Omega-3 fatty acid ethyl ester supplementation decreases very-low-density lipoprotein triacylglycerol secretion in obese men


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Abstract

Dysregulated VLDL-TAG (very-low-density lipoprotein triacylglycerol) metabolism in obesity may account for hypertriacylglycerolaemia and increased cardiovascular disease. ω-3 FAEEs (omega-3 fatty acid ethyl esters) decrease plasma TAG and VLDL concentrations, but the mechanisms are not fully understood. In the present study, we carried out a 6-week randomized, placebo-controlled study to examine the effect of high-dose ω-3 FAEE supplementation (3.2 g/day) on the metabolism of VLDL-TAG in obese men using intravenous administration of d5-glycerol. We also explored the relationship of VLDL-TAG kinetics with the metabolism of VLDL-apoB-100 and HDL (high-density lipoprotein)-apoA-I. VLDL-TAG isotopic enrichment was measured using gas chromatography-mass spectrometry. Kinetic parameters were derived using a multicompartmental model. Compared with placebo, ω-3 FAEE supplementation significantly lowered plasma concentrations of total (−14 %, P < 0.05) and VLDL-TAG (−32 %, P < 0.05), as well as hepatic secretion of VLDL-TAG (−32 %, P < 0.03). The FCR (fractional catabolic rate) of VLDL-TAG was not altered by ω-3 FAEEs. There was a significant association between the change in secretion rates of VLDL-TAG and VLDL-apoB-100 (r = 0.706, P < 0.05). However, the change in VLDL-TAG secretion rate was not associated with change in HDL-apoA-I FCR (r = 0.139, P > 0.05). Our results suggest that the TAG-lowering effect of ω-3 FAEEs is associated with the decreased VLDL-TAG secretion rate and hence lower plasma VLDL-TAG concentration in obesity. The changes in VLDL-TAG and apoB-100 kinetics are closely coupled.

Key words: fish oil supplementation, obesity, triacylglycerol-rich lipoprotein kinetics

INTRODUCTION

Hypertriacylglycerolaemia is the most consistent lipid disorder in visceral obesity and a risk factor for coronary artery disease [1,2]. In the postabsorptive state, this abnormality is due to alterations in the kinetics of TAG (triacylglycerol)-rich VLDL (very-low-density lipoprotein), including overproduction and/or delayed clearance of VLDL [3]. Expansion of the VLDL-TAG pool also dysregulates HDL (high-density lipoprotein) metabolism by enhancing CETP (cholesteryl ester transfer protein)-mediated hetero-exchange of neutral lipids among lipoproteins, and the subsequent increased catabolism of TAG-rich HDL [4]. Accumulation of TAG-rich lipoproteins can play a central role in atherogenesis, including foam cell formation, endothelial injury and inflammation [2,5]. Correction of hypertriacylglycerolaemia may reduce the risk of CVD (cardiovascular disease) in obese subjects.

Fish oils are rich source of long-chain ω-3 fatty acids, primarily EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Fish oil supplementation lowers plasma TAG, and may therefore be useful for treating for obesity-related dyslipidaemia [5]. The ethyl ester is potentially more effective than the TAG form of omega-3 fatty acids because of its higher bioavailability, sustained intestinal absorption and protection against oxidative stress [6]. We have reported previously that ω-3 FAEE (omega-3 fatty acid ethyl ester) supplementation decreased hepatic

Abbreviations: apo, apolipoprotein; BMI, body mass index; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FCR, fractional catabolic rate; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; NEFA, non-esterified ‘free’ fatty acid; ω-3 FAEE, omega-3 fatty acid ethyl ester; SR, secretion rate; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

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secretion of VLDL-apoB (apolipoprotein B)-100 [7]. However, the effect of ω-3 FAEEs on VLDL-TAG metabolism has not yet been examined in obese subjects.

In the present study, we examined the effects of ω-3 FAEE supplementation on VLDL-TAG kinetics in men with visceral obesity. We hypothesized that ω-3 FAEEs would decrease plasma TAG concentrations by concomitantly reducing the secretion and increasing the FCR (fractional catabolic rate) of VLDL-TAG. We also explored whether changes to VLDL-TAG kinetics were associated with changes in the metabolism of VLDL-apoB-100 and HDL-apoA-I.

