Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors[1–3]

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ABSTRACT

Background: Regular exercise and consuming long-chain n−3 fatty acids (FAs) from fish or fish oil can independently improve cardiovascular and metabolic health, but combining these lifestyle modifications may be more effective than either treatment alone.

Objective: We examined the individual and combined effects of n−3 FA supplements and regular exercise on body composition and cardiovascular health.

Design: Overweight volunteers [body mass index (BMI; in kg/m^2): >25] with high blood pressure, cholesterol, or triacylglycerols were randomly assigned to one of the following interventions: fish oil (FO), FO and exercise (FOX), sunflower oil (SO; control), or SO and exercise (SOX). Subjects consumed 6 g tuna FO/d (≈1.9 g n−3 FA) or 6 g SO/d. The exercise groups walked 3 d/wk for 45 min at 75% age-predicted maximal heart rate. Plasma lipids, blood pressure, and arterial function were assessed at 0, 6, and 12 wk. Body composition was assessed by dual-energy X-ray absorptiometry at 0 and 12 wk only.

Results: FO supplementation lowered triacylglycerols, increased HDL cholesterol, and improved endothelium-dependent arterial vasodilation ($P < 0.05$). Exercise improved arterial compliance ($P < 0.05$). Both fish oil and exercise independently reduced body fat ($P < 0.05$).

Conclusions: FO supplements and regular exercise both reduce body fat and improve cardiovascular and metabolic health. Increasing intake of n−3 FAs could be a useful adjunct to exercise programs aimed at improving body composition and decreasing cardiovascular disease risk. *Am J Clin Nutr* 2007;85:1267–74.

KEY WORDS n−3 Fatty acids, body fat, flow-mediated dilatation, lipids, dual-energy X-ray absorptiometry, DXA

INTRODUCTION

Obesity is a risk factor for cardiovascular disease (CVD), and its prevalence is increasing in Western society (1, 2). Moreover, obesity clusters with several other risk factors for CVD and diabetes in what is known as the metabolic syndrome (3). Hence, interventions for obesity should target multiple cardiovascular and metabolic risk factors.

Evidence from several studies supports an inverse relation between the intake of n−3 fatty acids (FAs) from fish or fish oil and the rate of mortality from coronary artery disease (4). Furthermore, regular consumption of n−3 FAs, particularly docosahexaenoic acid (DHA), has the capacity to ameliorate several cardiovascular risk factors, including elevated blood pressure and triacylglycerols, platelet aggregation, endothelial dysfunction, and arrhythmia (5). Evidence also suggests that n−3 FAs may have a favorable effect on metabolism by modulating gene expression (6). Indeed, animal studies have found that fat mass deposition is reduced after feeding with n−3 FAs (7, 8). In human studies, however, the evidence is less clear: some (9, 10) but not all studies indicate that n−3 FAs can reduce body fat with (11) or without (12) concomitant dietary restriction.

Physical activity is often recommended for weight loss, although most studies find that physical activity alone produces relatively small changes in body weight (13–15). The extent of weight loss that can be achieved through exercise may be small, but it is clear that physical activity plays a key role in preventing weight gain; however, as much as 60–90 min/d of moderate-intensity physical activity can be required to maintain body weight (16). In addition, much evidence suggests that exercise with or without weight loss may independently improve several risk factors for CVD, including lowering blood pressure, favorably altering blood lipid profiles (17), and improving blood vessel function (18).

Although several studies have investigated the potential for regular aerobic exercise to independently improve body composition and CVD and metabolic risk factors, few properly controlled studies have investigated the effect of n−3 FA supplementation on these risk factors, particularly body composition. Only 2 studies have previously investigated these 2 interventions in combination (19, 20), and those study designs were such that it could not be determined whether this combined intervention

