

Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance

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Abstract Omega-3 fatty acids (FAs) reduce postprandial triacylglycerol (TG) concentrations. This study was undertaken to determine whether this effect was due to reduced production or increased clearance of chylomicrons. Healthy subjects ($n = 33$) began with a 4-week, olive oil placebo (4 g/d) run-in period. After a 4-week wash-out period, subjects were randomized to supplementation with 4 g/d of ethyl esters of either safflower oil (SAF), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA) for 4 weeks. Results for EPA and DHA were similar, and therefore the data were combined into one ω -3 FA group. Omega-3 FA supplementation reduced the postprandial TG and apolipoprotein B (apo B)-48 and apoB-100 concentrations by 16% ($P = 0.08$), 28% ($P < 0.001$), and 24% ($P < 0.01$), respectively. Chylomicron TG half-lives in the fed state were reduced after ω -3 FA treatment (6.0 ± 0.5 vs. 5.1 ± 0.4 min; $P < 0.05$), but not after SAF (6.9 ± 0.7 vs. 7.1 ± 0.7 min). Omega-3 FA supplementation decreased chylomicron particle sizes (mean diameter; 293 ± 44 vs. 175 ± 25 nm; $P < 0.01$) and increased pre-heparin lipoprotein lipase (LPL; 0.6 ± 0.1 vs. 0.9 ± 0.1 μ mol/h/ml; $P < 0.05$) activity during the fed state, but had no effect on postheparin LPL or hepatic lipase activities. The results suggest that ω -3 FA supplementation accelerates chylomicron TG clearance by increasing LPL activity, and that EPA and DHA are equally effective.—Park, Y. and W. S. Harris. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *J. Lipid Res.* 2003. 44: 455–463.

Supplementary key words eicosapentaenoic acid • docosahexaenoic acid • lipoprotein lipase

There is growing evidence that abnormal postprandial lipemia is associated with coronary artery disease (1–5). Thus, reduction of postprandial triacylglycerol (TG) concentration may be cardioprotective. Studies have shown that chronic ω -3 fatty acid supplementation significantly lowers postprandial TG concentrations regardless of the type of fat in test meal (6–10). Since the consumption of test meals containing fish oil or vegetable oil produce sim-

ilar rises in postprandial TG levels when subjects are consuming their usual background diets, fish oil itself is absorbed and cleared normally (11). There are only two mechanisms that can explain the reduced postprandial lipemia of chronic ω -3 fatty acid administration: slowed chylomicron secretion into the circulation or faster chylomicron clearance.

Several investigators reported that ω -3 fatty acids do not enhance lipoprotein lipase (LPL) or hepatic lipase (HL) activity measured in vitro in human postheparin plasma (11–14). It has also been shown that clearance of an intravenous bolus of lipid emulsion was not enhanced by fish oil feeding despite a reduction in the fasting TG concentration (9). On the other hand, others reported that ω -3 fatty acid supplementation increased pre- (15) and post-heparin (16) LPL activities. As regards slowed secretion, since ω -3 fatty acids retard hepatic secretion of VLDL TG (17), they might have the same effect on intestinal chylomicron secretion.

The objective of this study was to determine the extent of and mechanism responsible for reduced postprandial lipemia following supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in normal subjects. We tested the hypothesis that ω -3 fatty acids accelerate chylomicron TG clearance.

SUBJECTS AND METHODS

Subjects

Volunteers (age 21–70) with body mass indices of 22–30 kg/m², fasting serum LDL cholesterol (LDL-C) concentrations less than 160 mg/dl, HDL-C concentrations greater than 35 mg/dl, and TG concentrations less than 200 mg/dl were recruited. Individuals with known hepatic, renal or gastrointestinal diseases, lactose intolerance, or those taking medications known to affect lipid metabolism or fat absorption were excluded. Subject char-

Abbreviations: Chol, cholesterol; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acid; SAF, safflower oil; SFA, saturated fatty acid; TG, triacylglycerol.

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TABLE 1. Subject characteristics

| | SAF (n = 11) | EPA (n = 11) | DHA (n = 11) |
|--------------------------|--------------|--------------|--------------|
| Age (y) | 37 ± 3 | 41 ± 4 | 40 ± 5 |
| BMI (kg/m ²) | 26 ± 1 | 25 ± 1 | 27 ± 1 |
| Male/Female | 7/4 | 6/5 | 5/6 |

SAF, safflower oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Values are mean ± SEM.

acteristics are shown in **Table 1**. The study was approved by Saint Luke's Hospital Institutional Review Board, and informed, written consent was obtained from all participants.

Diets

Subjects were asked to refrain from fish (or fish oil) intake throughout the study and counseled to maintain a stable dietary and exercise pattern. Subjects were given low-fat meals (<30% energy from fat) the night before, and were asked to refrain from alcohol and strenuous exercise for 48 h before each study visit.

Protocol

This was a randomized, double-blind, parallel group, placebo-controlled study that began with a 4-week placebo (4 g/d of olive oil ethyl esters) run-in period to establish a baseline steady state. There was then a 4-week wash-out period followed by randomization to either safflower oil (SAF), ethyl esters (4 g/d), EPA ethyl esters (4 g/d; 95% pure), or DHA ethyl esters group (4 g/d; 95% pure) for 4 weeks. The oils were provided by the Fish Oil Test Material Program of the Department of Commerce and the NIH. Subjects reported to the Metabolic Research Unit on the morning after an overnight (12 h) fast. During the last week of the placebo and active treatment periods, oral fat tolerance tests (OFATs), intravenous fat clearance tests (IVFCT), and heparin injection tests for LPL activity were performed (**Fig. 1**).

