Early Kinetic Abnormalities of ApoB-Containing Lipoproteins in Insulin-Resistant Women With Abdominal Obesity

Frédéric Pont, Laurence Duvillard, Emmanuel Florentin, Philippe Gambert, Bruno Vergès

Objective—The kinetic abnormalities of apolipoprotein B (apoB)-containing lipoproteins in abdominally obese insulin-resistant individuals remain poorly understood. To determine the influence of insulin resistance, linked with abdominal obesity, on apoB metabolism at an early stage, we performed a stable isotope kinetic study of apoB in very low density lipoproteins (VLDLs), intermediate density lipoproteins (IDLs), and low density lipoproteins (LDLs) in 5 abdominally obese insulin-resistant women with normal fasting triglyceride levels and without impaired glucose tolerance and in 5 age-matched control women.

Methods and Results—Each subject received an intravenous injection of a 0.7 mg/kg bolus of L-[1-13C]leucine, immediately followed by a 16-hour constant infusion at 0.7 mg/kg per hour. Compared with control women, insulin-resistant women with abdominal obesity showed a significant 84% increase of the VLDL apoB production rate (27.18±11.53 versus 14.80±1.94 [control] mg/kg per day, P=0.009), a significant 54% increase of the IDL apoB production rate (20.63±3.66 versus 13.39±3.99 [control] mg/kg per day, P=0.009), and a significant 63% increase of the LDL apoB production rate (18.49±1.70 versus 11.33±3.79 [control] mg/kg per day, P=0.009), leading to significantly higher VLDL, IDL, and LDL apoB concentrations. The fractional catabolic rates of VLDL, IDL, and LDL apoB were not significantly different between abdominally obese insulin-resistant women and control women.

Conclusions—Our study shows that patients at an early stage of insulin resistance linked with abdominal obesity (without glucose intolerance) already have an altered metabolism of the VLDL-IDL-LDL cascade (increased VLDL, IDL, and LDL apoB production rates), which is consistent with the augmented risk of atherosclerosis observed in this population. (Arterioscler Thromb Vasc Biol. 2002;22:●●●–●●●.)

Key Words: insulin resistance ■ abdominal obesity ■ insulin ■ stable isotopes ■ apolipoprotein B ■ lipids

Abdominal obesity, which is frequent in the general population, is associated with insulin resistance1–2 and with an increased risk of cardiovascular disease.3 The insulin-resistant state is commonly associated with lipoprotein abnormalities, such as hypertriglyceridemia, high levels of VLDL, and low levels of HDL cholesterol.4 Insulin plays a central role in regulating lipoprotein metabolism, and many studies have suggested that insulin resistance may be a factor causing dyslipidemia.5,6 Among the lipid abnormalities observed in insulin-resistant patients with abdominal obesity, the changes of apoB-containing lipoprotein metabolism are likely to play a key role in the development of atherosclerosis. Kinetic studies using radiolabeled tracers examining apoB metabolism in obesity have been conflicting.7–9 In a stable isotope study of VLDL apoB, an increased hepatic secretion of VLDL apoB-100 has been demonstrated in obese insulin-resistant subjects.10 However, that study focused on VLDL metabolism in markedly hyperlipidemic obese subjects. The discrepancies between the different results concerning VLDL, IDL, and LDL apoB metabolism in obesity may be due to patients’ characteristics and to the method used to label the lipoproteins. Particularly, all studies of IDL and LDL apoB metabolism have been performed with the use of exogenous labeling.7–8 The exogenous labeling may modify the proteins under investigation.11 In addition, with this method, LDLs, which have a slow turnover rate, are preferentially labeled compared with lipoproteins with faster turnover rates, such as VLDL and IDL, which can lead to erroneous kinetic results.12 Furthermore, the previous studies were performed in obese patients but were not limited to subjects with abdominal obesity, and no assessment of insulin resistance was performed. Moreover, obese subjects from these studies had significantly increased fasting plasma triglyceride (TG) levels. So far, no data are available concerning apoB metabolism at an early stage of insulin resistance in individuals with abdominal obesity before the onset of fasting hypertriglyceridemia and glucose intolerance.

