Contribution of Hyperinsulinemia to Modulation of Lipoprotein Lipase Activity in the Obese Zucker Rat

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This study was designed to assess the contribution of hyperinsulinemia to the maintenance of high adipose and low muscle lipoprotein lipase (LPL) activity in the obese Zucker *fa/fa* rat. Insulinemia in obese Zucker rats was reduced for 4 days with a single injection of low-dose streptozotocin (STZ). Saline-injected intact obese (obese-INT) and STZ-injected obese (obese-STZ) rats were compared with a lean *Fa/?* reference group. LPL activity was assessed after a 12-hour fast, with or without a 1-hour refeeding period. Fasting serum insulin levels were 17-fold higher in obese-INT versus lean rats and were reduced to 60% of obese-INT levels in obese-STZ animals. In the postprandial state, serum insulin levels remained low in obese-STZ rats and were similar to the values in lean animals, whereas insulinemia increased in the obese-INT group to 18-fold the levels in lean rats. Serum glucose, nonesterified fatty acid (NEFA), and triglyceride levels, which were higher in obese-INT versus lean rats, were further increased in the obese-STZ group. Tissue weights of obese rats were unaffected by STZ treatment. Fasting LPL specific activity was higher in white adipose tissue ([WAT] +87%) and brown adipose tissue ([BAT] +167%) of obese-INT versus lean rats. Reducing the insulinemia in obese-STZ rats reduced fasting enzyme activity to the levels in lean animals in both WAT and BAT. Insulinemia and adipose LPL activity were positively correlated in the fasted state. Acute food intake increased WAT LPL activity in lean animals, but not in obese animals. Soleus LPL activity was lower in obese-INT compared with lean rats and was further decreased in obese-STZ animals. Heart LPL was decreased only in obese-STZ rats compared with the lean group. LPL in muscle tissue was not correlated with insulinemia, but an inverse relationship was found between serum NEFA levels and enzyme activity. It is concluded that in the obese Zucker rat, hyperinsulinemia is responsible for the maintenance of elevated basal LPL activity in adipose tissue independently of fat mass, whereas muscle enzyme activity appears to be more strongly and inversely related to the availability or tissue utilization of lipid substrates.

PLASMA LEVELS of triglycerides and their partitioning among tissues are partly determined by the activity of lipoprotein lipase ([LPL] EC 3.1.1.34).[1,2] LPL activity is chronically increased in adipose tissue in obese humans[2] and animal models of obesity such as the Zucker rat,[3,4] whereas muscle LPL is generally unchanged, decreased, or abnormally responsive to modulators such as insulin.[5-8] Tissue-specific alterations in LPL activity may thus constitute metabolic adaptations that would favor the development and maintenance of obesity through increased channeling of lipids toward storage rather than oxidation.[9]

Insulin is clearly required for the maintenance of normal tissue levels of LPL, as total insulin deficiency produces a large reduction in LPL in both adipose tissue and muscle.[10] Over and above this general action of insulin on LPL, the hormone modulates the activity of the enzyme in a tissue-specific fashion. Thus, food intake and insulin infusion acutely increase adipose LPL activity in rats and humans that are normal,[1,5,11] moderately obese,[12-15] insulin-resistant, or diabetic.[16-18] In fact, we have shown that insulin is a necessary and probably sufficient condition for the tissue-specific changes in LPL caused by food intake.[19] It has been reported that adipose LPL is resistant to insulin action in massively obese humans,[20,21] and in vitro experiments have suggested that the enzyme's responsiveness to insulin may be blunted in the presence of sustained hyperinsulinemia.[20] In muscle tissue, LPL activity is reduced by the intake of an insulinogenic meal,[1,16,22] and it has been negatively correlated with insulinemia.[23] However, the responsiveness of muscle LPL to insulin may vary according to the obesity status and divergent findings have been reported in this regard.[24-27] It has also been suggested that in addition to insulin, the availability of energy substrates may influence muscle LPL,[22,25,27] and high circulating levels of lipids have been found to reduce its activity.[28] Thus, insulin may modulate muscle LPL activity either directly or indirectly through its action on energy substrates.

