The Plasma and Lipoprotein Triglyceride Postprandial Response to a Carbohydrate Tolerance Test Differs in Lean and Massively Obese Normolipidemic Women

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ABSTRACT The goal of the present study was to compare the plasma lipid responses of massively obese and lean women to a fat load and a carbohydrate load. For this purpose, 11 lean [body mass index (BMI), 21.6 ± 2 kg/m²] and 8 obese (BMI, 50.8 ± 7 kg/m²) normolipidemic women were given, in random order, either a dietary carbohydrate load (3.43 MJ, 166 g carbohydrates, 38 g proteins) or a dietary fat load (3.35 MJ, 70 g fat, 36 g proteins). Blood samples were collected hourly for 9 h after the test meal for measurements of triglyceride-rich lipoprotein (TRL)-lipid, apolipoprotein (apo)B-48 and apoB-100. Triglycerides (P < 0.0001), TRL triglycerides (P < 0.0001), TRL cholesterol (P < 0.04) and apoB-48 (P < 0.0001) peaked 3 h after the fat meal and returned progressively to baseline values in both obese women and lean controls. These lipid and apolipoprotein changes did not differ between the two groups. In contrast, after the carbohydrate load, the plasma triglyceride (P < 0.0001) and TRL triglyceride (P < 0.0001) increments were significantly greater in obese women than in lean controls. This carbohydrate-induced TRL triglyceride increment was half of that following the isocaloric fat load. The carbohydrate load did not affect apoB-100 and apoB-48 levels. These findings suggest that postprandial triglyceride metabolism is impaired after a carbohydrate load in normolipidemic massively obese women.


KEY WORDS: • triglycerides • lipoprotein • apolipoprotein • obesity • carbohydrate • lipid • humans

Several studies have suggested that low fat/high carbohydrate diets increase plasma triglyceride levels (1–3). The importance of triglyceride changes has been ascribed to the amount of dietary carbohydrate, the type of sugar (i.e., monosaccharide or disaccharide as opposed to oligo- or polysaccharides) and the underlying metabolic disorders (i.e., dyslipidemia, insulin resistance or obesity). In addition, the effects of dietary carbohydrates on plasma triglycerides vary with time (3–10).

The mechanisms of carbohydrate-induced plasma triglyceride changes have been investigated in lean and obese subjects (11–13). Sidossis et al. (11) showed that glucose infusion in healthy volunteers inhibited splanchnic fatty acid oxidation, thereby increasing fatty acid availability for triglyceride secretion. In their study, plasma VLDL-triglyceride elevation was associated with increased VLDL-triglyceride secretion. Marques-Lopes et al. (12) reported that a single low fat, high carbohydrate meal stimulated postprandial hepatic fat synthesis and lowered fat oxidation to a greater extent in overweight subjects than in lean controls. However, in long-term dietary experiments, fatty acid synthesis did not differ between lean and obese subjects, suggesting some kind of adaptation (13).

Disorders of postprandial lipid metabolism have been demonstrated in obese subjects (14–19). For example, Lewis et al. (19) found a larger 24-h postprandial triglyceride response in obese subjects than in lean controls. Moreover, Couillard et al. (16) showed that visceral adipose tissue accumulation was associated with an impaired postprandial triglyceride-rich lipoprotein (TRL)3 clearance. However, in these studies, substantially higher basal triglyceride levels in obese subjects confounded the interpretation of the results. Although low fat, high carbohydrate diets are recommended to reduce body weight, little is known about the effect of such diets on postprandial lipoproteins in patients with massive or moderate obesity. Therefore, the goal of the present study was to compare the plasma lipid responses of massively obese and lean women to a fat load and a carbohydrate load.

SUBJECTS AND METHODS

Subjects. Eight obese women (aged 35 ± 8 years) were recruited from the Clinic of Lille University Hospital through personal contacts. The

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inclusion criteria were body mass index (BMI) ≥ 30 kg/m² and stable weight (Δ weight < 2 kg in the last 3 mo). Eleven healthy women (aged 22 ± 2 y) were recruited for the study from the University of Lille II through advertisements and posters. The inclusion criteria were BMI < 25 kg/m² and stable weight (Δ weight < 2 kg in the last 3 mo). Both obese women and control subjects had a fasting blood sample for screening of diabetes, hyperlipidemia and impaired thyroid, hepatic or renal function. Exclusion criteria were glucose > 5.5 mmol/L, triglycerides > 2.8 mmol/L, LDL cholesterol > 4.1 mmol/L, high blood pressure (systolic > 140 mm Hg and/or diastolic > 90 mm Hg), smoking, drug consumption or chronic use of medications.

