Influence of n−3 fatty acid supplementation on the endogenous activities of plasma lipases1–3

William S Harris, Gouping Lu, Gro S Rambjor, Ann I Wilén, Joseph A Ontko, Qi Cheng, and Sheryl L Windsor

ABSTRACT The aim of these studies was to explore the possibility that enhanced triacylglycerol clearance may contribute to the hypotriacylglycerolemic effect of n−3 fatty acids in humans. Healthy subjects (n = 20) and hypertriacylglycerolemic patients (n = 6) were given a placebo (olive oil, OO) or a fish-oil concentrate (FOC; 41% eicosapentaenoic acid and 23% docosahexaenoic acid) in two, independent, randomized, blind trials. For the healthy subjects, the FOC treatment period was 3 wk long and FOC intakes were 5 g/d. For the patients, treatment periods were 4 wk long and dosages were 5 g · 70 kg body wt−1 · d−1. Washout periods were 2–4 wk for both groups. Blood samples were drawn at the end of each phase and analyzed for plasma lipids, lipoproteins, and endogenous (nonheparin-stimulated) activities of lipoprotein lipase (LPL) and hepatic lipase (HL). In the healthy subjects the FOC decreased plasma triacylglycerol concentrations by 18% (P < 0.01), whereas in the patients concentrations were reduced by 35% (P < 0.05). Low-density-lipoprotein-cholesterol concentrations increased by 25% in the latter group (P = 0.06). FOC increased the endogenous activities of LPL and HL by 62% and 68%, respectively (P < 0.0001), in the healthy subjects, but only LPL in the patients (65%, P < 0.005). These data suggest that endogenous lipase activities may be altered by nutritional interventions, and further, that accelerated lipolysis could contribute, at least in part, to the observed effects of n−3 fatty acids on human lipoprotein metabolism. Ann J Clin Nutr 1997;66:254–60.

KEY WORDS Plasma phospholipid fatty acids, lipoprotein lipase, hepatic lipase, eicosapentaenoic acid, docosahexaenoic acid, humans, n−3 fatty acids

INTRODUCTION

It has long been known that fish oils lower triacylglycerol concentrations by ∼25–35% (1–4). Several studies have suggested that this is caused by reduced hepatic triacylglycerol output (5, 6) and not by accelerated clearance of very-low-density-lipoprotein (VLDL) triacylglycerol. n−3 Fatty acid feeding does not increase the activities of lipoprotein lipase (LPL) or hepatic lipase (HL) in postheparin plasma (7–9), nor does it accelerate the clearance of intravenous lipid emulsions (10). On the other hand, there is recent evidence that dietary n−3 fatty acids accelerate chylomicron triacylglycerol clearance in rats (11), and the frequent increases in low-density-lipoprotein (LDL) cholesterol seen in humans fed fish oil (4) may result from increased conversion of VLDL to LDL, which is lipolytically mediated. Even though LPL and HL activities in postheparin plasma are usually not altered by n−3 fatty acid supplementation, the effect of these fatty acids on endogenous, nonheparin-stimulated LPL and HL activities has not been evaluated. If they were to be increased, it would suggest that n−3 fatty acids enhance lipolytic capacity in vivo.

SUBJECTS AND METHODS

Subjects

Two separate studies were conducted, one in healthy subjects and another in hypertriacylglycerolemic patients. The healthy subjects (n = 20) were recruited from the University of Kansas Medical Center staff and student population. They were normolipidemic but with triacylglycerol concentrations in the higher end of normal (50th-90th percentile for their age and sex). The type IV patients (n = 6) were recruited from the Lipid Clinic population at the Medical Center. None of the patients was taking any medicines known to affect lipid metabolism. The characteristics of the study groups are shown in Table 1. Each participant gave informed consent before beginning the trials, which had been approved by the Human Subjects Committee of the Medical Center.