In the present study, we report the first apoB kinetic study through the VLDL→IDL→LDL cascade with endogenous stable isotope labeling in insulin-resistant women with abdominal obesity but with normal fasting TG levels and without glucose intolerance. The aim of the present study was...
to determine the first abnormalities of lipid metabolism in the insulin-resistance state linked with abdominal obesity before the onset of fasting hypertriglyceridemia and glucose intolerance.

**Methods**

**Subjects**

Five insulin-resistant women with abdominal obesity and 5 lean, normolipidemic, age-matched control women were studied. All abdominally obese insulin-resistant women had an increased body mass index (BMI, >38 kg/m²) and waist/hip ratio (>1.02). They all featured a waist circumference >88 cm, confirming abdominal obesity according to the criteria of the National Cholesterol Education Program, Adult Treatment Panel III. Insulin-resistant women with abdominal obesity were selected according to their insulin sensitivity by using the homeostasis model assessment (HOMA) method and the insulin suppression test, as explained below. All insulin-resistant women with abdominal obesity presented with HOMA levels >2 and steady-state plasma glucose (SSPG) values (during the insulin suppression test) >8 mmol/L. Insulin-resistant women with abdominal obesity were not glucose intolerant, with fasting plasma glucose levels <6.1 mmol/L and plasma glucose levels (after a 75-g oral glucose load) <7.8 mmol/L. Furthermore, they were normotriglyceridemic, inasmuch as their fasting TG levels were within the normal limits of our laboratory (<1.60 mmol/L), ranging from 0.69 to 1.47 mmol/L. No patient was taking any medication known to affect lipid metabolism.

All control subjects were in good health, with normal glucose tolerance and normal plasma lipid levels. They were not taking any medication. The protocol was approved by the Dijon University Hospital ethics committee, and written informed consent was obtained from each subject before the study.

**Experimental Protocol**

The kinetic study was performed with subjects in a fed state. Food intake, with a leucine-poor diet (1700 kcal/d, 55% carbohydrates, 39% fats, and 7% proteins), was fractionated in small equal portions that were provided every 2 hours, starting 6 hours before the tracer infusion up to the end of the study, to avoid important variations in apolipoprotein plasma concentration, as previously performed by other groups. To determine the kinetics of apoB-100, the subjects received an intravenous injection of a 0.7 mg/kg bolus of L-[1-13C]leucine (99% 13C, Eurisotop) immediately followed by a 16-hour constant infusion at 0.7 mg/kg per hour. Blood samples were collected at hours 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16. Serum was separated by centrifugation and stored at 4°C. Inhibitors of protease (17 mg/L aprotinin) and bacterial growth (500 and 16. Serum was separated by centrifugation and stored at 4°C. The kinetic study was performed with subjects in a fed state. Food intake, with a leucine-poor diet (1700 kcal/d, 55% carbohydrates, 39% fats, and 7% proteins), was fractionated in small equal portions that were provided every 2 hours, starting 6 hours before the tracer infusion up to the end of the study, to avoid important variations in apolipoprotein plasma concentration, as previously performed by other groups. To determine the kinetics of apoB-100, the subjects received an intravenous injection of a 0.7 mg/kg bolus of L-[1-13C]leucine (99% 13C, Eurisotop) immediately followed by a 16-hour constant infusion at 0.7 mg/kg per hour. Blood samples were collected at hours 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16. Serum was separated by centrifugation and stored at 4°C. Inhibitors of protease (17 mg/L aprotinin) and bacterial growth (500 mg/L sodium azide) were added to each sample.

**Analytical Procedures**

Analytical procedures were performed as previously described in detail.

**Isolation and Measurement of Apolipoproteins**

VLDL (density [d]<1.006 g/mL), IDL (1.006 g/mL<d<1.019 g/mL), and LDL (1.019 g/mL<d<1.063 g/mL) were isolated from plasma by sequential flotation ultracentrifugation with the use of a 50.4 rotor in a L7 apparatus (Beckman Instruments). IDL and LDL fractions were then dialyzed against a 10 mmol/L ammonium bicarbonate buffer, pH 8.2, containing 0.01% EDTA and 0.013% sodium azide. VLDL, IDL, and LDL fractions were delipidated with diethyl ether–ethanol, and apoB-100 from each lipoprotein fraction was isolated by preparative SDS-PAGE (3%). After staining with Coomassie blue R-250, apoB-100 bands were excised from the polyacrylamide gels and hydrolyzed in 6 mol/L HCl at 110°C for 16 hours under nitrogen vacuum. Samples were then lyophilized in a Speed Vac (Savant Instrument). Lyophilized samples were dissolved in 50% acetic acid and applied to an AG-50W-X8 200 to 400 mesh cation exchange resin (Bio-Rad), and amino acids were recovered by elution with 4N NH₄OH.

