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# Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men[1–3]

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**ABSTRACT**

**Background:** Regular consumption of n−3 fatty acids of marine origin can improve serum lipids and reduce cardiovascular risk.

**Objective:** This study aimed to determine whether eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have differential effects on serum lipids and lipoproteins, glucose, and insulin in humans.

**Design:** In a double-blind, placebo-controlled trial of parallel design, 59 overweight, nonsmoking, mildly hyperlipidemic men were randomly assigned to receive 4 g purified EPA, DHA, or olive oil (placebo) daily while continuing their usual diets for 6 wk.

**Results:** Fifty-six men aged 48.8 ± 1.1 y completed the study. Relative to those in the olive oil group, triacylglycerols fell by 0.45 ± 0.15 mmol/L (≈20%; $P = 0.003$) in the DHA group and by 0.37 ± 0.14 mmol/L (≈18%; $P = 0.012$) in the EPA group. Neither EPA nor DHA had any effect on total cholesterol. LDL, HDL, and $HDL_2$ cholesterol were not affected significantly by EPA, but $HDL_3$ cholesterol decreased significantly (6.7%; $P = 0.032$). Although HDL cholesterol was not significantly increased by DHA (3.1%), $HDL_2$ cholesterol increased by ≈29% ($P = 0.004$). DHA increased LDL cholesterol by 8% ($P = 0.019$). Adjusted LDL particle size increased by 0.25 ± 0.08 nm ($P = 0.002$) with DHA but not with EPA. EPA supplementation increased plasma and platelet phospholipid EPA but reduced DHA. DHA supplementation increased DHA and EPA in plasma and platelet phospholipids. Both EPA and DHA increased fasting insulin significantly. EPA, but not DHA, tended to increase fasting glucose, but not significantly so.

**Conclusions:** EPA and DHA had differential effects on lipids, fatty acids, and glucose metabolism in overweight men with mild hyperlipidemia. *Am J Clin Nutr* 2000;71:1085–94.

**KEY WORDS** Eicosapentaenoic acid, docosahexaenoic acid, EPA, DHA, hyperlipidemia, fish oil, n−3 fatty acids, lipids, LDL particle size, glucose metabolism, insulin metabolism, men

## INTRODUCTION

There is considerable evidence to support a protective effect of dietary n−3 polyunsaturated fatty acids against atherosclerotic heart disease (1). The 2 principal n−3 fatty acids in marine oils, eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3), have a wide range of biological effects (1–3). Those relevant to heart disease include influences on lipoprotein metabolism (4, 5), platelet and endothelial function, vascular reactivity, neutrophil and monocyte cytokine production, coagulation, fibrinolysis, and blood pressure (1–3, 6, 7). In addition, the effect of n−3 fatty acids may be dependent, to some extent, on the presence of underlying disorders such as dyslipidemia, hypertension, diabetes mellitus, and vascular disease.

n−3 Fatty acid supplementation in animals and humans results in substantial increases in plasma and tissue EPA and DHA as well as variable incorporation in different phospholipid classes in different tissues (8–10). These differences may be important to the subsequent utilization and metabolism of EPA and DHA. Although both fatty acids are considered to be biologically active, most studies have focused on the relative importance and effects of EPA, primarily because of its predominance in marine oils and fish species. The recent availability of purified EPA and DHA, however, has enabled studies of the independent biological effects of these fatty acids.

Evidence from in vitro studies suggests differential effects of EPA and DHA (11, 12). In vitro (13) and animal (10, 14, 15) studies have also suggested that EPA may be primarily responsible for the hypotriglyceridemic effect of n−3 fatty acids. Rambjor et al (16) concluded that EPA is responsible for the triacylglycerol-lowering effect of fish oils in humans, but their study had small numbers of subjects and was of short duration. In contrast, a hypotriglyceridemic effect of DHA was shown in healthy subjects (17) and in patients with combined hyperlipidemia (18).

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Grimsgaard et al (19) reported that EPA and DHA have similar triacyglycerol-lowering effects compared with corn oil placebo. However, DHA significantly increased HDL cholesterol, whereas EPA significantly lowered both total cholesterol and apolipoprotein (apo) A-I concentrations (19). Neither fatty acid altered LDL-cholesterol concentrations significantly.

