Abstract  Dietary n–3 fatty acids (FAs) reduce postprandial triacylglycerol concentrations in humans by unknown mechanisms. Our goals were to reproduce this phenomenon in the rat, and then to determine the mechanism. In an oral fat tolerance study two groups of rats were fed diets containing 2.1% ethyl esters of n–3 FA or olive oil for 2 weeks. After gavaging with emulsified soybean oil, the postprandial chylomicron triacylglycerol levels in the n–3 FA group were reduced by 40% (P < 0.05). The hypothesis that n–3 FA feeding reduced chylomicron production/secretion from the gut was tested by blocking chylomicron removal with Triton WR1339 before gavaging the rats with the fat load. This completely eliminated significantly lower fractions of dose remaining at slower chylomicron formation, faster chylomicron removal, or a combination of these factors, further studies were conducted to examine the interaction between the fat in the background diet and the fat in the test meal. Similar observations were reported by Weintraub et al. (3). Later studies (4–8) showed that n–3 FA supplementation (3–4 g/d) could also blunt the normal chylomicronemic response to a fat load. Therefore, chylomicron metabolism was significantly impacted by chronic, relatively low intakes of n–3 FAs.

There are only two general mechanisms that can explain reduced postprandial lipemia: slowed entrance of chylomicrons into the blood (production) or accelerated removal from it (clearance). Several investigators have shown that n–3 FAs do not enhance postheparin plasma lipoprotein lipase (LpL) or hepatic lipase activities when measured in vitro (2, 3, 8–12). In addition, the clearance of a fat emulsion is not accelerated in n–3 FA-fed subjects (5). These observations argue against the accelerated clearance hypothesis, and by default, for the slowed production hypothesis. Also favoring the latter is the n–3 FA-induced reduction in triacylglycerol secretion from the liver, a finding supported by human kinetic, animal, and cell culture studies (13). If n–3 FAs inhibit triacylglycerol synthesis and/or secretion from hepatocytes, they might have the same effect in enterocytes. On the other hand, chylomicron secretion into their usual background diets. However, when the background diets contained large amounts of n–3 FAs (10–25 g/d), postprandial triacylglycerol levels were significantly lowered regardless of the type of fat in the test meal.

In 1980, Harris and Connor (1) reported that postprandial lipemia was reduced after an oral load of fish oil in subjects on an n–3 fatty acid (FA)-rich background diet. As this blunted response could have been caused by inefficient n–3 FA digestion and absorption, slower chylomicron formation, faster chylomicron removal, or a combination of these factors, further studies were conducted to examine the interaction between the fat in the background diet and the fat in the test meal. Those studies (2) revealed that test meals containing 50 g of fish oil produced a normal rise in postprandial triacylglycerol levels when subjects were consuming

N–3 fatty acids and chylomicron metabolism in the rat

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In 1980, Harris and Connor (1) reported that postprandial lipemia was reduced after an oral load of fish oil in subjects on an n–3 fatty acid (FA)-rich background diet. As this blunted response could have been caused by inefficient n–3 FA digestion and absorption, slower chylomicron formation, faster chylomicron removal, or a combination of these factors, further studies were conducted to examine the interaction between the fat in the background diet and the fat in the test meal. Those studies (2) revealed that test meals containing 50 g of fish oil produced a normal rise in postprandial triacylglycerol levels when subjects were consuming

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LpL, lipoprotein lipase; FA, fatty acid; OFT, oral fat tolerance; TAG, triacylglycerol; PL, phospholipid; SAAM, simulation, analysis, and modeling.

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the lymph was not inhibited in fish oil-fed rats (14), nor was increased fat excretion noted (15). Thus, there is circumstantial evidence supporting both hypotheses.

The n-3 FA-induced reduction in postprandial lipemia has been documented several times in humans but never in animals. Therefore, the first objective of this study was to determine whether the rat could serve as an animal model for this phenomenon. Our second objective was to determine the mechanism responsible. We measured postprandial chylomicron levels after injection of Triton WR1339 to block chylomicron lipolysis, and we measured the rate of chylomicron clearance by injecting labeled chylomicrons into control and n-3 FA-fed rats. These experiments supported accelerated chylomicron lipolysis as the mechanism of reduced postprandial lipemia in the n-3 FA-fed rat.

MATERIALS AND METHODS

Animals

Male Wistar rats (Mollegaard Breeding Center, Ejby, Denmark) weighing initially between 200 and 240 grams were used in all experiments. They were kept individually in wire-bottom cages in a room with controlled temperature (23 ± 1°C) and humidity (60 ± 3%). The light cycle was reversed (dark from 10:00 to 22:00 h). The animals were allowed to acclimate to the animal care facility for 5 days before starting the study. During this time they were fed the control diet described below. All studies were carried out at the University of Oslo, and all procedures to which the animals were subjected conformed to institutional guidelines.

