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Effect of \( \omega-3 \) Fatty Acid Ethyl Esters on Apolipoprotein B-48 Kinetics in Obese Subjects on a Weight Loss Diet: a New Tracer Kinetic Study in the Postprandial State

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ABSTRACT

Context: Dysregulated chylomicron metabolism may account for hypertriglyceridemia and increased risk of cardiovascular disease in obese subjects. Omega-3 fatty acid ethyl ester (ω-3 FAEE) supplementation decreases plasma triglyceride. However, its effect on postprandial chylomicron metabolism in obese subjects on a weight loss diet has not yet been investigated.

Objective: We aimed to examine the effect of ω-3 FAEE supplementation on apolipoprotein (apo) B-48 kinetics in obese subjects on a weight loss diet.

Design, Setting and Patients: We carried out a 12-week, randomized trial of a hypocaloric diet plus 4g/day ω-3 FAEE supplementation (46% eicosapentaenoic acid and 38% docosahexaenoic acid) (n=13) compared with a hypocaloric diet alone (n=12) on postprandial apoB-48 kinetics in obese subjects following ingestion of an oral load. ApoB-48 kinetics were determined using stable isotope tracer kinetics and multi-compartmental modeling.

Outcomes Measures: Plasma total and incremental apoB-48 0-10hr area-under-the-curves (AUC), as well as apoB-48 secretion and fractional catabolic rate.

Results: Weight loss with or without ω-3 FAEE supplementation significantly reduced body weight, total fat mass, HOMA score, fasting triglyceride concentration, postprandial triglyceride AUC, and increased plasma HDL-cholesterol concentration (P<0.05 in all). Compared with weight loss alone, weight loss plus ω-3 FAEE significantly (all P<0.05) decreased fasting triglyceride (-11%), apoB-48 (-36%) concentrations, postprandial triglyceride (-21%) and apoB-48 (-22%) total AUCs, as well as incremental postprandial triglyceride AUCs (-32%). ω-3 FAEE also significantly decreased apoB-48 secretion at basal state, without significant effect during the postprandial period (3-6hr). The fractional catabolic rate of apoB-48 increased, with both interventions with no significant independent effect of ω-3 FAEE supplementation.

Conclusion: Addition of n-3 FAEE supplementation to a moderate weight loss diet in obese subjects can significantly improve chylomicron metabolism by independently decreasing the secretion of apoB-48.
INTRODUCTION

Elevated plasma triglyceride concentrations are directly related to risk of cardiovascular disease (CVD) (1). Hypertriglyceridemia is the most consistent lipid disorder in obesity and may contribute to increased risk of CVD in these subjects (2). Humans consume multiple meals during the day and, hence, are continually in a dynamic state of postprandial lipid and lipoprotein metabolism (3).

After ingesting a meal, dietary triglycerides are packaged and transported into the circulation by intestinally-derived apolipoprotein (apo) B-48 containing chylomicrons (CMs) (4). Accumulating evidence suggests that chylomicron remnants play a central role in development of atherosclerosis via effects on endothelial dysfunction, inflammation and oxidative stress, and foam cell formation (5). Using a new compartmental model, we recently demonstrated that obese individuals have increased fasting and postprandial apoB-48 concentration owing to an overproduction and impaired catabolism of apoB-48-containing lipoproteins (6). These abnormalities may be consequent on the central obesity and insulin resistance. Hence, regulation of these abnormalities via lifestyle or pharmacotherapy is important to reduce the associated risk of CVD.