The contribution of sustained hyperinsulinemia, which frequently accompanies obesity, to the tissue-specific modulation of LPL remains uncertain. In the present study, we evaluated the effects of a short-term (4 days) reduction in insulinemia on glycemia, lipemia, and tissue LPL activity in obese hyperinsulinemic Zucker *fa/fa* rats, to test the hypothesis that hyperinsulinemia is responsible for the maintenance of high adipose and low muscle LPL activity typical of the obese state. Obese *fa/fa* rats in which insulinemia was reduced by a streptozotocin (STZ) injection, as well as intact obese and lean *Fa/?* rats, were studied after a 12-hour fast or 2 hours after the intake of a meal. Low-dose STZ was used to avoid total insulin depletion, and the short treatment duration prevented overt changes in adiposity, thereby allowing a dissociation of the effects of insulinemia and obesity.

MATERIALS AND METHODS

Animals and Diet

Thirty-four male Zucker obese *fa/fa* rats and 20 lean *Fa/?* controls (Charles River, St. Constant, Quebec, Canada) aged 6 to 9 weeks were housed individually in stainless steel cages in a room maintained at 22° ± 1°C and lighted between 8 PM and 8 AM. They had free access to a nonpurified diet (rat chow no. RMH420; Charles River) and tap water.

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The animals were cared for and handled in accordance with the Canadian Guide for the Care and Use of Laboratory Animals, and the protocols were approved by our institutional Animal Care Committee.

Protocol

The animals were allowed to acclimate to the environmental conditions and diet for at least 1 week before the experiments were initiated. Half of the obese rats received an intraperitoneal injection of STZ (45 mg/kg body weight in 50 mmol/L citrate buffer, pH 4.5) and the remaining animals received the citrate buffer alone. Three groups were thus formed: lean, intact obese (obese-INT), and STZ-treated obese (obese-STZ). Four days later, food was removed at the beginning of the light period. The following morning, half of the animals in each experimental group were killed by decapitation in the fasted state. The remaining rats were killed by decapitation 2 hours after the onset of a 1-hour feeding period during which they had access to their habitual nonpurified diet. The experiment was performed for a total period of 4 weeks to ensure that all rats were killed at the same age.

Serum and Tissue Sampling

Blood was collected and kept on ice until centrifugation (1,500 *g* at 4°C for 15 minutes), and serum was stored at −70°C until later biochemical measurements. The retroperitoneal white adipose tissue (WAT), interscapular brown adipose tissue (BAT), soleus muscle, and heart were excised. BAT was cleaned of adhering white fat and muscle, and the heart was washed in saline. The tissues were weighed, and approximately 50 mg tissue was taken from the WAT, BAT, soleus, and apex of the heart and then homogenized using a glass tissue grinder (Kontes, Vineland, NJ). Samples of WAT and BAT were homogenized in 1 mL of a solution containing 0.25 mol/L sucrose, 1 mmol/L EDTA, 10 mmol/L Tris hydrochloride, and 12 mmol/L deoxycholate, pH 7.4. Soleus and heart samples were homogenized in 1 mL of a solution containing 1 mol/L ethylene glycol, 50 mmol/L Tris hydrochloride, 3 mmol/L deoxycholate, 10 IU/mL heparin, and 5% (vol/vol) aprotinin (Trasylol; Miles Pharmaceuticals, Rexdale, Ontario, Canada), pH 7.4. These homogenizing media yielded optimal LPL activity in the individual tissues. Homogenates of soleus and heart were quickly frozen at −70°C until later LPL measurements. Homogenates of WAT and BAT were centrifuged at 12,000 *g* at 4°C for 20 minutes. The fraction between the upper fat layer and the bottom sediment was removed, diluted with 4 vol homogenization solution without deoxycholate, and stored at −70°C until later measurement of LPL activity.

Tissue LPL Activity

LPL activity was measured in tissue homogenates as previously described.[29] Samples (100 μL) of tissue homogenates were incubated for 1 hour at 28°C under gentle agitation with 100 μL substrate mixture consisting of 0.2 mol/L Tris hydrochloride buffer, pH 8.6, containing 10 MBq/L [carboxyl-^{14}C]-triolein (Amersham, Oakville, Ontario, Canada) and 2.52 mmol/L cold triolein emulsified in 50 g/L gum arabic, as well as 20 g/L fatty acid–free bovine serum albumin, 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 Universol (New England Nuclear, Montreal, Quebec, Canada) and sample radioactivity was determined in a scintillation counter. LPL activity was calculated by subtracting the lipolytic activity determined in a final NaCl concentration of 1 mol/L (non-LPL lipolytic activity) from the total lipolytic activity measured in a final NaCl concentration of 0.1 mol/L. In the present conditions, 1 mol/L NaCl inhibited 82% to 91% of total lipolytic activity in all tissue homogenates. LPL activity is expressed as microunits (1 μU = 1 μmol nonesterified fatty acids [NEFAs] released per hour of incubation at 28°C). The interassay coefficient of variation was 4.8% as determined using bovine skim milk as a standard source of LPL. The protein content of the homogenates was measured by the method of Lowry et al.[30] To account for the large difference in adipose tissue mass between lean and obese animals, LPL activity was expressed relative to tissue protein (specific activity).