Possible benefits of n−3 fatty acids have to be weighed against the potential for impairment of glycemic control, particularly in patients with type 2 diabetes (20–23). However, studies in healthy subjects, in patients with dyslipidemia (24), and in patients with untreated hypertension (25) showed no adverse effects of n−3 fats on plasma glucose concentrations. To our knowledge, there have been no studies in which the effects of pure EPA were compared with those of DHA on indexes of glucose and insulin metabolism in humans.

In view of the increasing use of n−3 fatty acids in the diet as food additives or as therapeutic substances, it is important to determine the extent of any differential effects of EPA and DHA. This study examined the independent effects of EPA and DHA on fatty acid and lipid metabolism, as well as on fasting glucose and insulin concentrations. The study also aimed to determine whether EPA and DHA differ in their effects on HDL-cholesterol subfractions and LDL particle size.

## SUBJECTS AND METHODS

### Study population

Mildly hypercholesterolemic but otherwise healthy, nonsmoking men aged 20–65 y were recruited from the general community by media advertising. Entry criteria included a serum cholesterol concentration >6 mmol/L, a triacylglycerol concentration >1.8 mmol/L, or both; a body mass index (BMI; in kg/m$^2$) between 25 and 30; and no recent (previous 3 mo) symptomatic heart disease, diabetes, or liver or renal disease (plasma creatinine >130 μmol/L). None of the subjects were regularly taking nonsteroidal antiinflammatory, antihypertensive, or lipid-lowering drugs or other drugs known to affect lipid metabolism. All of the men had a usual weekly consumption of not more than one fish meal and drank <210 mL ethanol/wk. Fifty-nine of the 136 subjects screened satisfied the entry criteria. The study was approved by the ethics committee of the Royal Perth Hospital and all subjects gave written consent.

### Dietary education and intervention

All subjects maintained their usual diets and alcohol intakes during a 3-wk familiarization period. Baseline measurements were collected and the men were stratified for age and BMI before being randomly assigned to 1 of 3 groups: 4 g daily of EPA, DHA, or olive oil (placebo) capsules for 6 wk. Capsules contained either purified preparations of EPA ethyl ester (≈96%), DHA ethyl ester (≈92%), or olive oil (≈75% oleic acid ethyl ester). All participants were instructed to maintain their usual diets, alcohol intakes, and physical activities, and not to make any changes to their lifestyle throughout the intervention period.

At an initial interview, subjects were given written and verbal instructions by a dietitian on how to keep diet records, with food weighed or measured. The same dietitian monitored the dietary intake of all the volunteers at 2-wk intervals and ensured that usual eating habits were maintained. A 3-d diet record (2 weekdays and 1 weekend day) was completed by the volunteers at baseline and postintervention.

### Lifestyle assessment and anthropometry

Alcohol intakes, physical activities, and any medications taken were monitored every second week during the intervention by using 7-d retrospective diaries. Weight was measured every second week with an electronic scale.

### Serum lipids, glucose, and insulin

Fasting serum lipids, lipoproteins, glucose, and insulin were measured twice at baseline and twice at the end of the intervention. Serum glucose was measured with an automated Technicon Axon Analyzer (Bayer Diagnostics, Sydney, Australia) by using a hexokinase method within 12 h of collection. The assay precision for serum glucose at 4.9 mmol/L was 3.1%. Serum insulin was measured by radioimmunoassay with an automated immunoassay analyzer (Tosoh Corporation, Tokyo). The CV for serum insulin at 21 and 102 pmol/L was 14.0% and 8.0%, respectively. The precision in the range of 234–720 pmol/L was 7.0%.

Serum total cholesterol and triacylglycerols were determined enzymatically on the Cobas MIRA analyzer (Roche Diagnostics, Basel, Switzerland) with reagents from Trace Scientific (Melbourne). The assay CVs were 2.2% at 4.2 mmol/L and 1.4% at 10.5 mmol/L for total cholesterol, and 1.6% at 4.0 mmol/L and 2.5% at 1.2 mmol/L for triacylglycerol. HDL cholesterol was determined on a heparin-manganese supernate (26); the CV at 1.1 mmol/L was 1.9%. $HDL_2$ and $HDL_3$ cholesterol were determined by using a single precipitation procedure (27). LDL cholesterol was calculated by using the Friedewald formula (28). Serum for the analyses of lipids, lipoproteins, and insulin was snap-frozen in liquid nitrogen and stored at − 80°C. Samples obtained at baseline and at the end of the intervention were measured in a single assay to minimize interassay variation.