MATERIALS AND METHODS

Subjects
A total of 24 non-smoking abdominally obese men (waist circumference >100 cm with BMI (body mass index) ranging from 27 to 46 kg/m², plasma TAG >1.2 mmol/l and cholesterol >5.2 mmol/l) were recruited from the community. VLDL-TAG kinetics are only reported in 22 subjects because of insufficient sample available in two subjects. The clinical characteristics and treatment effects on lipids and lipoproteins in these 22 subjects did not differ significantly from the parent group [7]. None of the subjects had diabetes mellitus, APOE2/2 genotype, macroproteinuria, creatinemia (>120 μmol/l), hypothyroidism, abnormal liver enzymes, consumed >30 g alcohol/day, history of CVD, or were on agents known to affect lipid metabolism. The study was approved by the Ethics Committee of the Royal Perth Hospital.

Study design and clinical protocols
The study presented is a component of a large placebo-controlled 2×2 factorial designed intervention trial with ω-3 FAEE supplementation and other agents on lipoprotein metabolism. The aim of the overall study was to examine aspects of the mechanisms of action of ω-3 FAEE supplementation and atorvastatin on lipoprotein metabolism, including the metabolism of apoA-I, apoA-II, apoB and chylomicron remnants [7,8]. Briefly, volunteers entered a randomized double-blind placebo-controlled trial involving a 3-week run-in period with body weight variations of <2%. After the run-in period they were randomized to Lovaza (4 g/day, consisting of 46% EPA and 38% DHA, equivalent to 3.2 g of ω-3 FAEE) or 4 g/day placebo corn oil. Compliance was assessed by capsule count at weeks 3 and 6. All subjects were admitted to a metabolic ward in the morning after a 14-h fast. Venous blood was collected for measurements of biochemical analytes. Plasma volume was determined by multiplying body weight by 0.045 and by a correction factor to adjust for the decrease in relative plasma volume associated with an increase in body weight [9]. A single bolus of d3-leucine (5 mg/kg of body weight) and d5-glycerol (10 mg/kg of body weight) was administered intravenously and blood samples were taken at baseline and after isotope injection at 5, 10, 20, 30 and 40 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 24 h. Additional fasting blood samples were collected in the morning on the next 4 days of the same week. Dietary intake was assessed for energy and major nutrients using at least two 24 h dietary diaries in the week prior to the kinetic studies before and after 6-week intervention. Diets were analysed using DIET 4 Nutrient Calculation Software (Xyris Software).

Isolation and measurement of isotopic enrichment of plasma and VLDL-TAG
VLDL (density <1.006 kg/l) was separated by ultracentrifugation from 2 ml of whole plasma (50000 rev/min, 16 h, 20°C). The VLDL fraction from each time point was mixed with equal volumes of isopropanol. Heat-activated zeolite was added to the VLDL/isopropanol mixture to remove phospholipids. The sample was mixed and centrifuged for 30 min at 2000 g and 4°C. The supernatant was aspirated and evaporated to dryness under nitrogen. The samples were saponified with KOH (2 h at 60°C). Lipids were extracted by the addition of chloroform/water, mixed and centrifuged (2000 g, 20 min, 4°C). The top aqueous layer was aspirated and dried down under nitrogen. Glycerol was derivatized with MTBSTFA and pyridine (1:1) for 4 h at 70°C. Plasma glycerol was isolated from plasma by precipitating with 6% (v/v) perchloric acid, followed by ion exchange chromatography and derivatized as described above. Isotopic enrichment was determined by selected ion monitoring of derivatized samples at a mass/charge ratio of 377 and 382. The SAAM II program (The Epsilon Group) was used to fit the model to the observed tracer data. VLDL-TAG metabolic parameters, including FCR and SR (secretion rate) were derived following a fit of the compartment model, to the glycerol tracer/tracee ratio data [10]. This model was built on the back of the VLDL apoB-100 model, described below.

Isolation of apo and measurements of leucine enrichments
Laboratory methods for isolation and measurement of isotopic enrichment have been detailed previously [11,12]. Briefly, apoB-containing lipoproteins were precipitated from 250 μl of plasma using heparin and MnCl₂. HDL was subsequently separated by ultracentrifugation, isolated using SDS/PAGE and transferred to the PVDF membrane. The apoA-I band was excised from the membrane, hydrolysed with 200 μl of 6M HCl at 110°C for 16 h and dried for derivatization. For apoB isolation, VLDL were isolated from 3 ml of plasma by sequential ultracentrifugation at density (d) of 1.006 g/ml. ApoB-100 in the VLDL fraction was then precipitated by isopropanol, delipidated, hydrolysed and derivatized. Isotopic enrichment of apoA-I and apoB-100 were then determined by selected ion monitoring of derivatized samples using gas chromatography-mass spectrometry. Tracer-to-tracee ratio was derived from isotopic ratios for each sample.