Procedures

OFATs. Subjects consumed a test drink (1 g fat/kg body weight) made up of 87 parts of light whipping cream (half-and-half) and 13 parts of chocolate syrup. The supplements were not taken on the day of the test. Blood was drawn at 0, 2, 3, 4, 5, 7, and 9 h.

IVFCTs. This test measured chylomicron TG clearance in both the fasting and the fed states, assuming that clearance processes might be affected by physiological state. The IVFCTs were kinetic studies that required stable background chylomicron TG levels in both the fasting and the fed states. In order to establish steady state chylomicronemia in the fed state, subjects consumed a priming dose (350 mg fat/kg) of the test drink described above, and then 2 h later began to drink a small portion of the mixture every 15 min for the next 5.5 h. This regimen produced

| Treatment | Olive Oil | | | | SAF, EPA, or DHA | | | |
|---------------------------|-----------|----|----|----|------------------|----|----|----|
| | 1 | 22 | 25 | 28 | 57 | 78 | 81 | 84 |
| OFAT | | x | | | | | | x |
| IVFCT | | | x | | | | | x |
| Pre-heparin LpL (fed) | | x | | | | | x | |
| Pre-heparin LpL (fasting) | | | x | | | | | x |
| Post-heparin LpL | | | | x | | | | x |

Fig. 1. Timing of study procedures. × indicates the day of procedure. Olive oil was the placebo. SAF, safflower oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; OFAT, oral fat absorption test; IVFCT, intravenous fat clearance test; LpL, lipoprotein lipase.

an overall fat ingestion rate of 175 mg · kg⁻¹ · h⁻¹ (18, 19). As with the OFAT, no supplements were given on the day of the IVFCT.

Since ω-3 fatty acid supplementation reduces postprandial TG levels, and since chylomicron TG kinetics are influenced by background TG levels, it was important to achieve similar levels of chylomicronemia during the placebo and active treatment phases. Thus, the ingestion rate was adjusted for each individual based on the change (from placebo to active treatment) in the OFAT-TG area under the curve (AUC). For example, if the OFAT AUC was reduced by 10% from the placebo to the active treatment periods, the rate of fat ingestion during the active IVFCT (which was conducted 3 days after the OFAT) was increased by 10%, i.e., from 175 mg to 183 mg · kg⁻¹ · h⁻¹.

Once steady-state chylomicronemia was established, chylomicron TG clearance was measured by injection of a commercial lipid emulsion (see below) containing 4 μCi [³H]triolein. An intravenous sampling cannula was placed in a forearm vein and an injection cannulae in a contralateral vein; both were kept patent with infusions of 0.9% NaCl. The emulsion (containing 140 mg of TG) was injected once before (in the fasting state), and three times in the fed state (at 5, 6, and 7 h after ingestion of the priming dose) (19). Blood samples (6 ml each) were drawn at 0, 1, 3, 5, 7, 9, 13, 17, 20, and 30 min after each bolus injection.

Laboratory methods

Lipid emulsion. The emulsion was prepared and sterilized as described previously (18, 19). Briefly, The [9,10-³H(N)]triolein (30 μCi/200 μl in ethanol; American Radiolabeled Chemicals, St. Louis, MO) was added to 1 ml of 10% Liposyn® (Abbott Laboratory, Chicago, IL). The labeled emulsion was purified by HPLC (Millipore Waters, Milford, MA) with three size-exclusion columns (TOSO Haas, Montgomeryville, PA) in series to isolate a fraction containing TG-rich particles ~340 nm in diameter (about the size of human chylomicrons) (18, 19).

Fasting lipid profile. Plasma was analyzed for total cholesterol, TG, and HDL-C concentrations using a Cobas Fara II with enzymatic reagents from Boehringer Mannheim; VLDL and LDL-C concentrations were estimated by the Friedewald equation (20). Whole plasma and the d > 1.006 g/ml TG concentrations were used to calculate VLDL-TG concentrations. Ongoing quality control was provided by the CDC NIH lipid standardization program and the Excel program from Pacific Biometrics in Seattle, WA.

Fasting plasma phospholipid (PL) and chylomicron TG and PL were determined by gas chromatography of their constituent fatty acids (GC; GC9A, Shimadzu, Columbia, MD) as described previously (18). Glyceryltriheptadecanoate and diheptadecanoylphosphatidylcholine were added as TG and PL internal standards, respectively. Triacylglycerol and PL were separated by TLC and methylated in boron trifluoride (BF₃) methanol-benzene. Fatty acid methyl esters were analyzed with a 30 m SP2330 capillary column (Supelco, Bellefonte, PA). Fatty acids were identified by comparison with known standards.

Triacylglycerol-rich lipoprotein isolation. Plasma (2 ml) was layered below 1 ml of saline and spun in a TLA 100.3 rotor for 2 h at 100,000 rpm in a Beckman TL-100 ultracentrifuge (21). The top 0.4 ml of TG-rich lipoprotein fraction was collected by aspiration.

Chylomicron isolation. Plasma (2 ml) was layered below 8 ml of distilled water and spun in a SW41 rotor for 30 min at 25,000 rpm in a Beckman L7-65 ultracentrifuge as previously described (18, 19). This procedure was repeated two more times to remove as much VLDL as possible from the chylomicron fraction.

Radioactivity of chylomicrons. Chylomicron fractions were transferred to vials containing 10 ml Opti-fluor (Packard, Meriden, CT) and counted in a Wallac 1410 liquid scintillation counter (Pharmacia, Gaithersburg, MD) using appropriate quench

curves established. Background counts at time zero were subtracted from each data point for each of the four injections.