**Determination of Leucine Enrichment by Gas Chromatograph/Combustion/Isotope Ratio Mass Spectrometry**

Amino acids were converted to N-acetyl O-propyl amino acid esters and analyzed with a Delta C isotope ratio mass spectrometer (Finnigan Mat) coupled to an HP 5890 series II gas chromatograph (Hewlett Packard), as previously described.

**Modeling**

Apolipoprotein kinetics data were expressed as tracer-to-tracee mass ratios, z(t), calculated as follows:

\[
z(t) = \frac{e(t)}{e_i(t)}
\]

where \(e_i\) is the tracer enrichment, and \(e(t) = a(t) - a_s\), where \(a(t)\) and \(a_s\) indicate the isotope abundance of the labeled and the unlabeled species, respectively.

The data were analyzed with the Simulation Analysis and Modeling II program (SAAM Institute, Inc) by using a multicompartamental model. Development of compartmental models in stable isotope experiments has been previously described in detail.

The models chosen to describe the data are shown in Figure 1. Plasma VLDL apoB kinetic data are represented by compartments 1 and 2, and plasma IDL apoB kinetic data are represented by compartments 11 and 12. Compartment 2 and 12 turned over more slowly than did compartments 1 and 11, respectively. These compartments were required in most subjects for optimal model fit of the data. Although the majority of IDL apoB was derived from VLDL, a direct IDL apoB input was required to fit the data in some subjects. Because the experiment was performed in the steady state, the fractional synthetic rate equaled the fractional catabolic rate (FCR). The direct FCRs of VLDL apoB and FCRs from VLDL to IDL or LDL expressed in pools per day, were calculated as follows:

\[
\text{Direct FCR}_{\text{VLDL}} = \frac{M_k(0,2)}{M_i + M_j}
\]

**Production rates (PRs) of the apoB-100 in each lipoprotein fraction were normalized to body weight and calculated as follows:**

\[
\text{PR} = \text{FCR}_{\text{TFCR}} \times \text{apoB pool size/body weight}
\]

where apoB pool size is calculated by multiplying the apoB concentration in the lipoprotein fraction (VLDL, IDL, or LDL) by the estimated plasma volume.

For normal-weight control subjects, plasma volume was determined as 4.5% of body weight. In abdominally obese subjects, a correction of plasma volume was performed as previously reported by many authors. The plasma volume was modified by multi-

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plying by a correction factor to take into account the decrease in relative plasma volume associated with an increase in body weight.28

**Analytical Methods**

**Apolipoprotein and Lipid Assays**
ApoB-100 concentrations were measured by immunoturbidimetry (Boehringer-Mannheim). Coefficient variation of apoB measurement was 5%.

All chemical assays were performed on a Cobas-Fara Centrifugal Analyser (Hoffmann-La Roche). Total cholesterol and unesterified cholesterol concentrations were measured by enzymatic methods with the use of Boehringer-Mannheim reagents. TG concentration was measured by enzymatic methods with the use of Roche reagents. The lipid and apolipoprotein values during the kinetic experiment, with subjects in the fed state, are the means of 4 time points during the 16-hour infusion time.

**Insulin Resistance Evaluation**

The insulin resistance level was estimated by using the HOMA method14 and the insulin suppression test.15 The HOMA was calculated with the following formula14:

\[
\text{HOMA} = \frac{\text{[glucose]} \times \text{[insulin]}}{22.5}
\]

where [glucose] is the fasting glucose millimolar concentration, and [insulin] is the fasting insulin concentration expressed in milliunits per liter.

Plasma insulin was measured by radioimmunoassay (CIS Bio International).

