Hyperinsulinemia Is Associated With Increased Production Rate of Intestinal Apolipoprotein B-48–Containing Lipoproteins in Humans

Hélène Duez, Benoît Lamarche, Kristine D. Uffelman, René Valero, Jeffrey S. Cohn, Gary F. Lewis

Objectives—Whereas postprandial hyperlipidemia is a well-described feature of insulin-resistant states and type 2 diabetes, no previous studies have examined intestinal lipoprotein production rates (PRs) in relation to hyperinsulinemia or insulin resistance in humans.

Methods and Results—Apolipoprotein B-48 (apoB-48)–containing lipoprotein metabolism was examined in the steady-state fed condition with a 15-hour primed constant infusion of [D3]-L-leucine in 14 nondiabetic men with a broad range of body mass index (BMI) and insulin sensitivity. To examine the relationship between indices of insulin resistance and intestinal lipoprotein PR were analyzed in 2 ways: by correlation and by comparing apoB-48 PRs in those whose fasting plasma insulin concentrations were above or below the median for the 14 subjects studied (60 pmol/L). ApoB-48 PR was significantly higher in hyperinsulinemic, insulin-resistant subjects (1.73 ± 0.39 versus 0.88 ± 0.13 mg/kg per day; P < 0.05) and correlated with fasting plasma insulin concentrations (r = 0.558; P = 0.038), despite great heterogeneity in apoB-48 kinetic parameters, particularly among the obese subjects. There was no significant difference in clearance of apoB-48 between the 2 groups, nor was there a significant correlation between apoB-48 fractional clearance rate and fasting insulin or homeostasis model assessment-insulin resistance.

Conclusions—These are the first human data to conclusively demonstrate that intestinal apoB-48–containing triglyceride-rich lipoprotein PR is increased in hyperinsulinemic, insulin-resistant humans. Intestinal lipoprotein particle overproduction is a newly described feature of insulin resistance in humans. (Arterioscler Thromb Vasc Biol. 2006;26:1357-1363.)

Key Words: lipoprotein ■ intestinal ■ insulin resistance ■ hyperinsulinemia ■ stable isotope kinetic ■ triglyceride
utable to decreased lipoprotein lipase activity, (2) modified lipoprotein composition, (3) reduced remnant recognition by hepatic receptors, or (4) an expanded pool of VLDL leading to competition for removal between VLDL and chylomicrons.\textsuperscript{13-15} Whether exaggerated postprandial lipemia also involves intestinal overproduction of chylomicrons and remnants in addition to a delayed clearance has not been fully investigated, and little information is available regarding the factors that regulate apoB-48–containing lipoprotein production in insulin-resistant humans. We have recently shown that diet-induced insulin resistance in Syrian Golden hamsters is associated with a marked increase in intestinal lipoprotein production rate (PR) in both the fasting and the fed states,\textsuperscript{16-18} and insulin sensitization partially reversed apoB-48–containing lipoprotein oversecretion.\textsuperscript{17,18} However, to date, the relevance of these findings to humans is not known, and there is no evidence that insulin resistance is associated with increased intestinal apoB-48–containing lipoprotein particle overproduction in humans. Therefore, the aim of the present study was to determine whether intestinal TRL–apoB-48 production or clearance is perturbed in men with features of hyperinsulinemia and insulin resistance.

Materials and Methods

Subjects

Fourteen healthy, normoglycemic men, 31 to 60 years of age, with a broad range of body weights (from 64.3 to 134.3 kg), BMIs (from 20.0 to 41.6 kg/m\(^2\)), and waist girths (from 77 to 135 cm) participated in the study. Subjects were included if their total plasma cholesterol was ≤ 5.5 mmol/L, HDL cholesterol ≥ 0.8 mmol/L, LDL cholesterol ≤ 4.0 mmol/L, and TGs ≤ 4.0 mmol/L. All participants were non-smokers, and none had a previous history of cardiovascular disease or systemic illness. None had any surgical intervention within 6 months before the studies. No subject was taking medications, and all had a normal 75-g oral glucose tolerance test performed immediately before enrollment in the study. An index of insulin sensitivity was derived from fasting insulin and glucose concentrations using the homeostasis model assessment-insulin resistance (HOMA-IR) method, as described previously.\textsuperscript{19} The research ethics board of the University Health Network, University of Toronto, approved the study, and all subjects gave written informed consent before their participation.

