Feeding Minipigs Fish Oil for Four Weeks Lowers Postprandial Triacylglycerolemia

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ABSTRACT

We wanted to establish a minipig model for the study of postprandial lipemia and plasma lipid clearance after fish oil consumption. Seven minipigs were fed a fish oil–enriched nonpurified diet and a control diet for 4 wk in a randomized cross-over study. After each intervention period, each pig was challenged with a gastric fat load (2 g fat/kg body) and an intravenous fat bolus (0.1 g/kg body) on separate days. Frequent blood samples were collected for 6 h after the gastric fat load and for 40 min after the intravenous bolus. The fish oil–enriched diet was associated with lower triacylglycerol, glycerol and nonesterified fatty acid concentrations in the hours after the gastric fat load than the control diet (P < 0.05). In contrast, the triacylglycerol disappearance rate after the intravenous fat bolus was not affected by fish oil (P = 0.19). In conclusion, dietary fish oil supplementation attenuates postprandial lipemia in minipigs similarly to what occurs in humans. Minipigs could serve as a useful model for future studies of this phenomenon. We observed no significant effect of fish oil supplementation on plasma triacylglycerol clearance and thus were unable to identify the mechanism explaining the attenuated lipemia in minipigs.

KEY WORDS: • lipid clearance • pigs • triacylglycerol

The very long–chained (n-3) PUFA [(n-3) VLCPUFA], abundantly present in fish oil, can correct hypertriacylglycerolemia and lower postprandial plasma triacylglycerol concentrations in humans (1–9). The daily consumption of 1 g (n-3) VLCPUFA was shown to lower postprandial triacylglycerolemia by ~30% (6,10). This effect of (n-3) VLCPUFA may be explained by increased clearance of lipid from the blood and/or altered intestinal lipid absorption.

In a rat model, we demonstrated previously that a delayed efflux of triacylglycerol from the enterocyte into the blood stream contributes to the lowering effect of (n-3) VLCPUFA on postprandial triacylglycerolemia (11). However, this observation does not exclude an effect of fish oil consumption on plasma lipid clearance. Harris and co-workers demonstrated that long-term fish oil feeding increases lipid clearance in rats (9), and long-term intake of fish oil has been shown to increase endogenous nonheparin–induced lipoprotein lipase (LPL) activity in humans (12). On the other hand, daily intake of (n-3) VLCPUFA intake did not affect postheparin LPL activity and triacylglycerol clearance in several other human trials (1–3,13–15). Whether long-term (n-3) VLCPUFA consumption affects postprandial plasma lipid clearance therefore remains unclear.

Minipigs may be a suitable model for studying fish oil effects on postprandial lipemia because of the similarity in lipid metabolism between humans and minipigs, i.e., both are mammals with LDL (16). In the present study, we therefore used a recently established minipig model to investigate the effect of consumption of (n-3) VLCPUFA for 4 wk on postprandial triacylglycerolemia and plasma triacylglycerol clearance.

MATERIALS AND METHODS

Animals. Male castrated minipigs (n = 7; 1 y old; Ellegaard Göttingen Minipigs, Dalme, Denmark) health monitored according to the Federation of European Laboratory Animal Science Associations guidelines (17) and with a median weight of 23.8 kg were maintained at 19–21°C and a humidity of 55–80%. The study was performed in accordance with the Danish Animal Experimentation Act on a license granted by the Ministry of Legal Affairs. All housing and procedures were as a minimum performed according to the Convention ETS 123 of the Council of Europe.

Diets. The pigs were fed a standard nonpurified diet for minipigs during an acclimation period of 1 wk. The diet was ground and enriched with either cod liver oil [approximate fatty acid composition: saturated fatty acids (SFA): 17 g/100 g; monounsaturated fatty acids (MUFA): 48 g/100 g; polyunsaturated fatty acids (PUFA): 40 g/100 g], fish oil diet, or a mixture of sunflower, palm, and olive oil (1:1:5) (control diet). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%). The vegetable oils were purchased from Aarhus, Denmark (fish oil diet) or a mixture of sunflower, palm, and olive oil (%).

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composition of the diet is shown in Table 1. Because α-tocopherol was added to the applied fish oil, it was also added to the oil mixture used for the control group (0.01 g/100 g; α-tocopherol acetate, Sigma-Aldrich, Copenhagen, Denmark). Each minipig was offered 2 × 220 g of the experimental diet (total fat: 4.1 g/100 g) when consuming the fish oil diet, each minipig was therefore offered ~2.5 g/d (n-3) VLCPUFA (20:5 + 22:5 + 22:6). The diets were prepared in 10-kg batches and kept frozen until used. The two diets were coded with colored labels and the codes were not broken until results were analyzed.