Diets

All animals were fed a semipurified diet containing 21% fat by weight. The fat was composed of lard (17%), soybean oil (1.5%), and ethyl esters of either olive oil or n-3 FAs (2.5%). This intake of n-3 FAs was determined from preliminary experiments in which it was found to significantly reduce postprandial triacylglycerol levels. The olive oil ethyl esters were provided by the Biomedi-
cal Test Materials Program, US Department of Commerce, and the n-3 FA ethyl esters (K-85; 51% eicosapentaenoic acid [EPA], 34% docosahexaenoic acid [DHA]) by Pronova Biocare AS, Oslo, Norway. The EPA + DHA content of the diet was 2.1% by weight. The fatty acid compositions of the two ethyl ester preparations and of the test diets are given in Table 1.

Other dietary ingredients were sucrose (20%), cornstarch (31.5%), casein (20%), cellulose (1%), and vitamin (1.5%) and salt mixes (5%) (ICN Pharmaceuticals, Cleveland, OH). The diet contained 40% energy from fat, 17% from protein, and 43% from carbohydrate. Vitamin E intakes were 6–7 mg/d on all diets. The animals were given 20 g of fresh diet daily; any uneaten food was discarded. Diets were prepared so as to minimize exposure to light and air (i.e., the food was mixed in a darkened room under a nitrogen atmosphere and remained frozen (−20°C) until the day before it was fed when it was thawed at 4°C). Food was dispensed just before the dark cycle began.

In all experiments a high fat test meal was given by gavage to induce postprandial hyperlipidemia. The meal was an emulsion containing 1 g of fat in a volume of 3 mL including the following components (% by weight): 38%-fat cream (48%); powdered milk (12%); sugar (2%); blended eggs (14%); soybean oil (10%), and water (13%). Fat provided 83% of energy in the meal, protein 5%, and carbohydrate 12%. The meal contained no marine n-3 FAs (Table 1).

Protocols

Study 1: Oral Fat Tolerance (OFT). The animals in this study were fed the control and n-3 FA diets for 14 days before the experiments began. Postprandial lipemia was induced by gavaging with the test emulsion after an overnight fast. At 2, 3, 4, and 6 h after gavaging, 5 to 7 animals/group were killed by decapitation. Ten animals (5 from each group) were killed at zero time to establish baseline values. The sera obtained from these

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Olive Oil</th>
<th>K-85</th>
<th>Control</th>
<th>N-3 FAs</th>
<th>Oral Fat Load</th>
</tr>
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<tbody>
<tr>
<td>18:0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>16:0</td>
<td>12</td>
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<td>24</td>
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<tr>
<td>16:1 n-7</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
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<td>5</td>
<td>6</td>
<td>16</td>
<td>12</td>
<td>10</td>
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<tr>
<td>18:1 n-9</td>
<td>73</td>
<td>0</td>
<td>39</td>
<td>39</td>
<td>25</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>7</td>
<td>1</td>
<td>13</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>18:4 n-3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20:4 n-6</td>
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<td>0</td>
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<tr>
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<td>34</td>
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<tr>
<td>EPA mg/d</td>
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<td>—</td>
<td>0</td>
<td>227</td>
<td>—</td>
</tr>
<tr>
<td>DHA mg/d</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>141</td>
<td>—</td>
</tr>
<tr>
<td>n-3 FA mg/d</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>370</td>
<td>—</td>
</tr>
<tr>
<td>n-3 FA mg/kg/d</td>
<td>—</td>
<td>—</td>
<td>25</td>
<td>1233</td>
<td>—</td>
</tr>
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</table>
animals were analyzed for total, chylomicron, and nonchylomicron triacylglycerols. Chylomicron triacylglycerol to phospholipid ratios were determined in samples drawn at 2, 3, and 4 h post gavaging.

Study 2: Chylomicron Production. This study was done to test the hypothesis that n–3 FA treatment slows the entry of chylomicron triacylglycerol into the bloodstream (by slowing gastric emptying, accelerating intestinal transit, inhibiting fat absorption and/or retarding chylomicron formation or secretion into the lymph). After 2 weeks of consuming the test diets, the animals were fasted overnight and gavaged with 3 mL of emulsion (1 g of fat) as described above. The emulsion in this study also contained 2 μCi of tri[9,10(n)-3]H0leylglycerol. Immediately prior to gavaging, each animal was injected via the dorsal vein of the hind foot with 250 mg/kg of Triton WR1339 (tyloxapol; Sigma Chemical Co., St. Louis, MO; about 400 μL of a 20% solution in sterile saline). This compound blocks catabolism of triacylglycerol-rich lipoproteins (16). Two and 3 h after gavaging with the test meal (times of demonstrable hypothyroidism seen in the OFT study), 5–7 rats from each group were killed by decapitation. Blood was collected, and the gastrointestinal tract was immediately ligated above and below the stomach and at the ileocecal junction, removed and placed in ice-cold saline. Stomach contents were recovered, and the small intestine (wall and contents) was homogenized. These samples, along with the chylomicron and non-chylomicron fractions of serum, were analyzed for triacylglycerol mass and radioactivity. The fractions of the gavaged dose present in the bloodstream, the stomach, and the intestine were determined.

