Plasma apolipoprotein B-48 transport in obese men: A new tracer kinetic study in the postprandial state

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**Context:** The mechanisms responsible for impaired chylomicron metabolism have not been adequately investigated in obese subjects.

**Objective:** We aimed to compare apolipoprotein (apo) B-48 kinetics in obese and lean men by developing a new model to describe the kinetics of apoB-48 particles in the postprandial state.

**Design, Setting, and Patients:** Seven obese and 13 age-matched lean men were given an oral fat load. apoB-48 tracer to tracee ratios were measured after intravenous d3-leucine administration using gas chromatography-mass spectrometry. Kinetic parameters were derived using a multi-compartmental model.

**Outcomes Measures:** Plasma total and incremental apoB-48 0–10 hour areas under the curve as well as apoB-48 secretion and fractional catabolic rate.

**Results:** Compared with lean men, fasting plasma triglyceride (±148%) and apoB-48 (±110%) concentrations as well as plasma total and incremental triglycerides (±184% and ±185%, respectively) and apoB-48 (±182% and 224%, respectively) areas under the curve were significantly higher in obese men (P < .05 for all). The obese men also had significantly (P < .05 for all) higher secretion rates of apoB-48 in the fasted state (±145%) as well as at 3 hours (±70%), 4 hours (±82%), 5 hours (±82%), 6 hours (±76%), and 8 hours (±61%) in response to the fat load. This was associated with a greater number of apoB-48-containing particles secreted over the 10-hour study period in the obese men, compared with lean men (±125%, P < .01). The fractional catabolic rate of apoB-48 was significantly lower in the obese men compared with the lean men (±33%, P < .05)

**Conclusion:** We demonstrate that postprandial hypertriglyceridemia in central obesity relates to an overproduction and impaired catabolism of apoB-48-containing lipoproteins. These findings are based on a new, physiologically relevant, kinetic model, which describes the non-steady-state postprandial metabolism of apoB-48. *(J Clin Endocrinol Metab 99: E122–E126, 2014)*

Elevated plasma triglyceride concentrations are directly related to risk of cardiovascular disease (CVD). The prevalence of hypertriglyceridemia (>1.7 mmol/L) is more than 30% in the adult US population (1). Hypertriglyceridemia is the most consistent lipid disorder in obesity and may contribute to increased risk of CVD in these subjects. Humans consume multiple meals during the day and hence are continually in a dynamic state of postprandial lipid and lipoprotein metabolism.

After ingesting a meal, dietary triglycerides are packaged and transported into the circulation by intestinally-derived apolipoprotein (apo)-B-48 containing chylomicrons.
crones. There is an accumulating body of evidence to suggest that apoB-48 plays a central role in the development of atherosclerosis, contributing to endothelial dysfunction, inflammation and oxidative stress, and foam cell formation (2–4). We previously demonstrated that obese individuals have increased fasting and postprandial apoB-48 concentrations (5). However, it is unclear whether a defect in secretion and/or catabolism of these particles is responsible for the concomitant hypertriglyceridemia. No investigation has specifically studied postprandial apoB-48 kinetics in obesity.

In the present study, we applied a new stable isotope protocol to test the hypothesis that the intestinal secretion and catabolism of apoB-48 is disturbed in obese men in response to a high-fat meal.

Materials and Methods

Subjects

Seven obese men with a waist circumference greater than 92 cm and 13 age-matched normotriglyceridemic lean men (waist circumference < 90 cm and plasma triglycerides < 1.2 mmol/L) while consuming ad libitum, weight-maintaining diets were recruited. None of the subjects were smokers; consumed more than 30 g alcohol per day; or had familial hypercholesterolemia or type 2 diabetes, APOE2/E2 genotype, proteinuria (≥30 mg/L), creatininemia (≥120 μmol/L) with low glomerular filtration rate (<60 mL/min), hypothyroidism, elevated hepatic aminotransferase, or a history of CVD. One obese subject was on antihypertensive drug treatment. All subjects were requested to maintain their usual diets and level of physical activity 2 weeks prior to the study. The study was approved by the Ethics Committee of the Royal Perth Hospital.

Clinical protocols

All subjects were admitted to the metabolic ward in the morning after a 14-hour fast. They were studied in a semirecumbent 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 Dinamap 1846 SX/P monitor (Critikon Inc.). Body composition was estimated using a Wedderburn body composition analyzer (Wedderburn Pty Ltd) from which total body fat and fat-free mass (FFM) were derived. Dietary intake was assessed for energy and major nutrients using FoodWorks 7 Pro (Xyris).