Each subject was informed about the nature and purpose of the investigation and was enrolled in this study after giving an informed consent. The hospital ethics committee (CCPRB de Lille, CHR et U de Lille) approved the protocol according to the current regulations in France. During the 24 h preceding each experimental session, the subjects were reminded to refrain from exercise and alcohol consumption.

To avoid possible effects of menstrual cycle on lipid and lipoprotein variables, all women (lean and obese) had to be on monophasic oral contraception for at least 4 wk. Each test-meal experiment was conducted when women had been on active contraception for at least 2 d.

Experimental design. The study was performed in the Clinical Investigation Center of Lille University Hospital. The experiment used a repeated-measure design in which each subject served as her own control. There were two subsets of dietary experiments named: “carbohydrate load” or “fat load.” The order of presentation was balanced. There was an interval of at least 5 d between sessions. The two test loads were isocaloric and were composed of 1) fat-free cottage cheese (500 g) strawberry jam (90 g) and dextrin-maltose mixture (90 g) for the “carbohydrate load,” or 2) fat cream (200 g; 94.5% saturated, 5.5% polyunsaturated) and a protein mixture of cow’s milk origin (35 g of Protifar Plus; Nutricia, Rueil Malmaison, France) for the “fat load” (Table 1). These loads were designed to compare the effect of fat and carbohydrate on postprandial triglyceridaemia. Because the amount of fat in the meal influences the level of postprandial lipemia, it was necessary to provide these loads were isocaloric and were composed of a similar amount of fat (or carbohydrate) to lean and obese women to allow comparisons between groups.

On the day preceding each test, the subjects were asked to finish their last meal before 2000 h. On the test day, the subjects arrived at the research center at 0730 h. They were weighed after voiding their bladder. An indwelling venous cannula was inserted into an antecubital vein before the test load. At 0800 h, a baseline blood sample was taken and the participants were given the test load and instructed to consume it completely within a maximum time of 20 min. Then, blood samples were drawn hourly for 9 h.

Biochemical measurements. Blood was collected into EDTA tubes. Plasma was separated by centrifugation at 3300 × g for 20 min at 4°C. Lipoproteins were separated under standard procedure by a combination of ultracentrifugation (at d = 1006 kg/L) and Mg²⁺ “phosphotungstate precipitation. HDL cholesterol was measured after precipitation with Mg²⁺–phosphotungstate using commercially available reagents (Cholesterol HDL, CHOD/PAP, Boehringer Mannheim, Mannheim, Germany). TRL were separated by ultracentrifugation, using a Beckman TL100 (Beckman France, Gagny, France), from 0.5 mL of plasma by a single spin at density 1006 kg/L with modifications (20,21). Briefly, 0.5 mL of 9 g/L NaCl was added to 0.5 mL of plasma and spun in a polycarbonate tube (400,000 × g, 20°C) in a Beckman 100.2 Ti rotor for 3 h. The tube was cut in two and the remaining 0.5 mL infranatant was analyzed for lipids. The TRL (d < 1006 kg/L) fraction was measured by subtracting infranatant values from total plasma concentrations. Thus, during the postprandial phase the so-called TRL fraction contains both VLDL and chylomicrons. Cholesterol and triglycerides were measured using Boehringer Mannheim reagents (ref. 1.040.1839 and 1.058.550, respectively). The intra- and interseries CV were 1 and 1.3% for cholesterol, and 1.1 and 1.8% for triglycerides. Glucose was determined enzymatically (Glucose, hexokinase Method, Randox Laboratories, Crumlin, UK). C-peptide levels were determined by RIA (RIA-coat C-Peptid, ByK-Sangtec Diagnostica, Dietzenbach, Germany).