Fish oils are a rich source of long-chain ω-3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Compelling evidence suggests that fish oil supplementation protects against CVD and is partly mediated by improvement in hypertriglyceridemia (7). However, two recent clinical outcome trials with fish oil supplementation failed to show a significant CVD benefit in high risk subjects including diabetics, possibly owing to a low dose of fish oil supplementation (~1g/day) being employed (8, 9). Data from a meta-analysis of 20 randomised trials also suggest that fish oil supplementation was not associated with a lower risk of major CVD events (10). However, most of these studies were conducted using low dose fish oil supplementation (<2g/days). We have previously
reported that a 6-week of high dose ω-3 fatty acid ethyl ester (ω-3 FAEE) supplementation resulted in reductions in VLDL-apoB-100 and VLDL-triglyceride concentrations (11). This could be related to reductions in VLDL-apoB-100 and VLDL-triglyceride secretion rates in the postabsorptive state. Recent evidence also suggests that ω-3 FAEEs could regulate chylomicron metabolism via an effect on apoB-48 synthesis (12-14). However, fasting apoB-48 concentration was not altered with ω-3 FAEE supplementation (Chan et al unpublished observation), probably owing to persistent insulin resistance in obese subjects. Weight loss through dietary restriction is the first-line approach to correct dyslipidemia and insulin resistance in obesity (15). We previously demonstrated that intensive weight loss (>10%) with a low caloric diet effectively lowers plasma concentrations of triglyceride-rich lipoproteins (TRLs), including VLDL-apoB-100 and apoB-48, and also improves insulin sensitivity (16). Hence, inclusion of a weight loss component may enhance the effect of ω-3 FAEE supplementation on apoB-48 metabolism in insulin resistant, obese subjects. However, such intensive weight loss is difficult to achieve and maintain. Therefore, in a clinical setting, the combination of ω-3 FAEE supplementation and moderate weight loss (3-5%) could be an optimal approach to improve postprandial hypertriglyceridemia, especially chylomicron metabolism. To date, no kinetic study has examined the effect of ω-3 FAEE supplementation on chylomicron metabolism in obese subjects on a moderate weight loss diet.

In the present study, we employed a new compartmental modelling to investigate the combined effects of ω-3 FAEE supplementation and moderate weight loss on apoB-48 kinetics in a postprandial, non-steady state setting (6). We hypothesized that ω-3 FAEE would decrease fasting and postprandial apoB-48 concentrations chiefly by reducing apoB-48 secretion in obese subjects who were on a moderate weight loss diet.
METHODS

Subjects

Twenty-seven obese men and post-menopausal obese women aged between 18-75 (body mass index >30kg/m² or waist circumference >94 cm for men and >80cm for women), who were consuming ad libitum, weight maintaining diets were recruited from the community. None of the subjects had familial hypercholesterolemia, APOE2/E2 genotype, macroproteinuria, raised creatinine (>120µmol/L) with low glomerular filtration rate (<60 mL/min), hypothyroidism, intolerance to fish oil or elevated hepatic aminotransferase or a history of CVD. Two subjects dropped out due to time commitment. This study was approved by the Ethics Committee of the Royal Perth Hospital, and informed consent was obtained in all subjects.

Clinical Protocol

This study was a randomised, single-blind intervention trial. All eligible patients entered a 4-week run-in diet stabilising period, at the end of which they entered a hypocaloric diet for 12 weeks immediately followed by a 4-week weight maintenance period. During the 16-week intervention period, subjects were randomised into one of the two groups, weight loss alone group or weight loss plus 4g/day Omacor (ω-3 FAEEs). Compliance with n-3 FAEE supplementation was checked by capsule count at weeks 6, 12 and 16 post randomisation. For the 12 weeks of weight loss, subjects were advised to reduce portions sizes in order to achieve energy deficit of at least ~1900kJ. At the 4 weeks of weight maintenance period, energy intake increased by ~460kJ. Dietary intake was assessed for energy and major nutrients using FoodWorks 7 Pro (Xyris, Queensland Australia). Dietary intake, alcohol and exercise diaries were completed at week 0, 6, 12 and 16. All subjects were reviewed fortnightly and requested to
maintain their usual level of physical activity. All dietary assessments and recommendations were conducted by a registered dietitian.

Postprandial and stable isotope protocol

All subjects were admitted to the metabolic ward in the morning after a 14-hour fast. They were studied in a semi-recumbent position and allowed to drink only water after the test-meal. Arterial blood pressure was recorded after 3 minutes in the supine position using a Dinamap1846 SX/P monitor (Critikon Inc, Tampa, FL, USA). Body composition was estimated using a Wedderburn Body Composition Analyzer (Wedderburn Pty Ltd, Australia) from which total body fat and fat free mass (FFM) were derived.

