absorptiometry as a reference technique (27).

Trained venipuncturists drew blood samples and sent them to a quality-assured pathology laboratory (Southern IML Pathology). Total plasma antioxidant capacity was measured spectrophotometrically using the Randox Total Antioxidant Status assay kit (Randox Laboratories, Crumlin, U.K.) and erythrocyte fatty acid composition determined by gas chromatography. Erythrocyte membrane lipids were isolated by ultracentrifugation (28) and then the fatty acids derivatized using a direct transesterification method (29). The resulting fatty acid methyl esters were analyzed by gas chromatography using a Shimadzu GC-17A equipped with a 30-m × 0.25-mm capillary column (FAMEWAX, Restek) with hydrogen as a carrier gas. Fatty acid identification was based on the retention time of authentic fatty acid methyl ester standards (Sigma-Aldrich, Castle Hill, Australia).

Statistical analysis

All data were analyzed using SPSS (version 11.0.0; SPSS, Chicago, IL) and STATA (version 7; STATA, College Station, TX). For baseline subject characteristics, non-Gaussian data were log transformed before analysis. Data were presented as means and SDs before trans-

formation to assist with interpretation. Differences between groups were assessed using one-way ANOVA with post hoc analysis performed using Tukey's test. Nonparametric analysis was conducted using the Kruskal-Wallis test. Changes in clinical outcomes were analyzed with an intention-to-treat model using repeated-measures ANOVA. For baseline, dietary data assumptions of normality were made, and differences between groups were assessed by ANOVA. Effects of treatment on dietary intake over time were assessed by repeated-measures ANOVA based on data from those completing the trial. Spearman's correlation coefficient was determined to assess the relationship between reported changes in dietary PUFA and erythrocyte cell membrane levels in subjects.

RESULTS— After advertising, 101 subjects volunteered for the study, and 58 of those meeting the inclusion criteria attended the information session, producing a group randomization of 21:20:17. One subject dropped out from each group during the study. There were no significant differences between groups for clinical characteristics except total cholesterol at baseline (Table 1). There was no significant difference in the proportion of men and women randomized to each group. Percent body fat was significantly greater in women (41.75 ± 4.78 vs. 27.73 ± 6.69 , $P = 0.00$), but after adjusting for sex, there was no significant difference between groups for percentage of body fat (ANCOVA, $P = 0.165$).

Anthropometry

Throughout the trial, and after adjustments for sex, there were no significant changes in body weight for all study groups (Table 1). There was a trend toward a significant interaction in body fat percentage ($P = 0.057$). Post hoc follow-up indicated a significant increase in body fat in the control group only, but there were no other significant changes over time. At 3 months, there was no significant overall change in body fat over time, but the change in body fat in the walnut group was significantly different compared with the control group ($P < 0.04$), remaining below the baseline measurement.

Lipids

The total cholesterol levels of the walnut group remained lower than the other two groups at each time point ($P = 0.021$) (Table 2). There was also a significant time effect for changes in this variable ($P = 0.037$), but univariate analysis failed to show a significant increase in any individual group. In contrast, although LDL cholesterol levels of the walnut group were not significantly lower than the other two groups at baseline, the continued lower levels produced a significant group effect in the trial ($P = 0.032$), and in univariate analysis the walnut group LDL levels decreased significantly over time ($P = 0.036$), with no change observed in the other two groups (Fig. 1).

HDL cholesterol levels increased significantly in all three groups, producing a time effect ($P < 0.001$). Post hoc analysis indicated a significant increase in each arm ($P < 0.001$), but the walnut group increased at a greater rate in the second 3 months, noting the significant time-by-group effect ($P = 0.046$) (Table 2). There was a significant time-by-group effect for HDL cholesterol-to-total cholesterol ratios ($P = 0.049$). Univariate analysis found significant effects for the control ($P = 0.014$), modified fat ($P = 0.09$), and walnut group ($P = 0.000$), but post hoc analysis of the interaction showed that the control group results were only significant for baseline to 3 months ($P = 0.012$) and not for 3–6 months ($P = 0.971$). Changes in HDL cholesterol-to-total cholesterol ratio for the modified-fat groups were significant for the first 3 months ($P = 0.006$) and in the second 3 months ($P = 0.048$), but this effect was much stronger in the walnut group, par-

ticularly in the second 3 months ($P = 0.000$ and 0.009 , respectively).