After a baseline fasting blood sample, a bolus of d3-leucine (5 mg/kg of body weight) was administrated iv, within a 2-minute period, into an antecubital vein via a 21-gauge butterfly needle. A liquid-formulated, high-fat test meal consisting of 100 mL of full cream milk, 150 mL of pure cream, 70 mL of corn oil, 90 g of whole egg, and 10 g of sugar (a total of 4800 kJ, 130 g fat, 17 g protein, and 21 g carbohydrate) was consumed over several minutes. Additional blood samples were obtained after 30 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, and 10 hours.

Isolation and determination of apoB-48 leucine enrichment

Triglyceride-rich lipoproteins (TRLs) were isolated from 3.5 mL plasma by ultracentrifugation at density of 1.006 kg/L (40 000 rpm, overnight, 4°C). apoB-48 was isolated using SDS-PAGE and hydrolyzed with 200 μL 6 M HCl at 110°C overnight. Derivatization of leucine to the oxazolinone derivative was described previously (6). Tracer to trace ratio was determined using gas chromatography-mass spectrometry with selected ion monitoring of each sample at a mass to charge ratio of 212 and 209.

Model of apoB-48

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 (7) and that the increase in plasma apoB-48 concentration was due to an increase in apoB-48 secretion after the 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 the number of apoB-48 secreted in response to the fat meal. Details of multicompartmental models are given in the Supplemental Materials, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org.

Biochemical measurements

The laboratory methods for measurements of biochemical analytes were previously described (8). Plasma apoB-48 levels were measured by enzyme immunoassay (EIA) kit (Fujirebio). Plasma apoC-III was determined using a Hydragel LP CIII electroimmunodiffusion kit (Sebia). The TRL-apoB-48 particle size was estimated by the ratio of plasma triglyceride to apoB-48 concentrations. Postprandial metabolism was quantified by calculating the area under the curve (AUC) and incremental area under the curve (iAUC), respectively, for plasma triglyceride and apoB-48 (0–10 h) 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 hours.

Statistical analyses

All analyses were performed using SPSS 21 (SPSS, Inc). Group characteristics were compared by unpaired t tests, with logarithmic transformation of skewed variables where appropriate. Statistical significance was defined at the 5% level.

Results

Table 1 shows the clinical and biochemical characteristics of obese and lean men. Age and blood pressures were not
significantly different between the groups. As anticipated, the obese men had a significantly higher body weight, body mass index, and waist circumference ($P < .001$) compared with the lean men. Although plasma glucose was not significantly different between the groups, the obese subjects had significantly elevated fasting insulin concentrations, and homeostasis model assessment score ($P < .05$, for both). The obese group had significantly higher fasting plasma triglyceride and apoA-I ($P < .01$ for both) compared with the lean group. Plasma triglyceride to apoA-I ($P < .05$ for both) but lower high-density lipoprotein-cholesterol and apoB-48 ($P < .01$ for both) were also significantly higher in the obese than the lean men ($P < .01$). There were no statistically significant differences in the proportion of energy intake derived from carbohydrate (45% vs 46%), protein (18% vs 19%), fat (32% vs 31%), and alcohol (5% vs 4%) between the obese and lean groups.

The concentration curves for plasma triglycerides and apoB-48 are shown in Figure 1, A and B, respectively. Compared with lean men (Table 1), postprandial triglyceride and apoB-48 AUCs, in response to the fat meal, were significantly higher in obese men by +180% and +181%, respectively ($P < .001$ for both). Moreover, the iAUCs for plasma triglycerides and apoB-48 were also found to be significantly higher in obese men compared with lean men (+190% and +245%, respectively, $P < .001$ for both).