Apolipoproteins (apo)B-48 and B-100 measurements. ApoB-48 and B-100 were assayed by ELISA methods. ApoB-100 was measured using a noncompetitive sandwich ELISA. Briefly, 96-well microtiter plates (Nunc Maxisorp, Polylabo, France) were coated with 2G8 monoclonal anti-apoB-100 antibody (Mona, Moscow, Russia) that does not crossreact with apoB-48 (22). Plasma samples were added to the wells and the apoB-100 quantified after addition of an anti-apoB-100 polyclonal antibody labeled with peroxidase (Calibiochem, Meudon, France) by colorimetry. ApoB-100 containing LDL were used as the standard. ApoB-48 were assayed using a competitive ELISA assay with a specific anti-apoB-48 antibody. This polyclonal antibody was obtained from rabbit immunized with a C-terminal specific heptapeptide (23). A 96-well microtiter plate (Nunc Maxisorp) was coated with the anti-apoB-48 antibody. Plasma samples were incubated with an appropriate detergent to allow competition between immobilized antigen and plasma apoB-48. ApoB-48 containing chylomicrons were used as the standard. Peroxidase-labeled anti-rabbit immunoglobulins and their substrate allowed color development. The absorbency was read at 450 nm. The intra-assay CV were 5.4 and 7.9%, respectively. The interassay CV were 5.5 and 8.3%, respectively (n = 12).

Statistical analysis. Student's t-test and paired Student's t-test were used to compare means of independent and dependent variables, respectively. The effects of fat and carbohydrate were analyzed separately. The lipid response was measured as an increment from baseline and was considered significant when

### TABLE 1

<table>
<thead>
<tr>
<th>Meal</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy, kJ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3373</td>
<td>3448</td>
</tr>
<tr>
<td>Fat</td>
<td>2671</td>
<td>38</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>100</td>
<td>2775</td>
</tr>
<tr>
<td>Protein</td>
<td>602</td>
<td>635</td>
</tr>
<tr>
<td><strong>Fat, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>94.5</td>
<td>—</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>5.5</td>
<td>—</td>
</tr>
<tr>
<td><strong>Carbohydrate, g</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>—</td>
<td>22</td>
</tr>
<tr>
<td>Fructose</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>Saccharose</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>Maltose</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.9</td>
<td>14</td>
</tr>
<tr>
<td>Pectite</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Oligosaccharide</td>
<td>—</td>
<td>78</td>
</tr>
</tbody>
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kg/m²). Body weight (P < 0.0001) and age (P < 0.001) were greater in obese women than in lean subjects (Table 2).

**Glucose and C-peptide.** After the fat load, glucose (P < 0.05) and C-peptide (P < 0.006) levels were higher in obese subjects than in lean controls (Fig. 1). After the carbohydrate load, glucose and C-peptide levels rose between 1 and 2 h after and then returned progressively to baseline values (Fig. 1). This effect was more pronounced in obese women than in lean controls for glucose and C-peptide (P < 0.0001).

**Lipid and lipoprotein variables.** Baseline triglycerides (P < 0.0013) and TRL triglycerides (P < 0.0011) were higher and HDL-cholesterol (P < 0.0001) was lower in obese women than in lean controls (Table 3). The other lipid variables did not differ between groups.

In lean women, the TRL triglyceride peak was higher after the fat load than after the carbohydrate load (0.55 ± 0.32 vs. 0.13 ± 0.13 mmol/L; P < 0.001) and the time of the peak did not differ between the fat and carbohydrate loads (2.82 ± 2.92 vs. 3.18 ± 0.98 h). In obese women, the TRL triglyceride peak also was higher after the fat load than after the carbohydrate load (0.69 ± 0.40 vs. 0.33 ± 0.14 mmol/L; P < 0.04) and the times of peaks did not differ (4.0 ± 1.0 vs. 4.4 ± 1.7 h).

Because fasting triglyceride concentration is a major determinant of postprandial lipid and lipoprotein levels (24), the remaining statistical analyses were performed on lipid and lipoprotein responses assessed as the increment (Δ) above the baseline fasting value. Δ-Triacylglycerides (P < 0.0001), Δ-TRL triglycerides (P < 0.0001) and Δ-TRL cholesterol (P < 0.04) peaked 3 h after the fat meal and returned progressively to baseline values in both obese women and lean controls (Figs. 2 and 3). Two-way ANOVA did not reveal any significant interactions between “weight” and “time” nor any effects of “weight,” suggesting similar lipid changes in both groups of women. Dietary fat did not affect on postprandial Δ-LDL-cholesterol levels (Fig. 3).