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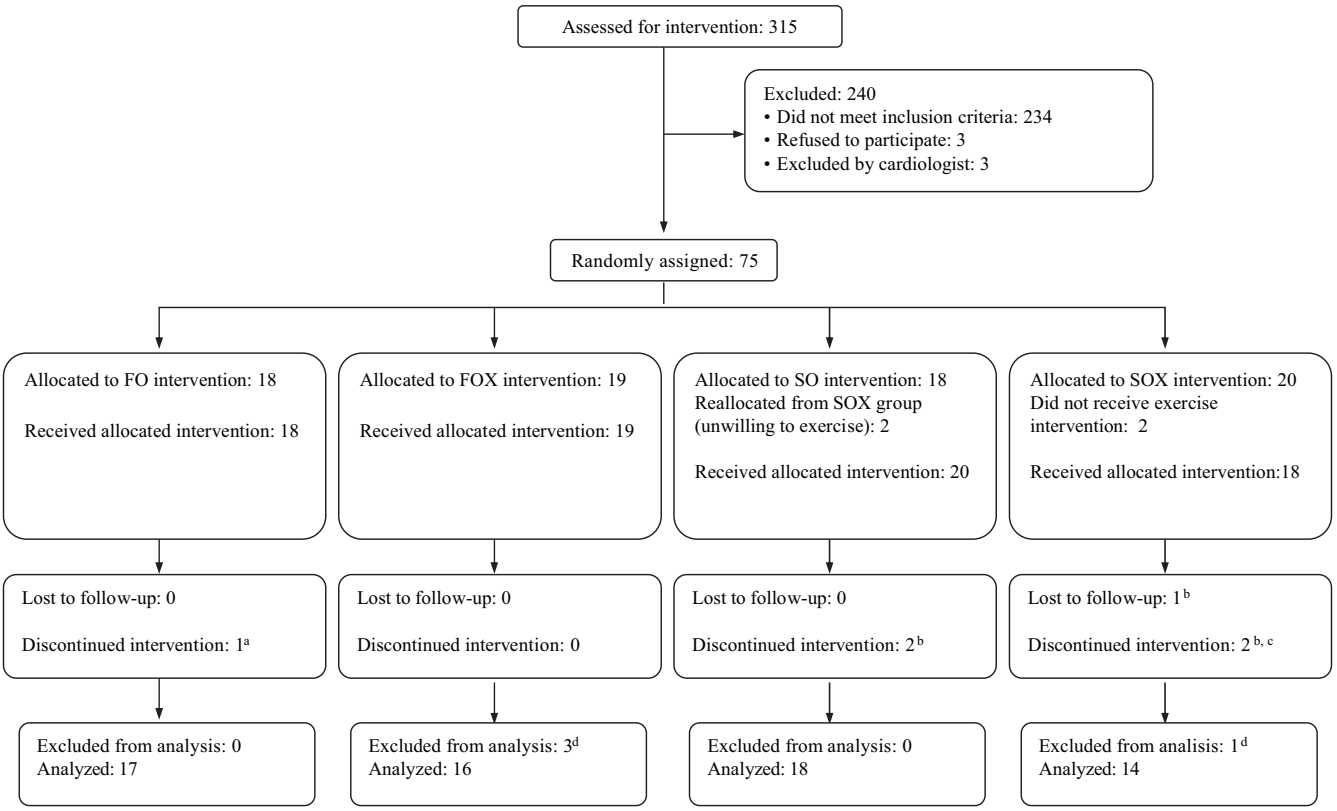

FIGURE 1. Flow chart showing numbers of subjects recruited and their attrition patterns during the study. FO, fish oil; FOX, FO and exercise; SO, sunflower oil; SOX, SO and exercise. Reasons for subject withdrawal were as follows: [a]did not take required number of capsules; [b]change in work or family circumstances; [c]could not meet exercise intensity; and [d]did not comply with exercise protocol.

was effective in reducing cardiovascular risk and improving body composition in overweight volunteers. In the present study, we made a placebo-controlled comparison of the effects of 3 mo of n−3 FA supplementation and regular aerobic exercise, alone and in combination, on body composition and cardiovascular risk factors in overweight subjects with characteristics of the metabolic syndrome.

SUBJECTS AND METHODS

Subjects

We recruited for the study adult volunteers aged 25–65 y who had a body mass index (BMI; in kg/m^2) > 25 and ≥1 of the following cardiovascular risk factors: mild hypertension (140/90–160/100 mm Hg), elevated plasma triacylglycerols (>1.6 mmol/L), or elevated total cholesterol (>5.5 mmol/L). Initial suitability for the study was determined by completion of a simple diet and lifestyle questionnaire. Volunteers were then invited to attend a screening appointment to confirm eligibility for study entry. During screening, height, weight, blood pressure, and fasting triacylglycerols and total cholesterol in venous blood were measured. Subjects also completed a medical screening, which included electrocardiogram monitoring (Nihon Kohden, Tokyo, Japan) during a graded exercise test, to confirm their suitability for exercise training.

Subjects were excluded if they exercised >1 time/wk for the purposes of improving their health; took fish-oil (FO) capsules or ate >1 fatty fish meal/wk; had diabetes, liver disease, or CVD; took blood pressure or lipid-lowering medication; were pregnant or lactating; or were following a weight-reduction diet. Twenty-eight men and 53 women were enrolled.

Written informed consent was obtained from all subjects before their participation. Ethical approval was obtained from both the University of Adelaide and the University of South Australia.

Study design and intervention

Subjects were allocated to 1 of 4 groups, and each group was balanced for sex, BMI, and triacylglycerols. The groups were then randomly assigned to 1 of 4 treatments. Two groups took 6 g tuna fish oil/d (Hi-DHA; Nu-Mega Ingredients Pty Ltd, Brisbane, Australia), which provided 260 mg DHA and 60 mg eicosapentaenoic acid (EPA) in each 1-g capsule, and 2 groups took 6 g placebo oil (sunflower oil; SO)/d for 12 wk. All capsules were identical in color, shape, and flavor and were administered in a double-blind fashion. One of the groups assigned to each oil treatment also participated in a program of regular physical activity. The 4 groups were therefore identified as SO (n = 20), FO (n = 18), SO and exercise (SOX; n = 18), and FO and exercise (FOX; n = 19) (**Figure 1**). Subjects allocated to an oil plus exercise group (FOX and SOX) were required to run or walk 3 times/wk for 45 min at a heart rate (HR) that corresponded to 75% of their age-predicted maximum [208 − (0.7 × age)] (21). Subjects were provided with individual HR monitors (Polar F1; Polar

Electro, Kempele, Finland) to facilitate their exercising at the appropriate HR.