**Feeding regimen.** After the acclimation period, the minipigs were fed the fish oil diet and the control diet for periods of 4 wk in a randomized, blinded cross-over study. The feeding periods were separated by a washout period of 3 wk during which the pigs were fed the standard diet. At the beginning of the study, the seven pigs were randomly assigned to two groups with three or four pigs, and placed in two pens with free access to water and straw. The pigs remained in the same pen throughout the study. The pigs were fed twice each day (morning and afternoon). One of the minipigs was fed in a separate pen because it was kept from feeding by the other minipigs. After each feeding period, each pig was challenged with a gastric fat load and then 2 d later an intravenous fat bolus. The pigs were weighed once each week throughout the experiment.

**Gastric fat load.** A model for studying postprandial lipemia was developed in an earlier study (18). Minipigs were fed 220 g of the standard diet 1 h before gastric fat administration via a gastric tube. Fat was given as Intralipid (200 g soy oil/L, 12 g egg yolk/L, and 21.3 g standard diet 1 h before gastric fat administration via a gastric tube. Developed in an earlier study (18). Minipigs were fed 220 g of the fish oil diet, each minipig was offered 2 g fat/kg body. A total of 48–68 g of fat (soy oil) was given; each pig received the same amount of fat after both feeding periods. One third was given at time 0 h, and the remaining two thirds at time 1.5 h. Blood samples were collected immediately before the first fraction (baseline, t = 0 h) and then at t = 3, 3 1/2, 4, 5, and 6 h. Blood was sampled from the cranial vena cava in tubes containing EDTA (final concentration: 0.004 mol/L) and was kept on ice until centrifuged at 3000 × g for 15 min at 4°C. Chylomicrons were isolated from 3 mL freshly isolated plasma (see below). The remaining plasma was frozen in aliquots at −80°C until analysis.

**Intravenous fat bolus.** As the final test of each experimental period, each minipig had 0.5 mL/kg body of Intralipid (200 g fat/L, Fresenius Kabi AB) injected intravenously through a catheter in the carotid vein over a period of 30 s. Blood samples (1 mL) were collected in syringes by puncture of the cranial vena cava before and 2, 4, 6, 8, 10, 15, 20, 30 and 40 min after infusion of the fat emulsion. Serum was separated by centrifugation (3000 × g in 15 min at 4°C) and frozen at −80°C until analysis. The fat bolus was always given in the morning −45 min after the usual morning feeding.

**Analytical methods.** Chylomicrons were separated from VLDL, LDL and HDL as described previously (20). Triacylglycerol concentrations were analyzed in chylomicrons. Commercial enzymatic methods were applied for analyses of triacylglycerol (GPO-PAP, Boehringer Mannheim, Mannheim, Germany), total cholesterol (CHOD-PAP, Boehringer Mannheim), HDL cholesterol (HDL-C plus, Boehringer Mannheim), nonesterified fatty acids (NEFA) (NEFA C, Wako Chemicals GmbH, Neuss, Germany) and free glycerol (Randox Laboratories, Crumlin, United Kingdom) on a Cobas Mira S (Roche Diagnostics, Basel, Switzerland). LDL cholesterol concentrations were calculated as total cholesterol − (total triacylglycerol/2.2 + HDL cholesterol) (21). Fatty acid compositions of the experimental diets were determined by GLC as described previously (11).

**Statistical methods.** Due to the non-Gaussian distributions of some variables, nonparametric statistics were applied except for the fatty acid composition of the experimental diets. Within-group changes over time were tested with Friedman’s two-way ANOVA, whereas between treatment effects were tested with Wilcoxon matched pairs signed rank sum test. Areas under the curve were calculated with the trapezoidal method described by Matthews et al. (22). Differences were considered significant at P < 0.05.

### Table 1

<table>
<thead>
<tr>
<th>Fatty acid composition in the lipid fraction of the control and the fish oil diets</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control diet</strong></td>
<td><strong>Fish oil diet</strong></td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>36.0 (33.2–36.9)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>3.9 (3.6–4.1)</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>ND</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>ND</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>ND</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>ND</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>18.5 (18.3–18.6)</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>39.5 (38.5–42.9)</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>40.1 (39.6–40.9)</td>
</tr>
<tr>
<td>Σ (n-6) PUFA</td>
<td>36.0 (32.3–36.8)</td>
</tr>
<tr>
<td>Σ (n-3) PUFA</td>
<td>3.9 (3.6–4.1)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>2.1 (1.8–3.1)</td>
</tr>
<tr>
<td></td>
<td>3.2 (3.1–3.4)</td>
</tr>
</tbody>
</table>

1 Values are means (ranges) of determinations of aliquots collected during the whole study (n = 5).
2 Abbreviations: ND, not detectable; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

### RESULTS

Plasma triacylglycerol, total cholesterol, HDL cholesterol, LDL cholesterol, NEFA and free glycerol concentrations in morning blood samples of minipigs that had been deprived of food overnight did not differ between treatments. Median (mmol/L; 25–75 percentiles) plasma concentrations were triacylglycerol: 0.56 (0.46–0.64); total cholesterol 1.52 (1.30–1.70); HDL cholesterol: 0.58 (0.49–0.66); LDL cholesterol: 0.68 (0.51–0.86); NEFA: 0.02 (0.02–0.03); and free glycerol: 21.8 μmol/L (11.6–28.2 μmol/L).