Lipoprotein Kinetic Studies

After a 14-hour overnight fast, an intravenous catheter was inserted into a superficial vein in each forearm: 1 for infusion and 1 for sampling. After the withdrawal of a baseline sample, the subject was instructed to ingest 15 identical hourly volumes of a liquid food supplement called Boost (Mead Johnson Nutritional), each equivalent to one fifteenth of their total daily caloric needs, using the Harris Benedict equation to determine the total energy requirements (based on height, weight, age, and activity factors).\textsuperscript{20,21} Boost contains 20% of the total energy derived from fat, 25% was polyunsaturated fat, 65% monounsaturated fat, and 13% saturated fatty acid. Three hours later (at \(10\) AM), all subjects received a primed constant infusion (10 \(\mu\)mol/kg bolus followed by 10 \(\mu\)mol/kg per hour for 12 hours) of deuterium-labeled leucine\textsuperscript{22} ([\(L-5,5,5\)-\(D\)]-leucine; 98%; Cambridge Isotope Laboratories) to enrich apoB-48 in intestinally derived lipoprotein particles and to calculate the production and clearance rates of the particles as described previously (see the online supplemental methods, available at http://atvb.ahajournals.org).\textsuperscript{23} Blood samples were collected at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 5 hours, 7 hours, 9 hours, 10 hours, 11 hours, and 12 hours into sterile tubes containing 0.1% EDTA and 10 \(\mu\)L preservative (containing 17 \(\mu\)g/ml aprotinin and 0.2 mg sodium azide) and placed immediately on ice.

Sample Processing, Laboratory Measurements, Analysis of Lipoprotein Production and Clearance Rates, and Statistical Analysis

Please see the online data supplement, available at http://atvb.ahajournals.org.

Results

Demographic and Biochemical Characteristics of Subjects

As a method of analyzing the data to determine whether apoB-48 PRs are increased in those with features of insulin resistance, subjects were divided into 2 groups based on their fasting plasma insulin concentration. The second method of data analysis was to examine correlations between apoB-48 PR and indices of insulin resistance, as reported below. By design, subjects in the higher-insulin group (above the median of 60 pmol/L) had a mean (±SEM) insulin level of 128.9±8.9 pmol/L compared with the lower-insulin group, which had a mean insulin of 45.4±4.4 pmol/L (\(P=0.000001\)). Data in the tables are presented separately for these 2 arbitrarily segregated groups of subjects. Subjects with higher fasting plasma insulin concentrations had significantly elevated waist circumference and HOMA-IR compared with those with lower insulin levels (Table 1). BMI and body weight were elevated in the hyperinsulinemic group, but the difference was not significant. Fasting blood glucose and free fatty acid (FFA) levels were not significantly different.

Plasma and TRL Lipid and Apolipoprotein Concentrations

Plasma total cholesterol, HDL cholesterol, and LDL cholesterol were not significantly different between individuals with fasting insulin levels above or below the median (Table 2). Fasting TG levels tended to be higher (1.6-fold higher) (high-insulin group 1.59±0.26 versus low-insulin group 1.09±0.08 mmol/L; \(P=0.121\)), although the difference was not statistically significant. In the fasting state, TRL TGs (TRL-TGs) tended to be higher (1.6-fold) in the high-insulin group and were positively correlated with plasma insulin concentrations (\(r=0.692\); \(P=0.006\)) and HOMA-IR (\(r=0.697\); \(P=0.006\); Table 2). TRL apoB-48 concentrations tended to be higher in the higher versus the lower-insulin group, but the difference was not statistically significant (0.66±0.15 versus 0.39±0.05 mg/dL; \(P=0.15\); Table 2).