Mean triglyceride levels dropped in the first 3 months, which was a trend that continued for all but the control group, in which they rose again in the second 3 months. This produced a significant time effect ($P = 0.006$), but the degree of variation in the data meant that no significant differences were found between groups in these changes. The levels were not high, with mean values ranging from 1.55 to 2.18 mmol/l.

Glycemic control

HbA_{1c} levels decreased at 3 months then increased, leaving a time effect for overall increase in HbA_{1c} ($P < 0.001$). The level, however, remained at or below 7% for each group (Table 1). Total antioxidant status increased significantly in all groups during the intervention ($P = 0.000$), with no significant difference between groups.

Dietary intake

At baseline, there were no significant differences between groups for energy and macronutrient intakes. Except for changes in fatty acids, reported intakes for macronutrients remained constant throughout the study in all groups, with no time or group differences in energy intake, the average percentage of energy from protein and total fat remaining on target and, in the case of carbohydrate, slightly below target (Table 2).

Significant differences emerged

among groups at 3 months in reported PUFA and ω -3 fatty acid intakes ($P < 0.001$) and in the polyunsaturated fat-to-saturated fat ratio (P:S ratio) of the overall diet ($P < 0.05$). There was a significant interaction effect for SFA intake ($P = 0.005$), PUFA intake, P:S ratio, ALA intake ($P < 0.000$), eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) ($P = 0.006$), and ω -6-to- ω -3 ratio ($P = 0.028$) (Table 2). The walnut group consumed more PUFA, had a higher dietary P:S ratio, and reported significantly higher ALA intakes ($P < 0.001$)—approximately double that of the other two groups—and had the lowest mean ω -6-to- ω -3 ratio for the diet.

Reported energy intakes from the diet history were within $\sim 10\%$ of estimated requirements at all times. Dietary intake of very-long-chain ω -3 PUFA (DHA + EPA) was strongly related to the levels seen in erythrocyte membranes at baseline, 3 months, and 6 months ($P < 0.01$). At the completion of the intervention, significant correlations between reported intakes and corresponding erythrocyte levels were also seen for the ω -6-to- ω -3 ratio ($P < 0.05$) and total ω -3 fatty acids ($P < 0.02$). These compared with food intakes where the entire walnut group reported consuming 30 g of walnuts per day (31% of total fat intake and 50% of ω -3 PUFA). Analysis of the food intake pattern indicated that the walnut group consumed 350 g of fish per week (6% total fat

and 17% ω -3 PUFA), compared with 500 g of fish in the modified low-fat group (8% total fat and 42% ω -3 PUFA).

CONCLUSIONS— This study has demonstrated the novel finding of a significant increase in HDL cholesterol-to-total cholesterol ratio in patients with diabetes from manipulating dietary PUFA. This was achieved through the provision of “whole of diet” advice inclusive of 30 g of walnuts per day in addition to the recommendation for fish consumption received by all groups. The Strong Heart Study has found the ratio of total and HDL cholesterol to be a good predictor of cardiovascular risk in both men and women with type 2 diabetes, although it was a stronger predictor in men (30).

The finding contrasts with other observations involving nuts and coronary heart disease risk, showing a reduction in HDL cholesterol levels (31–34) because most of those studies used a supplemental rather than whole-diet approach. One found an increase in HDL (7.9% compared with our 18%) (35), whereas those using fish or fish oils found no changes in total HDL cholesterol but divergent changes within HDL subclasses (36,37). Similarly there is little evidence in the literature to suggest that ALA may be responsible for the increase in HDL cholesterol (35). Bearing in mind that new recommendations target lower levels of total cholesterol for diabetes manage-