Study 3: Chylomicron Clearance. To determine whether chylomicron clearance was accelerated in rats fed n–3 FAs, we first obtained dual radiolabeled lymph from rats fed the control or n–3 FA diets as follows. The lymph donor animals (one from each group) were anesthetized with Hypnorm®/Dormicum® (1:1 mixture of each in 50% water solutions: 3 mL/kg) and the mesenteric lymph duct was cannulated as described previously (17). A second cannula was placed through the stomach wall into the duodenum and secured with a drop of tissue glue. Once lymph flow began, the rats were allowed to recover in a 30°C room (to counteract post-operative hypothermia) and saline was infused into the intestine (2 mL/h) to keep the animals hydrated. The rats were given an analgesic (Temgesic®, 0.2 mg/kg, ip) during this post-operative period. Soon after lymph flow was established, the saline was replaced by an emulsion consisting of 1 g of soybean oil (containing 250 μCi of [11,12(n)-3]Hretinol and 38 μCi of tri[1-14C]oleyl glycerol) sonicated in 7 mL of 10 mM sodium taurocholate. This was infused into the small intestine at the rate of 2 mL/h over 4 h to mimic the absorption of the 1 g of fat in the OFT meal. Once the fat infusion was complete, the saline infusion was reestablished. Lymph was collected at 30°C overnight in a plastic tube containing a preservative mixture composed of gentamicin (0.1 mg/mL), ethylenediamine tetraacetic acid (2.7 mmol/L), thimerosal (0.2 mmol/L), and glutathione (1.5 mmol/L) (18), and shielded from light with aluminum foil. Clots were removed with a wooden stick, the lymph was centrifuged at 2,500 rpm for 10 min to sediment blood cells, and the supernatant lymph was stored at room temperature until injected within 48 h (see below). The concentration of triacylglycerol, and the 3H and 14C radioactivity were determined in both control and n–3 FA lymph samples. The one with the higher triacylglycerol concentration was diluted with saline to match that of the more dilute. In this way equal volumes of lymph containing equal masses of triacylglycerol could be injected. One day after the lymph collection the clearance studies were started, and they continued for 2 days. The recipient animals were fasted overnight and then gavaged with the test meal (that used in Studies 1 and 2) 3 h before the clearance study began to establish the same physiological milieu as was present during the OFT tests.

The clearance studies were done in awake animals as anesthesia markedly prolongs chylomicron clearance (19). The studies began by injecting 300 μL of lymph (about 19 μg of triacylglycerol) into the dorsal vein of one hind foot. All rats received homologous lymph (i.e., lymph obtained from a donor fed the same diet as the recipient). At regular intervals after injection (1, 2, 4, 8, and 16 min) blood samples were obtained by capillary tube from a small cut in the dorsal vein of the contralateral foot. The blood was collected into pre-weighed capillary tubes which were weighed again after filling to determine the amount of blood collected. The capillary tubes were then immediately dropped into test tubes containing 7 mL of a mixture of chloroform–methanol–water 4:4:3.4 to extract lipids. A final blood sample was collected at 32 min by decapitation. The 14C and 3H counts were determined in lipid extracts of the blood. The liver was also removed and analyzed for accumulated 3H and 14C. Counts were converted to dpm with appropriate quench corrections. Chylomicrons isolated from the 32-min serum samples (3.5 h post gavaging) from 5 animals from each group were analyzed for phospholipid and triacylglycerol fatty acid composition as described below.

The fractions of the injected doses of 14C and 3H remaining in the blood at each time point (1–16 min) and those found in the liver at 32 min were used to test
the clearance hypothesis. The blood volume was estimated to be 6.3% and serum volume 3.65% of total body weight (20).

Compartmental modeling was used to analyze the tracer elimination curves. This method generates estimates for the fractional transfer coefficients which describe the flow of tracer(s) into and out of various “compartments.” The residence (or transit) time for a compartment in a non-recycling system is computed as the inverse of the sum of the transfer coefficients for tracer leaving that compartment (21). As a starting point for model-based compartmental analysis, we used previous models for chylomicron metabolism (22, 23) to postulate a physiologically reasonable model; chylomicron particles enter the blood, acquire apoC-II to activate LpL, bind to endothelial-bound LpL (margination: the particles disappear for a time from the circulation), are lipolyzed by LpL, are released from the endothelium as remnants, and finally removed from the circulation primarily by hepatic chylomicron remnant receptors. Although it has traditionally been assumed that retinyl esters remain with the chylomicron particle throughout its course in the plasma, being removed only as chylomicron remnants were cleared by the liver (24), this has recently been challenged (25–27). Accordingly we included compartment 40 (see below) in the model to allow for the possibility of intravascular removal of retinyl esters. Finally, it was assumed that once cleared, very little labeled retinol would be secreted by the liver (on retinol binding protein) over the 32-min study (25).