Plasma and TRL-TGs and TRL ApoB-48 Concentrations in Response to Feeding

TRL-TGs were higher in the higher-insulin group compared with the lower-insulin group throughout the kinetic study (Figure 1A; mean TRL-TGs 1.72±0.10 versus 1.12±0.08 mmol/L; \(P=0.0005\)). As expected, TRL-TGs during feeding were positively correlated with fasting plasma TGs (\(r=0.609\); \(P=0.021\)) and were significantly associated with fasting plasma insulin concentration (\(r=0.890\); \(P<0.001\)) and HOMA-IR (\(r=0.843\); \(P<0.001\)). As can be seen in Figure 1B, no statistically significant difference in steady-state–fed
Correlation of TRL–Apo-B48 PR With Plasma Insulin and HOMA-IR

TABLE 1. Demographic and Biochemical (Fasting Plasma Metabolites, Hormone, and Lipids) Characteristics of Subjects With Insulin Above and Below the Median

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Waist (cm)</th>
<th>FFA (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin &gt;60 pmol/L</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46</td>
<td>95.0</td>
<td>32.8</td>
<td>112</td>
<td>0.45</td>
<td>5.1</td>
<td>160.2</td>
<td>6.05</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>98.0</td>
<td>30.6</td>
<td>108</td>
<td>0.33</td>
<td>5.3</td>
<td>151.0</td>
<td>5.93</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>106.5</td>
<td>29.8</td>
<td>108</td>
<td>0.23</td>
<td>5.7</td>
<td>123.0</td>
<td>5.19</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>83.2</td>
<td>28.4</td>
<td>97</td>
<td>0.27</td>
<td>4.7</td>
<td>120.0</td>
<td>4.18</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>112.5</td>
<td>33.6</td>
<td>111</td>
<td>0.42</td>
<td>4.9</td>
<td>114.0</td>
<td>4.14</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>123.5</td>
<td>39.0</td>
<td>135</td>
<td>0.50</td>
<td>5.5</td>
<td>105.0</td>
<td>4.28</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>48.5±4.7</td>
<td>103.1±5.8</td>
<td>32.4±1.5</td>
<td>112±5</td>
<td>0.37±0.04</td>
<td>5.2±0.1</td>
<td>128.9±8.9</td>
<td>4.96±0.36</td>
</tr>
</tbody>
</table>

| Insulin <60 pmol/L | | | | | | | | |
| 1       | 50          | 66.0        | 25.1        | 86         | 0.38         | 5.1             | 60.0            | 2.27    |
| 2       | 39          | 134.3       | 41.6        | 127        | 0.37         | 4.0             | 59.0            | 1.75    |
| 3       | 50          | 76.3        | 25.5        | 92         | 0.34         | 5.6             | 51.0            | 2.12    |
| 4       | 51          | 67.0        | 21.8        | 84         | 0.17         | 4.5             | 46.2            | 1.54    |
| 5       | 42          | 76.5        | 25.5        | 86         | 0.49         | 4.7             | 43.2            | 1.50    |
| 6       | 41          | 64.3        | 20.0        | 77         | 0.39         | 5.1             | 14.4            | 1.56    |
| 7       | 58          | 82.5        | 24.7        | 92         | 0.40         | 4.8             | 14.4            | 1.47    |
| 8       | 50          | 79.0        | 24.3        | 83         | 0.54         | 4.5             | 21.0            | 0.7      |
| Mean±SEM | 47.6±2.3    | 80.7±8      | 26.1±2.3    | 91±5       | 0.38±0.04    | 4.8±0.2         | 45.4±4.4        | 1.61±0.17 |

P value 0.859 0.056 0.058 0.019 0.77 0.11 <0.00001 <0.00001

The 14 subjects were divided into 2 groups based on their fasting insulin plasma levels. P values are by unpaired t test.