Model of apoA-I and apoB-100 metabolism and calculation of kinetic parameters
Models of apoA-I and apoB-100 and calculation of kinetic parameters have been detailed previously [11,12]. Briefly, the apoA-I model includes a four-compartment subsystem (compartments 1–4), which describe plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment 5 that accounts for the time required for the synthesis and secretion of apoA-I.
Fish oil and lipoprotein metabolism in obesity

into plasma. Compartments 6 and 7 describe the kinetics of apoA-I in the plasma HDL fraction and in a non-plasma compartment, respectively. The apoB-100 model includes a plasma leucine subsystem, intrahepatic delay compartment and five compartments (four-compartment delipidation cascade and a single compartment for slowly turning over VLDL). The SAAM II program (The Epsilon Group) was used to fit the model to the observed tracer data. HDL-apoA-I and VLDL-apoB metabolic parameters, including FCR and SR, were derived following a fit of the compartment model to the tracer/tracee ratio data.

Biochemical analyses

Plasma TAG and cholesterol concentrations were determined by standard enzymatic methods with the use of a biochemical analyser (model 917; Hitachi). VLDL-TAG was measured by an enzymatic method with the use of a commercial kit (Trace Scientific) after ultracentrifugation as described above. HDL cholesterol was measured by an enzymatic colorimetric method using a commercial kit (Boehringer Mannheim). Non-HDL cholesterol was derived as total cholesterol minus HDL cholesterol. LDL cholesterol was calculated by the Friedewald equation. Apo-AI and apoB concentrations were determined by immunonephelometry (Beckman Instruments). Plasma insulin was measured by radioimmunoassay (DiaSorin) and glucose concentration by a hexokinase method on a Hitachi 917 analyser. Insulin resistance was estimated by the HOMA (homeostasis model assessment) that uses the formula [fasting insulin (m-unit/l)×fasting plasma glucose (mmol/l)/22.5] [13].

Statistical analyses

Analyses were carried out using SPSS software version 15.0. Group characteristics were compared by Student’s t tests. Adjustment for differences in baseline covariates and changes in variables during the study were performed by ANCOVA (analysis of co-variance) using general linear models. Associations were examined by linear regression methods. Statistical significance was defined at the 5% level using a two-tailed test.

RESULTS

The subjects were middle-aged (mean ± S.D., 55 ± 9 years) normotensive (blood pressures 130 ± 12/76 ± 9 mmHg), centrally obese (waist circumference, 112 ± 8 cm; BMI, 33 ± 4 kg/m²), mildly dyslipidaemic [plasma TAG, 1.7 ± 0.6 (range, 1.2–2.7) mmol/l; total cholesterol, 5.8 ± 0.6 mmol/l]. Compared with a group of ten normolipidaemic non-obese subjects (age 53 ± 9 years; BMI, 25 ± 3 kg/m²; plasma TAG, 0.8 ± 0.3 mmol/l; and total cholesterol, 4.3 ± 0.3 mmol/l) from one of our previous studies [13], the obese subjects were insulin-resistant (HOMA score, 8.6 ± 3.7 against 5.7 ± 1.2; P < 0.05) and 11 of them had the metabolic syndrome [14]. Average daily energy and nutrient intake (mean ± S.D.) was 9834 ± 1454 kJ (33 ± 5% energy from fat, 41 ± 7% energy from carbohydrates, 16 ± 5% energy from protein and 6 ± 6% energy from alcohol); none of these variables differed between groups at baseline. Using data from our previous study [10], VLDL-TAG concentrations and production rates were significantly higher in the obese compared with lean subjects (VLDL-TAG, 1.05 ± 0.44 compared with 0.56 ± 0.12 mmol/l; and secretion rate, 357 ± 170 mmol/l 200 ± 70 mg/kg of body weight per day; P < 0.05 for both). Capsule counts confirmed that compliance with randomization to active intervention or placebo was >95%. As we reported previously [15], plasma EPA and DHA concentrations (% total fatty acid) also increased by 350 and 85% respectively, confirming therapeutic compliance with fish oil capsules. No subject reported side effects.