Serum Measurements

Serum glucose was determined using the Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA). Insulin was determined by radioimmunoassay using a reagent kit from Incstar (Stillwater, MN) with rat insulin as a standard. Plasma corticosterone was determined by a competitive protein-binding assay (sensitivity, 0.058 nmol/L; interassay coefficient of variation, 9.0%) using plasma from a dexamethasone-treated female rhesus monkey as a source of transcortin.[31] NEFA and triglyceride levels were measured enzymatically using reagents from Wako Chemicals (Richmond, VA) and Boehringer Mannheim (Montreal, Quebec, Canada), respectively.

Statistical Analyses

All data are expressed as the mean ± SEM. Main treatment effects and treatment interactions were analyzed by a 3 × 2 factorial ANOVA. One factor was the long-term condition, defined as the condition of the animals during the 4-day period between STZ injection and the end of the experiment, with 3 levels (lean, obese-INT, and obese-STZ); and the second factor was the nutritional status, with 2 levels (fasted and fed). Post hoc pairwise mean comparisons were performed using Fisher's protected least-squares difference (PLSD) test. Body and tissue weights were analyzed by a 1-factor ANOVA, as the nutritional status had no effect on these variables, and post hoc pairwise mean comparisons were performed using Fisher's PLSD test. When appropriate, data were logarithmically transformed before analysis to achieve homogeneity of variance, but untransformed data are presented. Linear correlation coefficients were calculated to assess possible relationships between tissue LPL activity and serum insulin, glucose, NEFA, and triglyceride levels. Differences were considered statistically significant at a P level less than .05.

RESULTS

Food intake during the final 1-hour feeding period was similar in all groups (Table 1). Final body weight and retroperitoneal WAT, interscapular BAT, and heart weight were higher in obese versus lean rats, and STZ treatment did not affect these variables. Soleus weight was comparable in all groups. STZ treatment of obese animals did not alter tissue protein content (data not shown).

Table 1. Meal Intake During the Final 1-Hour Feeding Period and Final Body and Tissue Weights of Lean, Obese-INT, and Obese-STZ Rats

Parameter	Lean	Obese-INT	Obese-STZ
Meal intake (g)	7.0 ± 0.5	8.9 ± 0.7	6.8 ± 1.1
Body weight (g)	288 ± 5[a]	417 ± 7[b]	399 ± 12[b]
Tissue weight (g)			
Retroperitoneal WAT	0.8 ± 0.1[a]	3.2 ± 0.3[b]	3.3 ± 0.3[b]
Interscapular BAT	0.40 ± 0.02[a]	1.5 ± 0.1[b]	1.4 ± 0.1[b]
Soleus muscle	0.124 ± 0.004	0.114 ± 0.008	0.106 ± 0.005
Heart muscle	0.92 ± 0.02[a]	1.07 ± 0.02[b]	1.04 ± 0.02[b]

NOTE. Nutritional status had no effect on body and tissue weights, and the collapsed mean ± SEM of fasted and fed rats are shown (n = 17-20 rats per group). Mean values not sharing the same superscript are significantly different ($P < .05$). The absence of a superscript indicates the lack of a significant main effect of the long-term condition.

Serum glucose concentrations in the fasted state were comparable in the lean and obese-INT groups, whereas fasting glycemia was 3-fold higher in the obese-STZ group versus the other two groups (Fig 1A). Food intake increased glycemia in all groups, but especially the obese-INT group, in which postprandial glycemia was higher than in lean rats. Glycemia remained higher in obese-STZ rats versus both the lean and obese-INT groups in the postprandial state. Differences in the serum glucose profile of the 3 long-term conditions according to nutritional status were confirmed by the significant treatment interaction. Figure 1B shows that the long-term condition and nutritional status also interacted with each other on insulin levels. In the fasted state, obese-INT and obese-STZ groups had comparable serum insulin values, which were 17- and 11-fold higher than the levels in lean animals, respectively. Food intake increased insulinemia nearly 8-fold in both lean (to 1.6 nmol/L) and obese-INT animals (to 26.8 nmol/L), whereas serum insulin in obese-STZ rats was not increased to a significant extent by acute food intake and was comparable to the postprandial level in lean rats. Figure 1C depicts serum corticosterone levels measured near the peak of the circadian rhythm. Serum corticosterone was unaltered by the long-term condition and was significantly reduced by acute food intake in all groups, albeit slightly less so in obese-STZ animals.

