Resistance of adipose tissue lipoprotein lipase to insulin action in rats fed an obesity-promoting diet

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ELEVATED POSTPRANDIAL TRIGLYCERIDEMIA is considered to be a condition of high atherogenic potential (20, 38). It has been suggested that the modulation of plasma triglyceride (TG) levels after a meal may be more closely related to the rate of TG clearance than to that of TG secretion (9, 10, 28, 30). Lipoprotein lipase (LPL, EC 3.1.1.34) bound to the vascular endothelium of capillaries is the key enzyme responsible for the hydrolysis of circulating TG (36). Therefore, postprandial LPL activity is likely to be an important factor in the modulation of plasma TG levels after a meal (8).

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The postprandial modulation of LPL activity is tissue specific (36). Meal intake increases LPL activity in adipose tissue by as yet incompletely defined post-translational mechanisms (14), whereas it decreases enzyme activity in muscle (26). We (26) have recently shown that the rise in insulin was a necessary and sufficient determinant of the meal-induced changes in LPL in both adipose and muscle tissues.

Resistance of glucose metabolism to the action of insulin, a condition termed insulin resistance, is associated with abnormal rates of TG clearance in the postprandial state (10, 28). Muscle LPL activity appears to respond normally to food intake or insulin in obese and/or insulin-resistant rats (5, 15, 26, 29), suggesting that in this tissue, LPL does not become resistant to the action of insulin. On the other hand, impaired stimulation of adipose tissue LPL activity has been reported in insulin-resistant obese humans compared with lean subjects 4 h after ingestion of a high-carbohydrate meal (23) or 6 h after a euglycemic-hyperinsulinemic clamp (13). However, another study showed that adipose LPL activity was increased to the same extent in lean and obese insulin-resistant Zucker rats after an insulinogenic meal (2). Because of these considerations, whether adipose LPL modulation becomes resistant to the action of insulin during the postprandial state remains unclear. This issue could be critical to explain the higher circulating TG levels (11) and impaired TG clearance seen in insulin-resistant individuals during feeding (10, 28), particularly in the face of a persistent decrease in muscle LPL activity.

The present study was aimed at assessing the response of adipose tissue LPL to refeeding in 24-h fasted control insulin-sensitive rats and in animals rendered overweight and insulin resistant by chronic (4 wk) ingestion of a high-sucrose, high-fat (HSHF) diet. The diet has been shown by us (15) and others (16) to induce overt insulin resistance in rats. Although a 24-h fast is obviously not representative of habitual ingestive behavior, the length of fasting was chosen for its ability to reduce both insulinemia and adipose LPL activity of obese rats to that of control insulin-sensitive
animals (27), since insulin levels remain higher in insulin-resistant than in normal rats after a shorter (overnight) fast (4, 15). This was deemed important since persistence of hyperinsulinemia in the fasted state may affect basal LPL activity and its ability to respond to a further postprandial increase in insulin. Finally, pelleted chow was given to both groups of rats during refeeding to allow comparison of the LPL response to a meal similar in size and nutrient composition.