ApoB-48 and apoB-100. Triacylglycerol-rich lipoprotein fractions (1.2 ml) prepared from 6 ml plasma were delipidated with 12 ml of ethanol-ether (3:1, v/v) overnight (22). Sample preparation and gel electrophoresis were performed as described previously (18). Briefly, the precipitated pellet was solubilized in a buffer containing sodium dodecylsulfate, dithiothreitol, mercaptoacetate, and bromophenol blue, and heated for 3 min at 100°C. The protein concentration of the sample was estimated using sample TG concentration (0.1 mg protein/mg TG) and then adjusted to ~1 mg/ml with sample buffer (23). Samples were loaded on 3–10% linear polyacrylamide slab gels; one gel was run for each OFAT. Electrophoresis was carried out at a constant voltage of 50 volts per gel for the first 30 min and then at 75 volts per gel for 90 min (SX 250/260, Hoefer, San Francisco, CA). ApoB-48 and apoB-100 were quantified by densitometric scanning (Sharp JX-330, Pharmacia Biotech, Japan) against a standard curve of apoB-100 prepared from narrow-cut LDL (1.03–1.04 g/ml). The chromogenicities of human apoB-48 and apoB-100 have been shown to be equal (24).

Preheparin plasma lipase assay. Lipase activity was measured by incubating plasma with emulsified triolein and then determining the amount of oleic acid liberated. Blood was collected into heparinized tubes, and the plasma was separated and stored at –80°C. Substrate was prepared fresh daily with 200 mg triolein and 5.68 ml of 90 g/l gum arabic in 50 mmol/l NH₄OH-NH₄Cl (buffer; pH 8.5) by sonication (series 4710, Cole-Parmer Instrument Co., Chicago, IL). Then, 1.375 ml of 200 g/l BSA in buffer and 1.375 ml of the internal standard solution were added to the mixture. The internal standard solution was made in advance as follows: Heptadecanoic acid (13.525 mg) was dissolved in 10 ml of methanol and 1 ml of 10 mol/l ammonium hydroxide, and then dried under nitrogen. Bovine serum albumin (20 ml of 200 g/l BSA in buffer) was added and the mixture was sonicated at amplitude of 40 for 2 h in an ice bath.

Plasma (100 µl), 20 µl of 880 mmol/l SDS solution, 180 µl of buffer, and 0.5 ml of substrate were added. The blank contained 50 mg of NaCl and no SDS to inhibit lipase activity (25, 26). The mixtures were vortexed and incubated for 2 h at 28°C. Triacylglycerol hydrolysis reaction was terminated by adding 5.33 ml of methanol-chloroform-heptane solution (5.6:5.0:4.0, v/v/v) and 1.5 ml of 0.1 mol/l carbonate-bicarbonate buffer in 1 mol/l NaCl (pH 10.5). After shaking and centrifugation, the supernatant (containing nonesterified fatty acids) was transferred, and 0.5 ml of 0.5 mol/l HCl and 3 ml of hexane were added. The mixture was shaken vigorously and centrifuged at 3,000 rpm for 45 min. The supernatant was transferred and dried under nitrogen. Samples were methylated by adding 1 ml of BF₃ and heating at 100°C for 3 min. The methylated fatty acids were extracted by adding 2 ml of distilled water and 2 ml of hexane. The supernatants were dried under nitrogen and analyzed by gas chromatography (injection temperature, 200°C; oven temperature, 210°C) with a 30 m SP2330 capillary column (Supelco). The amount of liberated oleic acid was determined (after subtracting appropriate blanks) and activity was expressed as µmol oleic acid released/h/ml plasma. This assay was found to be linear with time, substrate, and plasma enzyme concentration, and inhibited by known LPL inhibitors such as NaCl, guanidine HCl, protamine sulfate, paraoxon, and tetrahydrolipstatin (unpublished observations).

Postheparin plasma lipoprotein and HL assays. Blood was drawn 15 min after the injection of heparin (100 IU/kg body weight). The substrate described above was added to 20 µl of postheparin plasma mixed with buffer (1:1, v/v), 80 µl of human serum (as a source of apo C-II), and 200 µl of buffer. For the

blank, 50 mg NaCl, 20 µl of 880 mmol/l SDS solution, and 180 µl of buffer were used instead of 200 µl of buffer. For the HL assay, 3.6 mol/l NaCl solution was used instead of buffer. The rest of the procedure was the same as described for the preheparin samples.

Calculations

Chylomicron TG half-lives ($t^{1/2} = 0.693/k$) were estimated from the monoexponential radiolabeled lipid disappearance curves ($y = \alpha e^{-kx}$; Microsoft Excel, version 4.0) (19). Chylomicron mean diameters were estimated from the TG-PL ratio as described previously (27).

$$\text{Mean diameter} = 0.4626 \cdot ([0.211 \cdot (\text{TG/PL})] + 0.27) \cdot 100 + 7.2936$$

Statistical analysis

We used ANCOVA (controlling for baseline values) to compare lipid profile, plasma and chylomicron fatty acid composition, TG, apo B-100 and apo B-48 AUC, tracer-determined chylomicron half-lives, chylomicron TG and PL concentrations, chylomicron diameters, and lipase activities. The relationship between half-lives and plasma TG concentrations was analyzed using linear regression. A two-tailed *P* value of < 0.05 was required for statistical significance.