Supplements

A fish-oil concentrate (FOC) and an olive oil (OO) placebo were provided as ethyl esters in 1-g capsules by the Biomedical Test Materials Program, National Marine Fisheries Service Charleston Laboratory, Charleston, SC. The FOC contained 41% eicosapentaenoic acid (EPA) and 23% docosahexaenoic acid (DHA) (Table 2). Each capsule contained 3 mg vitamin E and 3 mg cholesterol.
TABLE 1  
Characteristics of the study groups

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Healthy subjects (n = 13 M, 7 F)</th>
<th>HTAG patients (n = 4 M, 2 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 ± 9</td>
<td>52 ± 11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 2.7</td>
<td>27.7 ± 3.7</td>
</tr>
</tbody>
</table>

Screening lipids (mmol/L)  
Cholesterol: 4.41 ± 0.53, 5.88 ± 1.01  
Triacylglycerol: 1.44 ± 0.34, 5.53 ± 0.84  
HDL cholesterol: 1.23 ± 0.33, 1.04 ± 0.31  
LDL cholesterol: 2.44 ± 0.70, 2.67 ± 0.75

Protocol

In both studies, participants were randomly assigned to receive the FOC or the OO placebo in a single-blind, randomized crossover design. Dosages for the healthy subjects were 5 g/d; dosages for the patients were adjusted for body weight (5 g · 70 kg body wt⁻¹ · d⁻¹) in anticipation of higher body weights in this group. In this way similar exposures to n-3 fatty acids would be achieved. Five grams of the FOC provided ~3 g EPA + DHA, amounts that are known to be both practical and effective (4). In the patient study, there were two 4-wk treatment phases separated by a 4-wk washout period. Because of time constraints of the students coordinating the trial and a limited number of capsules available, the study in the healthy subjects used a 2-wk placebo period, a 2–3-wk washout phase, and a 3-wk treatment phase. The 3-wk period was judged adequate for the purposes of this study because triacylglycerol concentrations in subjects taking n-3 fatty acids have been shown to stabilize within 2 wk (12). All participants were instructed to maintain their normal lifestyles and background diets throughout the study.

Fasting blood samples were drawn twice at the end of each supplementation period and analyzed for lipid and lipoprotein concentrations. The fatty acid composition of the plasma phospholipid fraction was also determined. Endogenous activities of LPL and HL were assessed in plasma samples drawn at the end of the second and third weeks of each treatment period in the healthy volunteer studies, and at the end of weeks 3 and 4 in the patient studies. When two data points from the end of any treatment period were available they were averaged before being subjected to statistical analysis.

Laboratory methods

Serum total cholesterol, triacylglycerol, and high-density-lipoprotein (HDL) cholesterol were measured enzymatically on a Cobas Mira analyzer (Roche Diagnostic Systems, Somerville, NJ) as described previously (13). VLDL- and LDL-cholesterol concentrations of the healthy subjects were calculated by using the Friedewald equation (14). For the patients, VLDL- and HDL-cholesterol concentrations were determined by β-quantitation as described previously (13). HDL₂ was precipitated from total HDL with dextran sulfate (15) and the cholesterol content of HDL₃ was calculated as the difference between total HDL and HDL₂-cholesterol concentrations. Plasma lipids were extracted and separated by thin-layer chromatography and the fatty acid composition in the phospholipid fraction was determined by combined thin-layer and gas chromatography as detailed previously (13). Our laboratory participates in the Lipid Standardization Program of the National Institutes of Health and Centers for Disease Control and Prevention and in the Pacific Biometrics ALERT proficiency survey.

Endogenous (nonheparin-stimulated) plasma lipase activities (LPL and HL) were measured by using a modification of the approach taken by others (10, 16, 17), which involved the isolation of the lipases from plasma by heparin affinity chromatography and their subsequent incubation with emulsified [¹³C]triolein. One advantage to this approach is that the natural lipase substrates (primarily VLDL) can be removed and the enzymes incubated with a known amount of labeled substrate. The intraassay CV was 5% for HL and 4% for LPL, and interassay CVs were 12% for each enzyme. All samples from each subject were analyzed in triplicate in the same run.