MATERIALS AND METHODS

Animals and treatments. One hundred twenty male Sprague-Dawley rats initially weighing 175–200 g were purchased from Charles River Laboratories (St. Constant, Canada) and housed individually in stainless steel cages in a room kept at 23 ± 1°C with a 12:12-h light-dark cycle (lights on at 2000). The animals were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, and the protocols were approved by our institutional animal care committee. Rats had free access to tap water and a stock diet (Charles River Rodent Diet no. 5075; Ralston Products, Woodstock, ON, Canada). Two days after their arrival, one-half of the rats was given the ground chow diet (chow), whereas the other one-half was fed a purified diet consisting of 41% energy as carbohydrate, 39% as fat, and 20% as protein. The composition of the diet was the following (in g/100 g diet): 45.0 sucrose, 10.0 corn oil, 10.0 lard, 22.5 casein, 0.3 DL-methionine, 1.2 vitamin mix (Teklad no. 40060; Teklad Test Diets, Madison, WI), 5.5 mineral mix (AIN-76; ICN Biochemicals, Montreal, QC, Canada), and 5.5 fiber (Alphacel; ICN Biochemicals). This HSHF diet has been shown to rapidly induce insulin resistance in peripheral tissues, including adipose tissues and muscles (15, 16). A subgroup of 36 rats was used after 2 wk of feeding to perform euglycemic-hyperinsulinemic clamp experiments (see below). Two weeks later (i.e., after a total period of 4 wk), the remaining 54 rats were killed 1 h after the beginning of the lighted period, either after a 24-h fast or after 1, 3, or 6 h of refeeding after 24 h of food deprivation. This period of fasting was chosen because of its ability to decrease both insulinemia and adipose LPL of insulin-resistant rats to levels observed in insulin-sensitive animals (4, 15, 27). During the refeeding period, both chow- and HSHF-fed rats were given pelleted rodent chow, since a preliminary study indicated that this procedure temporarily limited food intake of chow-fed rats to that of HSHF-fed rats, whose food intake was otherwise reduced because of the change in diet. This allowed comparison of changes in LPL activity independently of meal size and composition. Refeeding was not pursued beyond 6 h because of differences in food intake between dietary cohorts. The protocol also allowed comparison of groups at the same time of the circadian glucocorticoid rhythm. Rats were anesthetized with an intraperitoneal injection of 0.4 ml/kg of a ketamine (20 mg/ml)-xylazine (2.5 mg/ml) solution, and blood and tissues were harvested immediately thereafter (see below).

Euglycemic-hyperinsulinemic clamp. Thirty-six rats were cannulated in the right jugular vein and left carotid artery under isoflurane anesthesia. Two days after surgery, food was removed at 2200. The next day, after a 12-h fast, nine rats from each of the two dietary cohorts were infused for 2 h with the carrier solution [0.1% albumin (fraction V, fatty acid free); Sigma, St. Louis, MO] dissolved in 0.9% NaCl, whereas the remaining nine animals from both dietary cohorts were subjected to a 2-h euglycemic-hyperinsulinemic clamp. The clamp was performed essentially as described by Kraegen et al. (18). Rats were allowed to rest for 20 min after having been fitted for the infusions. Insulin (150 mU/ml) dissolved in the carrier solution was infused at a rate of 4.1 mU/kg body wt -1 min -1 in the jugular vein. Every 5 min, 20 μl of blood were taken from the carotid cannula to measure glycemia. The cannula was then flushed of blood with 3% sodium citrate dissolved in 0.9% NaCl. A 25% glucose solution dissolved in 0.9% NaCl was infused in the jugular vein from the beginning of the clamp and was adjusted thereafter to maintain glycemia near the fasting level. Sham infusions were performed similarly, except that no insulin was present in the NaCl-albumin solution and no glucose was infused. Blood was also collected before and after the clamp and was kept on ice until centrifuged (1,500 g, 4°C, 15 min). The separated plasma was stored at −70°C until later insulin measurement.

Rate of appearance of TG in plasma. To determine the rate at which exogenous and endogenous TG appear in the circulation, an additional protocol was carried out with 40 rats fed long-term chow or the HSHF diet, fasted, and refed exactly as described above. Either in the fasted state (10 rats/dietary cohort) or 2.5 h into the chow-refeeding period (10 rats/group), after an initial blood sample (0.15 ml) was withdrawn through the venous catheter, rats were injected through the catheter with 300 mg/kg body wt of Triton WR-1339 (Sigma), a detergent that prevents intravascular TG catabolism (25). Blood samples (0.15 ml) were then taken 20, 40, and 60 min after the Triton injection. The rate of TG appearance in the circulation was determined from regression analysis of TG accumulation in plasma vs. time. The rate of TG appearance was calculated by multiplying the slope of the regression line by plasma volume estimated from body weight and was expressed as micromoles per minute.