During the insulin suppression test, insulin-stimulated glucose uptake was estimated by measuring the SSPG concentrations achieved during the last 60 minutes of a 180-minute continuous infusion of somatostatin, insulin, and glucose. Somatostatin is used to suppress endogenous insulin production, and insulin and glucose are infused at a dose of 0.8 mU/kg per minute and 6 mg/kg per minute, respectively. Higher SSPG levels during the last 60 minutes of the continuous infusion are associated with higher insulin resistance. Normal subjects have SSPG levels <6.6 mmol/L.15

**Statistical Analysis**

Data are reported as mean±SD. Statistical calculations were performed by using the SPSS software package. The Mann-Whitney U test was used to compare clinical, biological, and kinetic characteristics between patients and control subjects. Correlation coefficients were calculated by the Spearman test. A 2-tailed value of \( P<0.05 \) was accepted as statistically significant.

**Results**

**Apolipoprotein and Lipid Concentrations**

Clinical and glucose metabolism characteristics of the studied subjects are presented in Table 1. The insulin-resistant women were significantly overweight-a compared with control women (BMI 40.5±3.9 versus 23.2±1.5 kg/m², respectively; \( P=0.009 \)). They had the clinical features of abdominal obesity with increased waist circumference (125±2 versus 75±6 cm, respectively; \( P=0.009 \)) and waist/hip ratio (1.04±0.01 versus 0.81±0.07, respectively; \( P=0.009 \)). Moreover, insulin resistance was confirmed in each subject by elevated HOMA (2) and SSPG values during the insulin suppression test (>8 mmol/L). The subjects were neither diabetic nor glucose intolerant, as assessed by normal fasting and postload glucose concentrations. Data for plasma apolipoprotein and lipid concentrations measured before and during the kinetic study are presented in Table 2. Because no significant variation was observed between measurements at 4 different infusion times (data not shown), it was considered that all subjects were in steady state throughout the study. Each insulin-resistant patient had fasting TGs within the normal range of our laboratory (<1.60 mmol/L), ranging from 0.69 to 1.47 mmol/L. Fasting TGs were not significantly different between insulin-resistant subjects and control subjects. Compared with control subjects, the insulin-resistant subjects with abdominal obesity had significantly

![Figure 1. Multicompartmental model for kinetic analysis of apoB-100 metabolism. A forcing function, corresponding to VLDL apoB-100 plateau enrichment, was used to drive the appearance of leucine tracer into the different lipoprotein fractions (compartment 3). Compartments 1 and 2 represent plasma VLDL apoB-100; compartments 11 and 12, plasma IDL apoB-100; and compartment 21, plasma LDL apoB-100. Although the majority of IDL apoB-100 was derived from VLDL, a direct IDL apoB-100 input was sometimes required. A shunt from compartment 1 to compartment 21 also improved the fit in some cases.](http://atvb.ahajournals.org/issue.jpg)
lower HDL cholesterol levels ($P=0.04$). During the fed state, insulin-resistant subjects with abdominal obesity had significantly higher plasma TG, VLDL TG, LDL TG, and apoB concentrations than did control subjects (Table 2).

ApoB-100 concentrations were significantly higher in insulin-resistant patients with abdominal obesity than in control subjects in VLDLs ($P=0.009$), IDLs ($P=0.009$), and LDLs ($P=0.016$, Table 3).

**Kinetic Data**

The kinetic curves of VLDL, IDL, and LDL apoB-100 are shown in Figure 2 for a control subject and an insulin-resistant subject with abdominal obesity.

Kinetic parameters concerning apoB-100 are shown in Table 3. The VLDL apoB PR was significantly higher in insulin-resistant women with abdominal obesity than in control women (27.18$\pm$11.53 versus 14.80$\pm$1.94 mg/kg per day, respectively; $P=0.009$), corresponding to an 84% increase of VLDL apoB PR. The IDL apoB PR was significantly augmented in insulin-resistant women with abdominal obesity compared with control women (20.63$\pm$3.66 versus 13.39$\pm$3.99 mg/kg per day, respectively; $P=0.009$), corresponding to a 54% increase of IDL apoB PR. The LDL apoB PR was significantly higher in insulin-resistant women with abdominal obesity than in control women (18.49$\pm$1.70 versus 11.33$\pm$3.79 mg/kg per day, respectively; $P=0.009$), corresponding to a 63% increase of LDL apoB PR. The FCRs of VLDL apoB, IDL apoB, and LDL apoB were not significantly different between insulin-resistant women with abdominal obesity and control women.

**Correlations**

In control and insulin-resistant abdominally obese women, VLDL apoB and IDL apoB PRs were positively correlated ($r=0.95, P<0.001$). The LDL apoB PR was also positively correlated with VLDL apoB ($r=0.84, P=0.002$) and IDL apoB ($r=0.84, P=0.002$) PRs.