Table 1 shows the anthropometric characteristics, plasma lipid, lipoprotein, apolipoprotein, glucose, insulin concentrations and HOMA score before and after 6 weeks on ω-3 FAEE supplementation and placebo. Compared with placebo, ω-3 FAEEs significantly lowered plasma TAG (−14%, P < 0.05) and VLDL-TAG (−32%, P < 0.05). Plasma concentrations of total cholesterol, non-HDL-cholesterol, LDL-cholesterol, apoB-100, apoC-III and NEFAs (non-esterified ‘free’ fatty acids) were also lowered; none of these changes were significant. There were no significant changes in body weight, blood pressures, glucose, insulin and HOMA score with ω-3 FAEEs. Daily energy and nutrient intake did not alter significantly during the study. The changes in plasma TAG (r = 0.621, P = 0.055) and VLDL-TAG (r = 0.706, P = 0.03) were associated with the change in VLDL-apoB-100 concentration on ω-3 FAEEs.

Compared with placebo (Figure 1), ω-3 FAEEs significantly lowered VLDL-TAG concentration (−32%, P < 0.05) and secretion rate (−32%, P < 0.05), without significant change in FCR.

Using the VLDL-apoB-100 and HDL-apoA-I kinetic data from the same subjects [7,8], the percentage reduction in VLDL-TAG secretion rate was significantly associated with decreased VLDL-apoB-100 secretion (Figure 2). However, no significant association was found between the change in VLDL-TAG secretion rate and percentage VLDL into LDL conversion (results not shown). The change in VLDL-TAG secretion was also not associated with a change in HDL-apoA-I FCR (r = 0.139, P > 0.05).

DISCUSSION

Our principal finding was that ω-3 FAEE supplementation decreased plasma VLDL-TAG concentrations in obese men by decreasing the hepatic secretion of VLDL-TAG without significantly altering the fractional catabolic rate. These were independent of changes in insulin resistance, body weight and dietary fat. We also demonstrated significant association between the secretion rates of VLDL-TAG and VLDL-apoB-100.

Fish oil supplementation decreased VLDL-apoB-100 concentration by reducing the secretion of VLDL, enhancing VLDL conversion into LDL [7,16] and, to a lesser extent, by increasing the FCR of VLDL-apoB-100 [17]. Using radiolabelling, Nestel et al. [18] showed in six non-obese subjects that 3 weeks of high-dose dietary fish oils (MaxEPA) decreased VLDL-TAG concentration chiefly by reducing its secretion. In an uncontrolled study of five non-obese subjects with severe hypertriglyceridaemia (range from 4.6 to 40 mmol/l), Sanders et al. [19] found that
Table 1  Changes in anthropometric characteristics, dietary intakes, plasma lipids, lipoproteins, apolipoproteins and biochemical characteristics after 6 weeks placebo and ω-3 FAEE supplementation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n = 12) Baseline</th>
<th>Placebo (n = 12) Week 6</th>
<th>ω-3 FAEEs (n = 10) Baseline</th>
<th>ω-3 FAEEs (n = 10) Week 6</th>
</tr>
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<tbody>
<tr>
<td>Age (year)</td>
<td>58 ± 3</td>
<td>51 ± 3</td>
<td>24 ± 2</td>
<td>24 ± 2</td>
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<tr>
<td>Weight (kg)</td>
<td>100 ± 2</td>
<td>100 ± 2</td>
<td>104 ± 6</td>
<td>104 ± 7</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>32 ± 1</td>
<td>32 ± 1</td>
<td>34 ± 2</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110 ± 2</td>
<td>110 ± 2</td>
<td>114 ± 3</td>
<td>114 ± 3</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>128 ± 3</td>
<td>130 ± 3</td>
<td>132 ± 4</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 3</td>
<td>80 ± 2</td>
<td>76 ± 3</td>
<td>75 ± 3</td>
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<tr>
<td>Energy intake (kJ)</td>
<td>9611 ± 274</td>
<td>9611 ± 274</td>
<td>9503 ± 386</td>
<td>9390 ± 314</td>
</tr>
<tr>
<td>Protein intake (%)</td>
<td>21 ± 2</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>22 ± 1</td>
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<tr>
<td>Fat intake (%)</td>
<td>34 ± 1</td>
<td>36 ± 1</td>
<td>34 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Carbohydrate intake (%)</td>
<td>38 ± 2</td>
<td>38 ± 3</td>
<td>43 ± 2</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Alcohol intake (%)</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Plasma TAG (mmol/l)</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.3 ± 0.2¹</td>
</tr>
<tr>
<td>VLDL-TAG (mmol/l)</td>
<td>1.07 ± 0.57</td>
<td>1.00 ± 0.57</td>
<td>1.31 ± 0.95</td>
<td>0.78 ± 0.47*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.8 ± 0.1</td>
<td>5.6 ± 0.1</td>
<td>5.9 ± 0.3</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.05 ± 0.06</td>
<td>1.03 ± 0.06</td>
<td>1.02 ± 0.07</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/l)</td>
<td>4.8 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>4.0 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>ApoA-I (g/l)</td>
<td>1.3 ± 0.05</td>
<td>1.3 ± 0.04</td>
<td>1.2 ± 0.04</td>
<td>1.2 ± 0.05</td>
</tr>
<tr>
<td>ApoB-100 (g/l)</td>
<td>1.3 ± 0.04</td>
<td>1.2 ± 0.03</td>
<td>1.3 ± 0.07</td>
<td>1.1 ± 0.05</td>
</tr>
<tr>
<td>VLDL-apoB-100 (mg/l)</td>
<td>99 ± 12</td>
<td>88 ± 10</td>
<td>77 ± 6</td>
<td>56 ± 6*</td>
</tr>
<tr>
<td>ApoC-III (mg/l)</td>
<td>156 ± 9</td>
<td>143 ± 7</td>
<td>145 ± 7</td>
<td>133 ± 6</td>
</tr>
<tr>
<td>Fasting NEFAs (mmol/l)</td>
<td>0.26 ± 0.03</td>
<td>0.27 ± 0.02</td>
<td>0.39 ± 0.06</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.4 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>Fasting insulin (m-unit/l)</td>
<td>32 ± 3</td>
<td>29 ± 2</td>
<td>38 ± 4</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Insulin resistance (HOMA score)</td>
<td>7.7 ± 0.7</td>
<td>7.4 ± 0.5</td>
<td>9.7 ± 1.5</td>
<td>11.0 ± 2.7</td>
</tr>
</tbody>
</table>