Plasma and tissue sampling. Immediately after the opening of the thoracic cage, blood was collected by cardiac puncture and centrifuged (1,500 g, 15 min at 4°C), and the separated plasma was stored at −70°C until later biochemical measurements. Inguinal, epididymal, and retroperitoneal white adipose tissues (WAT) were excised, and −50 mg from each tissue were homogenized with all-glass tissue grinders (Kontes, Vineland, NJ). Adipose tissue samples were homogenized in 1 ml of a solution containing 0.25 mol/l sucrose, 1 mmol/l EDTA, 10 mmol/l Tris-HCl, and 12 mmol/l deoxycholate, pH 7.4. Homogenates were centrifuged (12,000 g, 20 min at 4°C), and the fraction between the upper fat layer and the bottom sediment was removed, diluted with 4 vol of the homogenization solution without deoxycholate, and stored at −70°C until LPL activity measurement. LPL in tissue homogenates represents a pool of endothelium-bound and active intracellular enzyme. The measurement of this total pool to various physiological conditions generally parallels that of the heparin-releasable, endothelium-bound fraction, although not necessarily in an identical extent (22).

Plasma determinations. Plasma glucose concentrations were measured by the glucose oxidase method with a Beckman glucose analyzer. Insulin was determined by RIA using a reagent kit from Linco Research (St. Charles, MO) with rat insulin as the standard. Plasma TG were measured by an enzymatic method using a reagent kit from Boehringer Mannheim (Montreal, QC, Canada) that allows correction for free glycerol. Plasma nonesterified fatty acids (NEFA) were also determined by an enzymatic colorimetric technique (Wako Pure Chemical Industries, Richmond, VA).

Tissue LPL activity. Thawed tissue homogenates (100 μl) were incubated under gentle agitation for 1 h at 28°C with 100 μl of a substrate mixture consisting of 0.2 mol/l Tris-HCl...
buffer, pH 8.6, that contained 10 MBq/l [carboxyl-14C]triolein (Amersham, Oakville, ON, Canada) and 2.52 mmol/l cold triolein emulsified in 50 g/l gum arabic, as well as 20 g/l fatty acid-free BSA, 10% human serum as a source of apolipoprotein C-II, and either 0.2 or 2 mol/l NaCl. Free oleate released by LPL was then separated from intact triolein and mixed with Universal (NEN, Montreal, QC, Canada), and sample radioactivity was determined in a scintillation counter. LPL activity was calculated by subtracting lipolytic activity determined in a final NaCl concentration of 1 mol/l (non-LPL activity) from total lipolytic activity measured in a final NaCl concentration of 0.1 mol/l. LPL activity was expressed as microunits (1 μU = 1 μmol NEFA released/h of incubation at 28°C). The interassay coefficient of variation was 4.1% and was determined using bovine skim milk as a standard source of LPL. Protein content of the tissue extracts was measured by the method of Lowry et al. (19). To account for diet-induced differences in tissue TG content, data are expressed as LPL activity per gram total tissue protein.

Statistical analysis. Data are presented as means ± SE. The main and interactive effects of the chronic diet and time or nutritional status were analyzed by factorial ANOVA. When justified by the ANOVA analysis, differences between individual group means were then analyzed by Fisher’s protected least squares difference test. Differences were considered statistically significant at P < 0.05.

RESULTS

In the animals subjected to the hyperinsulinemic-euglycemic clamp, HSHF-fed rats were hyperinsulinemic compared with their chow-fed counterparts before the onset of the insulin infusion (1.9 ± 0.4 vs. 0.8 ± 0.1 nmol/l, P < 0.04), whereas insulinemia, which was increased significantly by exogenous insulin infusion, was comparable in both cohorts at the end of the 2-h infusion (3.6 ± 0.5 vs. 4.1 ± 0.1 nmol/l in HSHF- and chow-fed rats, respectively). Preinfusion glucose levels were slightly higher in the HSHF than in the chow-fed group (5.0 ± 0.2 vs. 4.3 ± 0.1 mmol/l, P < 0.003). Glycemia achieved during insulin infusion was comparable in the chow- and HSHF-fed rats (average levels between 60 and 120 min of infusion: 4.4 ± 0.2 and 4.6 ± 0.2 mmol/l, respectively). The variation in glycemia during the infusions never exceeded 10%. The sham infusion did not alter plasma glycemia (data not shown). The steady-state glucose infusion rate needed to maintain euglycemia in the HSHF-fed rats was approximately one-half of that in the chow cohort (average between 60 and 120 min: 14.2 ± 1.5 vs. 27.2 ± 0.4 mg·kg⁻¹·min⁻¹, P < 0.0001, n = 8 and 7, respectively), indicating frank whole body insulin resistance.