RESULTS

Fasting lipid profile

There was no significant effect of treatment on fasting plasma TG, and total, HDL, LDL, and VLDL-C concentration (Table 2). The effects of treatment on the fasting PL fatty acid composition are presented in Table 3. EPA treatment significantly increased EPA, docosapentaenoic acid (DPA), and total ω-3 fatty acid concentrations, and decreased linoleic acid, eicosatrienoic acid, arachidonic acid (AA), and total ω-6 fatty acids. DHA treatment significantly increased EPA, DHA, and total ω-3 fatty acid concentrations, and decreased linolenic acid, AA, and total ω-6 fatty acids.

Oral fat absorption test

DHA and EPA reduced (but not significantly) the TG-AUC in the OFAT (vs. baseline) by 17% and 15%, respectively as compared with SAF (4%) (Fig. 2). EPA and DHA significantly decreased OFAT apoB-48 and apoB-100 concentrations compared with SAF, and the reduction was greater at the later hours (Figs. 3, 4).

TABLE 2. Fasting lipid profile

| | Olive | SAF | Olive | EPA | Olive | DHA |
|-----------|----------|----------|---------|---------|----------|----------|
| | mg/dl | | | | | |
| TG | 107 ± 14 | 106 ± 13 | 74 ± 5 | 67 ± 6 | 90 ± 9 | 82 ± 8 |
| Chol | 172 ± 5 | 175 ± 6 | 163 ± 9 | 166 ± 8 | 166 ± 10 | 169 ± 11 |
| HDL-Chol | 42 ± 3 | 42 ± 3 | 45 ± 3 | 45 ± 4 | 50 ± 4 | 52 ± 4 |
| LDL-Chol | 109 ± 6 | 112 ± 6 | 103 ± 7 | 108 ± 6 | 97 ± 8 | 102 ± 9 |
| VLDL-Chol | 21 ± 3 | 21 ± 3 | 15 ± 1 | 13 ± 1 | 18 ± 2 | 16 ± 2 |

TG, triacylglycerol; Chol, cholesterol. Values are mean ± SEM. n = 11 in each group. Olive oil was used in the placebo run-in, baseline period whereas SAF (control), EPA and DHA were given during the treatment period.

TABLE 3. Fasting plasma phospholipid fatty acid composition

| | Olive | SAF | Olive | EPA | Olive | DHA |
|------------------|------------|------------|------------|-------------------------|------------|-------------------------|
| 14:0 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 |
| 16:0 | 27.5 ± 0.8 | 27.4 ± 0.7 | 28.8 ± 0.9 | 28.5 ± 0.9 | 27.6 ± 2.8 | 27.7 ± 2.7 |
| 16:1 ω -7 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 |
| 18:0 | 15.4 ± 0.4 | 16.0 ± 0.5 | 14.8 ± 0.3 | 15.4 ± 0.4 | 13.8 ± 1.4 | 13.8 ± 1.3 |
| 18:1 ω -9 | 10.1 ± 0.9 | 8.8 ± 0.6 | 10.9 ± 0.4 | 9.4 ± 0.4 | 9.6 ± 1.1 | 8.5 ± 1.0 |
| 18:2 ω -6 | 26.6 ± 1.3 | 27.4 ± 1.2 | 26.3 ± 1.1 | 22.0 ± 1.3 ^a | 24.0 ± 2.3 | 22.8 ± 2.1 |
| 18:3 ω -3 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.1 ± 0.0 ^a |
| 20:3 ω -6 | 3.3 ± 0.3 | 3.2 ± 0.4 | 3.1 ± 0.3 | 2.0 ± 0.2 ^a | 3.0 ± 0.4 | 2.3 ± 0.3 ^a |
| 20:4 ω -6 | 11.1 ± 1.0 | 11.5 ± 1.0 | 10.6 ± 0.5 | 9.1 ± 0.5 ^a | 9.2 ± 1.1 | 7.7 ± 0.8 ^a |
| 20:5 ω -3 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 6.2 ± 0.5 ^a | 0.4 ± 0.0 | 1.0 ± 0.2 ^a |
| 22:5 ω -3 | 1.6 ± 0.2 | 1.6 ± 0.2 | 1.5 ± 0.1 | 3.7 ± 0.3 ^a | 1.2 ± 0.1 | 0.9 ± 0.1 |
| 22:6 ω -3 | 2.7 ± 1.0 | 2.4 ± 0.2 | 2.4 ± 0.1 | 2.6 ± 0.2 | 2.0 ± 0.2 | 6.6 ± 0.6 ^a |
| SFA | 43.3 ± 1.0 | 43.9 ± 0.9 | 44.0 ± 1.1 | 44.2 ± 1.1 | 41.8 ± 4.0 | 42.0 ± 3.7 |
| MUFA | 11.0 ± 1.0 | 9.6 ± 0.6 | 11.7 ± 0.4 | 10.2 ± 0.4 | 10.5 ± 1.1 | 9.2 ± 1.0 |
| ω -6 PUFA | 41.0 ± 0.7 | 42.1 ± 0.6 | 40.0 ± 1.0 | 33.1 ± 1.4 ^a | 36.1 ± 3.4 | 32.7 ± 2.9 ^a |
| ω -3 PUFA | 4.8 ± 0.5 | 4.4 ± 0.3 | 4.4 ± 0.5 | 12.6 ± 0.8 ^a | 3.7 ± 0.4 | 8.5 ± 0.9 ^a |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values are mean ± SEM. n = 11 in each group.

^a The change from olive to SAF is different ($P < 0.05$) from the change from olive to EPA or DHA.