### LDL particle size

LDL particle size was determined from LDL isolated by vertical density-gradient ultracentrifugation of 4 mL plasma collected into EDTA (29). LDL particle diameter was determined by using a previously published method (30, 31) with use of commercially available 3–13% nondenaturing native gels (Gradipore, Sydney, Australia). Markers used were 28-nm latex beads (Duke, Palo Alto, CA) and high-molecular-weight standards (Pharmacia, Peapack, NJ). Gels were scanned by Tracktel video densitometry (Vision System Ltd, Adelaide, Australia) to provide a quantitative estimate of the dominant peak size. Particle diameter was obtained from a standard curve of the logarithm of the diameter of the standards (latex beads, 28 nm; thyroglobulin, 17 nm; and ferritin, 12.2 nm) against their positions on the scanned gel. A statistical package was used to derive a regression equation that allowed test samples to be sized. The CV of a 26.1-nm quality-control sample run on every gel was 0.8%.

### Plasma and platelet phospholipid fatty acids

Plasma (1 mL) and washed platelets prepared from blood collected into EDTA were extracted with chloroform:methanol (2:1 by vol, 5 mL). The phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography by using a solvent system of hexane:diethyl ether:acetic acid:methanol (170:40:4:4, by vol) on silica gel 60 $F_{254}$-precoated aluminum sheets (Merck, Darmstadt, Germany). Fatty acid methyl esters were prepared by

**TABLE 1**
Characteristics of participants in the 3 groups at baseline[1]

| | Olive oil (control) ($n$ = 20) | EPA ($n$ = 19) | DHA ($n$ = 17) |
|---|---|---|---|
| Age (y) | 48.4 ± 2.0 | 48.9 ± 1.7 | 49.1 ± 2.2 |
| Body weight (kg) | 88.7 ± 2.0 | 89.1 ± 2.3 | 90.8 ± 2.8 |
| BMI (kg/m$^2$) | 28.4 ± 0.5 | 29.0 ± 0.7 | 28.9 ± 0.7 |
| Waist-to-hip ratio | 0.94 ± 0.01 | 0.93 ± 0.01 | 0.94 ± 0.01 |

[1] $\bar{x}$ ± SEM. There were no significant differences by one-way ANOVA. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

treating phospholipid extracts with 4% $H_2SO_4$ in methanol at 90°C for 20 min and analyzed by gas-liquid chromatography with a model 5980A gas chromatograph equipped with a 3393A computing integrator (Hewlett-Packard, Rockville, MD). The column was a BPX70 (25 m × 0.32 mm, 0.25-μm film thickness; SGE, Ringwood, Australia) with a temperature programmed from 150 to 210°C at 4°C/min and with nitrogen as the carrier gas at a split ratio of 30:1. Peaks were identified by comparing them with a known standard mixture. Individual fatty acids were calculated as a relative percentage with the evaluated fatty acids set at 100%.

### Statistical analysis

Diet records were analyzed by using DIET/1 (version 4; Xyris, Brisbane, Australia), which is based on the Australian Food Composition Database NUTTAB 1995A (32). Data were analyzed by using SPSS (SPSS Inc, Chicago) with general linear models to assess the effects of EPA or DHA relative to the olive oil group. Significance levels were adjusted for multiple comparisons by using the Bonferroni method. Values are reported as means ± SEMs.

## RESULTS

### Study population

Fifty-six of the 59 subjects completed the study. Two subjects withdrew because they were unable to maintain the schedule of laboratory visits and one subject withdrew because of gastrointestinal symptoms. Baseline characteristics of the 3 groups confirmed that they were well matched for the entry criteria (**Table 1** and **Table 2**).

### Energy and macronutrient intakes

Evidence of adherence to the diets was from analysis of diet records and capsule counts. There was no significant difference in body weight between the groups at baseline (Table 1) and no significant change during the intervention. Weight changes in the 3 groups were as follows: 0.2, 0.2, and 0.3 kg in the control, EPA, and DHA groups, respectively. Analysis of diet records indicated that total energy and major macronutrient intakes were not significantly different between groups at baseline (**Table 3**) and did not change significantly in any of the groups during the intervention. Alcohol drinking and physical activity were unchanged during the intervention in all groups.