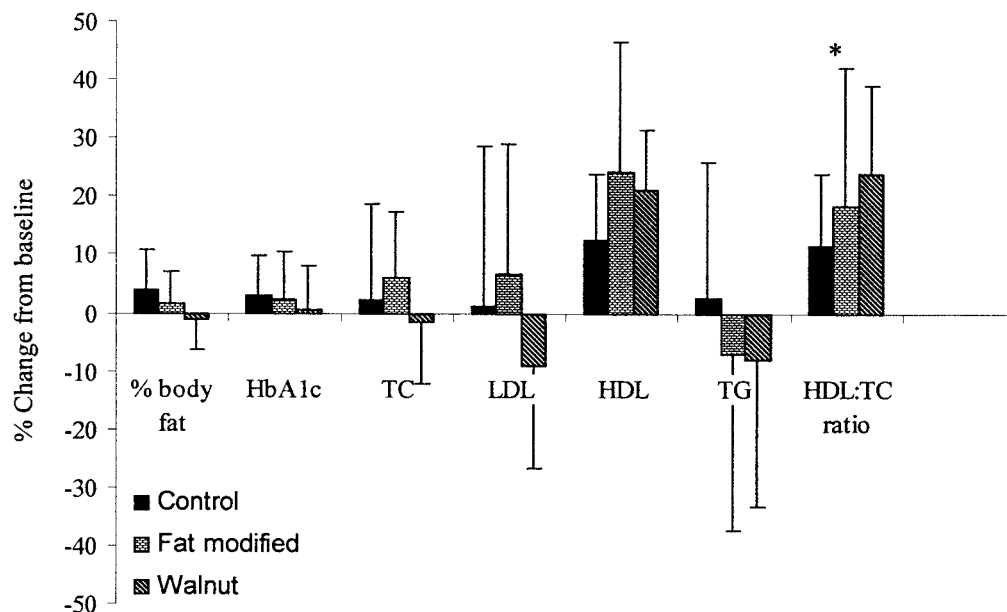


Figure 1—Percent change at 6 months from baseline of selected indicators. Data are means \pm SD. $P < 0.05$, difference between groups over time.

ment (38), our finding of a significant time ($P = 0.037$) but not interaction effect for total cholesterol changes may be explained by the concomitant significant rise in HDL levels by all groups. Furthermore, although a significant difference in total cholesterol could not be detected, the improvement in HDL total cholesterol and in HDL cholesterol-to-total cholesterol ratio suggests that the intervention provided benefits in a desirable direction. This observation was enhanced through univariate analysis showing a significant decrease in LDL cholesterol levels observed in the walnut group ($P = 0.036$), with no changes in the other groups and a significant group effect ($P = 0.032$).

The metabolic syndrome is characterized by low HDL levels and high triglycerides (39). Our success in increasing HDL levels may be attributed to the relative fatty acid composition of the diets (38,40) and the lack of detectable differences in triglyceride levels with carbohydrate intakes (38) sustained at 43% energy. However, PUFA intakes of the two modified-fat groups were both much higher than the control group, and pooling the data from the former in a post hoc analysis produced a significant interaction effect for triglycerides ($P = 0.045$), with the modified low-fat groups demonstrating reduced levels through the intervention (Table 1). The differences in lipids may also be due to reduced SFA intake or the food matrix provided by walnuts, which is an area for further research.

The trend toward a difference in changes in body fat between the control and walnut group was intriguing. The literature contains early suggestions that diets lower in saturated fats and higher in unsaturated fat may have a beneficial effect on body fat distribution and fat mass (41,42), possibly due to differences in fatty acid metabolism. Animal studies have shown that PUFA may protect against the development of obesity through regulatory mechanisms operating at the level of the hypothalamus (43), but more work needs to be done to explore this issue further. The stronger trend toward loss of body fat by the walnut group in the first 3 months compared with the second is also interesting, possibly reflecting an adaptive phenomenon or greater variation in the diet in the second phase. From a practice perspective, this study suggests that body fat may be a use-

Table 2—Energy and macronutrient intakes of all groups at the 0-, 3-, and 6-month interventions