Intravenous fat clearance test

As described in Subjects and Methods, fat ingestion rates were increased during the second IVFCT in proportion to the decrease in OFAT AUC for each subject in an attempt to achieve similar steady-state plasma TG concentrations pre- and post-treatment. The fat intake rates during the second IVFCT were increased from 175 to 190 ± 7, 207 ± 10 and 210 ± 10 mg · kg⁻¹ · h⁻¹ in the SAF, EPA,

and DHA treatment groups, respectively. This approach proved successful for the SAF and DHA groups, but steady-state fed TG levels were slightly lower with EPA treatment (Fig. 5). The rate of disappearance of chylomicron [³H]triolein was significantly increased by EPA and DHA supplementation (Fig. 6). Tracer-determined chylomicron TG half-lives in the fed state (average of all three

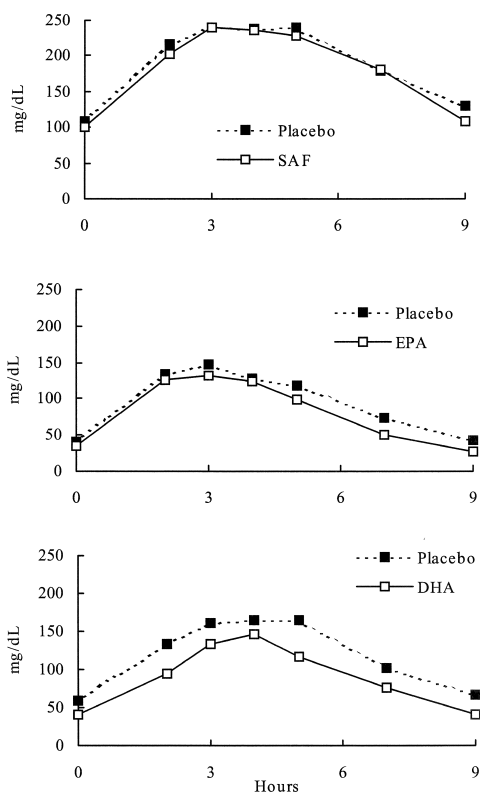


Fig. 2. Triacylglycerol (TG) concentrations of TG-rich lipoprotein fraction during the oral fat absorption test. Each point represents data from 11 subjects.

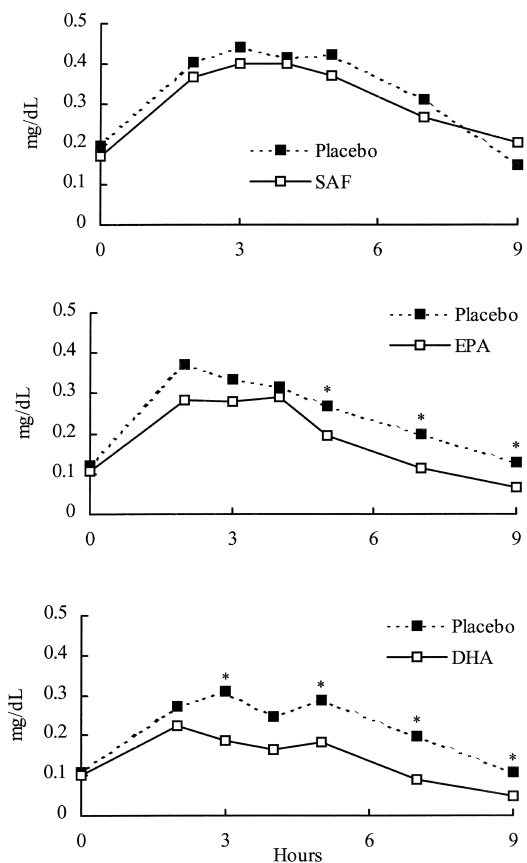


Fig. 3. ApoB-48 concentrations during the oral fat absorption test. Subject numbers are as in Fig. 2. * $P < 0.05$.

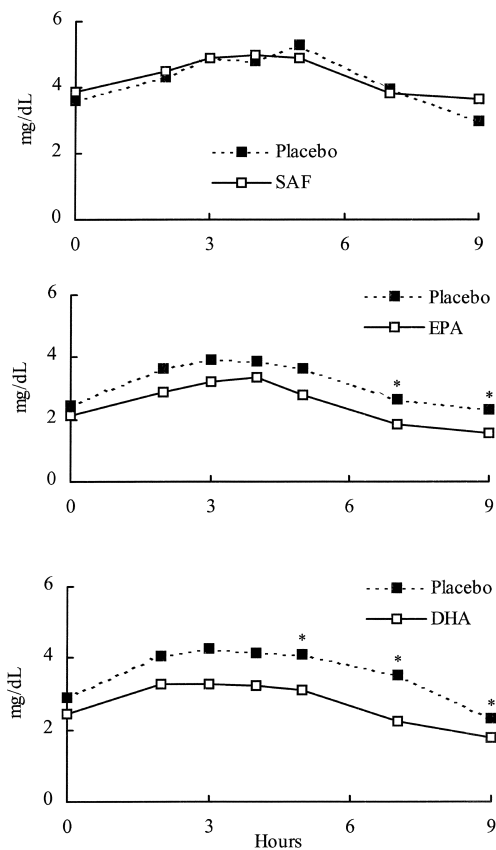


Fig. 4. ApoB-100 concentrations during the oral fat absorption test. Subject numbers are as in Fig. 2. * $P < 0.05$.

tests) were significantly ($P < 0.01$) shorter after EPA (6.1 ± 0.8 vs. 5.2 ± 0.7 min) and DHA (5.9 ± 0.7 vs. 4.9 ± 0.5 min) treatment than SAF (6.9 ± 0.7 vs. 7.1 ± 0.7 min). In contrast, supplementation had no effect on chylomicron TG half-lives during the fasting state (EPA, 4.6 ± 0.3 vs. 4.4 ± 0.3 min; DHA, 4.7 ± 0.4 vs. 4.6 ± 0.7 min; and SAF, 5.3 ± 0.5 vs. 5.2 ± 0.5 min). As expected, chylomicron TG half-lives were significantly longer during the fed than the fasting states (6.0 ± 0.4 vs. 4.8 ± 0.3 min; $P < 0.01$), regardless of treatment. There was a significant positive correlation between chylomicron TG half-lives and plasma TG concentrations during the fasting and the fed states (Fig. 7), although the relationship was stronger during the latter. There was virtually no carry over of chylomicron radioactivity from one clearance test to the next.