Figure 1C shows the postprandial secretion rate of apoB-48 in response to the fat meal in the subjects studied. Compared with lean men, the secretion rate of apoB-48 in the fasted state ($t = 0$ h) was 45% higher in the obese men ($P < .05$). Obese subjects also had significantly ($P < .05$ in all) higher postprandial secretion rates of apoB-48 at 3, 4, 5, 6, and 8 hours compared with lean men. This was consistent with a greater number of apoB-48-containing particles secreted in response to the fat meal in the obese men (+125%, $P < .01$). The FCR of apoB-48 was significantly lower in the obese men, compared with the lean

### Table 1. Clinical, Biochemical, and Lipoprotein Kinetic Characteristics of the Lean and Obese Men

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lean (n = 13)</th>
<th>Obese (n = 7)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61 ± 8</td>
<td>56 ± 6</td>
<td>.199</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71 ± 7</td>
<td>100 ± 11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 ± 2</td>
<td>32 ± 4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>84 ± 4</td>
<td>109 ± 8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucose, mmol/liter</td>
<td>5.3 ± 0.3</td>
<td>5.6 ± 0.5</td>
<td>.117</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>3.0 ± 1.2</td>
<td>8.5 ± 4.4</td>
<td>.016</td>
</tr>
<tr>
<td>HOMA score</td>
<td>0.7 ± 0.3</td>
<td>2.0 ± 1.0</td>
<td>.013</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130 ± 15</td>
<td>131 ± 10</td>
<td>.889</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75 ± 4</td>
<td>77 ± 7</td>
<td>.536</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.7 ± 0.2</td>
<td>2.0 ± 0.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.7 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>.871</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.5 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>.001</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>3.1 ± 0.5</td>
<td>3.1 ± 0.7</td>
<td>.994</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ApoB-100, g/L</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>.256</td>
</tr>
<tr>
<td>ApoB-48, mg/L</td>
<td>5.4 ± 1.6</td>
<td>11.3 ± 5.8</td>
<td>.034</td>
</tr>
<tr>
<td>ApoC-III, mg/L</td>
<td>153 ± 35</td>
<td>105 ± 43</td>
<td>.022</td>
</tr>
<tr>
<td>Triglyceride to apoB-48 ratio</td>
<td>0.15 ± 0.06</td>
<td>0.24 ± 0.08</td>
<td>.006</td>
</tr>
<tr>
<td>Triglycerides 0–10 hour AUC, mmol/L</td>
<td>13 ± 4</td>
<td>37 ± 16</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides 0–10 hour iAUC, mmol/L</td>
<td>6.3 ± 2.7</td>
<td>18 ± 11</td>
<td>.002</td>
</tr>
<tr>
<td>ApoB-48 0–10 hour AUC, mg/L</td>
<td>82 ± 23</td>
<td>231 ± 79</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ApoB-48 0–10 hour iAUC, mg/L</td>
<td>34 ± 15</td>
<td>118 ± 44</td>
<td>.002</td>
</tr>
<tr>
<td>Basal apoB-48 SR, mg/kg FFM per hour</td>
<td>0.20 ± 0.10</td>
<td>0.29 ± 0.08</td>
<td>.045</td>
</tr>
<tr>
<td>Basal apoB-48 FCR, pool/h</td>
<td>0.67 ± 0.20</td>
<td>0.45 ± 0.17</td>
<td>.021</td>
</tr>
<tr>
<td>Basal apoB-48 secreted, mg/kg FFM</td>
<td>0.81 ± 0.32</td>
<td>1.83 ± 0.59</td>
<td>.003</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; SR, secretion rate. Data are mean ± SD.

* apoB-48 secreted in response to the fat meal on the top of basal apoB-48 secretion.
men (−33%, \( P < .05 \)). In a pooled analysis, plasma apoC-III concentration was significantly and inversely associated with TRL-apoB-48 FCR (\( r = -0.534, P < .02 \)).

**Discussion**

We show that obese men have impaired chylomicron metabolism and increased plasma concentrations of triglycerides and apoB-48 in the fasted and postprandial states. These new findings support that this abnormality in apoB-48 metabolism is probably a consequence of both increased intestinal secretion and decreased catabolism of apoB-48 in the basal and postprandial periods.

Few studies have examined the metabolism of apoB-48 in the postprandial state (5, 9–11). Blackburn et al (10) found that fasting apoB-48 and postprandial apoB-48 AUCs were significantly higher in obese men with hypertriglyceridemia. We have also previously reported that the total and iAUCs for apoB-48 were significantly greater in obese subjects compared with lean controls (5). However, these studies did not investigate whether the higher AUCs were attributable to a defect in secretion and/or catabolism of these particles. Using a primed-constant infusion of \( \text{d}_{3}\text{-leucine} \) in the constant fed state, Hogue et al (11) found that in type 2 diabetic patients, the accumulation of apoB-48 in plasma was attributable to both increased production and decreased catabolism. We have previously reported that the accumulation of TRLs in the postabsorptive state may, in part, relate to defective triglyceride lipolysis and impaired clearance of TRL remnants due to elevated apoC-III concentration (12). In this study, we confirm that plasma apoC-III concentration was significantly and inversely associated with TRL-apoB-48 FCR. Our present data extend the previous studies by investigating apoB-48 metabolism after a fat meal in nondiabetic obese men using a stable isotope label and compartmental modeling.