Preparation of the column

The Sepharose 4B column (Pharmacia, Piscataway, NJ) was prepared as described by Wang et al. (16). Fifteen grams of cyanogen bromide–activated Sepharose 4B was allowed to swell in 200 mL of 1 mol HCl/L for 10 min. The gel was then washed on a fritted disc glass funnel with 1 L of 1 mol HCl/L and then placed into a beaker containing the 75 mL heparin buffer (1 g heparin dissolved in 75 mL of 0.1 mol NaHCO₃/L and 0.5 mol NaCl/L, pH 8.3). This mixture was rotated end-over-end for 16 h at 4 °C. The slurry was then put back in the funnel and washed with 200 mL of 0.1 mol NaHCO₃ and 0.5 mol NaCl/L to remove unbound heparin. The gel was then placed in a beaker containing 200 mL of 1.0 mol ethanolamine/L for 4 h at 20 °C. The heparin-Sepharose gel was washed in the funnel sequentially with the following solutions: 1 L of 0.1 mol Na₂H₂O₂ · 3H₂O and 1.0 mol NaCl/L (pH 4.0) and 1 L of 0.1 mol Na₂B₄O₇ · 10H₂O/L and 1.0 mol NaCl/L (pH 8.0). The gel was washed a final time with 3 L distilled water and stored at 4 °C in 100 mL 50 mmol NH₄OH-HCl/L, pH 8.5 (hereafter referred to as the “buffer”). Chromatography was carried out at 4 °C in 7-mm diameter Pipette Droppers (Dyntatech Lab, Inc, Chantilly, VA). Glass wool (40 mg) was placed in the bottom of the dropper. 2 mL slurry was added to

TABLE 2  
Fatty acid composition of the supplements

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>FOC</th>
<th>OO</th>
<th>mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>0</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>16:3 n-4</td>
<td>45</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16:4 n-1</td>
<td>44</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>0</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>0</td>
<td>667</td>
<td></td>
</tr>
<tr>
<td>18:1 n-7</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>0</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>18:4 n-3</td>
<td>90</td>
<td>0</td>
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<tr>
<td>20:4 n-6</td>
<td>16</td>
<td>0</td>
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<tr>
<td>20:5 n-3</td>
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<td>21:1 n-3</td>
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<tr>
<td>22:5 n-3</td>
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</tr>
<tr>
<td>22:6 n-3</td>
<td>229</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

FOC, fish-oil concentrate; OO, olive oil placebo; FOC, fish-oil concentrate. Analyses were conducted by the National Marine Fisheries Service Charleston Laboratory, Charleston, SC.
achieve a 2-cm column height, and the column was washed with 2 mL buffer before addition of the plasma sample.

Sample preparation and chromatography

Fasting blood was drawn into iced EDTA-containing tubes. Unlike Eckel et al (18), under our experimental conditions we found no difference in endogenous lipase activity between EDTA and sodium heparin as the anticoagulant (data not shown); therefore, the former was used throughout. Plasma was isolated by centrifugation at 1500 × g at 4 °C and frozen at −80 °C until assayed. Before application to the column, the plasma was thawed at 4 °C and centrifuged at the same temperature for 2 min to allow precipitated proteins to form a sediment. The plasma supernate was mixed 1:1 with 2 mL buffer and this 4-mL sample was placed on the column. The sample tube was rinsed with 2 mL fresh buffer and this was also applied to the column. The column was then washed with 2 mL buffer, with 4 mL of 0.3 mol NaCl/L in buffer, and then with 0.6 mL 1% heparin buffer (these eluates were discarded). Finally, 2 mL heparin buffer was added and the eluate containing the lipases was collected and stored at 4 °C until used. The column was discarded.

Substrate preparation

The procedure used was modified from that of Nilsson-Ehle and Schotz (19). The materials used were as follows: triolein (500 mg dissolved in 50 mL heptane:isopropanol 3:7) to give a final concentration of 11.3 mmol/L (10 g/L); Sigma, St Louis; labeled triolein (glycerol tri-[9,10-3H]oleate; NEN-Dupont, Wilmington, DE; 37 MBq dissolved in 20 mL hexane to give 1.85 GBq/L); lecithin [dioleylphosphatidyl choline, Sigma; 500 mg dissolved in 50 mL heptane:isopropanol 3:7) to give a final concentration of 27 mmol/L (10 g/L); bovine serum albumin (Fraction V, fatty acid free; Sigma), 20% solution in buffer; and pooled human serum (a source of apolipoprotein C-II; apo C-II). For each milliliter of substrate needed (each assay tube required 50 μL), 50 μL lecithin, 500 μL cold triolein, and 80 μL labeled triolein solutions were combined and the solvents evaporated under a stream of nitrogen. The dry components were mixed with 900 μL buffer and sonicated for 3 min with an Ultrasonic homogenizer with a microtip (Cole-Parmer Instrument Co, Chicago). Finally, 100 μL of the bovine serum albumin solution was added and mixed with a vortex. The substrate was kept at 4 °C and was used within 1 h of sonication. The specific activity of [3H]triolein was 1.4 MBq/mmole.