Compared with chow, chronic ingestion for 4 wk of the HSHF diet resulted in larger increases in body weight (P < 0.004) and adipose tissue weight (P < 0.0001) for all depots studied (Table 1), which reflected the larger energy intake (average: 413 ± 11 vs. 258 ± 14 kJ/day, P < 0.0001), as previously reported (15, 21). Pelleted chow intake during refeeding after a 24-h fast was similar in both chow- and HSHF-fed animals at all times during refeeding (Table 2).

At the end of the fasting period, no difference in plasma insulin (Fig. 1A), glucose (Fig. 1B), NEFA (Fig. 1C), or TG (Fig. 1D) concentrations was observed between chow- and HSHF-fed rats. As expected, food intake increased glycemia and insulinemia of both groups. Despite similar plasma insulin concentrations, HSHF-fed rats had higher levels of glucose than chow-fed animals at 3 and 6 h after the beginning of the refeeding period (P < 0.0001), which indicates resistance of glucose metabolism to the action of insulin in the former group. The reduction in plasma NEFA was slower in HSHF-fed animals than in chow-fed rats (P = 0.003 between groups at 1 h); however, NEFA concentrations had reached ad libitum values (data not shown) from the 3rd h of refeeding in both groups. Triglyceridemia was significantly higher in HSHF-fed rats than in controls at 3 h upon refeeding (P < 0.01).

In the three adipose depots studied (inguinal, retroperitoneal, and epididymal; Fig. 2, A, B, and C, respectively), LPL activity was not significantly different between the two dietary groups after the 24-h fast. Refeeding increased LPL activity in all three depots in both the inguinal and epididymal depots, and further increased at 6 h in the retroperitoneal depot (P < 0.05). In HSHF-fed animals, food intake brought about a slight increase in LPL activity that did not reach beyond 30% over fasting values. Three hours into the refeeding period, LPL activity was at least twofold higher in chow-fed than in HSHF-fed animals (P < 0.003 for all depots studied), which coincided with the time point at which triglyceridemia was higher in the HSHF-fed than in the chow-fed rats.

The refeeding-induced changes in LPL activity in the soleus and vastus lateralis muscles (VLM) are depicted in Fig. 3. The postprandial excursion of soleus LPL activity was relatively modest and did not reach sig-

<p>| Table 1. Body and WAT weights of rats chronically fed either rodent chow or an HSHF diet for 4 wk |</p>
<table>
<thead>
<tr>
<th></th>
<th>Chow Diet</th>
<th>HSHF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt</td>
<td>184.1 ± 1.0</td>
<td>183.4 ± 1.1</td>
</tr>
<tr>
<td>Final body wt</td>
<td>361.5 ± 4.3</td>
<td>379.1 ± 3.9*</td>
</tr>
<tr>
<td>Inguinal WAT</td>
<td>1.93 ± 0.8</td>
<td>3.06 ± 0.17*</td>
</tr>
<tr>
<td>Epididymal WAT</td>
<td>2.27 ± 0.10</td>
<td>3.37 ± 0.16*</td>
</tr>
<tr>
<td>Retroperitoneal WAT</td>
<td>1.90 ± 0.13</td>
<td>3.22 ± 0.16*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 42 (chow) or 37 (high-sucrose, high-fat (HSFH)) animals. Units are g. WAT, white adipose tissue. *Different from chow-fed rats at P < 0.05.

