Markers of Triglyceride-rich Lipoprotein Remnant Metabolism in Visceral Obesity

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Background: Triglyceride-rich lipoprotein remnants are atherogenic, and this may be particularly important in visceral obesity. We investigated remnant metabolism in obese men by measuring remnant-like particle-cholesterol (RLP-C), apolipoprotein (apo) B-48, apoC-III, and the clearance of a labeled remnant-like emulsion.

Methods: Fasting RLP-C, apoB-48, and apoC-III concentrations were measured in 48 viscerally obese men and 10 lean controls. RLP-C was determined by immunoseparation assay, apoB-48 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and enhanced chemiluminescence, and apoC-III by immunoturbidimetric assay. The catabolism of chylomicron remnants was measured by intravenous injection of a remnant-like emulsion containing cholesteryl [13C]oleate, with isotopic enrichment of 13CO2 in breath determined by isotope-ratio mass spectrometry and a multicompartmental model to estimate fractional catabolic rate (FCR) of the emulsion.

Results: Compared with controls, obese men had significantly increased plasma concentrations of RLP-C, apoB-48, and apoC-III (P < 0.001 for all). Plasma total apoB-100, non-HDL-cholesterol, LDL-cholesterol, triglycerides, and insulin resistance (HOMA score) were also significantly higher in the obese group (P < 0.001 for all). Obese men had a significantly lower FCR of the remnant-like emulsion compared with controls (P = 0.020).

Conclusions: Viscerally obese individuals have insulin resistance and increased plasma concentrations of triglyceride-rich lipoprotein remnants, which may be attributable to decreased catabolism of these particles.

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Hypertriglyceridemia attributable to increased plasma concentrations of hepatic apolipoprotein (apo) B-100 and intestinal apoB-48 is the most consistent lipid disorder in visceral obesity and a risk factor for coronary artery disease (1, 2). The underlying mechanism may relate to oversecretion, reduced hydrolysis, and/or impaired clearance of triglyceride-depleted remnants by high-affinity pathways.

Measurement of triglyceride-rich lipoprotein (TRL) remnants is complex because of difficulties in separating lipoproteins of hepatic and intestinal origin. An immunoseparation method for measurement of remnant-like particle-cholesterol (RLP-C) has recently been proposed as a simple test for remnants (3, 4), but this assay may lack specificity. Increased plasma RLP-C concentrations have nevertheless been associated with coronary disease, diabetes mellitus, and other lipid disorders (5–11).

Plasma apoB-48 is a specific marker of chylomicrons and their remnants, and in the fasting state predicts postprandial lipemia (12). apoC-III is also a marker of TRL metabolism and has been shown to be strongly associated with plasma triglyceride concentrations and progression of coronary artery disease (13–15). There is little information concerning the relationship between plasma RLP-C, apoB-48, and apoC-III in nondiabetic obese individuals with insulin resistance. We have also previously described and validated a “breath test” that provides a functional measurement of chylomicron remnant metabolism (16, 17). The chylomicron remnant breath test may be a useful tool for assessing the kinetics of TRL metabolism in vivo.

In this study we investigated TRL remnant metabolism in obese individuals, using the aforementioned tests.

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Nonstandard abbreviations: apo, apolipoprotein; TRL, triglyceride-rich lipoprotein; RLP-C, remnant-like particle-cholesterol; HOMA, homeostasis model assessment; and FCR, fractional catabolic rate.
Materials and Methods

Participants
Participants included 48 obese men (body mass index > 29 kg/m²; waist circumference > 100 cm; waist-to-hip ratio > 0.97) and 10 normolipidemic lean controls of similar age (plasma triglycerides < 1.2 mmol/L and total cholesterol < 5.2 mmol/L). None had diabetes mellitus (excluded by oral glucose tolerance test), apoE2/E2 genotype, macroproteinuria, creatinemia (> 120 µmol/L), hypothyroidism, or abnormal liver and muscle enzymes, and none consumed > 30 g alcohol/day. None reported a history of cardiovascular disease or familial hyperlipidemia or was taking agents known to affect lipid metabolism. The study was approved by the local Ethics Committee.

Clinical Protocols
All participants were studied in the morning, after a 14-h fast, in a semirecumbent position. Venous blood was collected for biochemical measurements. The sterile isotopically labeled chylomicron remnant-like emulsion (14 mL) was injected intravenously into an antecubital vein via a 21-gauge butterfly needle. End-expiratory breath samples were collected into a Vacutainer Tube at baseline and postinjection every 10 min for the first hour, every 20 min for the second hour, every 30 min for the next 5 h, and hourly for another 3 h. Participants were then given a snack and allowed to go home. The following day, participants provided two additional breath samples. During the first 10 h of collection of breath samples, participants sat quietly in a chair and were allowed to drink only water.