The conversational version (28) of the Simulation, Analysis and Modeling computer program [SAAM (29)] was used to iteratively adjust the model structure and its parameter values to obtain the best fit of the model prediction to the tracer data. For purposes of weighting the non-linear regression analyses, a fractional standard deviation of 0.05 was assigned to each data point.

**Laboratory analyses**

Blood was collected in plastic tubes and allowed to clot. Serum was isolated by centrifugation at 3,000 rpm for 10 min at 4°C. In all studies serum triacylglycerols were analyzed in duplicate by an enzymatic kit (BioMerieux), March-Etoile, France). Chylomicrons were isolated from the serum by centrifugation for a total of 1 h at 20,000 rpm at 4°C in a Sorvall THT-45.6 rotor. These conditions produced a total gravitational exposure of 1.6 × 10^6 g·min and isolated particles of approximately S_ri > 400. The chylomicron and non-chylomicron fractions were recovered by tube slicing and, along with the whole serum, analyzed for triacylglycerol concentration. Phospholipids (choline-containing) were analyzed in the 2-, 3- and 4-h chylomicron samples from the OFT study by an enzymatic kit (BioMerieux). Mean chylomicron diameters were estimated from triacylglycerol (TAG) to phospholipid (PL) mass ratios by the equation: diameter (nm) = 60 × ([0.211 × TAG/PL] + 0.27) as modified from Fraser (30). Mmoles were converted to mg by multiplying triacylglycerol values by 886 and phospholipid by 775.

The fraction of the dose of ^14C remaining in the stomach and intestine at 2 and 3 postmeal was measured in the chylomicron production study, as was the fraction of [3H]retinyl esters and ^14C (originally from labeled triolein) accumulating in the liver after 32 min in the chylomicron clearance studies. The stomachs were cut open and their contents were washed out with 10 mL of a 10 mmol/L solution of sodium taurocholate. An aliquot of this mixture was counted for ^14C. The small intestines (including contents) were homogenized (PT 10/35, Brinkman Instruments, Westbury, NY), an aliquot was extracted for total lipids with chloroform–methanol 2:1 (31), dried under a stream of N_2, and counted in InstaGel (Packard, Downers Grove, IL) using a Packard Tricarb 1900 liquid scintillation counter. Livers were homogenized 1:5 in phosphate-buffered saline and an aliquot was extracted for retinol/retinyl esters and triacylglycerol with hexane (32). Retinol and retinyl esters were separated by reversed-phase HPLC (33) using a 5-μm Supercosil LC8 column (Supelco, Bellefonte, PA) with a Waters 590 pump (Waters Associates, Milford, MA). Absorbance was monitored at 326 nm with a Waters 440 detector.

In six preliminary clearance studies, serum chylomicrons were isolated to confirm that the decay of whole serum radioactivity accurately reflected chylomicron removal. For these analyses 35 to 50 μL of serum was layered under 125–140 μL of saline and centrifuged at full speed (30 psi) for 5 min in an Airfuge (Beckman Instruments, Palo Alto, CA). The bottom fraction (plasma volume plus 20 μL) was carefully removed with a Hamilton syringe and the top fraction (tube and contents) containing the chylomicrons was placed in a scintillation vial and counted. The chylomicron counts were compared to those in whole serum. As decay rates were virtually identical (data not shown), the disappearance of serum radioactivity was used in the clearance study and assumed to reflect chylomicron clearance.

The FA composition of chylomicron lipids was determined in the chylomicron clearance study. Lipids were extracted (31) and the phospholipid and triacylglycerol fractions were isolated by thin-layer chromatography on silica gel G (15). FAs were transmethylated in methanolic HCl (34) and injected into a Shimadzu GC-14A equipped with a polar capillary column (SGE BPX70, diameter 0.33 mm, length 25 m). Fatty acids were iden-
ified by comparison with known standards. Dietary triacylglycerols were also extracted and their FA composition was determined by gas chromatography.

Statistical analysis

Student’s t-tests were used for all comparisons between the control and n-3 FA groups. A one-tailed table was used when testing our major hypotheses (that n-3 FA feeding would reduce fasting or postprandial triacylglycerol levels, accelerate clearance, or slow absorption), and a two-tailed table was used for other comparisons that were not direct tests of hypotheses. Data are presented as means ± standard deviations unless otherwise noted.