Table 2. Pelleted chow intake during refeeding after a 24-h fast in rats chronically fed either rodent chow or an HSHF diet for 4 wk

<table>
<thead>
<tr>
<th>Hours of Refeeding</th>
<th>Chow Diet</th>
<th>HSHF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6 ± 0.5</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>7.6 ± 0.7</td>
<td>8.0 ± 0.6</td>
</tr>
<tr>
<td>6</td>
<td>11.3 ± 1.3</td>
<td>11.0 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–6 animals. Units are g.
nificance relative to fasting values. However, soleus LPL was consistently lower in the HSHF- than in the chow-fed cohort, and significantly so at the 1- and 3-h time points. In the VLM, LPL activity increased significantly after refeeding in the chow-fed, but not in the HSHF-fed animals. As was the case for the soleus, VLM LPL tended to be lower in the HSHF-fed than in the chow-fed rats at all time points postrefeeding and was approximately fourfold lower 1 h after the onset of refeeding.

In the fasted state, the rate of TG appearance in the circulation (essentially very low density lipoprotein from hepatic origin) was identical in the two dietary cohorts (Fig. 4). As expected because of the postprandial input of intestinal chylomicrons, the rate of TG appearance increased by 30–50% during refeeding (P < 0.0002), without a significant effect of the chronic diet.

DISCUSSION

The present study demonstrates that, after 24 h of food deprivation, the short-term activation of adipose tissue LPL on refeeding is impaired in insulin-resistant rats and that an HSHF diet alters the postprandial modulation of LPL by insulin. The findings further support the notion that impairment of adipose LPL modulation in rats with diet-induced insulin resistance exacerbates postprandial hypertriglyceridemia through attenuated TG clearance.

The HSHF diet, which mimics to some extent the high-energy diets frequently consumed by many individuals, has been previously used by us (15) and others (31) to induce insulin resistance in rats. The sucrose component of the diet potentiates the deleterious effect of high-fat diets on insulin-stimulated glucose uptake (16, 17, 37). In rats fed the HSHF diet for 2 wk, preclamp hyperinsulinemia and glucose infusion rates during the clamp confirmed the well-established production by such diets of whole body insulin resistance of glucose metabolism (16, 17). Defective peripheral insulin-mediated glucose disposal in rats fed a high-fat diet for 3 wk has been localized to skeletal muscle, WAT, and the liver in studies performed with glucose tracers and an insulin infusion rate similar to that used herein (35).

The length of fasting (24 h) was chosen because of its ability to decrease both insulinemia and adipose LPL of insulin-resistant rats to levels observed in insulin-sensitive animals (27). In diet-induced insulin-resistant rats (15) and in obese Zucker rats (4), hyperinsulinemia persists after overnight (12-h) food deprivation, which was also demonstrated in the present study (see RESULTS). Although direct measurement of insulin sensitivity was not performed in rats fed during 4 wk, the higher postprandial glycemia in HSHF-fed rats, which was not caused by differences in acute food consumption, is an indication of glucose intolerance and insulin resistance and corroborates the
clamp studies performed 2 wk earlier. The fact that refed chow to the HSHF rats resulted in hyperglycemia without hyperinsulinemia relative to chow-fed rats suggests a transient defect in insulin secretion. In fact, a recent study has reported a defect in glucose-induced insulin secretion by islets isolated from high-fat-fed rats (7). Such a secretory defect appears to be transient and may conceivably be specific to chow refeeding, because HSHF-fed rats are hyperinsulinemic compared to chow-fed rats when assessed after an overnight fast or after refeeding of their habitual (HSHF) diet (15). In the present refeeding paradigm, the fact that fasting and postprandial insulinemia were similar in both dietary cohorts throughout the refeeding period had the advantage of allowing assessment of the acute modulation of LPL in response to an identical insulin load.