Preparation of Stable Isotope-Labeled Remnant-Like Emulsions
Stable-isotope-labeled remnant-like emulsions were prepared as described previously (16). Briefly, pure lipid mixtures containing triolein (135 mg), phosphatidylcholine (75 mg), cholesterol (24 mg; Nu-Chek Prep), and cholesteryl [13C]oleate (70 mg) were emulsified by sonication for 1 h in 22 mL/L glycerol in water. After sonication, the mixture was centrifuged for 10 min to remove titanium fragments and then filtered into sterile vessels. All emulsion preparations were confirmed to be sterile and pyrogen-free by the Pharmacy Department of the Royal Perth Hospital. Uniformly labeled [13C]oleate was purchased from Novachem Pty. Ltd., and cholesteryl [13C]oleate was synthesized from cholesterol and [13C]oleic acids as described previously (17).

RLP-C Assay
RLP-C was determined from plasma with a JIMRO-II (Japan Immunoresearch Laboratories) assay using an immunoaffinity mixed gel containing monoclonal antibodies to human apoA-I and apoB100. The mixture was subsequently shaken for 2 h at room temperature to bind lipoproteins containing apoA-I and apoB100 to Sepharose 4B. After incubation, the gel was allowed to settle for 15 min, and 200 µL of the supernatant was placed into sample cups. The cholesterol concentration in the supernatant was measured on a Hitachi 917 Biochemical Analyzer (Hitachi Ltd) with Boehringer Mannheim cholesterol reagents (Boehringer Mannheim Diagnostics). Interassay CVs were < 6%.

Quantification of apoB-48
apoB-48 was measured as described previously (12) with the exception that apoB-48 was assayed directly from plasma rather than from the 1.063 kg/L lipoprotein fraction. Values obtained by either method are identical (J.C.L. Mamo and A.P. James, unpublished observations). Apolipoproteins were separated from other lipoproteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a Novex MiniCell Electrophoresis system (Novex) with precast NUPAGE 4–8% Tris-aceate gels. After electrophoresis, separated apolipoproteins were electrophoretically transferred to a PVDF membrane (0.45 µm; Immobilon P; Millipore Corporation). apoB-48 bands were identified and visualized by enhanced chemiluminescence (ECL; Amersham) using an antibody to apoB (Dako A/S) followed by an anti-rabbit IgG antibody (horseradish peroxidase conjugated; Amersham Pharma- cia Biotech). Densitometric scanning of apoB-48 bands and standardization to the known purified apoB-48 protein mass allowed quantification of the protein. Interassay CVs were < 10%.

Biochemical Analyses
Plasma triglyceride and cholesterol concentrations were determined by standard enzymatic methods on a Hitachi 917 Biochemical Analyzer. HDL-cholesterol was measured by an enzymatic colorimetric method using a commercial reagent set (Boehringer Mannheim). Non-HDL-cholesterol was derived as total cholesterol minus HDL-cholesterol. LDL-cholesterol was calculated using the Friedewald equation, apoA-I and apoB were determined by immunonephelometry; apoC-III was measured by immunoturbidimetric assay (Daichi). Plasma nonesterified fatty acids were measured by an enzymatic, colorimetric method using a commercial reagent set (Randox Co.). Plasma insulin was measured by RIA (DiaSorin, s.r.L.), and the glucose concentration was measured by a hexokinase method on a Hitachi 917 analyzer. Insulin resistance was estimated by the homeostasis model assessment (HOMA), as described by Matthews et al. (18). apoE genotyping was performed according to the method of Hixson and Vernier (19). Interassay CVs were < 6%.

Expired CO₂ was analyzed by isotope-ratio mass spectrometry using a Finnigan BreathPlus instrument (Thermoquest Systems Pty. Ltd). The ratio $^{13}$CO₂/$^{12}$CO₂ was referenced to PeeDeeBelemnite standard values, and the delta unit value was calculated using Breathmat software. The delta units reference a sample of limestone, a stan-
The compartmental model was fitted to the observed program (SAAM Institute) as described previously. Fasting NEFAs, Insulin resistance, HOMA score 5.70, Systolic blood pressure, mmHg 122, Waist-to-hip ratio 0.92, Waist, cm 91, Age, years 53.1.

STATISTICAL ANALYSIS
Two-group comparisons were carried by independent t-tests and the χ² test. Obese participants were divided into low triglyceride (<1.69 mmol/L), borderline-high triglyceride (1.69–2.23 mmol/L), and high triglyceride (>2.23 mmol/L) groups (20), which were compared by ANOVA with the Bonferroni adjustment. Associations were examined by Pearson correlation analyses.