RESULTS

Body weight and food intake

In all studies, food intake was about 17 g/d for both groups over 14 days of feeding. Beginning and ending body weights in the control and n-3 FA groups were 213 ± 15 g to 280 ± 28 g, and 208 ± 21 to 273 ± 32 g, respectively. These were not different between groups.

Study 1: Oral fat tolerance

Fasting triacylglycerol concentrations were unaffected by n-3 FA feeding (0.70 ± 0.2 vs. 0.65 ± 0.2 mmol/L, control vs. n-3 FA groups; Fig. 1). Thus, a 2.5% n-3 FA diet did not reduce fasting triacylglycerols significantly in this model. Despite the lack of difference in fasting levels, the animals fed the n-3 FA diet had reduced chylomicron triacylglycerol levels at each timepoint after the fat load, with the difference between groups being statistically significant at 3 h. The cumulative mean chylomicron triacylglycerol level after 2 to 6 h for the 24 control animals was 1.39 ± 1.07 and for the 23 n-3 FA animals, 0.87 ± 0.64 mmol/L (−40%; P < 0.05). There was no significant effect of the fat load on postprandial non-chylomicron triacylglycerol levels; however they did appear to rise slightly at 2 h and then to fall back below baseline thereafter in both groups.

Chylomicron sizes were estimated from their ratio of triacylglycerol to phospholipid, the former being exclusively a core component and the latter a surface component. At 2 h postprandially, the mean chylomicron diameters were 12% larger in the n-3 FA-fed rats than in the control animals (Table 2) whereas at 3 and 4 h there were no significant differences in chylomicron size. The mean, overall size from a pool comprised of equal amounts of 2, 3-, and 4-h chylomicrons was 99.5 ± 22.4 nm for the control animals and 99.3 ± 17.2 nm in the n-3 FA animals. This is the approximate size of particles between 400 and 1,000 Svedberg units (30).

The FA composition of the oral fat load (Table 1) was largely conserved in the chylomicron triacylglycerols isolated 3.5 h post-gavaging (32 min post-injection in the chylomicron clearance study). At this time, less than 5% of the injected chylomicron triacylglycerol remained in circulation (see below). Both EPA and DHA levels were increased in the phospholipid and triacylglycerol fractions from the n-3 FA-fed rats (Table 3). Enrichment was not extensive, however, with EPA + DHA in phospholipids rising from 2.8 to 6.4% and in triacylglycerol from 1.4 to 3.0%.

TABLE 2. Effects of n-3 fatty acid prefeeding on circulating chylomicron diameter determined from triglyceride to phospholipid ratio at various post-meal times (mean ± SD)

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>N-3 Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Diameter</td>
</tr>
<tr>
<td>2 h</td>
<td>7</td>
<td>100 ± 9</td>
</tr>
<tr>
<td>3 h</td>
<td>7</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>4 h</td>
<td>5</td>
<td>111 ± 36</td>
</tr>
<tr>
<td>Overall</td>
<td>19</td>
<td>100 ± 22</td>
</tr>
</tbody>
</table>
Fig. 2. Chylomicron production study. The accumulation of tritium (A) and triacylglycerol (B) in serum of rats fed control (open bars) or n-3 FA (shaded bars) diets 2 and 3 h after gavaging with the standard high fat test meal. All rats were injected with Triton WR1339 prior to gavaging.

Study 2: Chylomicron production

This study examined the effects of n-3 FA pretreatment on chylomicron production/secrection by blocking chylomicron lipolysis with Triton WR1339. We measured the accumulation of labeled triacylglycerol from the test meal in plasma and that which remained in the stomach and small intestine 2 and 3 h post-gavaging. There were no differences between groups in either the fraction of dose or the absolute mass of triacylglycerol in the chylomicron or non-chylomicron fractions at either time point (Fig. 2). The presence of a small amount of labeled material in the “non-chylomicron” fraction was probably “intestinal VLDL” (35).

The fractions of dose recovered in the stomach (33 ± 10% vs. 29 ± 9%) and small intestine (29 ± 4% vs. 27 ± 5%) at 3 h were not different between control and n-3 FA-fed animals, respectively. All of the administered dose could be accounted for by that recovered in the stomach, small intestine, and blood (107 ± 10% vs. 102 ± 9%, control vs. n-3 FA) indicating that Triton blockade was virtually complete for up to 3 h under these conditions.

Consistent with the observation that fasting triacylglycerol levels were not reduced by n-3 FA feeding in this model was the finding that there was no significant difference between groups with respect to non-chylomicron triacylglycerol mass at 2 or 3 h after the meal. This fraction would contain mostly VLDL secreted from the liver, the clearance of which the Triton WR1339 would also have blocked. This suggests that the liver was not secreting significantly less VLDL triacylglycerol in the n-3 FA-fed rats than in the controls.