Assay procedure

The enzyme assay was done in triplicate, with samples from both the placebo and n-3 fatty acid treatment periods for the same person assayed blindly within the same run. For the measurement of total lipase activity each tube contained the following components in a total volume of 200 μL: 35 μL buffer, 50 μL substrate, 15 μL pooled serum previously diluted 1:5 with buffer, and 100 μL enzyme solution. Blanks were prepared with buffer instead of the enzyme solution. HL activity was measured in parallel assay tubes by doubly inhibiting LPL with high salt (1 mol NaCl/L) and omission of serum (source of apo CII). For these incubations, 100 μL enzyme (or buffer for blanks) was incubated with 50 μL substrate and 50 μL buffer containing 4 mol NaCl/L. After adding all components, each tube was capped and placed in a rotating water bath at 37 °C for 1 h. The reaction was stopped and labeled fatty acids extracted as described by Boberg et al (17) with the addition of 3.2 mL chloroform:heptane:methanol (5:4:5.6, by vol) and 1 mL 200 mmol NaOH/L. After vortexing each tube three times, they were centrifuged at 1500 × g for 30 min at 20 °C to separate the phases. One milliliter of the supernate containing released oleic acid was removed and counted in a TriCarb 1500 Liquid Scintillation Counter in 10 mL Poly-Fluor (both from Packard Instrument Co, Downers Grove, IL) by using appropriate quench curves. Lipase activity was expressed as μmol fatty acid released · L plasma−1 · h−1 incubation. LPL activity was taken to be the difference between total lipase activity and HL activity.

Statistics

Although the studies reported here were conducted concurrently, they were designed and carried out as two separate, independent trials by two different coordinators in the laboratory. Thus the data from each study were analyzed separately by using a paired t test with each individual serving as his or her own control. When a comparison was made between the two studies (e.g., baseline lipase activities), an unpaired t test was used. A difference with a P value < 0.05 was considered to be statistically significant. Data are presented as means ± SDs.

RESULTS

Compliance was assessed by capsule count and was > 95% for the OO and FOC treatments in both groups of subjects. Body weights were not affected by treatment.

Oleic, linoleic, and arachidonic acid concentrations decreased significantly with the FOC treatment in the healthy subjects but not in the patients (Table 3). FOC supplementation increased plasma phospholipid EPA and DHA concentrations in both healthy subjects and patients, indicating good compliance with the FOC supplementation.

FOC supplementation decreased triacylglycerol and VLDL-cholesterol concentrations by 18% and 31%, respectively, in the healthy subjects and by 35% and 42%, respectively, in the patients (Table 4). The FOC did not affect LDL-cholesterol concentrations significantly in the healthy subjects but raised it by 25% in the patients (P = 0.06). Total HDL-cholesterol concentrations did not change significantly with FOC supplementation in either group, but HDL2-cholesterol concentrations increased by 36% in the healthy subjects (P < 0.01).

LPL and HL activities (20–40 μmol fatty acids released · L−1 · h−1) agreed well with those published by other investigators, regardless of whether isolated lipases (10) or whole plasma (20, 21) were assayed (Table 5). In the healthy subjects FOC treatment increased the endogenous activity of both LPL and HL by 62% and 68%, respectively (both P < 0.01). In the patients LPL activity increased by 65% (P < 0.05), but there was no significant change in HL activity. Baseline (placebo) LPL activity was not different between the healthy subjects and the patients whereas HL activity was significantly higher in the patients.

Although there was a negative correlation (r = −0.44, P < 0.05) between serum triacylglycerol concentrations and LPL
activity in the healthy subjects during placebo treatment, the correlation between triacylglycerol and HL was strongly positive \( (r = 0.75, P < 0.0002); \textbf{Figure 1}.\) During the \( n-3 \) fatty acid treatment periods the correlation between triacylglycerol and LPL became stronger \( (r = -0.60, P < 0.005) \) but that between triacylglycerol and HL disappeared \( (r = 0.06, P = 0.79; \textbf{Figure 1}) \) as might be expected with the significant increase in HL activity noted above. The inclusion of the patients’ data in these analyses diminished all correlations due, in part, to the markedly increased spread of serum triacylglycerol concentrations (data not shown). Among the six patients alone there were too few data points to make meaningful correlations.