Discussion

In the present study, apoB-48 PRs were found to be positively correlated with fasting insulin concentrations, despite great heterogeneity in apoB-48 kinetic parameters, particularly among the obese subjects. We also demonstrated a higher PR of intestinally derived apoB-48–containing lipoprotein particles in men with hyperinsulinemia and insulin resistance compared with those with lower insulin levels and greater insulin sensitivity. The clearance of apoB-48–containing lipoproteins in those with hyperinsulinemia and insulin resistance tended to be higher than in those with normoinsulinemia, although this difference was not significant, and there was no significant correlation between apoB-48 FCR and fasting insulin or HOMA-IR. Overproduction of intestinally derived apoB-48–containing lipoproteins appears to be a component of the dyslipidemia of hyperinsulinemic/insulin-resistant individuals.

An interesting finding of the present study was the lack of association between the clearance of apoB-48–containing TRL and insulin sensitivity indices. Other studies have
suggested defective catabolism of intestinally derived TRL and remnant lipoprotein TG in insulin-resistant and obese individuals.\textsuperscript{24–26} However, the design of those studies differed from ours in a number of respects, not least of which was that they examined TRL particle clearance in a non–steady-state condition after ingestion of an oral fat load. In that setting, the large influx of chylomicrons derived from the rapidly ingested fat load compete for clearance by lipoprotein lipase with endogenous VLDL, resulting in greater impairment in chylomicron clearance in those with even mild fasting hypertriglyceridemia, such as occurs with abdominal obesity and insulin resistance. In the present study, we assessed TRL apoB-48 production and clearance at steady-state plasma TRL–apoB-48 and TRL-TG concentrations induced by repeated, small-quantity liquid meals. Different findings between the studies may also be explained by the use of different methodological and modeling approaches. Most other studies have labeled the TG moiety of lipoprotein particles and examined TRL-TG clearance or modeled the area under the curve of plasma apoB-48 or plasma TG concentration after an oral fat load. TGs are contained in both intestinally derived as well as hepatically derived plasma lipoproteins, and there is no way of distinguishing the origin of TGs by this in vivo methodology. In the present study, we used a primed continuous infusion of deuterated leucine to label the apolipoprotein (apoB-48) moiety of the intestinally derived lipoprotein particles, allowing us to examine apoB-48 particle clearance rather than TRL-TG clearance. Others have used different approaches such as the measurement of \textsuperscript{13}CO\textsubscript{2} appearance in breath after ingestion of a labeled chylomicron-like emulsion, which requires not only uptake of TRL-TG and remnant TG by the liver but also, and different from our method, subsequent metabolism of hydrolyzed fatty acids.\textsuperscript{25,27} Using this method, Watts et al\textsuperscript{28} failed to find a significant correlation between hydrolyzed fatty acids.\textsuperscript{25,27} Using this method, Watts et al\textsuperscript{28} failed to find a significant correlation between.

### Table 2. Plasma Lipids and TRL TG and ApoB Concentrations in the Fasted State and Postprandial TRL ApoB-48 PR and FCR in Subjects With Insulin Above and Below the Median