Disturbances in chylomicron metabolism may relate to insulin resistance and abdominal fat accumulation in obesity. First, insulin resistance stimulates de novo lipogenesis, increases microsomal triglyceride transfer protein, and enhances intracellular apoB-48 stability in the intestine (13). Second, increased free fatty acid load delivered to the enterocyte, especially during the postprandial period, may further impair insulin signaling. Collectively these effects would increase enterocytic secretion of apoB-48 (14). Visceral fat accumulation marks the liver in the portal vein to the liver (15). This impairs the hepatic extraction of insulin and also stimulates triglyceride synthesis, thereby increasing very low-density lipoprotein (VLDL) secretion, consistent with our previous (16). Such effects would result in increased competition between chylomicron and VLDL remnants for hepatic receptors, thereby disturbing or delaying the uptake of chylomicron remnants by this pathway. Insulin resistance may also decrease lipoprotein lipase production and down-regulate low-density lipoprotein receptor expression, limiting remnant lipolysis and removal (17, 18).

Consistent with the aforementioned mechanisms, we found that the principal abnormalities driving elevated fasting levels and postprandial response of apoB-48 include the oversecretion and delayed clearance of apoB-48. Whether obese subjects exhibit similar kinetic defects in the postprandial metabolism of TRL apoB-100 remains to be elucidated. Hogue et al (11) demonstrated that elevated TRL-apoB-100 concentration was attributable to both increased secretion and impaired catabolism in patients with type 2 diabetes.

In this study despite ingesting the same fat load, the plasma triglyceride concentration/time curve in lean subjects (Figure 1A) did not rise to the same extent as it did in obese patients. This implies that lean subjects are better able to hydrolyze TRL triglycerides, resulting in a faster rate of catabolism (hydrolysis and conversion) compared with the obese patients. Consistent with this, we have previously observed that obese individuals with hypertriglyceridemia have reduced VLDL-triglyceride FCR compared with those without this phenotype (19). It is important to note that plasma triglyceride concentration includes triglycerides derived from the intestines in chylomicrons and those derived from the liver in VLDL particles. Hence, future kinetic studies should examine the postprandial responses of chylomicron and VLDL triglycerides in lean and obese subjects.

The uniqueness and novelty of our study is the evaluation of apoB-48 metabolism after a fatty meal. Previous apoB-48 kinetics were studied in the constant fed state to increase plasma apoB-48 concentration to permit accurate measurement of isotope enrichment (11, 14). However, this may result in the formation of smaller, less triglyceride-rich apoB-48-containing lipoproteins different to those present in the postprandial state. Moreover, a test of apoB-48 metabolism in a constant, fed state does not physiologically reflect usual daytime eating patterns. In this study, we developed a simple model to describe the kinetics of apoB-48 in nonsteady state. The current protocol also allows the examination of postprandial triglyceride and apoB-48 AUCs simultaneously in response to the fat meal. A bolus dose administration of \( \text{d}_{3}\text{-leucine} \) permits the better capture of the fast dynamics of apoB-48. We tested 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 in
which FCR was time invariant and apoB-48 secretion increased as a consequence of the test meal fitted the tracer data, allowing the FCRs to be determined with an acceptable level of precision. We therefore chose the latter model to analyze the apoB-48 tracer and concentration data over the non-steady-state period. To validate the model, further study to determine apoB-48 transport directly by coadministration of labeled apoB-48 with the fat meal is warranted. Nevertheless, there is evidence to support our results showing increased apoB-48 secretion in insulin-resistant subjects (11).

In conclusion, we have developed a new kinetic model that is more physiologically relevant to study the non-steady-state, postprandial metabolism of apoB-48 and with which we demonstrate elevated apoB-48 secretion and reduced apoB-48 catabolism in men with obesity. Further work is required to confirm whether our findings also apply to women and to patients with type 2 diabetes mellitus as well as to subjects from other ethnic groups. Therapeutic agents that inhibit the secretion and enhance the catabolism of apoB-48 may be particularly important in CVD prevention in this condition.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

References