After the carbohydrate load, Δ-triglycerides and Δ-TRL triglycerides increased between 2 and 5 h postprandially and then decreased progressively in obese women but not in lean controls (Fig. 2). This effect was consistent among obese women because they all showed a positive variation of Δ-TRL triglycerides (range: 0.18 to 1.24 mmol/L; median: 0.95 mmol/L) in lean women, the carbohydrate load did not affect Δ-triglyceride or Δ-TRL triglyceride responses. Two-way ANOVA revealed a significant interaction between “weight” and “time” for Δ-triglycerides (P < 0.005) and Δ-TRL triglycerides (P < 0.002), indicating that lean and obese women had different postprandial responses to the carbohydrate load. The Δ-TRL triglyceride peak (P < 0.0001) and the Δ-TRL triglyceride area under the curve (P < 0.02) were significantly greater in obese women than in lean controls (data not shown). In contrast, there was no evidence of “weight” or “weight” and “time” interactions on Δ-LDL-cholesterol and Δ-TRL cholesterol after the carbohydrate load (Fig. 3). There was a significant effect of “time” on Δ-LDL-cholesterol.

**Apolipoprotein B-48 and B-100.** To assess the contribution of liver and intestine lipoproteins to plasma TRL triglyceride changes, the levels of apoB-100 and apoB-48 were measured (Fig. 4). As expected, Δ-apoB-48 rose after the fat load in both lean and obese women (P < 0.0001). Two-way ANOVA did not show an effect of “weight” or an interaction between “weight” and “time,” suggesting a similar response in

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**TABLE 3**

Baseline plasma and lipoprotein lipid concentrations of lean and obese women at baseline

<table>
<thead>
<tr>
<th>Before fat load</th>
<th>Before carbohydrate load</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.31 ± 0.27</td>
<td>1.69 ± 0.61</td>
</tr>
<tr>
<td>TRL triglycerides</td>
<td>0.82 ± 0.17</td>
<td>1.14 ± 0.50</td>
</tr>
<tr>
<td>TRL cholesterol</td>
<td>0.95 ± 0.51</td>
<td>1.00 ± 0.26</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.62 ± 1.26</td>
<td>4.98 ± 0.95</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.84 ± 1.1</td>
<td>2.89 ± 0.80</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.80 ± 0.44</td>
<td>1.00 ± 0.26</td>
</tr>
</tbody>
</table>

1 Values are means ± sd.

2 ANOVA with repeated measures was used to test for differences among baseline values; NS, P > 0.05.

3 M is for main effect of “Meal”: fat vs. carbohydrate; W is for main effect of “Weight”: lean vs. obese; M × W is for interaction between “Meal” and “Weight.”
obese and lean controls. There was a significant main effect of “time” on \( \Delta\text{-apoB-48} \) \( (P < 0.04) \) after the carbohydrate load without evidence of a main effect of “weight” or an interaction between “weight” and “time.” This suggests a similar effect of carbohydrate on \( \Delta\text{-apoB-48} \) in both groups of subjects. The fat and carbohydrate loads did not affect \( \Delta\text{-apoB-100} \) levels in either lean or obese women.

**FIGURE 2** Mean plasma triglyceride and triglyceride-rich lipoprotein (TRL) concentration increments after a fat load or a carbohydrate load in lean \( (n = 11) \) and obese \( (n = 8) \) women. For the sake of clarity, the errors bars were omitted. \( P \)-values are for two-way ANOVA with repeated measures. \( ^{1}T \times W: \) interaction term between “time” (repeated factor 10 levels: 0–9 h) and “weight status” (2 levels: lean/obese); \( ^{2}T: \) main effect “time”; \( ^{3}W: \) main effect of “weight status.” Post-hoc analysis was performed when the interaction was significant: \( P < 0.05; ^{**}P < 0.001; ^{***}P < 0.0001. \)

**DISCUSSION**

The aim of the present study was to compare the plasma lipid responses of massively obese and lean women to fat and carbohydrate loads. Postprandial triglyceride and TRL triglyceride levels rose after the carbohydrate load to a greater extent in obese women than in lean controls. The magnitude of this response was half of that following the isocaloric fat load. In contrast, there were no differences in triglyceride, TRL triglyceride or apoB-48 increments between obese women and lean controls in response to the fat load. These findings suggest that postprandial TRL triglyceride metabolism is impaired after a carbohydrate load in normolipidemic massively obese women.