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma Total Cholesterol (mmol/L)</th>
<th>Plasma HDL-c (mmol/L)</th>
<th>Plasma LDL-c (mmol/L)</th>
<th>Plasma TG (mmol/L)</th>
<th>TRL TG (mg/dL)</th>
<th>TRL ApoB (mg/dL)</th>
<th>TRL ApoB-48 PR (mg/kg per day)</th>
<th>TRL ApoB-48 FCR (pool per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin &gt;60 pmol/L</td>
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</tr>
<tr>
<td>1</td>
<td>4.23</td>
<td>1.09</td>
<td>1.94</td>
<td>2.69</td>
<td>1.84</td>
<td>16.7</td>
<td>0.92</td>
<td>1.30</td>
</tr>
<tr>
<td>2</td>
<td>5.42</td>
<td>0.97</td>
<td>3.26</td>
<td>1.96</td>
<td>1.49</td>
<td>16.0</td>
<td>0.61</td>
<td>2.05</td>
</tr>
<tr>
<td>3</td>
<td>4.09</td>
<td>0.95</td>
<td>3.05</td>
<td>1.31</td>
<td>0.70</td>
<td>7.0</td>
<td>0.33</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>4.31</td>
<td>0.97</td>
<td>2.92</td>
<td>0.88</td>
<td>0.44</td>
<td>8.2</td>
<td>0.47</td>
<td>2.75</td>
</tr>
<tr>
<td>5</td>
<td>4.76</td>
<td>0.89</td>
<td>3.25</td>
<td>1.21</td>
<td>0.74</td>
<td>12.2</td>
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<td>1.73</td>
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<td>4.45</td>
<td>1.06</td>
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<td>1.11</td>
<td>10.5</td>
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<td>1.50</td>
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<tr>
<td>Mean ± SEM</td>
<td>4.54 ± 0.20</td>
<td>0.99 ± 0.03</td>
<td>2.90 ± 0.20</td>
<td>1.59 ± 0.26</td>
<td>1.05 ± 0.22</td>
<td>11.8 ± 1.6</td>
<td>0.66 ± 0.15</td>
<td>1.73 ± 0.39</td>
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<tr>
<td>Insulin &lt;60 pmol/L</td>
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<tr>
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<td>0.78</td>
<td>14.1</td>
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<td>1.40</td>
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<td>4.79</td>
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<td>2.70</td>
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<td>0.81</td>
<td>16.1</td>
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<td>0.70</td>
<td>9.4</td>
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<td>12.1</td>
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<td>10.5</td>
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<td>7.7</td>
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<tr>
<td>Mean ± SEM</td>
<td>4.47 ± 0.23</td>
<td>1.12 ± 0.08</td>
<td>2.81 ± 0.22</td>
<td>1.09 ± 0.08</td>
<td>0.67 ± 0.04</td>
<td>11.5 ± 1.1</td>
<td>0.39 ± 0.05</td>
<td>0.88 ± 0.13</td>
</tr>
</tbody>
</table>

\textsuperscript{P} (High vs low insulin levels)\textsuperscript{r} 0.84 0.14 0.77 0.121 0.14 0.909 0.15 0.040 0.297

Correlation with insulin levels (P value)\textsuperscript{r} nd nd nd r = 0.709 (0.005) r = 0.692 (0.006) r = 0.296 (0.30) r = 0.468 (0.063) r = 0.558 (0.038) r = 0.295 (0.306)

Correlation with HOMA-IR (P value)\textsuperscript{r} nd nd nd r = 0.691 (0.006) r = 0.697 (0.006) r = 0.242 (0.40) r = 0.411 (0.144) r = 0.515 (0.059) r = 0.314 (0.273)

Subjects (n = 14) were divided into 2 groups based on fasting insulin plasma levels. P values are by unpaired t test.

HDL-c indicates HDL cholesterol; LDL-c, LDL cholesterol; nd, not determined.

Figure 1. TRL-TG and TRL apoB-48 concentrations over the time course of the kinetic study. TRL-TG (A) and TRL apoB-48 (B) were measured throughout the 15-hour lipoprotein turnover study in subjects with low (<60 pmol/L; \# n = 8) or high (>60 pmol/L; \#; n = 6) insulin levels. Time 0 hours was fasting, immediately before starting hourly ingestion of liquid formula as described in methods. Analysis of the kinetics of apoB-48 was performed between 3 and 15 hours (ie, over a 12-hour period). Values are mean ± SEM for each group. Asterisks indicate difference between high- and low-insulin groups for each time point: *P < 0.005 and **P < 0.001 as analyzed by ANOVA (the overall significance over the time being achieved for TRL-TG: P < 0.0001; and for TRL apoB-48: P = 0.0298).
study by no means negates the finding that chylomicron and chylomicron remnant clearance is delayed after ingestion of a high-fat meal in those with even mild degrees of fasting hypertriglyceridemia, such as occurs in individuals with insulin resistance and type 2 diabetes. The fact that we did not demonstrate a defective clearance of apoB-48–containing lipoproteins in the present study may relate to the slow and modest rate of fat delivery to the intestine with frequent small liquid formula ingestion, which perhaps was insufficient to impair the clearance from the circulation of intestinally derived lipoproteins in those with hyperinsulinemia/insulin resistance. To our knowledge, PR of apoB-48–containing TRL has not been assessed directly in insulin-resistance states in humans to date, and our results represent the first human data to demonstrate higher apoB-48–TRL PRs in hyperinsulinemic individuals. We suggest that overproduction of TRL–apoB-48 in hyperinsulinemia may be an important contributor to postprandial hyperlipidemia in these conditions and may be an important cause of the accumulation of remnant lipoproteins even in the fasted state. This does not necessarily imply that it is the lipids secreted from the intestine per se that contribute directly to the hyperlipidemia of insulin-resistant states. An equally plausible explanation is that the increased numbers of circulating intestinally derived lipoprotein particles that result from higher PRs compete with hepatically derived lipoproteins for clearance mechanisms that become saturated postprandially, thereby impairing their removal and resulting in accumulation of lipoprotein particles of both hepatic and intestinal origin. The relative contributions of overproduction and defective clearance of intestinally derived lipoproteins to postprandial lipemia in insulin-resistant states is currently not known.