The postprandial modulation of LPL is mediated by insulin (26). The rise in insulinemia upon feeding is a sufficient and necessary condition to stimulate LPL activity in WAT and to decrease it in muscle (26). In WAT, the increase in LPL activity is induced by as yet poorly understood mechanisms that do not involve changes in LPL mRNA or total mass during at least the first 8 h after food intake (1, 12, 14, 23, 24, 33). The activity of LPL assessed here therefore constitutes the major modulated variable in the present conditions. Such a modulation probably involves several steps, including the release of active LPL, activation of an inactive pool present in the fasted state (1, 6), and possibly a reduced rate of LPL degradation (3, 34). The present findings clearly demonstrate that, in diet-induced insulin resistance, the postprandial modulation of WAT LPL is impaired. Indeed, acute food intake resulted in a two- to fourfold increase in adipose tissue LPL activity in chow-fed rats during the first 6 h of refeeding, as opposed to a small (<30%) elevation in animals chronically fed the HSHF diet. This diet-induced difference was observed despite similar increases in insulinemia during refeeding. Thus it can be concluded that insulin resistance brought about by chronic ingestion of an HSHF diet extends to LPL activation in WAT during the first hours of feeding.

Despite the association between chronic hyperinsulinemia and high adipose LPL activity in obesity-associated insulin resistance, which suggests an intact responsiveness of LPL to insulin, a few previous studies have indirectly suggested that LPL may become insulin resistant under acute conditions. In overnight-fasted humans, the dose-response curve of adipose tissue LPL to insulin infusion was shown to be shifted to higher insulin concentrations in obese subjects compared to lean individuals (13). Also, the stimulation of LPL activity measured 4 h after an insuligenic meal was partially blunted in obese

Fig. 2. Activity of lipoprotein lipase in inguinal (A), retroperitoneal (B), and epididymal (C) white adipose depots of rats chronically fed chow or the HSHF diet for 4 wk and killed after a 24-h fast, or at various times after subsequent refeeding with pelleted chow. Symbols represent means ± SE of 6 animals. *Different from chow-fed rats (P < 0.05). †Different from fasting (0-h time point) in same dietary cohort (P < 0.05).
men compared with their lean counterparts (23). In addition, we have recently shown that the postprandial activation of adipose LPL is delayed in obese Zucker rats despite frank postprandial hyperinsulinemia (27). Finally, stimulation of adipose LPL activity in older, less insulin-sensitive guinea pigs relies on slow mRNA changes, whereas that of young animals occurs rapidly (~1 h) after food intake (32). The present findings extend these studies with the novel finding that a defect in the acute feeding-induced activation of adipose LPL develops in rats chronically fed a diet that deteriorates insulin action on glucose metabolism. The results also demonstrate that, although transient, this defect persists for several hours after the onset of feeding.

The postprandial hypertriglyceridemia that was observed in the HSHF-fed rats was the result of impaired clearance. Indeed, in response to the intake of the same amount of chow, both dietary cohorts displayed the same rate of appearance of TG in the circulation. In the present study, muscle LPL most likely contributed to such an impaired clearance, as its availability was lower postprandially in HSHF-fed than in chow-fed rats. In fact, muscle LPL was decreased upon refeeding in insulin-resistant rats, much as it normally is after a shorter period (12 h) of fasting in insulin-sensitive rats (26), suggesting maintenance of the sensitivity of muscle LPL to insulin. In animals chronically fed chow, muscle LPL was increased postprandially, rather than decreased. We have shown in lean and obese Zucker rats that such an increase is mediated by the β-adrenergic system, which is activated upon refeeding after 24 h of fasting in insulin-sensitive, but not in insulin-resistant, rats (27) and which overcomes insulin action. In the present study, this resulted in a lower muscle LPL activity in insulin-resistant compared with insulin-sensitive animals, which most likely contributed to impaired TG clearance.

In conclusion, this study demonstrates that WAT LPL becomes resistant to the stimulatory action of insulin during refeeding in rats with diet-induced insulin resistance of glucose metabolism. The findings also strongly support the notion that, under certain nutritional conditions such as refeeding after prolonged fasting, impairment of adipose LPL modulation associated with insulin resistance, together with maintenance of the reduction in muscle LPL activity, exacerbates postprandial hypertriglyceridemia through attenuated TG clearance.

Fig. 4. Rate of appearance of triglyceride in the circulation in rats chronically fed chow or the HSHF diet for 4 wk and measured after a 24-h fast, or 2.5 h into subsequent refeeding with pelleted chow. Bars represent means ± SE of 8–10 animals. *Different from fasting of corresponding diet (P < 0.05).
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