### Plasma and platelet phospholipid fatty acids

At baseline, there were no significant differences between groups in plasma and platelet phospholipid fatty acid composition. The changes in plasma (**Figure 1**) and platelet (**Figure 2**) phospholipid fatty acids in each group indicated compliance with capsule intake. There were no significant changes in fatty acid composition in the control group.

*Plasma fatty acids*

In plasma phospholipids, EPA supplementation increased EPA by 494% ($P < 0.01$) and docosapentaenoic acid (DPA; 22:5n−3) by 87% ($P < 0.01$), without significantly changing DHA (9% change; NS). In the DHA group, DHA and EPA increased by 167% ($P < 0.01$) and 52% (NS) respectively, whereas DPA was not affected significantly. Oleic acid (18:1n−9) concentrations were significantly decreased by both EPA (by 11%; $P < 0.01$) and DHA (by 11%; $P < 0.01$) supplementation. There was a significantly larger ($P < 0.01$) decrease in linoleic acid (18:2n−6) in the EPA group (by 21%; $P < 0.01$) than in the DHA group (by 12%; $P < 0.01$). EPA and DHA decreased arachidonic acid (20:4n−6) by 25% ($P < 0.01$) and 22% ($P < 0.01$), respectively, and decreased 20:3n−6 by approximately the same extent, 36% ($P < 0.01$) and 28% ($P < 0.01$), respectively.

*Platelet fatty acids*

EPA supplementation significantly increased platelet phospholipid EPA by 370% ($P < 0.01$) and DPA by 56% ($P < 0.01$), but also significantly decreased DHA by 28% ($P < 0.01$). DHA supplementation significantly increased DHA by 155% ($P < 0.01$) and EPA by 54% (NS). DPA, however, unlike in plasma phospholipids, decreased significantly by 34% ($P < 0.01$). Both EPA and DHA significantly decreased stearic acid (18:0) ($P < 0.01$), whereas only EPA decreased 20:3n−6 (by 25%; $P < 0.01$). Similar to plasma phospholipids, 20:4n−6 decreased significantly more ($P < 0.01$) after EPA (by 15%; $P < 0.01$) than after DHA (by 7%; $P < 0.01$).

### Serum lipids

There were no significant differences in fasting serum lipids at baseline between groups (Table 2). Changes in fasting lipids and lipoproteins are shown in **Figures 3** and **4**. There were no significant changes in lipids with olive oil supplementation. Neither EPA nor DHA supplementation had an effect on serum total cholesterol concentrations. After adjustment for baseline values, fasting triacylglycerols decreased significantly by 18.4% with EPA ($P = 0.012$) and by 20% with DHA ($P = 0.003$), relative to the placebo group. Serum LDL cholesterol increased significantly with DHA (by 8%; $P = 0.019$), but not with EPA (by 3.5%; NS). In the EPA group, the nonsignificant 3% decrease in HDL cholesterol was attributable to a significant 6.7% reduction in $HDL_3$ cholesterol ($P = 0.032$) and no change in $HDL_2$ cholesterol. A small, albeit nonsignificant increase (3.1%) in HDL cholesterol after DHA supplementation was due to a significant increase (29%) in the $HDL_2$-cholesterol subfraction ($P = 0.004$) with no significant change in the $HDL_3$-cholesterol subfraction.

### LDL particle size

LDL particle size was not significantly different between groups at baseline (Table 2). Neither olive oil nor EPA had a significant effect on LDL particle size, whereas DHA supplementation significantly increased LDL particle size ($P = 0.002$) after adjustment for baseline values (Table 2 and **Figure 5**). At baseline, LDL particle size was inversely correlated with triacylglycerol ($r = -0.58$, $P < 0.0001$) and positively correlated with