The volume of the liquid formula administered to maintain a constant fed state during the lipoprotein turnover study was adjusted for height, weight, and activity factors. Previous apoB-48 turnover studies performed in the constant fed state have also calculated and adjusted calorie/fat intake during the study based on the usual daily intake requirement of the subjects or using the Harris Benedict equation as we did. This allows us to study intestinal lipoprotein turnover during a fed state that is simulated to match the daily caloric intake of each study participant, making it closer to that subject’s “normal” physiology. Consequently, the total calorie and fat content ingested by the more obese hyperinsulinemic subjects was greater than that ingested by those with normoinsulinemia. Although we cannot exclude the possibility that this difference may have contributed to the observed differences in apoB-48 PRs between hyperinsulinemic and normoinsulinemic individuals, the caloric intake values and fat content of ingested liquid supplements were not correlated with fed TRL–TG levels or apoB-48 PRs between hyperinsulinemic and normoinsulinemic individuals.

Figure 2. TRL apoB-48 production and FCRs in subjects with high- vs low-insulin levels or high vs low HOMA-IR. A) apoB-48 PR and FCR were determined at steady-state in subjects with low (<60 pmol/L) insulin levels and HOMA-IR (<2.3; n=8) or high (>60 pmol/L) insulin levels and HOMA-IR (>2.3; n=6).

\[\begin{align*}
\text{ApoB-48 Production Rate (mg/dL/hr)} & \quad \text{FCR (mg/dL/hr)} \\
\text{Low insulin/ HOMA-IR} & \quad 1.0 \quad 1.0 \\
\text{High insulin/ HOMA-IR} & \quad 2.5 \quad 2.5 \\
\end{align*}\]

\[\begin{align*}
\text{Low Insulin/ HOMA-IR} & \quad 0.04 \quad 0.029 \\
\text{High Insulin/ HOMA-IR} & \quad 0.024 \quad 0.935 \\
\end{align*}\]
We have shown that intestinal lipid synthesis and transfer to lipoprotein particles is increased in insulin-resistant hamsters, at least in part via increased microsomal transfer protein (MTP) mass and activity and enhanced lipoprotein assembly and secretion.\textsuperscript{16} MTP has also been shown to be increased in diabetic rats,\textsuperscript{29} New Zealand rabbit,\textsuperscript{41} and the desert gerbil \textit{Psammomys obesus}.\textsuperscript{42} In addition, humans carrying a common MTP gene polymorphism leading to increased MTP expression were found to have elevated accumulation of small apoB-48–containing lipoproteins in the postprandial state.\textsuperscript{43} Along the same line, Lundahl et al have shown that polymorphism in the MTP promoter leading to increased transcriptional activity of the gene was associated with increased apoB-48 in the small TRL fraction after a fat meal. These results indicate that MTP polymorphisms may be linked to the generation of small TRL from the intestine.\textsuperscript{44} Although we did not characterize the MTP promoter in our subjects, it is plausible that variation in the intestinal expression of MTP may influence postprandial TRL–apoB48 metabolism. Overproduction of hepatic VLDL in fructose-fed insulin-resistant hamsters has been associated with reduced hepatic insulin signaling as documented by increased protein–tyrosine phosphatase 1B levels, decreased phosphorylation of insulin receptors IRS-1 and IRS-2, as well as Akt and reduced phosphatidylinositol-3 kinase activity,\textsuperscript{45,46} and this may determine whether apoB is targeted for secretion or degradation. Impaired insulin signaling in primary cultured enterocytes is associated with enhanced apoB-48 stability, lipoprotein particle assembly, and secretion.\textsuperscript{47} Therefore, it appears that the mechanism leading to increased apoB-48–containing TRL in insulin resistance is complex and multifactorial.