Following a baseline fasting blood sample, a single bolus of d3-leucine (5mg/kg of body weight) was administered intravenously, within a 2-minute period into an antecubital vein via a 21-gauge butterfly needle. A liquid formulated high fat test meal was the consumed within 2 minutes (a total of 4800kJ, 130g fat, 17g protein and 21g carbohydrate), following with blood samples were obtained after 5, 20, 30, 40 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 hours. Subjects were then given a snack and allowed to go home. All the procedures were repeated after 16-week intervention period with the fat-test meal energy adjusted for change in body weight.

Isolation of apoB-48 and measurement of isotopic leucine enrichment

TRL fraction was isolated from 3.5ml plasma by ultracentrifugation (Optima XL-100K, Beckman Coulter, Australia) at density of 1.006 (40,000 rpm, 16 h, 4°C). The TRL samples were then prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The apoB-48 band was excised from the membrane, hydrolyzed with 200μL 6M HCl at 110°C for 16
hours. Derivatization of leucine to the oxazolinone derivative was described previously. Isotopic enrichment was determined using gas chromatography-mass spectrometry (GCMS) with selected ion monitoring of samples at a mass to charge ratio (m/z) of 212 and 209 and negative ion chemical ionization. Tracer to tracee ratios were derived from isotopic ratios for each sample.

Model of apoB-48

A non-steady compartment model was developed using the SAAM II program (The Epsilon Group, VA) to account for changes in plasma apoB-48 concentration following consumption of the fat load (6). Briefly, two separate, but linked, models were developed, one to account for the leucine tracer data, including plasma leucine and apoB-48 leucine enrichment, and the other model for apoB-48 concentration data. The leucine compartment model consists of a four-compartment subsystem that describes plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment that accounts for the time required for leucine tracer to be incorporated into apoB-48 and subsequently secreted into plasma. The apoB-48 concentration compartment model consists of a delay compartment that represents four compartments in series and an additional compartment represents plasma apoB-48 particles. The model assumed that catabolism was time invariant, similar to the kinetic studies by Le et al (17), and that the increase in plasma apoB-48 concentration was due to an increase in apoB-48 secretion following consumption of the fat meal. The model could be used to estimate apoB-48 secretion in the fasted state, in the postprandial state, apoB-48 fractional catabolic rate (FCR) and number of apoB-48 secreted in response to the fat meal. Details of multicompartmental models are given in the supplemental materials.

Biochemical measurements
Plasma lipid and glucose concentrations were determined by enzymatic methods using a Hitachi 917 Biochemical Analyser (Hitachi Ltd, Japan). HDL-cholesterol was measured enzymatically (Boehringer Mannheim, Germany). LDL-cholesterol was calculated by Friedewald equation, and non-HDL-cholesterol as total cholesterol minus HDL-cholesterol. Total plasma apoB-100 concentrations were determined by immunonephrelometry (Dade Behring BN2 Nephelometer). VLDL-apoB-100 was measured using ELISA kit (Mabtech, Nacka, Sweden). Plasma apoB-48 levels were measured by enzyme immunoassay kit (Fujirebio, Tokyo, Japan). Plasma insulin was measured by solid phase two site sequential chemiluminescent immunometric assay (Diagnostic Products Corporation, LA) and glucose by hexokinase method (Hitachi, Tokyo, Japan). Insulin resistance was estimated by the homeostasis model assessment (HOMA) score [fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5]. Plasma adiponectin was determined using enzyme immunoassay kits (Quantikine, R&D Systems, Minneapolis). Postprandial metabolism was quantified by calculating the area- and incremental under-the-curve (AUC) and iAUC, respectively, for plasma triglyceride and apoB-48 (0-10hr) using the trapezium rule. The iAUC was estimated as the difference between the area defined below the baseline concentration and the area under the plasma curve between 0 and 10 hour.