**Discussion**

Abdominal obesity is associated with insulin resistance in men and women$^{1,2,29}$ and with an increased risk of cardiovascular disease. The abnormalities of lipoprotein metabolism are likely to play an important role in the development of

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**TABLE 1. Clinical and Biological Characteristics of the Insulin-Resistant and Control Women**

<table>
<thead>
<tr>
<th></th>
<th>Control Women (n=5)</th>
<th>Insulin-Resistant Women (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.0±11.5</td>
<td>39.0±8.0</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.2±1.5</td>
<td>40.5±3.9</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>75±6</td>
<td>125±2</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.81±0.07</td>
<td>1.04±0.01</td>
<td>0.009</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.46±0.52</td>
<td>4.60±0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Post load plasma glucose, mmol/L</td>
<td>4.34±0.44</td>
<td>6.27±0.98</td>
<td>0.009</td>
</tr>
<tr>
<td>SSPG, mmol/L</td>
<td>—</td>
<td>15.48±5.38</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>4.5±1.1</td>
<td>20.3±13.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Postload insulin, mU/L</td>
<td>15.2±4.2</td>
<td>108.7±63.5</td>
<td>0.016</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.9±0.2</td>
<td>4.1±2.6</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Values are mean±SD. NS indicates not significant.

**TABLE 2. Lipid and Apolipoprotein Concentrations of the Insulin-Resistant and Control Women**

<table>
<thead>
<tr>
<th></th>
<th>Control Women (n=5)</th>
<th>Insulin-Resistant Women (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening values (in fasting state)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma chol, mmol/L</td>
<td>5.03±0.98</td>
<td>4.92±0.85</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>0.75±0.27</td>
<td>1.04±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>HDL chol, mmol/L</td>
<td>1.70±0.28</td>
<td>1.03±0.43</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL chol, mmol/L</td>
<td>2.99±0.75</td>
<td>3.43±0.82</td>
<td>NS</td>
</tr>
<tr>
<td>Experimental values (in fed state)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma chol, mmol/L</td>
<td>4.72±0.87</td>
<td>4.56±1.23</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma free chol, mmol/L</td>
<td>1.44±0.23</td>
<td>1.41±0.21</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>1.03±0.19</td>
<td>1.43±0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>VLDL-TG, mmol/L</td>
<td>0.30±0.10</td>
<td>0.81±0.37</td>
<td>0.009</td>
</tr>
<tr>
<td>LDL-TG, mmol/L</td>
<td>0.20±0.04</td>
<td>0.50±0.31</td>
<td>0.027</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>64±9</td>
<td>104±23</td>
<td>0.009</td>
</tr>
<tr>
<td>Apo A-1, mg/dL</td>
<td>122±12</td>
<td>119±34</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD. Chol indicates cholesterol.
atherosclerosis in insulin-resistant subjects with abdominal obesity. The present study is the first stable isotope kinetic study of VLDL, IDL, and LDL apoB-100 in obese insulin-resistant subjects with abdominal obesity at an early stage of insulin resistance, before the onset of impaired glucose tolerance or increased fasting plasma TGs. Our results show that such patients already have important alterations of apoB metabolism, with a significant increase of apoB PR in each apoB-containing lipoprotein fraction: VLDL (84%), IDL (54%), and LDL (63%).

TABLE 3. Kinetic Parameters of VLDL, IDL, and LDL Apo B-100 in the Insulin-Resistant and Control Women