Outcome measures were assessed and compared across each intervention group at 0, 6, and 12 wk, except body composition, which was assessed at 0 and 12 wk only. Subjects attended 2 clinic visits at each of these timepoints when fasting blood samples and cardiovascular and anthropometric data were collected. All subjects were instructed to maintain their normal diet during the study. If not asked to exercise as part of the intervention, subjects were instructed to maintain their normal level of physical activity. Subjects completed a 3-d physical activity diary [adapted from Bouchard et al (22)] and a weighed food record [analyzed with FOODWORKS PROFESSIONAL EDITION (version 3.02; Xyris Software, Highgate Hill, Australia)] on 2 weekdays and 1 weekend day before attending the clinic at each of the 3 timepoints. Compliance was assessed by capsule count, erythrocyte FA analysis, monitoring of regular participation in supervised exercise sessions, and completion of weighed food records and physical activity diaries.

Clinical assessments

Arterial compliance, heart rate, and endothelial function

Blood pressure, HR, and compliance of large and small arteries were measured with the use of the HDI/Pulsewave CR-2000 Cardiovascular Profiler (Hypertension Diagnostics Inc, Eagan, MN) while subjects were in a supine position after a minimum 10-min rest period. Endothelial function was assessed with the use of flow-mediated dilatation (FMD) (23). The diameter of the brachial artery was measured by a single operator with the use of 2-dimensional B-mode ultrasound (LOGIQ 5; GE Medical Systems, Waukesha, WI). Optimal imaging of the artery has been described by Raitakari and Celermajer (24). For the production of reactive hyperemia, a sphygmomanometer cuff was placed around the midpoint of the forearm (ie, distal to the scanned part of the artery) and inflated to a pressure of 200 mm Hg for 5 min. Images of the artery were taken before cuff inflation, 10 s before cuff release, 10 s after cuff release, and then every 30 s for an additional 3 min. For the assessment of endothelium-independent vasodilatation, 300 μg glyceryl trinitrate (Anginine; Sigma Pharmaceuticals Pty Ltd, South Croydon, Australia) was administered sublingually after baseline images were recorded. Images were then taken every minute for 10 min. Arterial diameter was measured as the maximum perpendicular distance between the intima with the use of digital calipers (LOGIQ software, version 5 1.1X; GE Medical Systems).

Anthropometry and body composition

Each subject's height and weight were recorded to calculate the person's BMI at each laboratory visit. Body composition was assessed in all subjects with the use of dual energy X-ray absorptiometry [(DXA) Lunar Prodigy; General Electric, Madison, WI] at 0 and 12 wk. Subjects were scanned while wearing a hospital gown and while in a supine position, in accordance with the manufacturer's instructions. The DXA scanner was calibrated according to the standard procedures recommended by the manufacturer.

Laboratory analyses

Blood sample collection and plasma lipid analysis

Fasting blood was obtained at each visit by venipuncture. Plasma concentrations of triacylglycerols (including second-day repeats) and total cholesterol were measured on an automated centrifugal analyzer (Cobas-Bio, Rotkreuz, Switzerland) with the use of reagents from Roche Diagnostica (Indianapolis, IN). HDL-cholesterol concentrations were measured by using an enzymatic colorimetric test on a Hitachi Autoanalyser system 911 (Hitachi, Tokyo, Japan) with the use of Roche Diagnostica test kits. LDL-cholesterol concentrations were calculated with the Friedewald equation (25).

Erythrocyte fatty acids

Erythrocytes were isolated, washed with isotonic saline (0.9%), frozen, thawed, and then lysed in hypotonic 0.01 mol Tris EDTA buffer/L (pH 7.4), and the membrane lipids were extracted and transmethylated according to the method of Lepage and Roy (26). FA methyl esters were measured with the use of a gas chromatograph 20A (Shimadzu Corp, Kyoto, Japan) fitted with a flame ionization detector and a 50-m BPX70 column (0.32-mm internal diameter and 0.25-μm film thickness; SGE, Ringwood, Australia). Individual FAs were identified by comparison with known standards for FA methyl ester (Nuchek Prep Inc, Elysian, MN) and expressed as a percentage of total FAs quantified from peak areas.

Statistical analysis

Statistical analysis was performed with the use of STATISTICA for WINDOWS software (version 5.1; StatSoft Inc, Tulsa, OK). On the basis of previous estimates of variance in triacylglycerol assessment, 80 subjects provided 80% power at $P < 0.05$ for detection of a 0.2-mmol/L (10%) change in triacylglycerols. Baseline comparisons of age, sex, and height were made with one-factor analysis of variance (ANOVA) (ie, FO compared with FOX compared with SO compared with SOX). The effects of the treatments on the dependent measures were analyzed by using a 3-factor repeated-measures ANOVA with oil treatment (FO or SO) and exercise treatment (exercise or nonexercise) as the between-group factors and time (0, 6, or 12 wk) as the within-subject repeated measurement. In the case of FMD, because the magnitude of the dilatory response is inversely related to the resting brachial artery diameter (27), and, because resting arterial diameter varied at 0, 6, and 12 wk, the resting diameter measured at 0, 6, and 12 wk was included in the model as a changing covariate. When the ANOVA indicated a significant main effect, differences between individual means were identified with the use of Tukey's procedure. To optimize the analysis of differences between treatments, when appropriate, a nested ANOVA design was used to examine changes in dependent variables from baseline with the oil and exercise treatments nested in time. Pearson's product-moment correlation coefficients were used to identify relations between variables. $P \leq 0.05$ was considered significant. Results are presented as means ± SEMs.