After the carbohydrate meal, the TRL triglyceride increment was greater in obese women than lean controls. The reasons for this different response are unknown. However, we suggest that the impairment of insulin sensitivity in obese women, indicated by the greater C-peptide response, contributes to this effect. This hypothesis is supported by in vitro and in vivo experiments showing that insulin suppresses VLDL-triglyceride secretion, stimulates adipose tissue lipoprotein lipase activity and inhibits fatty acid release by adipose tissue (25–27). An increase in VLDL-triglyceride secretion is usually observed, whereas adipose tissue lipoprotein lipase activity is not stimulated and hormone sensitive lipase is not inhibited by insulin (27,29–32). The consequence is an increment in fatty acid availability for triglyceride synthesis and a decrease in VLDL-triglyceride clearance, both of which could promote postprandial hypertriglyceridaemia. In the present study, the carbohydrate load did not affect \( \Delta\text{-apoB-48} \) and \( \Delta\text{-apoB-100} \) in obese women, suggesting that the carbohydrate load has no major effect on plasma apoB-48 and apoB-100 levels. However, these findings do not exclude the possibility that opposite changes in TRL apo-B and LDL-apo-B occurred after the carbohydrate meal.

The carbohydrate load did not elevate postprandial triglyc-
eridemia in the lean controls. This seems to contradict some studies that showed a rise in postprandial TRL concentrations after carbohydrate meals (4–7). In these studies, however, TRL rose as the result of consumption of fructose or sucrose (5,6), whereas glucose and complex sugars had no effect on postprandial TRL (6,7). In the present experiment, oligosaccharides were the major sources of carbohydrates, which might explain the lack of a TRL triglyceride–raising effect in lean controls.

After the fat load, absolute levels of postprandial TRL triglycerides were significantly higher in obese women than in lean controls (data not shown). This finding agrees with those of other studies that showed elevated postprandial TRL triglyceride levels in obese subjects (14–19). In those studies, however, fasting triglycerides were higher in obese individuals than in lean controls, thereby contributing to higher postprandial triglyceridemia (24). In contrast, Mekki et al. (15) showed that the postprandial chylomicron increment did not differ between normotriglyceridemic obese women and lean controls. In the present study, postprandial triglyceride increment was similar in obese and lean women, suggesting that these massively obese women had an appropriate capacity to handle the fat load. In fact, although obese subjects had higher baseline triglycerides and lower HDL-cholesterol than lean controls, they were selected for normal lipid levels. Because chylomicrons and VLDL share the same catabolic pathway (33), this compensation very likely applies to postprandial TRL triglyceride metabolism.

The present study has several limitations. First, it was performed in massively obese women who are not representative of the majority of overweight patients. Moreover, due to massive obesity it was not possible to assess the effect of distribution, which is an important determinant of postprandial triglyceridemia (16). Second, the obese women were older than the lean controls, which may have affected lipid and lipoprotein metabolism. However, this difference was relatively small and both lean and obese women were still young. Moreover, the lack of significant differences in plasma lipid responses between lean and obese women after the fat load argues against a systematic bias due to age. Third, women were selected for normal plasma lipid levels. Therefore, the conclusions apply to this particular group of normolipidemic obese women. Finally, it can be argued that on the basis of individual energy needs, obese women received a relatively lower energy load than lean subjects. However, because the fat content of a meal influences postprandial lipid levels (24), it was mandatory to provide an identical amount of dietary fat (and carbohydrate) to lean and obese women to be able to compare their postprandial responses. Had the fat and carbohydrate meals been adjusted to body weight, greater postprandial lipid and carbohydrate responses would have been demonstrated in obese women. Therefore, the finding of a larger response to carbohydrate, despite a relatively lower energy intake, further highlights the carbohydrate-induced alteration of lipid metabolism in obese women.

In conclusion, we compared separately the plasma lipid responses to dietary carbohydrate and fat between massively obese normolipidemic women and lean controls. Postprandial triglycerides and TRL triglycerides rose after the carbohydrate load in obese women but not in lean controls, suggesting that obese women are more sensitive to carbohydrate-induced postprandial triglyceridemia than their lean counterparts. Because postprandial triglyceridemia is associated with coronary artery disease (34) the effect of carbohydrate-induced lipemia on cardiovascular risk warrants further investigation.

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LITERATURE CITED