Statistical Analyses

All analyses were performed on SPSS 21 (SPSS, Inc., Chicago). Paired t-tests were used to assess changes within both treatment groups for all variables. Changes within group were compared between the two intervention groups by ANCOVA using baseline as covariate. Associations were examined using simple linear regression method. Statistical significance was defined at the 5% level.
RESULTS

Baseline characteristics

On average, the obese subjects were middle-aged (mean±SD 60±4 yrs), centrally obese, normotensive, dyslipidemic (elevated plasma triglycerides and low HDL-cholesterol) and insulin resistant. Six subjects were on lipid lowering therapy (four on atorvastatin and two on rouvastatin) and five were on antihypertensive treatment (two on angiotensin receptor blockers, two on angiotensin-converting enzyme inhibitors and one on B1-receptor blocker). Average daily energy and nutrient intake of the 25 obese subjects studied (mean ± SD) was: 8136 ± 2260 kJ, 33 ± 5% energy from fat, 44 ± 6% energy from carbohydrates, 19 ± 4% energy from protein and 4 ± 5% energy from alcohol.

Dietary adherence

Both groups achieved a significant reduction in energy intake during the weight loss period (weight loss only group -24%, p=0.016; weight loss plus ω-3 FAEE group -20% p=0.003). During the active study period, there were no significant differences in macronutrient composition and energy intake between the groups. Alcohol intake was also not significantly altered during week 0, week 6 and week 16 in the weight loss alone (3.7±1.4%, 2.1±0.9% and 2.7±1.3%, respectively) and weight loss plus ω-3 FAEE (4.1±1.3%, 4.4±1.3% and 3.9±1.7%, respectively) groups. That subjects in both the weight loss alone and weight loss plus ω-3 FAEE groups consumed an isocaloric diet from week 12 to week 16 was supported by the finding that body weight did not vary significantly during this period. No significant changes in physical activity levels were reported during the study in either group (data not shown). Capsule counts confirmed that compliance with randomization to ω-3 FAEE intervention was >95%. There were also no side effects reported with ω-3 FAEE supplementation.

Body weight, blood pressure and biochemical characteristics
The effects of weight loss alone and weight loss plus ω-3 FAEEs on body weight and biochemical characteristics are shown in Table 1. Both treatments resulted in significant reductions in body weight, body mass index, waist circumference, waist-to-hip ratio, total body fat mass, diastolic blood pressure, HOMA score and increase in plasma HDL-cholesterol and adiponectin concentrations (P<0.05 in all). However, these changes did not differ significantly between the groups. Systolic blood pressure, plasma triglyceride and VLDL-apoB-100 concentrations decreased significantly with weight loss alone and with weight loss plus ω-3 FAEEs (P<0.05). The reductions in systolic blood pressure (-8% vs -3%), plasma triglyceride (-36% vs -25%) and VLDL-apoB-100 (-34% vs -12%) concentration were significantly greater in the weight loss plus ω-3 FAEEs than in the weight loss alone groups (P<0.05 in both). There were no significant changes to fat free mass, fasting glucose, total cholesterol, LDL-cholesterol and non-HDL-cholesterol in both groups.