**TABLE 2**
Fasting serum lipids, glucose, and insulin at baseline and postintervention in the 3 groups[1]

| | Olive oil (control) ($n$ = 20) | EPA ($n$ = 19) | DHA ($n$ = 17) | Treatment effect ($P$)[2] | |
|---|---|---|---|---|---|
| | | | | EPA | DHA |
| Cholesterol (mmol/L) | | | | | |
| Baseline | 6.47 ± 0.21 | 6.20 ± 0.20 | 6.18 ± 0.18 | | |
| Postintervention | 6.22 ± 0.10 | 6.16 ± 0.11 | 6.34 ± 0.11 | −0.06 ± 0.15 (NS) | 0.11 ± 0.15 (NS) |
| Triacylglycerols (mmol/L) | | | | | |
| Baseline | 2.04 ± 0.19 | 2.01 ± 0.19 | 2.25 ± 0.40 | | |
| Postintervention | 1.95 ± 0.10 | 1.58 ± 0.10 | 1.50 ± 0.11 | −0.37 ± 0.14 (0.012) | −0.45 ± 0.15 (0.003) |
| LDL cholesterol (mmol/L) | | | | | |
| Baseline | 4.41 ± 0.19 | 4.28 ± 0.19 | 4.27 ± 0.17 | | |
| Postintervention | 4.31 ± 0.09 | 4.46 ± 0.10 | 4.64 ± 0.10 | 0.15 ± 0.13 (NS) | 0.34 ± 0.14 (0.019) |
| LDL particle size (nm) | | | | | |
| Baseline | 25.68 ± 0.14 | 25.64 ± 0.09 | 25.69 ± 0.11 | | |
| Postintervention | 25.72 ± 0.05 | 25.69 ± 0.05 | 25.96 ± 0.06 | 0.03 ± 0.07 (NS) | 0.25 ± 0.08 (0.002) |
| HDL cholesterol (mmol/L) | | | | | |
| Baseline | 1.12 ± 0.07 | 1.00 ± 0.04 | 0.96 ± 0.04 | | |
| Postintervention | 1.02 ± 0.02 | 0.99 ± 0.02 | 1.05 ± 0.02 | −0.03 ± 0.03 (NS) | 0.03 ± 0.03 (NS) |
| $HDL_2$ cholesterol (mmol/L) | | | | | |
| Baseline | 0.33 ± 0.05 | 0.25 ± 0.02 | 0.24 ± 0.03 | | |
| Postintervention | 0.26 ± 0.02 | 0.27 ± 0.02 | 0.33 ± 0.02 | 0.01 ± 0.02 (NS) | 0.07 ± 0.02 (0.004) |
| $HDL_3$ cholesterol (mmol/L) | | | | | |
| Baseline | 0.80 ± 0.03 | 0.74 ± 0.03 | 0.72 ± 0.03 | | |
| Postintervention | 0.76 ± 0.01 | 0.72 ± 0.02 | 0.72 ± 0.02 | −0.05 ± 0.02 (0.032) | −0.04 ± 0.02 (NS) |
| Glucose (mmol/L) | | | | | |
| Baseline | 4.95 ± 0.12 | 5.03 ± 0.09 | 5.15 ± 0.13 | | |
| Postintervention | 5.03 ± 0.08 | 5.24 ± 0.08 | 5.08 ± 0.09 | 0.21 ± 0.11 (0.062) | 0.05 ± 0.12 (NS) |
| Insulin (pmol/L) | | | | | |
| Baseline | 9.79 ± 1.24 | 8.78 ± 0.83 | 9.59 ± 0.99 | | |
| Postintervention | 8.76 ± 0.51 | 10.34 ± 0.52 | 11.38 ± 0.55 | 1.58 ± 0.73 (0.035) | 2.62 ± 0.74 (0.001) |

[1] $\bar{x}$ ± SEM. There were no significant differences between the 3 groups at baseline (by one-way ANOVA). Postintervention values were not adjusted. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] A general linear model was used to assess treatment effects on postintervention values adjusted for baseline values. Data represent the change and $P$ values for each of the 2 treatment groups relative to the olive oil group.

HDL-cholesterol ($r$ = 0.62, $P$ < 0.0001) concentrations. The change in LDL particle size from baseline to postintervention was inversely correlated with the change in triacylglycerols ($r$ = −0.35, $P$ = 0.009) and positively correlated with the change in HDL cholesterol ($r$ = 0.28, $P$ = 0.035). In regression analysis, DHA supplementation remained the strongest independent predictor of postintervention LDL particle size (adjusted $R^2$ = 0.776, $P$ = 0.002) after adjustment for baseline values and for the change in LDL particle size (adjusted $R^2$ = 0.216, $P$ = 0.023). DHA supplementation remained significant in regression models, which included changes in serum triacylglycerols and other lipids.