**DISCUSSION**

The main finding of this study was that \( n-3 \) fatty acid supplementation increased the activities of endogenous, circulating LPL in fasting plasma in both healthy subjects and hypertriacylglycerolemic patients. HL increased significantly as well but only in the healthy subjects. Because samples were obtained twice at the end of each treatment phase and were analyzed blindly, in triplicate, with appropriate blanks and in a paired fashion (placebo and FOC samples from the same person in the same run), it is unlikely that technical errors in the assay could have been responsible for the observed FOC-induced increases in lipase activities.

In 1943, Hahn (22) serendipitously observed that heparin injection would clear lipemic serum in dogs. Clearing factor, which was later named “lipoprotein lipase” by Korn (23), was shown by Engelberg (24) to be present even in nonheparin-stimulated plasma, suggesting that it was not simply an artifact produced by heparin injection but a true, physiologically significant factor that might play a role in lipoprotein metabolism. Despite marked progress in our understanding of the biology of LPL and HL over the past 40 y, little attention has been paid to endogenous lipase activity, and thus its physiologic significance remains obscure. Nevertheless, the possibility remains that, in some way, endogenous activity may reflect in vivo lipolytic potential. At present it is unclear whether endogenous LPL activity is a surrogate of heparin-releasable LPL; some investigators reported no correlation (10, 20, 21, 25) whereas others found a significant relation (18, 26). In the two studies that compared pre- and postheparin HL activity a positive correlation was found (20, 21). If future studies can confirm a close relation between endogenous and postheparin lipase activities, the need for heparin injection could be eliminated, opening the door to expanded clinical use of the assay.

Although most of the LPL released by heparin injection is active (dimeric) enzyme (25), the great majority of the endogenous, circulating LPL mass is inactive (monomeric) enzyme bound to remnants of triacylglycerol-rich lipoproteins (25, 27, 28). It is currently thought that LPL, through the carboxy terminal region of its monomeric form, interacts with cell-surface heparan sulfate and the LDL-receptor-related protein, resulting in the internalization and degradation of remnants of triacylglycerol-rich lipoproteins by receptor- (29) and possibly

**TABLE 3**

Effects of \( n-3 \) fatty acid supplements on selected fatty acids from the plasma phospholipid fraction

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects ( n = 20 )</th>
<th>HTAG patients ( n = 5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OO</td>
<td>FOC</td>
</tr>
<tr>
<td>% of total fatty acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>10.1 ± 1.6</td>
<td>9.0 ± 1.9(^j)</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>19.4 ± 2.0</td>
<td>17.4 ± 2.5(^j)</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>9.4 ± 1.5</td>
<td>7.9 ± 1.7(^i)</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>0.4 ± 0.1</td>
<td>2.9 ± 1.1(^i)</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>2.2 ± 0.5</td>
<td>4.0 ± 0.8(^i)</td>
</tr>
</tbody>
</table>

\(^{i,j}\) Significant differences from OO within group: \(^i P < 0.05, ^j P < 0.01, ^i P < 0.0001.\)

**TABLE 4**

Effect of \( n-3 \) fatty acid supplements on lipid and lipoprotein concentrations

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects ( n = 20 )</th>
<th>HTAG patients ( n = 6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OO</td>
<td>FOC</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.34 ± 0.61</td>
<td>4.33 ± 0.67</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>1.28 ± 0.35</td>
<td>1.05 ± 0.29(^2)</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.58 ± 0.50</td>
<td>0.43 ± 0.26(^4)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>2.54 ± 0.54</td>
<td>2.66 ± 0.55</td>
</tr>
<tr>
<td>HDL(_c) cholesterol</td>
<td>1.23 ± 0.35</td>
<td>1.26 ± 0.37</td>
</tr>
<tr>
<td>HDL(_l) cholesterol</td>
<td>0.97 ± 0.22</td>
<td>0.92 ± 0.19</td>
</tr>
<tr>
<td>HDL(_l) cholesterol</td>
<td>0.25 ± 0.15</td>
<td>0.34 ± 0.21(^4)</td>
</tr>
</tbody>
</table>