<table>
<thead>
<tr>
<th></th>
<th>VLDL Apo B</th>
<th>IDL Apo B</th>
<th>LDL Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apo B, mg/L</td>
<td>Production, mg·kg⁻¹·day⁻¹</td>
<td>Fractional Rate, pool/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Direct Catabolism</td>
<td>Transfer to IDL/LDL</td>
</tr>
<tr>
<td>Control women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>36.79</td>
<td>16.66</td>
<td>4.08</td>
</tr>
<tr>
<td>2</td>
<td>32.28</td>
<td>15.80</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>37.50</td>
<td>12.60</td>
<td>0.96</td>
</tr>
<tr>
<td>4</td>
<td>30.00</td>
<td>16.13</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>37.00</td>
<td>12.79</td>
<td>2.88</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>34.71±3.37</td>
<td>14.80±1.94</td>
<td>1.58±1.82</td>
</tr>
<tr>
<td>Insulin-resistant women with abdominal obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>104.62</td>
<td>33.25</td>
<td>3.60</td>
</tr>
<tr>
<td>2</td>
<td>112.47</td>
<td>44.70</td>
<td>4.32</td>
</tr>
<tr>
<td>3</td>
<td>49.44</td>
<td>19.97</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>59.25</td>
<td>18.02</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>83.33</td>
<td>19.97</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>81.82±27.48</td>
<td>27.18±11.53</td>
<td>1.73±2.07</td>
</tr>
<tr>
<td>P</td>
<td>0.009</td>
<td>0.009</td>
<td>NS</td>
</tr>
</tbody>
</table>

Figure 2. [¹³C]Leucine enrichment curves of human apoB-100 in 1 insulin-resistant subject (B) and in 1 control subject (A). Shown are tracer/tracee values (mean of triplicates) from VLDL apoB-100 (open circles), IDL apoB-100 (closed triangles), and LDL apoB-100 (closed circles) during the constant infusion experiment in the fed state. SDs were too low to be indicated. The curves were obtained by compartmental modeling as shown in Figure 1.
Egusa et al have reported, with use of a radiotracer, increased PRs of VLDL apoB and LDL apoB in obese subjects. However, most obese subjects in that study had increased fasting plasma TGs (up to 292 mg/dL). Furthermore, in that study, no information on the type of obesity (abdominal obesity or not) is available, and no assessment of insulin resistance was performed.

Our obese population was truly insulin resistant, as demonstrated by significantly increased HOMA (normal values <2),14 SSPG (normal values <6.6 mmol/L),15 plasma fasting, and postload insulin values. However, they were neither diabetic nor glucose intolerant, as assessed by normal fasting and postload glucose concentrations. Furthermore, our insulin-resistant subjects had fasting TG levels that were in the normal range of our laboratory and not significantly different from levels in control subjects. In the present study, we have been able to compare the kinetic data found in insulin-resistant subjects with the data obtained in sex- and age-matched control subjects. So far, no apoB kinetic data in such patients, at an early stage of insulin resistance, are available.

The insulin-resistant women with abdominal obesity in the present study were characterized by an important obesity, with a mean BMI of 40.5 kg/m². Thus, it is not possible to completely exclude an impact of obesity, per se, on the kinetic abnormalities of apoB-containing lipoproteins. However, we now have many data indicating that the increased cardiovascular risk and the metabolic abnormalities are associated with abdominal obesity but not with gluteofemoral obesity.1,2,30 Moreover, it has been shown that insulin resistance associated with abdominal obesity is a key factor of the metabolic disorders observed in patients with abdominal obesity.4–6 Thus, we surmise that the kinetic abnormalities observed in our obese women are mainly associated with their abdominal obesity–linked insulin resistance and that obesity, per se, is not likely to play a major role.

In our insulin-resistant women, the number of VLDL particles was increased, as assessed by the increased plasma concentration of VLDL apoB. The significant increase in VLDL apoB concentration in our abdominally obese women is explained only by the augmented VLDL apoB PR, because its FCR was not significantly modified.

Insulin resistance may be responsible for the increased VLDL apoB secretion. Indeed, insulin has been shown to inhibit VLDL apoB production. In vitro studies have demonstrated that insulin decreases VLDL apoB production in rat and human hepatocytes.31–33 In vivo studies have shown that insulin reduces VLDL apoB production in healthy humans33,34 and in diabetic subjects.35 Malmström et al36 have shown that under acute insulin action, the liver suppresses the production of VLDLapoB, leading to a decreased production of total VLDL particles. These authors have also demonstrated that the drop in VLDL apoB secretion in response to insulin is due entirely to a decrease in VLDLapoprotein B production, without any change in the production of VLDLapoB.36 This indicates that insulin acts mainly on VLDLapoprotein B secretion. In the present study, although VLDLapoB and VLDLapoprotein B kinetics were not studied separately, we may think that the increased VLDL apoB PR observed in our abdominally obese women is likely to be mainly an increase of VLDLapoB PR. It has also been demonstrated that the reduction of VLDLapoB production by insulin is due to not only the insulin-induced reduction of plasma free fatty acids (reducing the substrates for VLDL synthesis) but also a direct inhibitory effect of insulin in the liver.36 This direct inhibitory effect of insulin has been demonstrated in several studies.37–41 Indeed, it has been shown that insulin attenuates the rate of apoB mRNA translation38 and inhibits apoB secretion.39 Moreover, insulin is known to acutely reduce the mRNA level and the protein mass of the microsomal TG transfer protein, which is involved in the assembly and the secretion of VLDL particles, and an enhanced expression of the microsomal TG transfer protein has been observed in insulin-resistant hamsters.41 Thus, the increased VLDL apoB PR observed in the insulin-resistant subjects could be explained by a hepatic resistance to the inhibitory effect of insulin on VLDL apoB production.