### Glucose and insulin

At baseline there were no significant differences between groups in fasting serum glucose or insulin concentration (Table 2). Postintervention, however, there were significantly different responses between the EPA and DHA groups (**Figure 6**). Olive oil did not change either fasting glucose or insulin. After adjustment for baseline values, there was a trend toward increased fasting glucose concentrations with EPA ($P$ = 0.062), but not with DHA (NS), relative to the control group. Both EPA and DHA significantly increased fasting insulin, by 18% ($P$ = 0.035) and 27% ($P$ = 0.001), respectively. DHA supplementation also significantly decreased the glucose-insulin ratio by 0.13 ± 0.05 ($P$ = 0.018).

## DISCUSSION

This study addressed whether purified EPA and DHA have different effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. We found that DHA, but not EPA, improved serum lipid status, in particular a small increase in HDL cholesterol and a significant increase in the $HDL_2$-cholesterol subfraction, without adverse effects on fasting glucose concentrations. Neither EPA nor DHA affected total cholesterol and both fatty acids reduced

**TABLE 3**
Total energy and macronutrient intakes at baseline and changes during the intervention in the 3 groups[1]

| | Olive oil (control) ($n$ = 20) | EPA ($n$ = 19) | DHA ($n$ = 17) |
|---|---|---|---|
| Total energy intake (kJ/d) | | | |
| Baseline | 10441 ± 588 | 9516 ± 677 | 10550 ± 588 |
| Change | −471 ± 497 | 82 ± 844 | −188 ± 421 |
| Total fat (% of energy) | | | |
| Baseline | 34.2 ± 1.2 | 30.9 ± 1.6 | 32.6 ± 1.6 |
| Change | −0.4 ± 1.3 | 2.8 ± 1.6 | 2.3 ± 1.2 |
| Fatty acids (% of energy) | | | |
| Saturated fat | | | |
| Baseline | 13.6 ± 0.8 | 12.1 ± 0.9 | 13.6 ± 0.9 |
| Change | 0.0 ± 0.6 | 1.2 ± 0.9 | 0.8 ± 1.0 |
| Monounsaturated fat | | | |
| Baseline | 12.2 ± 0.6 | 11.1 ± 0.8 | 11.5 ± 0.7 |
| Change | −0.2 ± 0.6 | 1.4 ± 0.8 | 0.3 ± 0.6 |
| Polyunsaturated fat | | | |
| Baseline | 5.0 ± 0.3 | 4.2 ± 0.3 | 4.6 ± 0.2 |
| Change | 0.0 ± 0.4 | 0.2 ± 0.4 | 0.9 ± 0.5 |
| Protein (% of energy) | | | |
| Baseline | 18.0 ± 0.5 | 19.8 ± 0.9 | 18.1 ± 0.6 |
| Change | −0.5 ± 0.6 | −0.9 ± 0.8 | 0.1 ± 0.7 |
| Carbohydrate (% of energy) | | | |
| Baseline | 42.1 ± 1.7 | 44.6 ± 1.7 | 41.8 ± 1.8 |
| Change | 1.9 ± 1.6 | −1.0 ± 1.5 | −1.2 ± 1.4 |
| Fiber (g/d) | | | |
| Baseline | 30.8 ± 2.9 | 27.4 ± 1.8 | 26.1 ± 1.1 |
| Change | −3.3 ± 2.3 | −2.8 ± 2.1 | −0.1 ± 1.7 |

[1] $\bar{x}$ ± SEM. Baseline measures were compared by one-way ANOVA. A general linear model was used to test for treatment effects on postintervention values adjusted for baseline value. There were no significant differences between the groups in any of the dietary nutrients at baseline and no significant changes during the intervention. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

triacylglycerols and increased fasting insulin concentrations to a similar extent. DHA supplementation significantly increased LDL cholesterol; however, this was associated with an increase in LDL particle size, which may represent a shift to a less atherogenic LDL particle.