\(^{2,3,4}\) Significant differences from OO within group: \(^2 P < 0.001, ^3 P < 0.05, ^4 P < 0.01.\)
TABLE 5  
Effect of n-3 fatty acid supplements on endogenous, nonheparin-stimulated activities of lipoprotein lipase and hepatic triacylglycerol lipase.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 20)</th>
<th>HTAG patients (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OO</td>
<td>FOC</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 ± 10</td>
<td>40 ± 7</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Hepatic triacylglycerol lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 ± 7</td>
<td>28 ± 11</td>
<td>32 ± 13</td>
</tr>
</tbody>
</table>

1 ± SD. OO, olive oil placebo; FOC, fish-oil concentrate; HTAG, hypertriacylglycerolemic.
2 **P < 0.001.**

It is tempting to tie the increase in endogenous LPL activity observed here to the reduction in triacylglycerol concentrations, but our data do not causally link these observations. However, we did find a significant correlation between serum triacylglycerol concentrations and LPL activities in the healthy subjects during both the placebo and the n-3 fatty acid periods (Figure 1). The fact that the n-3 fatty acid correlation curve shifted upward instead of simply moving left up the placebo curve indicates that variations in endogenous LPL activity alone do not determine triacylglycerol concentrations.

In contrast with LPL, HL activity—which was strongly and positively correlated with serum triacylglycerol concentrations during the placebo period—ceased to be correlated with serum triacylglycerol when measured after n-3 fatty acid treatment (Figure 1). Because HL and LPL are believed to play a role in the conversion of intermediate-density lipoprotein to LDL (35), these enzymes could affect LDL concentrations. Heparin injection transiently raises LDL concentrations (36), and acute inhibition of LPL (37) or HL (38) lowers LDL. Genetic deficiencies in LPL (39) or HL (35) likewise reduce LDL concentrations. Indeed, the fibrate drugs, although effective at lowering triacylglycerols, often raise LDL concentrations, perhaps by stimulating LPL activity (40, 41). Data such as these suggest that LDL production is controlled to a significant extent by lipolytic enzyme activities. It is well-established that n-3 fatty acid feeding usually increases LDL concentrations in hypertriacylglycerolemic patients, but not in healthy subjects (4). This was our observation in the current study in which five of the six patients studied experienced an increase in LDL-cholesterol concentrations. The mechanism of this phenomenon is still unclear, but one might expect that, because LDL is derived from VLDL catabolism, reductions in VLDL would tend to lower LDL. The fact that LDL does not decrease with n-3 fatty acid feeding suggests that some factor is increasing its production (or inhibiting its clearance). To what extent HL and LPL play a role in these events is not known, but the observed increase in lipase activity is consistent with enhanced LDL production.

It is unlikely that the increases in LPL activity that we observed with n-3 fatty acid supplementation were simply due to increased amounts of active enzyme passively bound to lipoproteins, the concentrations of which were increasing for other reasons. Fish-oil feeding does not increase serum concentrations of VLDL remnants (42) [those particles thought to carry endogenous LPL activity in plasma (25, 27, 28)], and the small rise in LDL concentration observed here (5–20% in the subjects and patients, respectively) did not match the increase (> 60%) in LPL activity observed. If active LPL were carried on native VLDL particles, which appears unlikely (28), then LPL activities should have decreased, not increased, with n-3 fatty acid feeding. Basal LPL activity has been positively correlated with nonesterified fatty acid (NEFA) concentrations (21). This could reflect a higher rate of lipolysis of triacylglycerol-rich lipoproteins or, perhaps, a direct, local effect of NEFAs on LPL binding to heparan sulfate stalks on the endothelium. Saxena and Goldberg (43) showed that NEFAs differ in their ability to release LPL, with oleic acid being one of the most potent. Interestingly, these workers found that free EPA is a relatively poor releaser of LPL. Thus, the likely presence of higher-than-normal concentrations of EPA in the NEFA fraction in this study probably did not contribute to the increase in endogenous LPL noted. Therefore, our data are most consistent with the hypothesis that n-3 fatty acids directly increase circulating concentrations of LPL, which could lead to accelerated catabolism of VLDL. Further studies are clearly needed to examine this more thoroughly.