It has long been assumed that intestinally derived lipoproteins mainly transport exogenous TG derived from food absorption. However, there is evidence to suggest that the intestine constitutively synthesizes smaller TRL particles\textsuperscript{18} and maintains a basal level of apoB-48, even in the fasting state.\textsuperscript{2} The current thinking is that chylomicron formation involves the formation of small, phospholipid-rich, TG-poor primordial particles in the membrane of the smooth endoplasmic reticulum, with subsequent core lipid expansion and particle transfer from the smooth endoplasmic reticulum to the golgi for secretion.\textsuperscript{49} We have shown previously that hamster enterocytes have the capacity to secrete small, lipid-poor (HDL size) apoB-48–containing particles, and that in hamsters made insulin-resistant with fructose or high-fat feeding, there is a marked increase in TRL apoB-48 secretion not only in the fed but also in the fasted state.\textsuperscript{16,18} We would have liked to examine TRL apoB-48 particle production in fasted humans, but in pilot studies, we were unable to accurately quantify apoB-48 PRs in fasted normolipidemic humans because of their extremely low plasma concentrations of apoB-48.

In the present study, individuals with higher insulin levels were overweight compared with the low-insulin group, and we cannot exclude that higher body weights may have contributed to the observed increase in TRL apoB-48 particle production or may have affected its clearance. However, ANOVA did not reveal any significant contribution of body weight, BMI, or waist girth on the effect of insulin on TRL apoB-48 production or FCRs. In conclusion, the present report provides evidence that increased production of apoB-48 is characteristic of hyperinsulinemic men. Further studies are needed to explore the mechanisms underlying the increased production of intestinal lipoproteins in insulin-resistant states and to better determine the contribution of intestinal lipoproteins to atherosclerosis in this population.

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\textbf{References}


Hyperinsulinemia Is Associated With Increased Production Rate of Intestinal Apolipoprotein B-48–Containing Lipoproteins in Humans
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Sample Processing

Plasma was separated from blood samples, within 2 hours, in a refrigerated centrifuge at 2000 rpm for 15 min at 4°C. Triglyceride-rich lipoproteins (TRL) were isolated at each time point at d=1.006 for 16 hrs, 39,000 rpm at 12°C. Plasma IDL, LDL, and HDL were isolated by sequential ultracentrifugation, dialyzed and frozen for lipid and apolipoprotein analyses. Protein in the TRL fraction was determined by the method of Lowry \(^1\) and approximately 1000μg of protein were delipidated using methanol/diethyl ether to separate TRL lipids from protein. Delipidated proteins were separated by preparative 3.3% SDS-PAGE, stained with Coomassie R-250, destained and gel bands containing apoB-48 were excised and placed into 1.5 ml glass vials (Chromatographic Specialties Inc, Brockville, Ontario, Canada) for further processing.