Study 3: Chylomicron clearance

Having shown that the n-3 FA-induced hypochylomicronemia could be eliminated by blocking chylomicron clearance, we conducted a direct test of the clearance hypothesis by injecting dual-labeled lymph chylomicrons and following their removal from the blood. The lymph collected from control and n-3 FA-fed donors was of similar composition; virtually all (98%) of the radioactivity (both labels) in the lymph was found in the chylomicron fraction, and 97% of the [3H] retinol was esterified. As expected, triacylglycerol FA composition was very similar between treatments as the same oil (soybean) was infused in both control and n-3 FA-fed donor rats (Table 4). Phospholipid n-3 FA levels were increased in the lymph from the n-3 FA-fed rats reflecting the presence of EPA and DHA in biliary phospholipids as noted earlier.
Retinyl Esters

\[
\text{Time after injection (min)}
\]

Triacylglycerol

\[
\text{Time after injection (min)}
\]

\[0.2 - 0.1; \]

\[0.2 - 0.1; \]

\[0.2 - 0.1; \]

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Fig. 4. Working models for metabolism of chylomicron \([{}^{14}\text{C}]\)tri olein (top panel) and \([{}^{3}\text{H}]\)retinyl esters (bottom panel). Compartments are shown as circles; triangles represent the sum of two compartments at the site of sampling; arrows are fractional transfer coefficients \(L(I,J)\) = the transfer of tracer to compartment \(I\) from compartment \(J\). For processes affecting chylomicrons as particles, parameters in the parallel models were constrained to be equal: \(L(12,10) = L(2,1)\); \(L(35,12) = L(15,33) = L(3,23) = L(23,2)\); and \(L(14,13) = L(4,3)\). The \([{}^{14}\text{C}]\)tri olein- and \([{}^{3}\text{H}]\)retinyl ester-labeled chylomicrons were injected into compartments 1 and 10, respectively. \(IC(x) = 1.0\) means that the initial condition was that 100% of the tracer was introduced into compartment \(x\).

DISCUSSION

Chronic feeding of human volunteers with fish oils rich in n–3 FAs lowers not only fasting (13) but also postprandial triacylglycerol levels, even when the fat in the test meal contains no n–3 FAs (1–4,6,7). Investigations into the mechanism of this effect have concluded that enhanced chylomicron clearance was probably not responsible as n–3 FA feeding did not stimulate postheparin LpL activity when measured in vitro or did it accelerate the clearance of an intravenous fat emulsion (see below). As frank fat malabsorption has likewise not been observed, the reason for the postprandial hypochylomicronemia has remained obscure. Having an animal model of this effect would help unravel the mechanism.

In this investigation we were able to document that feeding n–3 FAs produced the same magnitude of postprandial hypochylomicronemia in rats as seen in humans. When chylomicron clearance was blocked, the effect vanished. When clearance was examined directly by injecting dual-labeled chylomicrons, rats fed n–3 FAs cleared chylomicron lipids faster than the controls. N–3 FA treatment did not markedly change the size of the chylomicron particles nor did it appear to accelerate hepatic clearance of chylomicron remnants. Taken together these findings suggest that n–3 FAs accelerate chylomicron lipid clearance by facilitating LpL-mediated lipolysis (although we cannot exclude enhanced non-LpL-mediated clearance by tissues such as spleen, muscle, adipose tissue, or kidney (37)). Whether the n–3 FAs affected the donor rats (i.e., the chylomicron particles) or the recipient rats (i.e., increased LpL activity) is currently not known.

Previous studies provide support both for n–3 FAs acting by enhancing chylomicron clearance or by slowing fat absorption (chylomicron production). Turning first to the latter, n–3 FAs are known to reduce fasting triacylglycerol levels by inhibiting hepatic VLDL triacylglycerol synthesis and/or secretion (13). ApoB-100 secretion from HepG2 cells is reduced by prior incubation with n–3 FAs (38) perhaps by inhibiting diacylglycerol acyltransferase (39), phosphatidate phosphohydrolase (40), or by increasing intracellular degradation of apoB (41). Three studies of VLDL kinetics in humans concluded that n–3 FAs slow VLDL production rates (42–44). If n–3 FAs can inhibit the secretion of hepatic triacylglycerol-rich lipoproteins, it is not unreasonable to hypothesize that they might have the same effect on the secretion of intestinal triacylglycerol-rich lipoproteins, although monoacylglycerol acyltransferases from these two tissues appear to be regulated differently (45). Murthy et al. (46) and Ranheim et al. (47) min whereas the residence time calculation extrapolates to infinity. The higher slope of the curve for the control group between 8 and 16 min is largely responsible for the similar extrapolated areas under the curves (residence times). Other pertinent model-predicted parameters of chylomicron metabolism are presented in Table 5. The time to particle margination appeared to be reduced by 30% with n–3 FA treatment, but there was no difference in the time spent at the endothelium.
provided potential evidence for this hypothesis by showing that n-3 FAs reduced triacylglycerol synthesis in and secretion from CaCo-2 cells, a human intestinal cell line. However, these findings must be viewed with caution as the major triacylglycerol synthesizing pathway in CaCo-2 cells is the 3-glycerol-phosphate pathway (48), not the mono-acylglycerol pathway that predominates in enterocytes postprandially (49). Thus, although EPA pretreatment does inhibit lipoprotein triacylglycerol output by these cells, this may not represent the mechanism operative in the intestine in vivo.