RESULTS

Eighty-one subjects were initially enrolled in the study; 75 subjects were randomly assigned to an intervention group. The subject enrollment and attrition patterns in the study are shown in Figure 1. Six subjects withdrew during the trial; 1 subject was unable to reach the required exercise intensity; 1 did not consume the required number of daily capsules; 3 subjects had a change in work or family circumstances, and 1 subject was lost to follow-up. Data for an additional 4 subjects were excluded from analysis because they failed to comply with the exercise requirements of the study. Thus, 24 men and 41 women (ie, 87% of those who began the trial) completed the trial requirements. Their characteristics at baseline (week 0) are shown in **Table 1**. No differences were observed between groups in any of these characteristics.

Effects on erythrocyte fatty acid concentrations

FA profiles of erythrocyte membranes were obtained from 46 subjects at 0, 6, and 12 wk. The percentage of long-chain n−3 FAs [EPA + docosapentaenoic acid (DPA) + DHA] in erythrocytes increased substantially in both groups treated with FO (from 10.7% to 13.7% in the FO group and from 10.4% to 13.5% in the FOX group), which resulted in a significant oil × time interaction ($P < 0.0001$; **Figure 2**). This increase was entirely attributable to the change in DHA, which increased by 85% in the FO group and by 86% in the FOX group but decreased by 6% in the SO group and by 9% in the SOX group ($P < 0.0001$ for oil × time interaction; Figure 2). No change was observed in EPA or DPA in any group as a result of supplementation.

Effects on plasma lipids

Plasma lipids were marginally high in all treatment groups at baseline (Table 1). The modest exercise intervention undertaken in this study did not improve lipids. However, nested analysis showed that FO reduced triacylglycerols (≈14%) significantly ($P < 0.05$) more than did SO (≈5% increase) (**Figure 3**). At the same time, FO increased HDL cholesterol (≈10%) significantly ($P < 0.05$ for oil × time interaction) more than did SO (≈3% increase) (Figure 3). Regression analysis showed that the changes in both total long-chain n−3 FAs and DHA content in erythrocytes from 0 to 12 wk were correlated with the changes in HDL cholesterol over this same period (n−3 FA: $r^2 = 0.16$, $P = 0.008$; DHA: $r^2 = 0.13$, $P = 0.02$) but not with changes in triacylglycerols (n−3 FAs: $r^2 = 0.06$, $P = 0.12$; DHA: $r^2 = 0.05$, $P = 0.17$). Exercise was associated with a marginal increase in total cholesterol ($P < 0.05$ for exercise × time interaction; Table 1), but post hoc analysis did not find any significant differences between means.

Effects on cardiovascular risk factors

Measures of blood pressure, HR, and arterial function were assessed in 65 subjects, except glyceryl trinitrate–mediated dilatation, which was assessed in 62 subjects (Table 1). ANOVA did not find any significant oil × exercise × time interactions for any of these risk factors. However, a significant ($P < 0.05$) oil × time interaction was observed for FMD when resting brachial artery diameter at each measurement at 0, 6, and 12 wk was used as a changing covariate (**Figure 4**). Post hoc analysis found a significantly ($P < 0.01$) greater improvement in FMD with FO than with SO by 12 wk. A significant ($P < 0.05$) exercise × time interaction was observed for small arterial compliance (SAC) (**Figure 5**), and post hoc analysis indicated a significant increase in SAC in subjects who undertook the exercise training program ($P = 0.05$): SAC increased by 26 ± 8% in the exercise group and by 1 ± 4% in the nonexercise group. Large artery compliance was not affected by either exercise or oil treatments.

Energy intake and effects on weight and body composition

As with the cardiovascular risk factors, no significant oil × exercise × time interaction was observed for energy intake after intervention. The average energy intake across all groups was 9785 ± 383 kJ at 0 wk, 9686 ± 433 kJ at 6 wk, and 9767 ± 469 kJ at 12 wk.

No significant oil × exercise × time interactions were observed for body weight or body composition, but a significant ($P < 0.05$) exercise × time interaction was observed for body weight (Table 1). Oil and exercise interactions were also evident for fat mass (exercise × time, $P < 0.05$; oil × time, $P < 0.05$; **Figure 6**), which indicated that both FO supplementation and regular aerobic exercise reduced fat mass. No significant effects of either exercise or oil treatments were observed on lean mass.

DISCUSSION

The results of this study confirm that regular supplementation with a moderate dose of DHA-rich FO can improve multiple cardiovascular risk factors (ie, plasma triacylglycerols, HDL cholesterol, and FMD). Regular moderate-intensity exercise, either alone or in addition to the FO supplementation, had no effect on these risk factors, although it improved the compliance of small resistance arteries. It is interesting, however, that both FO supplementation and regular exercise significantly reduced body fat, which indicates the potential benefit of a combined treatment strategy for optimizing body composition.