Although numerous studies have examined the effect of n−3 fatty acids on serum lipids, glucose, and insulin (1–5, 20–22), few have assessed the independent effects of EPA and DHA. In vitro, both EPA (13, 33–35) and DHA (13, 35, 36) inhibit triacylglycerol synthesis and secretion. In rats, EPA lowered triacylglycerols, whereas DHA lowered cholesterol (10, 14, 15). These studies, however, used very high doses (1–2 $g \cdot kg^{-1} \cdot d^{-1}$) of fatty acids, equalling 12–24 g/d in humans.

In humans, n−3 fatty acids reduce triacylglycerols (4, 5, 37), with more variable effects on total cholesterol, LDL cholesterol, and HDL cholesterol (4, 5). These contradictory findings may be explained, in part, by variations in the amount of n−3 fatty acids consumed, the manner in which they are presented (fish, fish oils, or purified oils), and the lipoprotein phenotype of the patients. Our own studies have shown that the background dietary fat intake influences serum lipid responses to n−3 fatty acids (37).

Trials in humans using mixtures enriched in EPA and DHA have suggested different effects of the 2 fatty acids on serum lipids (38, 39). In a placebo-controlled study, 4 g EPA/d reduced triacylglycerols by 35% (40). It was also shown in a single-blind crossover study that EPA reduced triacylglycerols and VLDL cholesterol, increased LDL cholesterol and HDL cholesterol, but had no effect on total cholesterol (16). DHA did not affect cholesterol, triacylglycerols, VLDL cholesterol, LDL cholesterol, or HDL cholesterol, but increased the $HDL_2$-cholesterol subfraction and reduced the $HDL_3$-cholesterol subfraction (16). That study, however, had only a small number of subjects in the DHA group, was short in duration, and included only a 2-wk washout period between treatments (16).

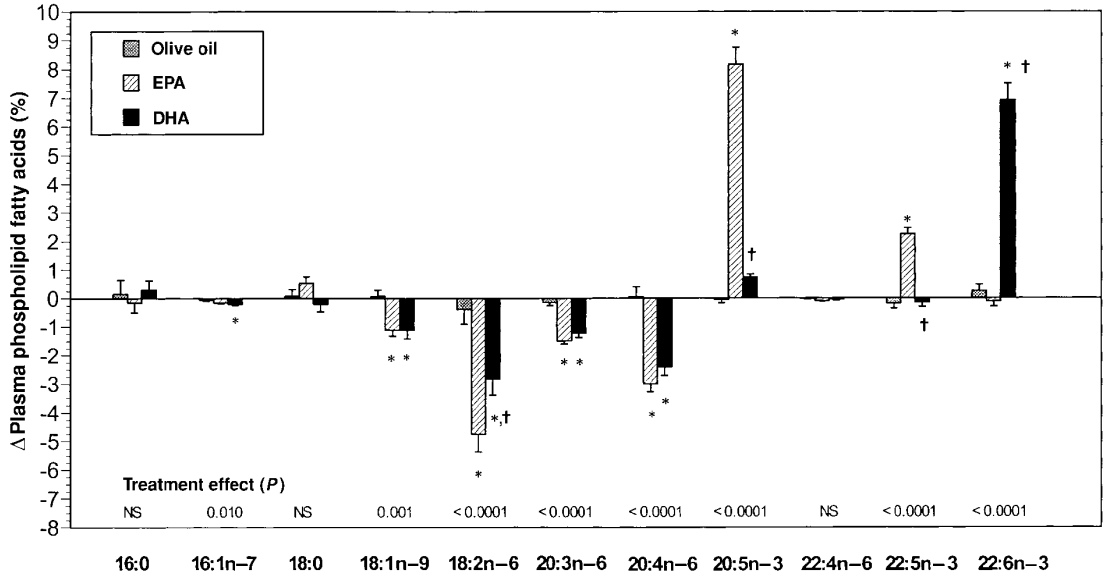


**FIGURE 1.** Mean (±SEM) changes in plasma phospholipid fatty acids from baseline to the end of the intervention in the olive oil (control; $n$ = 20), eicosapentaenoic acid (EPA; $n$ = 19), and docosahexaenoic acid (DHA; $n$ = 17) groups. ANOVA was used to assess treatment effects. *Significantly different from the olive oil group, $P < 0.01$. †Significantly different from the EPA group, $P < 0.01$.

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