Chylomicron size

Chylomicron PL concentrations were not significantly different before and after treatments, but chylomicron TG tended to be reduced by EPA and DHA. Mean chylomicron diameters decreased by 53% ($P < 0.01$) and 24% ($P < 0.05$) with EPA and DHA, respectively, compared with SAF (Table 4).

Lipase activities

Preheparin LPL activities during the fed state increased after DHA (47%; $P < 0.05$) and EPA (73%; $P < 0.05$) treatments compared with SAF, but no change was de-

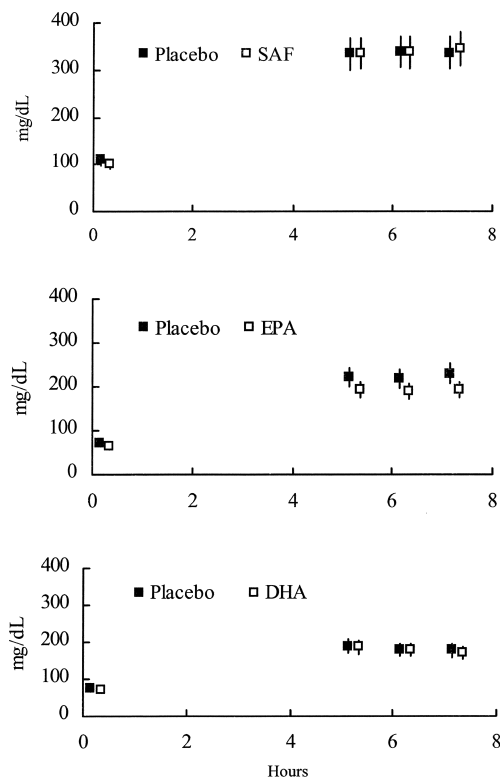


Fig. 5. TG concentrations during the IVFCTs. Each subject had four IVFCTs to measure chylomicron TG kinetics (once fasting and three times in the fed state) at the end of the both the placebo and active treatment periods. The values represent the average of ten measurements taken over 30 min after injection of the tracer. Each pair of symbols reflects the average (\pm SEM) TG levels at 0 h (fasting) and at 5, 6, and 7 h of constant feeding. Subject numbers are as in Fig. 2.

TECTED in the fasting state (Fig. 8). Postheparin LPL activities did not significantly differ pre- vs. postsupplementation with EPA (9 ± 1 vs. 10 ± 1 $\mu\text{mol/h/ml}$), DHA (12 ± 1 vs. 14 ± 1 $\mu\text{mol/h/ml}$), or SAF (8 ± 1 vs. 9 ± 1 $\mu\text{mol/h/ml}$). There was no significant effect of supplementation on postheparin HL activities (EPA, 11 ± 2 vs. 10 ± 1 ; DHA, 10 ± 2 vs. 11 ± 2 ; SAF, 19 ± 2 vs. 18 ± 2 $\mu\text{mol/h/ml}$).

DISCUSSION

Fish oil supplementation lowers not only fasting (17) but also postprandial TG concentrations, regardless of the nature of the fat in the test meal (6, 8, 11, 12, 28, 29). Since clinically significant fat malabsorption has not been observed with fish oil supplementation, the most likely explanation for the reduced postprandial chylomicronemia is accelerated chylomicron clearance. Indeed, fat absorption was not reduced (30, 31) and chylomicron lipids were cleared faster in fish oil-fed rats than in controls (32). However, the enhanced clearance hypothesis has never been directly tested in humans.

In this study, chylomicron clearance was determined by injecting a trace amount of radiolabeled lipid emulsion

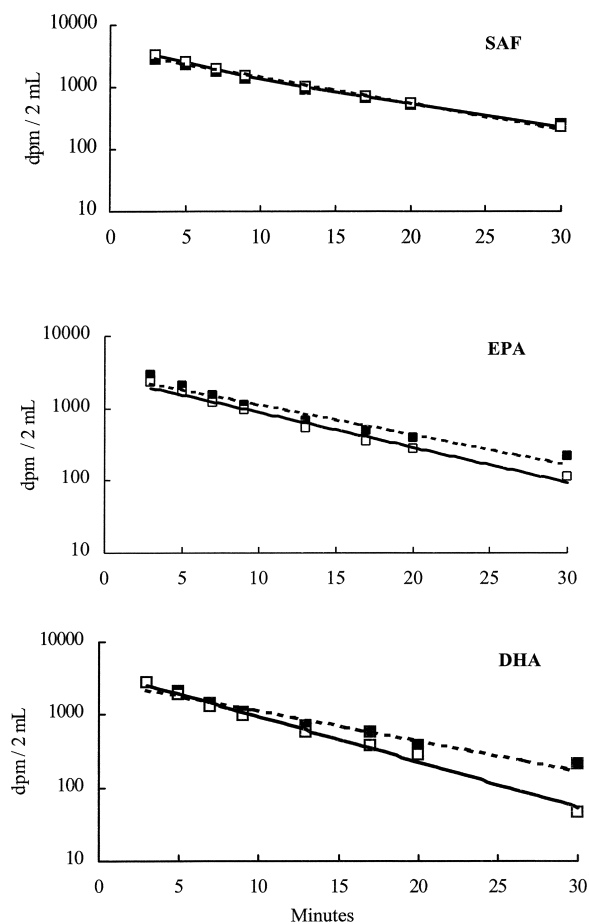


Fig. 6. Clearance curves of [^3H]triolein-labeled lipid emulsion from chylomicron fraction measured during the fed state (each point is the average of three tests administered at 5, 6, and 7 h of constant feeding). Subject numbers and symbols as in Fig. 2.