Evidence is limited that FO supplementation can reduce body fat in overweight or obese subjects, and, in the studies that do provide evidence, little effort was made to control for the confounding influence of physical activity. Only 2 trials have investigated the effect of n−3 FA supplementation from fish, FO, or both in combination with aerobic exercise training on body composition (19, 20). Warner et al (20) investigated the effect of 12 wk of aerobic exercise training (walk or jog 3 d/wk for 45–50 min at 75–80% maximal HR) in hyperlipidemic subjects allocated to 1 of 4 groups: FOX, FO, corn oil, or control. The percentage of body fat was reduced only in the combined FOX group. However, the design of that study did not make it possible to determine how much of this change was due to exercise, because the study did not include an exercise-only control. In their study of young, lean, healthy men, Brilla and Landerholm (19) combined fish and FO with exercise and had appropriate fish and FO–only and exercise-only control groups. They found no effect on body fat with a combination of fish and FO, but it is possible that this may be because the volunteers were relatively lean at the start of the study (ie, 15–22% body fat). The present study is the first properly controlled trial to show an improvement in body composition in overweight or obese subjects after intervention with n−3 FAs and regular aerobic exercise.

Both exercise and n−3 FAs have the potential to influence the mechanisms responsible for FA mobilization and its delivery to

TABLE 1
Effect of treatment on selected study risk factors after 12 wk of supplementation with 6 g docosahexaenoic acid–rich fish oil/d (FO), 6 g sunflower oil/d (SO), FO and exercise (FOX), or SO and exercise (SOX)[1]

	FO ($n = 17$)			FOX ($n = 16$)			SO ($n = 18$)			SOX ($n = 14$)		
	Week 0	Week 6	Week 12	Week 0	Week 6	Week 12	Week 0	Week 6	Week 12	Week 0	Week 6	Week 12
Sex												
Male (n)	6			5			7			6		
Female (n)	11			11			11			8		
Age (y)	52 ± 2[2]			47 ± 2			51 ± 2			51 ± 2		
Weight (kg)[3,4]	97 ± 4.7	97 ± 4.8	97 ± 4.8	98 ± 5.5	98 ± 5.4	96 ± 5.3	98 ± 4.8	101 ± 5.29	99 ± 4.8	96 ± 3.8	97 ± 3.8	96 ± 3.9
Height (m)	1.7 ± 0.03			1.7 ± 0.03			1.7 ± 0.03			1.7 ± 0.03		
BMI (kg/m^2)[3]	34 ± 1.5	34.2 ± 1.5	34 ± 1.5	34.5 ± 1.5	34.3 ± 1.5	33.9 ± 1.5	35.1 ± 1.2	35.8 ± 1.3	35.2 ± 1.2	32.7 ± 0.9	32.8 ± 0.9	32.7 ± 1
Energy intake (kJ)	8895 ± 679	8674 ± 632	9348 ± 804	9739 ± 921	9189 ± 925	10208 ± 1253	10549 ± 769	10329 ± 850	9334 ± 511	9937 ± 642	10703 ± 1053	10297 ± 1199
Body fat (%)[4,5]	43.9 ± 1.6		43.9 ± 1.6	43.5 ± 2.4		42.3 ± 2.3	43.7 ± 1.9		44.4 ± 1.9	41.5 ± 2.2		41.4 ± 2.1
TAG (mmol/L)[6]	1.66 ± 0.18	1.53 ± 0.19	1.43 ± 0.18	1.93 ± 0.43	1.52 ± 0.24	1.62 ± 0.36	1.73 ± 0.20	1.84 ± 0.18	1.71 ± 0.16	1.88 ± 0.22	1.82 ± 0.18	1.88 ± 0.26
HDL (mmol/L)[3,5]	1.34 ± 0.09	1.56 ± 0.14	1.47 ± 0.13	1.37 ± 0.10	1.50 ± 0.11	1.53 ± 0.13	1.35 ± 0.12	1.38 ± 0.15	1.38 ± 0.13	1.18 ± 0.11	1.22 ± 0.12	1.19 ± 0.09
TC (mmol/L)[4]	5.98 ± 0.21	6.34 ± 0.20	5.62 ± 0.36	5.94 ± 0.34	5.97 ± 0.39	6.27 ± 0.20	6.01 ± 0.23	5.93 ± 0.33	5.66 ± 0.22	5.65 ± 0.26	5.70 ± 0.25	5.92 ± 0.30
SBP (mm Hg)[3]	128.8 ± 3.5	128.8 ± 3.4	123.7 ± 3.3	131.9 ± 4.3	134.2 ± 4.6	126.1 ± 3.8	128.0 ± 3.1	130.1 ± 3.4	126.2 ± 3.4	132.4 ± 2.7	130.2 ± 3.3	128.5 ± 3.3
DBP (mm Hg)[3]	72.5 ± 2.5	73.9 ± 2.1	71.2 ± 2.3	77.6 ± 2.8	78.8 ± 3.1	73.0 ± 2.9	72.6 ± 1.8	72.5 ± 1.9	71.4 ± 2.1	75.9 ± 2.3	76.0 ± 2.7	73.2 ± 2.4
HR (bpm)	62.3 ± 1.6	61.9 ± 1.6	61.1 ± 1.6	62.0 ± 2.2	58.6 ± 2.4	58.6 ± 2.3	61.0 ± 1.8	61.4 ± 2.0	61.9 ± 1.4	62.0 ± 2.3	61.9 ± 2.4	61.0 ± 2.3
LAC (mL/mm Hg × 10)	15.7 ± 0.8	16.5 ± 1.1	17.2 ± 0.8	16.3 ± 1.0	16.6 ± 1.0	16.8 ± 1.0	16.6 ± 1.0	15.9 ± 0.9	15.8 ± 0.8	16.3 ± 1.0	16.8 ± 1.1	16.5 ± 1.2
SAC (mL/mm Hg × 100)[4]	7.8 ± 0.8	7.5 ± 1.0	7.9 ± 0.8	7.3 ± 0.9	7.7 ± 0.8	8.7 ± 1.0	6.9 ± 0.8	7.3 ± 1.0	6.4 ± 0.7	7.7 ± 1.1	8.1 ± 0.8	9.0 ± 0.8
FMD (mm)[7]	13.3 ± 2.6	21.4 ± 2.8	23.9 ± 3.0	14.6 ± 2.8	19.6 ± 1.6	20.8 ± 2.3	18.0 ± 2.3	20.0 ± 2.0	19.1 ± 3.0	19.8 ± 4.1	16.9 ± 2.7	20.2 ± 2.3
GTN-D (mm)	82.5 ± 3.4	83.9 ± 4.9	81.3 ± 3.6	85.1 ± 5.4	84.0 ± 4.9	85.8 ± 5.0	90.4 ± 7.2	96.1 ± 4.4	85.5 ± 4.4	84.3 ± 4.4	85.2 ± 6.2	87.1 ± 5.1