With respect to the clearance hypothesis, several investigators have shown that n-3 FAs do not enhance postheparin LpL or hepatic lipase activity measured in vitro in plasma from humans (2, 3, 8, 9), rats (10), chickens (11), or pigs (12). Muscle LpL activity may be increased in rats, however (50). This lack of effect does not necessarily rule out increased chylomicron clearance as the mechanism because: 1) chylomicrons made in a chronically n-3 FA-fed animal may be chemically different from, and catabolized more rapidly than, chylomicrons made in a control rat; 2) hepatic chylomicron remnant receptors may be upregulated by n-3 FA feeding; 3) reduced VLDL levels may make more LpL available for chylomicron clearance; 4) LpL in n-3 FA-fed rats may have a greater affinity for chylomicrons; 5)

TABLE 5. Model-predicted parameters of chylomicron metabolism* (mean ± SEM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calculation</th>
<th>Control</th>
<th>N-3 FAs</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to margination (min)</td>
<td>1/L(2,1)</td>
<td>4.45 ± 0.29</td>
<td>3.11 ± 0.31</td>
<td>0.0057</td>
</tr>
<tr>
<td>Delay at endothelium (min)^1</td>
<td>2 × [1/L(23,2)]</td>
<td>3.22 ± 0.95</td>
<td>2.45 ± 0.38</td>
<td>0.46</td>
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<tr>
<td>LpL-mediated triacylglycerol removal (%)</td>
<td>L(30,2) × 100</td>
<td>92.9 ± 0.28</td>
<td>94.7 ± 0.26</td>
<td>0.0002</td>
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<tr>
<td></td>
<td>L(30,2) + L(23,2)</td>
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<tr>
<td>LpL-mediated retinyl ester removal (%)</td>
<td>L(40,12) × 100</td>
<td>28.5 ± 2.7</td>
<td>41.4 ± 2.3</td>
<td>0.0017</td>
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<tr>
<td></td>
<td>L(40,12) + L(33,12)</td>
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<tr>
<td>Chylomicron particle (native + remnant) residence time (min)</td>
<td>1/L(2,1) + 1/L(4,3)</td>
<td>11.9 ± 0.79</td>
<td>12.8 ± 0.86</td>
<td>0.43</td>
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<tr>
<td>Chylomicron remnant residence time (min)</td>
<td>1/L(4,3)</td>
<td>7.41 ± 0.74</td>
<td>9.86 ± 0.98</td>
<td>0.064</td>
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</table>

*The model is shown in Fig. 4.
*As L(23,2) was set equal to L(3,23) in model development, the endothelial delay was the sum of these two identical residence times.

These parameters were calculated as indicated because the model included three compartments at the endothelium (2, 23, and 30 in the triacylglycerol model and 12, 33, and 40 in the retinyl ester model, Fig. 4). A fraction of each tracer was removed from the chylomicron particle (presumably by LpL action) to compartments 30 and 40, respectively, without returning to the circulation during the 16-min sampling time. That fraction was estimated as the fraction going to compartments 30 and 40 divided by the total fraction leaving compartments 2 and 12, respectively, per minute.

Fig. 5. The fit of the model prediction to data from one representative rat from each group.
chylomicrons in n–3 FA-fed animals may be larger in size, or 6) n–3 FA feeding may somehow increase postprandial blood flow through LpL-rich tissues.

Weintraub et al. (3) addressed the first possibility by incubating chylomicrons (generated by a saturated fat-rich meal administered during a fish oil background diet) with LpL in vitro. They found no effect of background diet on lipolytic rates. Although this argues against enhanced lipolysis, it is still possible that in vivo such chylomicrons might be cleared more rapidly, especially if their surface chemical composition [either phospholipid amount (51) or molecular species (52), or apoE epitope exposure (53)] is altered. In addition, chylomicron particle clearance is not accelerated by the injection of heparin in normal subjects (54, 55), and larger chylomicron particles are cleared faster than smaller ones (56). Thus, it is apparent that LpL activity (especially when measured in vitro with an artificial emulsion as substrate) is not the sole determinant of in vivo chylomicron removal rates.