previously shown to track native chylomicrons in humans (18, 19). We observed that in the fed state, EPA and DHA supplementation significantly reduced chylomicron TG half-lives and particle sizes, and increased preheparin LPL activities during the fed state. These findings were all consistent with the hypothesis that fish oils accelerate human chylomicron TG clearance, and that they do so by facilitating LPL-mediated lipolysis. The possibility that fish oil enhances non-LPL-mediated clearance by tissues such as spleen, muscle, adipose tissue, or kidney cannot be excluded, however (16, 33).

There has been evidence both for and against the enhanced clearance hypothesis. Fish oils are known to reduce fasting TG concentration by inhibiting hepatic VLDL-TG production (17). In human kinetic studies, fish oils slow hepatic VLDL production rate (34–37) and reduce apoB-100 secretion from HepG2 cells (38). Since they also lower TG synthesis in and secretion from CaCo-2 cells (39), it is not unreasonable to hypothesize that ω -3 fatty acids might reduce TG secretion from the intestine into the blood. However, the major TG synthesizing pathway in CaCo-2 cells is the 3-glycerol-phosphate pathway (40), not the monoacylglycerol pathway that predomi-

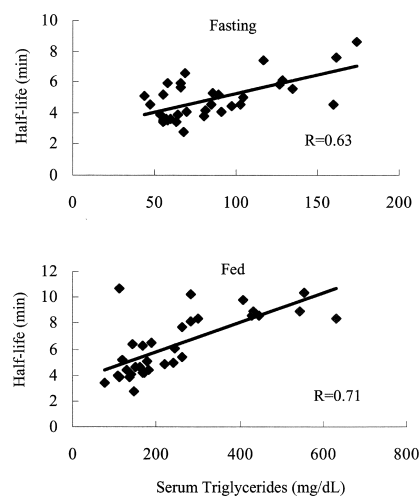


Fig. 7. The relationship between chylomicron TG half-lives and plasma TG concentration during the fasting (top) and the fed (bottom) states at the end of the placebo phase. $P < 0.001$ for both. $n = 33$.

nates in enterocytes postprandially (41) so the CaCo-2 findings may not reflect normal physiology. Fish oil feeding did not slow TG absorption or chylomicron secretion in rats (32) or secretion from rabbit enterocytes ex vivo (42). Together, these data suggest that fish oils do not affect intestinal chylomicron assembly and secretion as they do hepatic VLDL assembly.

Previously, Harris et al. (32) reported that fish oil feeding accelerated the removal of chylomicron TG in rats even though fasting TG levels were not reduced, much as observed here. However, in a previous study from our lab, the clearance of Intralipid (20%; 8 g of fat injected in the fasting state) was unaltered by fish oil supplementation despite a 24% reduction in fasting TG concentrations (9). Likewise, in the present study, ω -3 fatty acid supplementation did not affect the clearance of a trace amount of labeled lipid emulsion (140 mg of fat) in the fasting state. Clearance was, however, accelerated in the fed state. Since fasting TG clearance was not affected but fed clearance was, the ω -3 effect appears to be modulated by the physiological state (see below).

Fish oil supplementation has been shown to decrease the PL-TG ratio in the chylomicron particles, indicating reduced chylomicron size (9). This was observed in the present study and is consistent with increased fed-state LPL activity, since chylomicrons isolated from venous blood have already been processed to some degree by capillary LPL. We cannot, however, rule out the possibility that ω -3 fatty acid supplementation caused the intestine to secrete smaller chylomicrons.

The majority of studies have shown that fish oils do not increase postheparin LPL or HL activity measured in vitro in fasting plasma from humans (11–14, 43), rats (44), chickens (45), or pigs (46). However, we (15) reported that fish oil increased preheparin LPL activities of fasting plasma samples in healthy subjects and hypertriglyceridemic patients in a setting where fasting TG concentra-

TABLE 4. TG and PL concentrations, and mean diameters of chylomicrons obtained during the steady-state chylomicronemia of intravenous fat tolerance test

| | PL | | TG | | Mean diameter | |
|-----|-----------|-----------|--------------|------------|---------------|-----------------------|
| | Olive | Treatment | Olive | Treatment | Olive | Treatment |
| | | | <i>mg/dl</i> | | <i>nm</i> | |
| SAF | 1.0 ± 0.1 | 0.9 ± 0.1 | 26.8 ± 4.6 | 25.1 ± 6.2 | 365 ± 52 | 347 ± 47 |
| EPA | 0.8 ± 0.2 | 0.8 ± 0.1 | 18.2 ± 4.2 | 10.0 ± 2.4 | 319 ± 53 | 149 ± 20 ^a |
| DHA | 0.7 ± 0.1 | 0.5 ± 0.1 | 17.1 ± 3.7 | 9.1 ± 1.9 | 266 ± 35 | 201 ± 29 ^a |

PL, plasma phospholipid. Values are mean ± SEM. *n* = 11 in each group.