Variable	Target*	Control			Modified fat			Walnut		
		0 months	3 months	6 months	0 months	3 months	6 months	0 months	3 months	6 months
n	2,000	21	20	20	20	19	19	17	17	16
Energy (kcal)	2,033.77 ± 68.06	2,128.22 ± 04.84	2,145.95 ± 431.8	2,090.54 ± 504.09	2,015.56 ± 541.72	1,977.76 ± 500.14	2,074.69 ± 664.60	2,139.94 ± 574.54	2,006.59 ± 376.19	43.5 ± 3.8
Carbohydrates (% energy)	50	46.4 ± 6.6	45.2 ± 7.2	43.1 ± 8.1	44.1 ± 7.7	42.2 ± 6.4	41.4 ± 6.0	46.9 ± 6.5	43.9 ± 5.7	43.5 ± 3.8
Protein (% energy)	20	20.8 ± 2.8	21.0 ± 2.0	20.7 ± 2.7	20.6 ± 4.5	22.5 ± 3.5	22.6 ± 3.6	21.9 ± 3.4	20.9 ± 3.4	21.5 ± 2.5
Fat (% energy)	30	28.5 ± 6.4	29.4 ± 6.9	32.6 ± 8.5	31.2 ± 7.1	31.7 ± 5.9	32.7 ± 6.1	27.7 ± 7.2	32.2 ± 5.2	31.8 ± 4.1
SFA (% energy)	<10%	8.9 ± 2.5	8.6 ± 2.8	10.2 ± 4.1†	9.7 ± 3.1	7.9 ± 2.4	7.7 ± 2.3†	9.0 ± 2.8	7.8 ± 2.4	6.9 ± 1.6††
PUFA (% energy)	~7	5.3 ± 1.3	5.9 ± 1.3†	5.8 ± 1.8†	6.3 ± 2.8	8.0 ± 2.0†	9.4 ± 2.8†	5.4 ± 1.5	11.7 ± 1.6††	11.7 ± 1.8††
P:S ratio	1	0.6 ± 0.2	0.7 ± 0.3	0.6 ± 0.3	0.8 ± 0.6	1.1 ± 0.5	1.3 ± 0.5	0.7 ± 0.4	1.7 ± 0.8	1.8 ± 0.5
ALA (g)	2.22	1.3 ± 0.7	1.5 ± 1.1†	1.4 ± 0.7†	1.4 ± 1.0	2.0 ± 1.1†	1.7 ± 1.4†	1.4 ± 1.0	3.4 ± 1.7†	3.6 ± 1.9†
EPA + DHA (g)	0.65	0.5 ± 0.3	0.5 ± 0.2†	0.4 ± 0.2†	0.6 ± 0.5	1.2 ± 0.8†	1.2 ± 1.1†	0.4 ± 0.4	0.7 ± 0.4†	0.8 ± 0.6†
ω-6-to-ω-3 ratio	<10	6.6 ± 2.9	8.2 ± 5.7§	7.5 ± 3.6	8.2 ± 8.3	5.3 ± 2.4§	8.3 ± 5.0	8.6 ± 8.4	5.7 ± 1.4§	5.7 ± 1.4

Data are means ± SD. *Recommendations from the American Diabetes Association, the American Heart Association, the International Society for the Study of Fatty Acids and Lipids, and the literature (14a); †significantly different between groups at the given time point (one-way ANOVA, $P < 0.01$); ‡significantly different between groups at the given time point (one-way ANOVA, $P < 0.001$); §significantly different between groups at the given time point (one-way ANOVA, $P < 0.05$); ||significant effect due to treatment over time (repeated-measures ANOVA, control versus walnut group, $P < 0.01$); ††significant effect due to treatment over time (repeated-measures ANOVA, control versus walnut group, $P < 0.01$).

ful variable to assess consistently in the clinical context.

Diabetes control remained good, with HbA_{1c} $\sim 6.7\%$, which is well below the point at which insulin therapy may be instigated ($\sim 9\%$) and bearing in mind that nut supplementation is not likely to effect glucose homeostasis (19). The increase in dietary PUFA, however, has been a concern for antioxidant status in diabetes (12), but blood levels of antioxidants in people with diabetes appear related to serum lipids, not dietary intake, with products of oxidative damage more linked to intakes of SFA and cholesterol and to levels of endogenous antioxidants (44). In the case of large doses of fish oil supplements, a decreased oxidative stability of plasma and LDL has been demonstrated in healthy subjects, but this was offset by including five portions of fruit and vegetables in the daily diet (45). In our study, SFA intakes were kept low, and five portions of fruits and vegetables were included in the dietary advice.

Study limitations include open recruitment, where only $\sim 50\%$ of volunteers were able to participate, and this limits the generalizability of results; but the care taken in study design to accommodate research needs and clinical relevance may help in translating the results to practice (46). By the end of the trial, one subject was lost to each group, but the number was small and therefore unlikely to affect results. The lower cholesterol levels for the walnut group—an outcome of randomization—created bias that would have acted against our demonstrating the effect, but this proved not to be the case. The quality of the dietary data were favorable, which was an aspect reported in detail elsewhere (24). Changes in consumption of fatty acids over time were validated by biomarker analyses, and the clinical results were reasonably consistent with existing knowledge of dietary fat and metabolic syndrome (47). Thus, we can argue with good reason and confidence from our study that a whole of diet approach inclusive of specific advice on walnuts in addition to fish can help to improve the lipid profile of patients with type 2 diabetes.

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