15 g of MaxEPA decreased VLDL-TAG secretion without altering its catabolism. Using radionlabelled chylomicron emulsions, Park and Harris [20] showed that 4 weeks of ω-3 FAEEs (4 g/day) reduced postprandial plasma TAG and apoB-100 concentrations by accelerating chylomicron-TAG clearance in healthy subjects, but they did not examine VLDL-TAG kinetics. Only one study has examined the effects of fish oils on VLDL-TAG metabolism using stable isotope techniques [21]. Harris et al. found, in nine non-obese subjects, that a 3–5 week daily supplementation with 10–17g of MaxEPA decreased VLDL-TAG concentration by decreasing VLDL-TAG secretion rate and increasing its FCR. We have extended previous reports by using a highly purified form of ω-3 FAEEs in a placebo-controlled study design to investigate the effect of fish oil supplementation on VLDL-TAG metabolism in insulin-resistant obese subjects.

Obesity and insulin resistance alter the metabolism of VLDL particles [1,3]. Abdominal visceral adipocytes have increased β1-adrenergoreceptor sensitivity to catecholamine stimulation, but reduced sensitivity to the antilipolytic effect of insulin [22,23]. Hence there are some suggestions that these effects may lead to an increased delivery of NEFAs into the portal system [24]. This not only stimulates hepatic TAG synthesis, but also impairs hepatic extraction of insulin. Hepatic insulin resistance up-regulates the expression of sterol regulatory element-binding protein 1c, with activation of lipogenesis. The net effect is increased synthesis and accumulation of TAG in the liver, with oversecretion of VLDL particles. This is consistent with our observation that the obese subjects had higher VLDL-TAG concentration and secretion compared with controls. The decrease in VLDL-TAG secretion with ω-3 FAEEs could involve mechanisms that decrease hepatic TAG substrate availability [25], such as inhibition of de novo lipogenesis and TAG synthesis, and activation of PPARs (peroxisome-proliferator-activated receptors), with enhancement of mitochondrial and peroxisomal β-oxidation of fatty acids.

We have reported previously that ω-3 FAEE supplementation decreased VLDL-apoB-100 secretion, increased VLDL conversion into LDL and lowered HDL-apoA-I FCR in these subjects [7,8]. In the present study, we found that changes in the secretion rates of VLDL-TAG and VLDL-apoB-100 with ω-3 FAEEs were significantly correlated. This implies that these metabolic processes are coupled and the availability of core TAG substrate is central to the regulation of apoB-100...
Fish oil and lipoprotein metabolism in obesity

Figure 1 VLDL-TAG concentrations and kinetic parameters following placebo and ω-3 FAEE supplementation

Values are means ± S.E.M.; * P < 0.05 compared with baseline within group comparison. The lines indicating the significance level (above the bars) using general linear model line with baseline values as covariates.

secretion by the liver. However, only 50% of the variance in change of VLDL-apoB-100 secretion was explained by change in VLDL-TAG secretion, implying that beyond analytical and biological variation other factors independently regulate VLDL particle secretion. That changes in VLDL-TAG secretion and HDL-apoA-I FCR were not correlated suggests that additional mechanisms underlie these alterations in HDL catabolism following ω-3 FAEE supplementation.