[1] TC, total cholesterol; LAC, large artery compliance; SAC, small artery compliance; FMD, flow-mediated dilatation; GTN-D, glyceryl trinitrate dilatation; TAG, triacylglycerols; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute. For GTN-D, $n = 16$ at week 12 for FO; $n = 15$ at weeks 0 and 12 for FOX; $n = 17$ at weeks 6 and 12 for SO. There were no differences between treatment groups at baseline (1-factor ANOVA). Data were analyzed with a 3-factor repeated-measures ANOVA with interaction for fish oil, exercise, and time, with $P < 0.05$ considered significant. No 3-factor interactions were detected.

[2] $\bar{x}$ ± SEM (all such values).

[3] Significant main effect of time, $P < 0.05$.

[4] Significant exercise treatment × time interaction, $P \leq 0.05$.

[5] Significant fish oil treatment × time interaction, $P < 0.05$.

[6] Changes in TAG were analyzed by using nested ANOVA with oil and exercise nested in time. Significant effect of oil treatment, $P < 0.05$.

[7] FMD was analyzed by ANCOVA for fish oil status, exercise status, and time, with artery diameter as a changing covariate. Significant fish oil status × time interaction, $P < 0.05$.

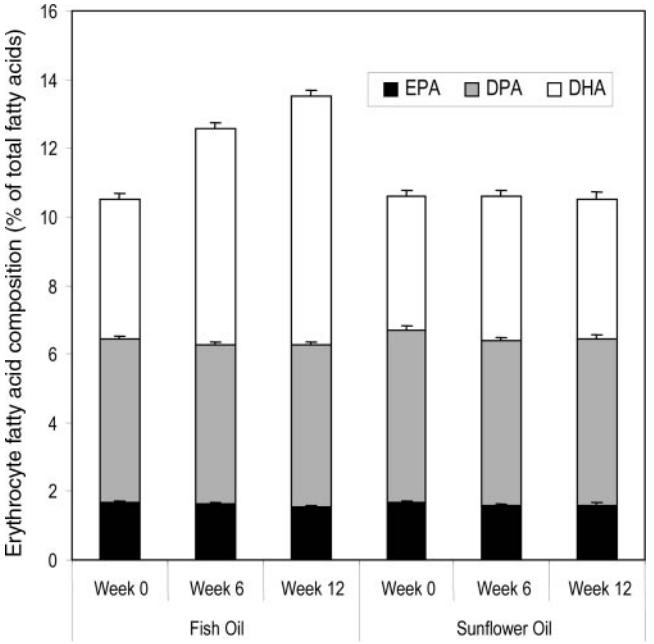

FIGURE 2. Mean (± SEM) erythrocyte fatty acid concentrations for eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) after supplementation with 6 g DHA-rich fish oil/d (n = 24) or 6 g sunflower oil/d (n = 19) for 12 wk. No differences were observed between groups for EPA, DPA, DHA, or long-chain n−3 fatty acids (EPA + DPA + DHA) at baseline ($P > 0.05$, ANOVA). A significant oil × time interaction was observed for DHA and total n−3 fatty acids, which increased in the fish oil–supplemented group, $P < 0.0001$.