ApoB-48 gel slices were hydrolysed and amino acids were purified by cation exchange chromatography before being derivatized to allow for the determination of plasma leucine isotopic enrichment, as previously described \(^2-5\). Enrichment of samples with deuterium-labeled leucine was measured by gas chromatography/mass spectrometry (Agilent 5973 GC/MS, Agilent Technologies Canada Inc, Mississauga, Ontario, Canada) using negative chemical ionization and methane as the moderator gas as previously described \(^2\). Selective ion monitoring at m/z =352 and 349 (ionic species corresponding to derivatized deuterium-labeled and derivatized non-deuterium-labeled leucine, respectively) was performed, and tracer-to-tracee ratios calculated from isotopic ratios for each sample according to the formula derived by Cobelli et al. (a representative curve is presented in Figure 2 of this manuscript) \(^6\). Tracer-to-tracee ratios were corrected for
background leucine in gel slices (i.e., trace amounts of leucine introduced during the amino acid purification and derivitization procedures) by estimating the amount of leucine in processed blank gel slices relative to the norleucine internal standard.

**Laboratory Measurements**

Triglycerides were measured using an enzymatic colorimetric kit (Roche Diagnostics, Mannheim, Germany) in which free glycerol was eliminated in a preliminary reaction, the TG hydrolysed and the liberated glycerol measured. Cholesterol was determined using the CHOD-PAP enzymatic colorimetric kit (Roche Diagnostics, Mannheim, Germany). FFAs were determined with the NEFA colorimetric method (Wako Industrials, Osaka, Japan). Plasma insulin concentrations were assayed by radioimmunassay using a human specific insulin kit (Linco Research, St Louis, MO, USA). Glucose was measured enzymatically using a Beckman Glucose Analyzer II (Beckman Instruments Corporation, Fullerton, CA). Total apoB in plasma and TRL was measured by electroimmunoassay as previously described. ApoB-48 mass in the TRL fraction was quantified using analytical SDS-PAGE as previously described. Briefly 100 μg TRL were delipidated and the precipitate dissolved overnight in sample buffer (0.05 M Tris-HCl, pH 6.8, 2% SDS, 2% 2-mercaptoethanol). An LDL standard (d=1.030 – 1.040) was also delipidated and treated in the same fashion as the TRL samples. Samples and standard dilutions were then run on 4-20% SDS-PAGE, stained and destained. Stained apoB-48 bands were scanned with a UMAX Image Scanner (Amersham Biosciences, Uppsala, Sweden) and the density of TRL bands were compared to known concentrations of LDL standard to give the mass of the apoB-100
and apoB-48 in TRL fractions. The coefficient of variation for the apoB mass assay was 7%.

**Analysis of lipoprotein production and clearance rates**

Stable isotope enrichment curves for apoB-48 were fitted to a three compartment model using SAAM II computer software (SAAM II institute, WA). Compartment 1 represented the plasma amino acids. Compartment 2 was an intracellular delay compartment, which accounts for the synthesis, assembly and secretion of apolipoproteins and compartment 3 represented circulating plasma lipoproteins. In those subjects where the TRL apoB-48 tracer data attained a plateau, we used this value as a measure of the precursor pool enrichment. In individuals among whom apoB-48 enrichment did not reach plateau (n=1), we let the model define the maximal enrichment rate. Each subject was in steady state with respect to apoB-48 concentrations so FCR (fractional clearance rate) was equivalent to fractional synthetic rate. Kinetic parameters were derived by analyzing individual enrichment curves and only those in which the cv for apoB-48 modeling was <25% were included.

Production rates were calculated using the FCR of TRL apoB-48 multiplied by pool size measured over the 12 hours of the study per kg body weight where pool size = average plasma concentration (mg/dl) between t3hr and t12h of the kinetic x plasma volume (0.045 liter/kg).

**Statistics**

Results are presented as mean ± SEM. Unpaired t-tests were used to compare patients with low vs high insulin levels or HOMA-IR score. A penalty factor was applied when test for homogeneity of variance gave a p-value greater than 0.05, so that the provided p-
values were from t-test not assuming equal variance (analyzed with the SPSS version13 software). Pearson correlation coefficients were determined to test the association between insulin levels and parameters of interest. Univariate analysis of variance (ANCOVA) were used to test the contribution of different parameters on the effect of insulin levels on TRL apoB-48 production and clearance rates. ANOVA was used to analyse TRL-TG and TRL-apoB-48 increase over the time. All analyses were performed with the SPSS version13. For all of the analyses, a p value < 0.05 was considered significant.

References