Our study has limitations. A cross-over design would reduce between subject variation. However, this design requires an extended study period for each subject and consideration of carry-over effects. Corn oil, used as a placebo oil, could possibly influence VLDL-TAG metabolism. However, we could not confirm its effect on plasma lipids nor VLDL-TAG concentration and kinetic variables in the present study. Between-group differences in pre-intervention VLDL-TAG secretion and FCR may appear to confound the effects of ω-3 FAEE. However, baseline differences were adjusted for in general linear modelling. The simultaneous infusion of remnant-like emulsion might have confounded the results [7]. However, our pre-intervention VLDL-TAG kinetic data are comparable with those reported by others [26,27]. Our findings are also consistent with the postulated mechanisms of action of ω-3 FAEE on VLDL-TAG metabolism [21]. Gender and other genetic factors (such as familial hypertriacylglycerolaemia and familial combined hyperlipidaemia) could possibly influence the effect of ω-3 FAEEs on VLDL metabolism in our subjects and this merits investigation.

The increased cardiovascular morbidity and mortality associated with obesity and type 2 diabetes may in part be a consequence of hypertriacylglycerolaemia [1,2]. A recent expert guideline recommends an optimal fasting TAG <1.13 mmol/l [2]. Hence the correction of hypertriacylglycerolaemia with ω-3 FAEE has potential to decrease the risk of coronary heart disease [5]. The recent ORIGIN trial failed to demonstrate any beneficial effect of low-dose (1 g/day) ω-3 fatty acids on cardiovascular outcomes in high-risk patients with dysglycaemia [28], but the dose employed was significantly lower than in the present investigation. Our study provides mechanistic insight into the effect of high-dose ω-3 FAEE supplementation on TAG metabolism in insulin-resistant obese men. Whether high-dose ω-3 FAEE supplementation improves clinical outcomes remains to be fully demonstrated in clinical trials. This is being addressed in one ongoing clinical trial (REDUCE-IT) to evaluate the effect of high-dose ω-3 FAEE (4 g of EPA/day) for preventing cardiovascular events in high-risk patients with hypertriglyceridaemia (http://clinicaltrials.gov/show/NCT01492361).

In conclusion, ω-3 FAEEs lower plasma TAG concentrations in dyslipidaemic obese subjects by decreasing the hepatic secretion of VLDL-TAG, with no significant effect on VLDL-TAG catabolism. Changes in VLDL-TAG secretion were tightly coupled with changes in VLDL-apoB-100 secretion; however, there was no association with changes in HDL-apoA-I catabolism. Given that ω-3 FAEE supplementation did not alter insulin sensitivity, further studies should determine the effect of weight loss or insulin sensitizers with ω-3 FAEE supplementation on VLDL metabolism.
CLINICAL PERSPECTIVES

- Hypertriacylglycerolaemia is a risk factor for coronary artery disease and the most consistent lipid disorder in visceral obesity. ω-3 Fatty acid ethyl esters decrease plasma TAG but the mechanisms are not fully understood.
- Compared with placebo, a high-dose ω-3 FAEE supplementation (4 g/day) decreased plasma TAG concentrations in obese men by decreasing the hepatic secretion of VLDL-TAG without significantly altering fractional catabolism.
- Our finding provides evidence on the mechanistic insight of ω-3 FAEE supplementation on TAG metabolism in insulin-resistant obese men. This may explain the cardioprotective effect of such a high dose of ω-3 FAEE supplementation.

AUTHOR CONTRIBUTION

Dick Chan, Hugh Barrett and Gerald Watts conceived and designed the study; Annette Wong, Dick Chan, Theodore Ng, Gerald Watts and Hugh Barrett supervised or conducted the research; Annette Wong, Dick Chan, Esther Ooi and Hugh Barrett analysed the data; Annette Wong, Dick Chan, Gerald Watts and Hugh Barrett wrote the paper. All authors read and approved the final manuscript.

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Fish oil and lipoprotein metabolism in obesity


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