Postprandial lipid responses

The postprandial responses for plasma triglycerides and apoB-48 to the fat load are shown in Figure 1. As seen in Table 2, both weight loss with or without ω-3 FAEE supplementation significantly reduced postprandial triglyceride total AUCs (-38% and -17%, P<0.05 in both). Postprandial triglyceride incremental AUCs (0-10hr) were significantly reduced by weight loss plus ω-3 FAEEs but not weight loss alone (-50% and -19%). The reductions in postprandial triglyceride total and incremental AUCs were significantly greater in the weight loss plus ω-3 FAEEs than in the weight loss alone groups (P<0.05). Compared with weight loss alone, weight loss plus ω-3 FAEEs also significantly decreased postprandial apoB-48 total AUC (-15% vs -37%, P<0.05). There were no significant changes to incremental apoB-48 AUCs and number of apoB-48-containing particles secreted in response to the fat load in both groups.
The effects of weight loss and weight loss plus ω-3 FAEEs on apoB-48 concentration and kinetics are shown in Figure 2. Compared with weight loss alone, weight loss plus ω-3 FAEEs significantly decreased apoB-48 concentration (-30%, P<0.01) and apoB-48 secretion in the fasted state (-37%, P<0.05). Moreover, both weight loss with or without ω-3 FAEE supplementation significantly increased the FCRs of apoB-48 by +22% and +39%, respectively (P<0.05 in both). When the FCR data from the two intervention groups were combined (n=25), the percentage change in apoB-48 FCR was inversely associated with change in VLDL-apoB-100 concentrations (r=-0.547, P=0.08). However, these changes in apoB-48 FCRs did not differ significantly between the groups. Compared with weight loss alone, weight loss plus ω-3 FAEEs had no significant, differential effect on apoB-48 secretion during the postprandial peak period (Figure 3), consistent with the lack of significant changes to number of apoB-48-containing particles secreted in response to the fat load (Table 2).
DISCUSSION

We provide new information based on a recently published compartmental model concerning the effect of ω-3 FAEEs additional to mild-to-moderate weight loss alone on postprandial chylomicron metabolism in subjects with obesity. Our results demonstrate that, relative to weight loss alone, weight loss plus ω-3 FAEEs decreased both fasting concentrations and total postprandial AUCs of triglyceride and apoB-48. These effects were chiefly attributed to a significant reduction in basal apoB-48 secretion rate, concomitant with the favorable effects of weight loss on apoB-48 FCR, insulin sensitivity and plasma concentrations of VLDL-apoB-100 and adiponectin.

This is the first study to demonstrate the combined effects of ω-3 FAEE supplement and weight loss on postprandial apoB-48 kinetics in obese subjects. Previous placebo controlled studies have examined the postprandial effects of ω-3 FAEEs on apoB-48 concentration only, with inconsistent results. Slivkoff-clark et al reported that in insulin resistant obese men, 4-week ω-3 FAEE supplementation (1.7g/day) did not alter fasting apoB-48 concentration or postprandial apoB-48 incremental AUC (18). Tinker et al found, in non-obese subjects with hypertriglyceridemia, that high dose fish oil supplementation (5.2g/day) for 6 weeks significantly reduced fasting apoB-48 concentration and total postprandial apoB-48 AUC (19). Park et al reported, in non-obese healthy subjects, that 4-week EPA or DHA supplementation (4g/day) reduced total postprandial apoB-48 AUC (20). The aforementioned discrepant findings might be explained by differences in subject characteristics, as well as the duration (4 to 6 weeks) and dose of fish oil interventions (1.7g to 5.2g/day). Importantly, these studies only measured plasma apoB-48 concentrations, a measure that does not provide information on rates of production or catabolism; a more detailed mechanism of action of ω-3 FAEE supplementation on apoB-48 metabolism is required by the present kinetic study. A recent study by Ooi et al reported that in non-obese healthy subjects the high fish diet (~1g/day) decreased apoB-48 concentration by reducing the secretion rate of apoB-48 compared with low fish diet (21); the study also found a concomitant reduction of apoB-48 FCR with high-fish diet.
Owing to a specific derangement in apoB-48 metabolism related to insulin resistance and visceral fat accumulation, treatment with ω-3 FAEE supplementation alone on apoB-48 metabolism may not adequately correct chylomicronemia and apoB-48 metabolism in obese subjects. Taken together, we have now extended these aforementioned studies by employing 16-week high dose ω-3 FAEE supplementation (4g/day) and examining insulin-resistant obese subjects with dyslipidemia against a background of weight loss. Another novelty of the present study is that apoB-48 kinetics are investigated in a postprandial, non-steady state by using a newly developed compartmental model to account for the administration of a fat load that increases triglyceride and apoB-48 secretion.