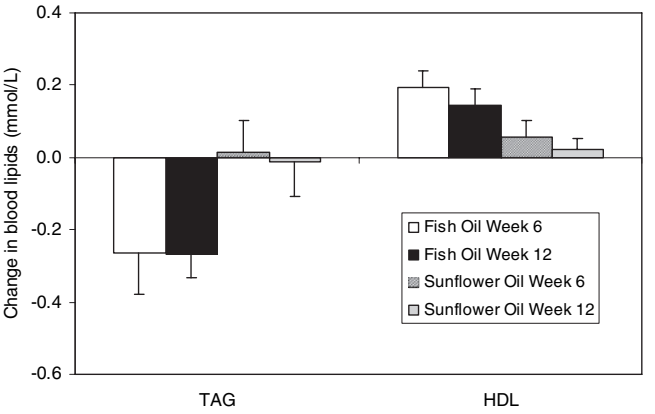

FIGURE 3. Mean (± SEM) changes in serum lipid concentrations by oil treatment after supplementation with 6 g docosahexaenoic acid–rich fish oil/d (n = 31) or 6 g sunflower oil/d (n = 31). Analysis of oil treatment nested in time showed a significant reduction in triacylglycerols (TAG) with fish oil supplementation, $P < 0.05$. A significant oil × time interaction was detected for HDL cholesterol, which increased in the fish oil–supplemented group, $P < 0.05$.

and oxidation in muscles. By up-regulating several nuclear receptors, n−3 FAs can effectively mediate a shift in fuel metabolism away from storage and toward oxidation (28). Evidence suggests that both exercise and n−3 FAs can increase the expression of genes that code for key enzymes involved in FA transport and β-oxidation, such as lipoprotein lipase, acetyl-CoA carboxylase-2 (29), FA translocase, carnitine palmitoyl transferase 1 (6, 30, 31), and mitochondrial uncoupling protein 3 (7). Indeed, several animal studies have shown a reduction in fat mass after supplementation with n−3 FAs (7, 8). However, few human studies have specifically investigated the effect of FO supplementation on body composition in overweight or obese adults, along with rigorous control of diet and physical activity. Couet et al (9) replaced 6 g visible dietary fat/d with 6 g FO/d for 3 wk and observed an increase in basal lipid oxidation and a reduction in fat mass in young, healthy adults. A limitation of that study was that the order of treatment was not randomized or balanced, and the changes in fat oxidation may have been due to seasonal variation or some other order effect. In a well-designed study, Groh-Wargo et al (10) reported that preterm infants fed a DHA-enriched formula for 12 mo accumulated significantly less fat mass than did their age-matched controls. In comparison, other studies that examined the effects of weight-loss diets supplemented with n−3 FAs have not observed any effects on body weight or fat mass beyond those induced by dietary restriction alone (11, 12). However, either those latter studies did not include n−3 FA control groups (11, 12) or they used the somewhat insensitive method of skinfold-thickness measurement to predict changes in body composition (11).

Exercise training was shown to improve FMD, albeit at higher intensities of exercise than was used in the present study (32). Although exercise appeared to have no effect on FMD in the present study, several studies have shown that n−3 FAs can independently improve endothelial function (33–37). This improved endothelial function may be due to increased endothelial production of nitric oxide (38), vasodilatory eicosanoids (39), or both. Recently, it was also shown that FO can increase arterial dilatation and blood flow to skeletal muscle during exercise (40), which has implications for FA delivery to sites of metabolism (ie, skeletal muscle) during physical activity. It is therefore possible that some of the change in body composition with FO supplementation in the present study may be due to improved blood flow that increased the delivery of fats to skeletal muscle, which, in conjunction with exercise and n−3 FA–induced changes in gene expression, may have facilitated fat oxidation during exercise. This proposition is indirectly supported by our own observation of an improvement in FMD, which indicated an

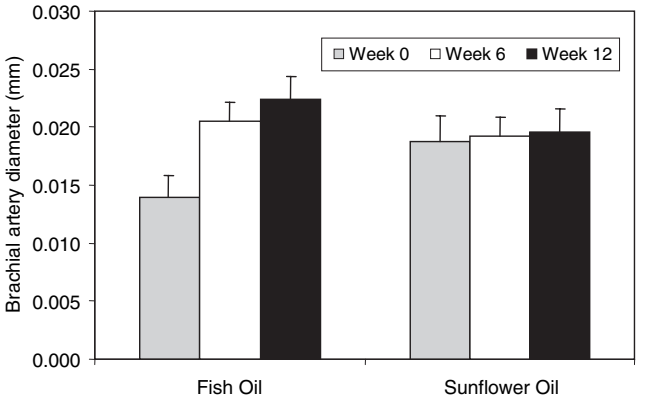

FIGURE 4. Mean (± SEM) change in endothelium-dependent dilatation of the brachial artery by oil treatment after supplementation with 6 g docosahexaenoic acid–rich fish oil/d (n = 33) or 6 g sunflower oil/d (n = 31). Endothelium-dependent dilatation was evaluated by ANCOVA (oil × exercise × time interaction) with resting artery diameter as a changing covariate. Dilatation increased significantly in the fish oil–supplemented group ($P <$ 0.05 for oil × time interaction).