^a The change from olive to SAF is different (*P* < 0.05) from the change from olive to EPA or DHA.

tions were reduced by 18% and 36%, respectively. Khan et al. (16) found that fish oils increased postheparin LPL activity (at 5 min but not at 15 min postinjection) and levels of LPL mRNA in adipose tissue of subjects with an atherogenic lipoprotein phenotype. In the present study, we found that EPA and DHA supplementation had no effect on pre- or postheparin LPL (at 15 min) or HL activity in the fasting state. Consistent with this finding, ω -3 fatty acids did not reduce fasting TG concentrations. [This is not uncommon in normolipidemic humans, occurring in about half of placebo-controlled trials (47).]

Chylomicrons (48) and lipid emulsions (49) are cleared more slowly in patients with elevated VLDL concentrations (likely because of increased competition for LPL), and reductions in endogenous VLDL concentrations accelerate chylomicron clearance (50). Therefore, fish oil-induced lowering of postprandial TG concentrations could be indirectly caused by diminished background VLDL concentrations. In the present study, neither EPA nor DHA significantly reduced fasting VLDL-C levels, but both reduced VLDL-apoB-100 levels in the fed state. Although it is possible that reduced postprandial competi-

tion from hepatic particles played a role in the enhanced chylomicron TG clearance, this seems unlikely for two reasons: 1) at plasma TG concentrations below 200 mg/dl, LPL is not saturated (51, 52), and 2) LPL prefers chylomicrons over VLDL as substrates (53). Thus, at these low TG levels, if competition was a factor, one would expect that VLDL clearance would suffer, not chylomicron clearance. As expected, chylomicron TG half-lives rose as background TG concentrations increased (Fig. 7). Thus, it is possible that the small decreases in background TG levels in the EPA group (215 mg/dl to 192 mg/dl) could have explained the shorter chylomicron half-lives observed during the constant-feeding IVFCT. However, the 23 mg/dl decrement would only be expected to reduce half-lives by 4.5% (based on data shown in Fig. 7), not the observed 17%. Therefore, it appears unlikely that the enhanced chylomicron TG clearance seen with ω -3 fatty acid supplementation resulted simply from reduced competition from VLDL.

There is growing evidence that EPA and DHA may have different effects on lipid metabolism. EPA was reported to be primarily responsible for the hypotriglyceridemic effect of ω -3 fatty acids in vitro (38), in rats (54–56), and in humans (57). Rambjor et al. (58) reported that EPA reduced TG concentration in humans but DHA did not. On the other hand, a hypotriglyceridemic effect of DHA has been reported in healthy subjects (59) and in patients with combined hyperlipidemic (60). Hansen et al. (61) found EPA to be less potent than DHA at reducing postprandial TG, but neither affected fasting TG concentrations. Grimsgaard et al. (62) and Mori et al. (63) observed significant reductions in fasting TG concentrations with both EPA and DHA. Taken together, these data suggest that the effects of EPA and DHA on plasma TG concentrations were not markedly different. Further studies are needed to determine whether EPA and DHA in combination (as they appear naturally in most fish oils) have a synergistic effect on reducing TG concentration (9, 11, 12).

We found that ω -3 fatty acids increased LPL activities and accelerated TG clearance only during the fed state. LPL is known to be stimulated in the fed state (64), presumably by insulin (65, 66). The extent of this stimulation can be modified by the presence of dietary fats (67); thus ω -3 fatty acids may, in some unknown manner, amplify the stimulatory effect of insulin on LPL. Second, feeding is

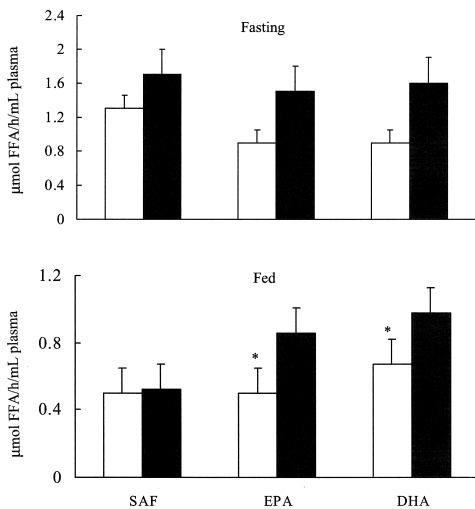



Fig. 8. Preheparin lipoprotein lipase activities during the fasting (top) and the fed (bottom) states. Open bars, placebo; filled bars, active treatment. Values are mean ± SEM. Subject numbers are as in Fig. 2. Abbreviations as in Fig. 1. **P* < 0.05 comparing the change in the SAF group to the change in the EPA and DHA groups.

known to affect blood flow to adipose tissue and skeletal muscle (68, 69). It is possible that ω -3 fatty acids, by virtue of their enhancement of endothelial function and vascular sensitivity to vasodilators (70–73), may allow for increased muscle and adipose tissue blood flow postprandially. Such an effect could expose chylomicrons to an increased surface area of LPL-rich capillary endothelium and thereby accelerate LPL-mediated clearance. Another possible explanation of our finding relates to the effect of ω -3 fatty acids on peroxisome proliferator-activated receptor γ (PPAR γ). Chambrier et al. (74) reported that adipose tissue mRNA levels for this transcription factor were positively correlated with plasma EPA concentrations. Since PPAR γ has been shown to increase LPL activity via a direct transcriptional effect on the LPL promoter (75), this mechanism may at least partly explain the increased LPL activity observed with ω -3 fatty acids.

In conclusion, supplementation with EPA or DHA accelerates human chylomicron TG clearance, apparently by increasing LPL-mediated lipolysis. Uncovering the mechanism responsible will require further investigation. 

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