RESULTS
The characteristics of the obese and lean men are shown in Table 1. Age and blood pressures were not significantly different between the groups. The obese group had a significantly higher body weight, body mass index, waist circumference, and waist-to-hip ratio (P < 0.001) compared with the lean group. Although plasma glucose and nonesterified fatty acids were not significantly different between the groups, the obese group had significantly higher insulin concentrations and HOMA scores (P < 0.001). There were no statistically significant differences in the frequency distribution of E alleles between the groups (data not shown).

The plasma concentrations of lipids and lipoproteins and the FCR of the remnant-like emulsion in the individuals studied are shown in Table 2. The obese group had significantly higher plasma triglycerides, cholesterol, non-HDL-cholesterol, LDL-cholesterol, and apoB-100 (P < 0.001), but lower HDL-cholesterol (P < 0.05) compared with the lean men. Plasma RLP-C, apoB-48, and apoC-III concentrations were significantly higher in the obese group compared with controls. The FCR of the remnant-like emulsion was significantly lower in the obese group compared with the lean group. Fig. 1 compares plasma RLP-C, apoB-48, apoC-III, and the FCR of the remnant-like emulsion in obese men according to the National Cholesterol Education Program cutoff points for triglycerides. With increasing plasma triglyceride concentrations, there were significant increases in plasma RLP-C, apoB-48, and apoC-III (P < 0.001). There was no significant difference in the FCR of the emulsion and other lipid and apolipoprotein variables among the obese groups.

In the obese group, plasma triglyceride concentrations significantly and positively associated with RLP-C (r = 0.796; P < 0.001), apoB-48 (r = 0.597; P < 0.001), apoC-III (r = 0.743; P < 0.001), and HOMA score (r = 0.301; P < 0.05) and inversely correlated with HDL-cholesterol (r = −0.315; P < 0.05) and LDL-cholesterol (r = −0.304; P < 0.05). There was a statistically significant association of plasma RLP-C with apoB-48 (r = 0.524; P < 0.001). RLP-C also correlated with apoC-III (r = 0.524; P < 0.001) and HOMA score (r = 0.508; P < 0.001), whereas apoB-48 correlated significantly with non-HDL-cholesterol (r = 0.453; P < 0.001), apoC-III (r = 0.478; P < 0.001), and HOMA score (r = 0.290; P < 0.05).

DISCUSSION
This is the first report to examine the metabolism of TRL remnants in obese individuals by measuring plasma RLP-C, apoB-48, and apoC-III concentrations. Increased plasma RLP-C has previously been associated with cardiovascular disease, type 2 diabetes mellitus, and dyslipidemia (5–11). Only one study has reported that the

**Table 2. Plasma lipids, lipoproteins, and apolipoproteins and the FCR of the remnant-like emulsion in the individuals studied.**

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 10)</th>
<th>Obese (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.33 ± 0.34</td>
<td>5.95 ± 0.75b</td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>0.77 ± 0.25</td>
<td>1.90 ± 0.77b</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.28 ± 0.30</td>
<td>1.04 ± 0.21c</td>
</tr>
<tr>
<td>Non-HDL-C, mmol/L</td>
<td>3.05 ± 0.42</td>
<td>4.91 ± 0.67b</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.70 ± 0.33</td>
<td>3.89 ± 0.68b</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.11 ± 0.03</td>
<td>0.41 ± 0.21b</td>
</tr>
<tr>
<td>apoB-100, mg/dL</td>
<td>78 ± 11</td>
<td>128 ± 19b</td>
</tr>
<tr>
<td>apoB-48, mg/L</td>
<td>12.3 ± 2.8</td>
<td>24.3 ± 8.8b</td>
</tr>
<tr>
<td>apoC-III, mg/L</td>
<td>118 ± 24</td>
<td>162 ± 34b</td>
</tr>
<tr>
<td>FCR, pools/h</td>
<td>0.096 ± 0.027</td>
<td>0.066 ± 0.004b</td>
</tr>
</tbody>
</table>

*a To convert from SI units to mg/dL, multiply the values by 38.61 for cholesterol and 88.5 for triglycerides.

*b P < 0.001 compared with lean controls by unpaired t-test.  

*c P < 0.05.

*d HDL-C and LDL-C, HDL- and LDL-cholesterol, respectively.
fasting RLP-C concentration is increased in obesity (21). Plasma concentrations of apoB-48 and apoC-III have previously been shown to be increased in viscerally obese individuals (22, 23). We also provide kinetic evidence, based on the remnant-like emulsion breath test, that increases in plasma concentration of remnants are partly related to decreased clearance of these particles.