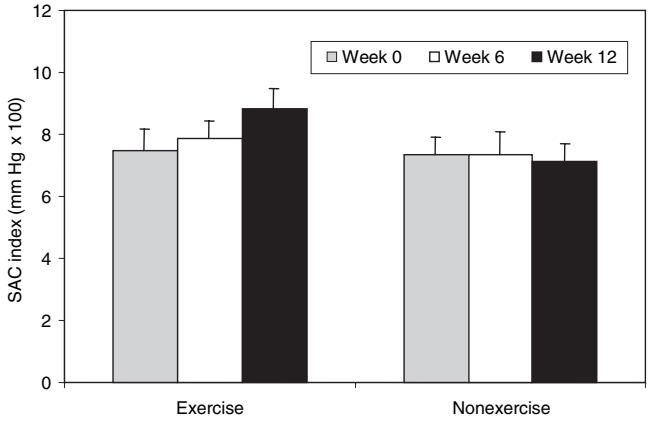

FIGURE 5. Mean (± SEM) changes in small artery compliance (SAC) index by exercise treatment after aerobic exercise training (n = 30) or no exercise (n = 34) for 12 wk. SAC increased significantly after aerobic exercise training ($P < 0.05$ for exercise × time interaction).

improvement in vasodilatory capacity, in response to n–3 FA supplementation.

It is well recognized that n–3 FAs lower triacylglycerols (41–43), and our results confirm this finding. Triacylglycerol reductions of 25–30% were seen with intakes of 3–4 g EPA and DHA (44), and the 14% reduction in triacylglycerols after daily supplementation with ≈1.9 g n–3 FAs in the present study is consistent with this magnitude of effect per gram of n–3 FAs. The 10% increase in HDL cholesterol in response to n–3 FA supplementation did not differ significantly from that reported from other studies, despite the fact that those studies used different DHA doses and durations of supplementation (42, 45). It is thought that exercise can also have a favorable effect on triacylglycerols and HDL cholesterol, but the data from the present study and the results of several meta-analyses indicate that this may not be the case, because most studies show great variability in the effects of exercise on triacylglycerols and HDL cholesterol between populations and individual persons (46, 47).

To date, only 2 trials have investigated the effect of n–3 FAs in combination with aerobic exercise training on blood lipids (19, 20). The combination of FO and 12 wk of exercise training was shown to reduce serum concentrations of LDL cholesterol and apolipoprotein B in hyperlipidemic persons more than FO alone (20). However, in healthy subjects, 10 wk of exercise training and fish and FO supplementation had no effect on HDL, LDL, or total cholesterol or triacylglycerols (19). We suggest that the lack of effect in the present study may have been attributable to the normal range of blood lipids seen in the subjects recruited for the trial.

The present trial is the first to evaluate the metabolic and cardiovascular benefits that can be achieved by combining n–3 FA supplementation and regular aerobic exercise in overweight or obese adults. We have confirmed the independent benefits of supplementation with DHA-rich FO for triacylglycerols, HDL cholesterol, and FMD and the independent benefit of regular moderate exercise for improving SAC. We also showed that both FO supplementation and regular moderate exercise reduced body fat in overweight or obese subjects who were at risk of CVD. The high compliance rate (>85%) within the present study indicates that this intervention is well tolerated, probably because of the modest level of physical activity required and because subjects did not need to change their background diet, and thus compliance may be sustainable in the longer term. Future research should evaluate the efficacy of this combined intervention over a longer duration and investigate the mechanism underlying the improvements in body composition.

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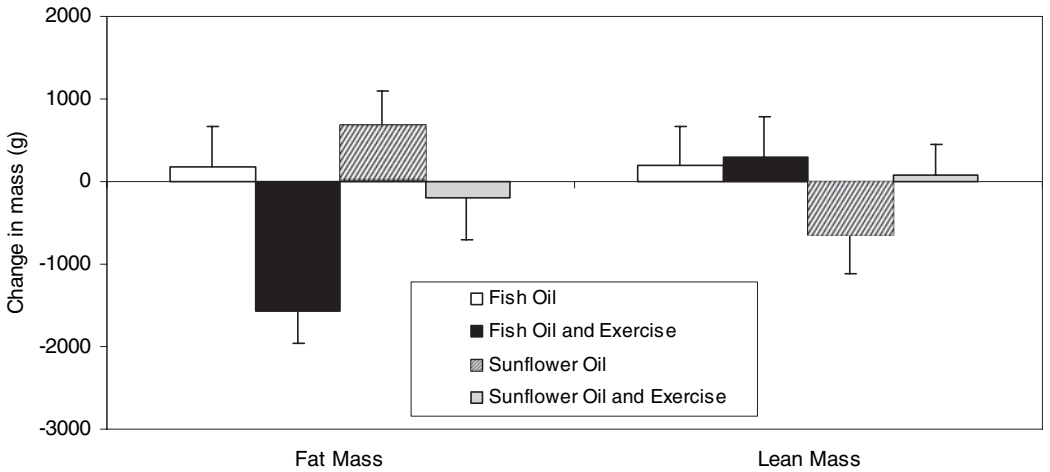

FIGURE 6. Dual-energy X-ray absorptiometry assessed changes in fat and lean mass after supplementation with 6 g docosahexaenoic acid–rich fish oil/d (n = 17), 6 g sunflower oil/d (n = 18), fish oil and exercise (n = 16), or sunflower oil and exercise (n = 14) for 12 wk. Significant oil × time and exercise × time interactions were detected for fat mass, $P < 0.05$. Fat mass was significantly reduced by both fish-oil supplementation (P = 0.043) and aerobic exercise (P = 0.005). The oil × exercise × time interactions were not significant.

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