There is a large body of evidence from cell culture experiments to in vivo VLDL kinetic studies in humans that n-3 fatty acids decrease serum triacylglycerol concentrations primarily by inhibiting the synthesis, secretion, or both of VLDL from the liver (3, 44). Although reduced production plays an important mechanistic role, these findings do not preclude a contribution from enhanced clearance as well. Past explorations of this alternate mechanism have focused on the effects of fish-oil feeding on postheparin LPL activity. Most studies showed no effect in humans (7–9), although one recent report found a slight but significant increase (45). Fish-oil feeding actually decreased postheparin LPL activity in rats (46) and swine (47, 48), although EPA alone was reported to increase activity in rats (49). The clearance rate of intravenous lipid emulsion was not increased by n-3 fatty acid supplementation in humans (13), and in three kinetic studies (5, 6, 50) slowed production rather than increased clearance explained the triacylglycerol-lowering effect. On the other hand, accelerated VLDL apolipoprotein B-100 clearance was reported in a recent study with fish-oil-fed swine (51). Taken together, the weight of published evidence supports reduced VLDL production over accelerated VLDL clearance as the primary mechanism. In light of this, the increased endogenous LPL activity reported here was unexpected. However, several potential explanations for the discrepancy between pre- and postheparin findings may be postulated. First, as noted above, postheparin and preheparin LPL activities have not correlated with each other frequently, and they appear to correlate with different lipoprotein patterns.
n-3 FATTY ACIDS AND PLASMA LIPASES

FIGURE 1. Correlations between fasting serum triacylglycerol concentrations and endogenous lipoprotein lipase (LPL) and hepatic lipase (HL) in healthy subjects (n = 20). Data from the olive oil placebo (O) and n-3 fatty acid fish-oil concentrate (●) periods were analyzed separately. The regression lines are labeled to indicate treatment period. All correlation coefficients were statistically significant except for HL on n-3 fatty acids (see text).

(10, 25). Second, the postheparin test reflects how much lipase can be released from tissues by heparin injection; it does not necessarily measure physiologically active enzyme concentrations (but as far as we know, neither does the endogenous assay). Finally, lipid emulsion clearance tests may not accurately reflect in vivo LPL activity, especially if the presence of n-3 fatty acids in the natural substrates (VLDL, chylomicrons) somehow enhances their interaction with or susceptibility to LPL. Thus, the failure of n-3 fatty acids to stimulate postheparin LPL activity measured in vitro does not exclude the possibility that they influence LPL-mediated lipolysis in vivo.

Although we could make no comparisons between the healthy subjects and the patients regarding the effects of n-3 fatty acid supplementation (because of differences in experimental designs), we were able to compare basal (placebo) lipase values. We observed that although LPL activity was not significantly different between groups (during the placebo phase), HL activity was higher in the patients than in the healthy subjects. This finding does not support the earlier observations of others (20, 25), who reported no significant difference in endogenous HL activities between healthy subjects and mixed populations of hyperlipidemic patients. On the other hand, Greten et al (52) reported that postheparin HL activity tended to be higher (37%, P = 0.1) in hypertriglyceridemic patients than in healthy control subjects. Confirmation of this finding awaits future trials.

We observed a rise in the HDL₂ cholesterol concentration in the healthy subjects and a tendency toward higher concentrations in the smaller, patient study. This is a relatively common observation in n-3 fatty acid trials (53-55), which may be mediated by an inhibition of lipid transfer protein activity (53). Such an inhibition could slow the exchange of HDL cholesteryl esters for VLDL triacylglycerol, resulting in higher HDL-cholesterol concentrations. Alternatively, lower concentrations of large, triacylglycerol-rich VLDL particles could reduce the substrate available for transfer mediated by lipid transfer protein and thereby allow HDL-cholesterol concentrations to increase. Whether such an effect of n-3 fatty acids has any effect on the risk for cardiovascular disease is unknown.

In conclusion, n-3 fatty acid supplementation increased the endogenous activity of LPL and HL in human plasma. Further studies are needed to determine whether this is merely an epiphenomenon or whether it is causally linked to the well-known triacylglycerol-lowering effect of fish oils.

REFERENCES