Disturbances in lipoprotein metabolism in visceral obesity may be attributable to insulin resistance and increased apoC-III (1). The principal abnormalities in our obese men included increased plasma TRLs, insulin, HOMA score, and to a lesser extent, LDL-cholesterol, with a reciprocal decrease in HDL-cholesterol. Insulin resistance increases hepatic synthesis of lipid substrates and the secretion of VLDL apoB-100 (24); it also downregulates LDL receptors (25). These effects potentially increase the plasma concentrations of remnant lipoproteins containing apoB-100 and increase competition for hepatic uptake between chylomicron and VLDL remnants (26). Moreover, increased apoC-III inhibits the lipolysis of VLDL-triglycerides by lipoprotein lipase (27) and interferes with the hepatic uptake of TRL remnants by LDL receptors (28).

The lower FCR values of the remnant-like emulsion in our obese participants suggest possible delayed catabolism of chylomicron remnants. The increased apoC-III in our study participants might also reflect disturbances in the lipolysis and clearance of TRL remnants. The independent relationship between the markers of TRL metabolism was supported by the highly significant direct correlations between plasma triglycerides, RLP-C, apoB-48, apoC-III, and insulin resistance in the obese group. However, <65% of variances in RLP-C, apoB-48, and apoC-III could be accounted for by plasma triglycerides. This suggests that plasma triglyceride concentrations only partially reflect the accumulation of remnant lipoproteins in obesity. The lack of a significant correlation between the FCR of the remnant-like emulsion and other markers of TRLs suggests that additional mechanisms may account for the accumulation of TRL remnants other than the delayed clearance of chylomicron remnant particles. Possible mechanisms include oversecretion of both hepatic and intestinally derived TRLs (29), and these require further investigation.

Measurements of apoB-48 may not discriminate between the nascent chylomicron and its remnant. Because participants fasted for at least 12 h to ensure minimal intestinal secretion of nascent chylomicrons, the concentration of apoB-48 was probably indicative of small, dense chylomicrons and their remnants. Fasting RLP-C is not a
specific marker of chylomicron and VLDL remnants because it quantifies apoE-rich lipoproteins of intestinal and some hepatic lipoproteins (4). Only 36% of the RLP-C could be accounted for by the plasma apoB-48 concentration, which suggests that the main source of variance in RLP-C may be endogenous remnant lipoproteins. The lack of a significant correlation between RLP-C and non-HDL-cholesterol might be the result of a confounding effect of LDL-cholesterol.

The breath test provides a specific functional assessment of chylomicron remnant metabolism and has previously been validated in patients with familial dyslipidaemias (16). However, it depends not only on the plasma clearance of RLPS, but also on the subsequent oxidation of fatty acids hydrolyzed from the emulsion of cholesteryl ester. Our unpublished data showed that plasma fatty acid pools do not confound the results. In a separate study, we also found that unselected obese individuals have delayed plasma clearance of the injected emulsion compared with lean individuals [0.77 ± 0.39 pools/h (n = 12) vs 1.55 ± 0.44 pools/h (n = 6); P <0.01]. Because apolipoproteins are ligands for hepatic lipoprotein receptors, transfer of apolipoproteins to the injected RLPS from endogenous lipoproteins in the circulation is a critical requirement for hepatic clearance of the emulsion. Accordingly, we previously reported that the FCR of RLPS measured by the breath test was significantly decreased in E2/E2 homozygous individuals (16) and apoE knockout mice (17).

Our data show that plasma RLP-C, apoB-48, and apoC-III concentrations are all significantly increased in obese individuals with borderline-high triglyceride concentrations compared with controls (Fig. 1). This finding supports the lowering of plasma triglyceride cutoff point for obese individuals to <1.69 mmol/L (20). In the future, measurement of RLP-C, apoB-48, and apoC-III may be helpful in risk assessment and treatment strategies.

In conclusion, we have demonstrated that viscerally obese individuals have insulin resistance and increased concentrations of TRL remnants, including RLP-C and apoB-48. The increases were more pronounced in obese individuals with increased plasma triglyceride concentrations. We suggest that accumulation of TRL remnants is partly attributable to defective lipolysis and impaired clearance of chylomicron remnants, as reflected by increased apoC-III concentrations and reduced FCR of the remnant-like emulsion. Further studies should examine the effect of weight loss or pharmacotherapeutic intervention on plasma RLP-C, apoB-48, and other markers of TRL metabolism.

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