Irrespective of the treatment (fish oil or control diet), the gastric fat load increased the plasma concentrations of triacylglycerol (Fig. 1, upper panel), chylomicron triacylglycerol (Fig. 1, lower panel), free glycerol (Fig. 2, upper panel), and NEFA (Fig. 2, lower panel). The fish oil diet attenuated the postprandial increases (P < 0.05).

The intravenous fat bolus resulted in a dramatic and sharp increase in serum triacylglycerol concentrations (maximum concentration ~3.7 mmol/L) followed by their rapid disappearance with no difference between diet treatments. After the fish oil diet, there was a tendency toward a more rapid disappearance of triacylglycerol during the first 15 min [median rate: 0.14 vs. 0.09 mmol/L (min) - min], but the difference was not significant (P = 0.19). Areas under the triacylglycerol clearance curves did not differ between diet treatments (P = 0.30); medians [mmol/L (min) - min] 25–75 percentiles, fish oil diet: 119.2 (107.6–155.1); control diet: 105.8 (86.5–129.4).

### DISCUSSION

The present study demonstrates that minipigs can be used for studying the effect of (n-3) VLCPUFA on postprandial triacylglycerolemia. An important advantage of minipigs compared with the more frequently used rats is that it is possible to draw a series of blood samples from the same minipig, which improves the possibility of studying the dynamics of postprandial lipemia and using cross-over designs. The similarities between human and porcine gastrointestinal physiology, lipoproteins and sensitivity to atherosclerosis in both species.
make the minipig a very attractive model for the study of postprandial lipemia (23,24).

Our study demonstrated that daily intake of fish oil lowers postprandial triacylglycerolemia in minipigs as it does in humans (2,3,6). As assessed from clearance kinetics of the intravenously administered lipid emulsion, the lower postprandial triacylglycerolemia could not be explained by the effects of fish oil on peripheral triacylglycerol clearance. However, it should be noted that our study had limited statistical power. Post-hoc power calculation on the triacylglycerolemia clearance data showed that with the applied design (7 minipigs in a cross-over design) we would be able to detect a 31% difference between the two diets (power: 0.80, significance level: 5%). The calculation also showed that for detection of a 20% difference, a study with 20 minipigs would have been required.

A single study suggested that endogenous LPL and hepatic lipase activities increase after daily (n-3) VLCPUFA intake, and in rats, an increased lipid clearance was indeed demonstrated (9,12). However, others were unable to confirm these findings (14,15). If fish oil increases LPL lipolysis, then postprandial plasma concentration of free glycerol and NEFA (products of lipolysis) would increase after the fish oil diet. We observed the opposite, i.e., significantly lower postprandial free glycerol and NEFA concentrations (Fig. 2). This observation could indicate a decreased flux of lipids from the intestine to the blood stream. This possibility is supported by our previous observation of a postprandial accumulation of triacylglycerol within the enterocytes and possibly also in the intestinal lumen after 4 wk of fish oil feeding of rats (11). Further support comes from in vitro studies with Caco-2 cells and hepatocytes, demonstrating that incubation with (n-3) VLCPUFA decreases triacylglycerol synthesis and secretion (25–27).

Intralipid is a homogenous protein-free triacylglycerol-rich lipid emulsion (average diameter of lipid particles: 1 μm). Chylomicrons are triacylglycerol-rich lipoproteins and are less homogeneous in composition and size. It is not known whether the intravenously infused lipid emulsion was metabolized in the same way as the endogenous chylomicrons, i.e., by LPL lipolysis and with remnant uptake in the liver. One study indeed indicated that triacylglycerol in emulsions was metabolized differently from chylomicron-triacylglycerol (28). However, Martins and co-workers (29) concluded that the metabolism of lipid emulsions and chylomicrons is comparable if the emulsions contain cholesterol. Intralipid is a cholesterol-containing emulsion (29–32). We therefore assert that the intravenous infusion of Intralipid is a relevant model for elucidating postprandial lipid clearance kinetics.

In summary, the present study demonstrated that minipigs can be used for studying the effect of (n-3) VLCPUFA on postprandial triacylglycerolemia. Further, the present study demonstrated that intake of (n-3) VLCPUFA lowers postprandial triacylglycerolemia in minipigs. We found no evidence that this phenomenon is explained by fish oil affecting peripheral triacylglycerol clearance.
LITERATURE CITED