There have been no reports on the effects of n–3 FAs on receptor-mediated chylomicron remnant removal, either by the LDL receptor or the LDL receptor-related protein (57). N–3 FA feeding was found to either decrease (58) or increase (59) the activity of the LDL receptor in rats, but how this receptor may affect chylomicron clearance is still ill defined.

Past workers have found a significant correlation between fasting triacylglycerol levels and the extent of postprandial lipemia (3, 60), suggesting that reduced competition from VLDL may play a role. However, these results could also mean that whatever caused the fasting triacylglycerols to be lower was also causing the postprandial triacylglycerols to be reduced. In considering this issue, two facts must also be appreciated. N–3 FAs lowered fasting triacylglycerol levels in normal subjects from about 0.89 to 0.67 mmol/L, and postprandial levels were reduced in the rat even though fasting levels were not (Fig. 1). The failure of n–3 FAs to significantly lower fasting triglyceride levels in normolipidemic humans is not uncommon, occurring in about half of all placebo-controlled trials reported to date (62).

Furthermore, chylomicrons are known to be better substrates for LpL than VLDL particles (63), and the former have recently been shown to delay the clearance of the latter and not vice versa (64). One minute after lymph injection, plasma triacylglycerol levels were 20% lower in the n–3 FA-fed rats owing to the reduced endogenous chylomicron levels. Did the lower chylomicron levels in these rats account for the more rapid removal of labeled chylomicrons? We believe not in light of data from Huttlin et al. (65) who showed that variations in injected chylomicron triacylglycerol mass between 4 and 64 mg (we injected 19 mg) did not alter clearance rates. In other words, these authors could find no evidence of LpL saturation across this wide range of injected chylomicron triacylglycerol. Therefore, a n–3 FA-induced reduction in background triacylglycerol levels may be insufficient to explain the hypochylomicronemia, especially in normolipidemic subjects and animals.

The finding that n–3 FA supplementation does not enhance the clearance of Intralipid triacylglycerol (5) also argues against a removal effect, but several points must be considered before drawing this conclusion. 1) LpL is known to be stimulated in the fed state (66), and our failure to see increased Intralipid clearance in fasting subjects may have been due to the absence of the normal hormonal milieu of the postprandial state; 2) Intralipid particles may behave differently than chylomicrons (e.g., chylomicron triacylglycerol half-life was 2.3 min compared to 13.7 min for Intralipid triacylglycerol measured in the same subjects (67)); and 3) it is not known whether Intralipid remnants are cleared by the hepatic chylomicron receptor or whether their removal from the circulation reflects normal chylomicron remnant kinetics.

Reduced plasma and hepatic levels of labeled retinyl esters in the treated animals suggested that peripheral removal of chylomicron-borne [3H] retinyl esters was accelerated. This finding supports the recent work of Blaner et al. (25) who reported that LpL-mediated hydrolysis of retinyl palmitate can occur, especially in highly delipidated remnant lipoproteins. If such loss is confirmed in future studies using physiological particles (not synthetic lipid emulsions), the use of retinyl esters as chylomicron “particle” markers will need to be re-evaluated as has been suggested by others (26, 27).

Compartmental modeling suggested that margination was accelerated by n–3 FA treatment. If true, then LpL in an n–3 FA-fed rat may have a greater affinity for chylomicron particles, or more LpL may be available to bind chylomicrons. Alternatively, n–3 FA feeding may increase chylomicron apoC-II/C-III ratios or increase the sensitivity of LpL to activation by apoC-II. Another possibility could be that feeding n–3 FAs causes a small diversion of blood flow from LpL-poor-tissues into LpL-rich tissues (e.g., from the GI tract to adipose tissue).
Finally, there have been suggestions that differences in chylomicron phospholipid fatty acid composition could alter particle clearance rates (52).

Despite the more extensive removal of chylomicron triacylglycerol and retinyl ester noted above, the plasma residence time for chylomicron particles (nascent+remnants) themselves did not appear to be affected by treatment; there was even a suggestion that remnant removal rates were prolonged by about 33\% in the n–3 FA group (see Table 4; also note the difference in retinol disappearance rates between 8 and 16 min in Fig. 3). It is important to note here that part of the power of model-based compartmental analysis lies in its ability to determine particle kinetics even if the labeled constituent leaves the particle. However, this requires that an estimate of the label departure rate be available, which in this case, was derived from the 0 to 4 min disappearance rates for both markers (having data available from two separate markers increased the power to make these estimates). Clearly, all of the hypotheses derived from the model will need to be tested directly in future studies.

In conclusion, the studies reported here suggest that n–3 FA-feeding affects chylomicron clearance, not production. The data raise the possibility that increased intravascular lipolysis may be the cause of n–3 FA-induced reduction in postprandial chylomicron triacylglycerol levels in the rat. It remains to be seen whether the same is true in humans.27

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