Interestingly, in our insulin-resistant abdominally obese women with normal fasting TG levels, a significant increase of VLDLapoB was observed with subjects in the fed state. This result is in agreement with data from the study by Guerci et al42 showing altered postprandial lipemia in abdominally obese insulin-resistant patients with normal fasting TGs. Thus, postprandial lipid abnormalities seem to occur early in insulin-resistant subjects, leading to increased levels of TG-rich lipoproteins that are likely to be atherogenic.

The apoB kinetic abnormalities observed in insulin-resistant women with abdominal obesity are not restricted to VLDL particles, as much as IDL apoB and LDL apoB PRs are significantly increased. The significant correlations found between the PRs of VLDL, IDL, and LDL indicate that kinetic abnormalities of apoB in VLDL, IDL, and LDL are tightly linked. Thus, we surmise that increased PR of IDL apoB is likely to be the consequence of increased PR of VLDL apoB and that increased PR of LDL apoB is likely to be the consequence of increased PR of IDL apoB. It seems that insulin resistance induces increased PR of VLDL apoB, leading to, as a consequence, increased PRs of IDL and LDL apoB.

In our insulin-resistant subjects, the number of VLDL, IDL, and LDL particles was increased, as assessed by the increased plasma concentrations of VLDL apoB, IDL apoB, and LDL apoB. Because the FCRs of VLDL, IDL, and LDL were not modified, the increased number of apoB-containing lipoproteins was entirely due to their augmented PRs. This increased number of VLDL, IDL, and LDL particles may be involved in the augmented cardiovascular risk observed in abdominally obese insulin-resistant subjects. Indeed, VLDL particles from hypertriglyceridemic subjects bind to macrophage receptors, promoting the lipid loading of macrophages and then their conversion into foam cells.43 IDL particles have been shown to have a high reactivity with human arterial wall proteoglycans.44 Thus, an excess of IDL particles is also likely to be harmful, as implied by some clinical data.45,46 The increased level of LDL particles, observed in the insulin-resistant women with abdominal obesity, may be suspected to play an important role in the development of atherosclerosis in these patients. Indeed, high levels of LDL particles increase the probability of penetration of LDLs in the arterial wall.
wall and thus promote the formation of the arteriosclerotic plaque.

An important result of the present study is the fact that these significant kinetic abnormalities of apoB-containing lipoproteins occur very early in the development of the metabolic syndrome of insulin resistance. Indeed, our abdominally obese insulin-resistant subjects, although they had normal fasting TG levels, presented significant abnormalities of apoB metabolism, such as an 84% increase of VLDL apoB PR, a 54% increase of IDL apoB PR, and a 63% increase of LDL apoB PR. These abnormalities occur at an early stage of insulin resistance, before the onset of fasting hypertriglyceridemia and glucose intolerance.

The present study is the first kinetic study of apoB in abdominally obese insulin-resistant patients with normal fasting TG levels. Our results indicate that in normoglycemic and fasting normotriglyceridemic insulin-resistant subjects with abdominal obesity, some abnormalities already exist in the VLDL→IDL→LDL cascade, with a significant increase of apoB PR in each apoB-containing lipoprotein (VLDL, IDL, and LDL). These abnormalities are responsible for the increased number of atherogenic apoB-containing particles (VLDL, IDL, and LDL) in the plasma and are likely to promote cardiovascular disease in abdominally obese insulin-resistant subjects.

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References


Early Kinetic Abnormalities of ApoB-Containing Lipoproteins in Insulin-Resistant Women With Abdominal Obesity
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