Elevated fasting levels and postprandial responses of apoB-48 have been shown in obese subjects (6, 22). Disturbances in chylomicron metabolism in obesity may be due to an effect of insulin resistance and the accumulation of visceral fat (23-26). Collectively, these conditions result in the oversecretion of TRLs, suppressed LDL receptor activity and increased competition with VLDL remnants. Weight loss improves insulin sensitivity and reduces visceral fat accumulation. We have previously reported that weight loss lowered fasting VLDL-apoB-100 concentration by reducing its secretion rate (27). Given apoB-48 and VLDL apoB-100 are competing for the same catabolic pathway, it is likely that the reduction in VLDL-apoB-100 concentration with weight loss alone or ω-3 FAEE plus weight loss might directly impact on apoB-48 catabolism. Such changes could collectively correct the abnormalities in apoB-48 metabolism by enhancing the uptake of chylomicron remnants by liver. Consistent with this, we found that the FCRs of apoB-48 increased with weight loss alone and weight loss plus ω-3 FAEE and that the percentage change in apoB-48 FCR was inversely associated with change in VLDL-apoB-100 concentration. Given that none of these changes differed between these two groups, it is conceivable that all these favourable changes were chiefly driven by the effects of weight loss. Our present results also found that mild-to-moderate weight loss alone had no significant effect on postprandial total apoB-48 AUC or apoB-48 secretion rate following a fat load. The findings might be different with a greater degree weight loss (16).
We also showed that adding \( \omega-3 \) FAEEs to weight loss resulted in a greater decrease in fasting triglyceride concentration and postprandial AUC responses of triglyceride compared with weight loss alone. This improvement is likely to be a consequence of reductions in triglyceride-rich VLDL-apoB-100 and chylomicron apoB-48 particles with \( \omega-3 \) FAEEs. We have previously reported that \( \omega-3 \) FAEE supplementation decreased the secretion of VLDL-apoB-100 and triglycerides in obese subjects (11, 28). Consistent with this, in the present study we showed that fasting VLDL-apoB-100 concentrations fell significantly with \( \omega-3 \) FAEE supplementation. Measurements of fasting and postprandial triglyceride content in chylomicron and VLDL particles are required to further clarify their contributions to the triglyceride-lowering effect of \( \omega-3 \) FAEE supplementation.

Several mechanisms of action of \( \omega-3 \) FAEEs on apoB-48 metabolism have been suggested. \( \omega-3 \) FAEEs increase posttranslational degradation of newly synthesized apoB-48 and decrease expression of apoB-48 m-RNA (12, 13), and can decrease monoacylglycerol acyltransferase and diacylglycerol acyltransferase activities, resulting in a net reduction in triglyceride synthesis and apoB-48 secretion in the intestine (14). Accordingly, we found that \( \omega-3 \) FAEE supplementation lowered fasting apoB-48 concentration and apoB-48 secretion in the basal state. However, the lack of changes in apoB-48 incremental AUC and apoB-48 secretion rates during the postprandial peak period suggests that the aforementioned mechanism of actions of \( \omega-3 \) FAEEs have no significant impact on apoB-48 synthesis during the postprandial period. The precise reasons for the lack of an acute effect on apoB-48 metabolism remain unclear.

In this study, we employed a compartmental model to describe the non-steady kinetics of apoB-48 based on a related study (6), similar to the kinetic studies by Le et al (17). The novelty of our approach has been previously described (6). Briefly, previous apoB-48 kinetic studies were conducted in a constant, fed state which does not reflect usual daytime eating patterns (29, 30). Moreover, this may also result in the formation of smaller, less triglyceride-rich apoB-48-containing lipoproteins different to those present in the postprandial state. The current protocol also permits measurements of postprandial triglyceride and...
apoB-48 AUCs, a common endpoint of many postprandial studies (17-19). In the current study, a bolus administration of d$_3$-leucine enables us to capture the fast dynamics of apoB-48 particles. We developed the model by assuming a constant rate of apoB-48 secretion, however, it failed to describe the apoB-48 tracer and concentration data. In contrast, a model where FCR was time invariant and apoB-48 secretion increased as a consequence of the fat load fitted the tracer data. This model permitted the precise estimation of apoB-48 FCR and secretion (6). To further validate the model in this setting, the intravenous administration of labeled apoB-48 concurrently with the fat load is warranted.

We also found that relative to weight loss alone, weight loss plus ω-3 FAEEs significantly reduced systolic blood pressure. This observation is consistent with the well-known effect of ω-3 FAEEs in lowering blood pressure. In a separate report in the same subjects (31), we have demonstrated that ω-3 FAEE supplementation improves large and small arterial elasticity independent of weight loss. These findings demonstrate the favorable vascular benefits of ω-3 FAEE supplementation beyond its hypotriglyceridemic effect in obesity.

Our study has limitations. The sample size was relatively small. The lack of significant effect of ω-3 FAEEs on postprandial apoB-48 incremental AUC may reflect lack of statistical power. Hence, the findings need to be confirmed in a larger study. We did not include a placebo oil capsule in weight loss group because placebo oil preparations, such as corn oil and olive oil, may affect chylomicron metabolism (32). Our study also pragmatically reflected the evaluation of the intervention under routine clinical practice conditions in which participants do not consume placebo. We could not strictly exclude the possibility that the increased performance in the ω-3 FAEE group could be biased by our open, single-blind design. Therefore, our results should be interpreted with caution. The observed differences in pre-intervention body weight, apoB-48 concentration and postprandial apoB-48 total and incremental AUCs might have confounded the results. However, the differences were not significant and the pre-
intervention variables had been used as covariates in general linear modeling, thereby adjusting for any group differences. We employed paired t-test within each intervention group to indicate the effect of weight loss on apoB-48 kinetics and associated lipid parameters. The true effect of weight loss requires an inclusion of a control group (i.e. weight maintenance group without ω-3 FAEE supplementation). Six subjects were using statin therapy throughout the study (three subjects in each group); the dosage was not altered during the study. Although this might have impacted upon the conclusion of the study, removal of the data associated with these subjects did not alter its outcome (data not shown). Currently, there is no standardised oral test-meal/fat-load recommended for postprandial lipemia studies. The composition of the test meal in the current study was essentially a fat meal. Such a meal may influence gastric emptying and potentially confound the effect of ω-3 PUFA supplementation on postprandial chylomicron metabolism. Hence, our results might have been different had we employed a test meal of different composition (e.g. low-fat or mixed meal). We did not measure fat absorption and the kinetics of apoB-100 and triglyceride which may otherwise help to clarify the mechanism of action of ω-3 FAEEs and/or weight loss on apoB-48 metabolism.

The increased rate of CVD in obesity and type 2 diabetes may be caused by the combination of insulin resistance and dyslipidemia (2). Correction of hypertriglyceridemia with ω-3 FAEEs has potential to decrease the risk of coronary heart disease. Our study provides mechanistic insight to the effect of high dose ω-3 FAEE supplementation on chylomicron metabolism in insulin resistant, obese men on weight loss. 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 of EPA (4g/day) for the prevention of cardiovascular events in high risk patients with hypertriglyceridemia.

In conclusion, our data support the hypothesis that addition of ω-3 FAEEs to moderate weight loss diet significantly improves chylomicron metabolism in obese subjects. This improvement is chiefly related to
an effect of ω-3 FAEEs in reducing apoB-48 secretion by the intestine, which is incremental to effects of weight loss in accelerating the catabolism of apoB-48.

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LEGENDS

Table 1. Clinical, biochemical and haemodynamic characteristics in relation to weight loss alone and to weight loss plus ω-3 FAEE supplementation

Table 2. Postprandial changes for plasma triglyceride and apoB-48 following the oral fat load in relative to weight loss alone and weight loss plus ω-3 FAEE supplementation

Figure 1. Plasma triglyceride and apoB-48 responses to the fat load in the weight loss alone (A and B) and weight loss plus ω-3 FAEE groups (C and D).

Figure 2. ApoB-48 concentration and kinetics in relation to weight loss alone and to weight loss plus ω-3 FAEEs

Figure 3. Changes in apoB-48 secretion rates in responses to the fat load in the weight loss alone (A) and weight loss plus ω-3 FAEE groups (B).