Serum NEFA concentrations in the fasted state were higher in obese-INT versus lean rats, and were further increased in

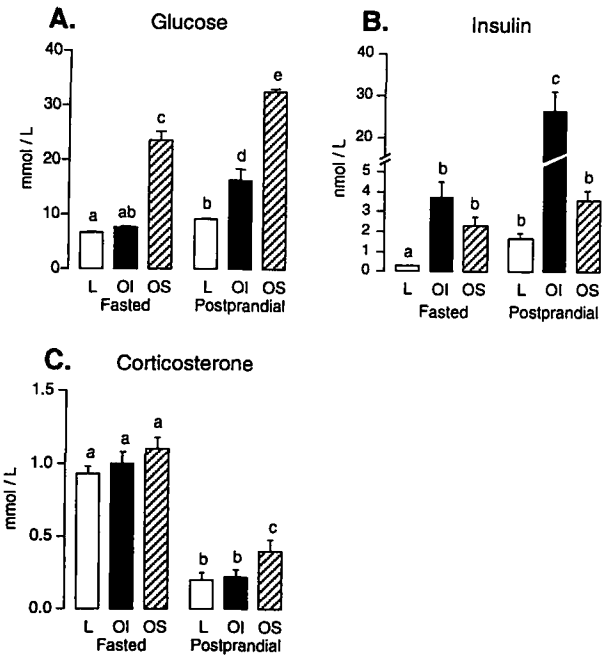

Fig 1. Serum concentrations of glucose (A), insulin (B; note break in scale of y=axis), and corticosterone (C) in lean (L), obese-INT (OI), and obese-STZ (OS) rats in the fasted and postprandial states. Bars represent the mean ± SEM of 7-10 rats. ANOVA for main effects of the long-term condition (LT) with 3 levels (lean, OI, and OS), nutritional status (N) with 2 levels (fasting and postprandial), and treatment interactions (LT × N): LT, $P < .0001$, .0001, and not significant (NS); N, $P < .0001$, .0006, and .0001; and LT × N, $P < .006$, .0003, and NS (for glucose, insulin, and corticosterone, respectively). Mean values not sharing the same superscript are significantly different ($P < .05$).

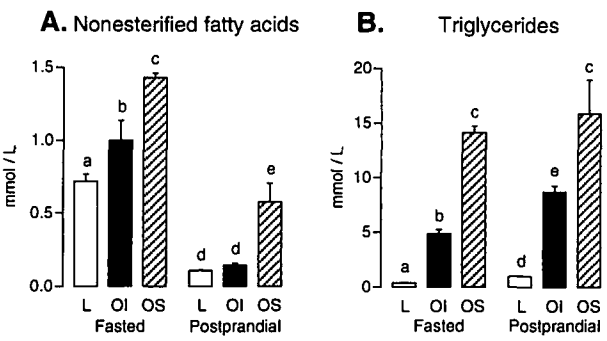

Fig 2. Serum concentrations of NEFAs (A) and triglycerides (B) in lean (L), obese-INT (OI), and obese-STZ (OS) rats in the fasted and postprandial states. Bars represent the mean ± SEM of 7-10 rats. ANOVA (described in Fig 1): LT, $P < .0001$ and .0001; N, $P < .0001$ and .0006; and LT × N, $P < .004$ and .003 (for NEFA and triglycerides, respectively). Mean values not sharing the same superscript are significantly different ($P < .05$).

obese-STZ animals (Fig 2A). Food intake decreased NEFAs to similar low levels in the lean and obese-INT groups, whereas the decrease was smaller in the obese-STZ group. Fasting serum triglyceride concentrations were 12-fold higher in obese-INT lean rats, and 3-fold higher in obese-STZ versus obese-INT rats (Fig 2B). Fasting NEFAs and triglycerides were closely and positively correlated ($r = .84$, $P < .0001$). Food intake increased triglyceridemia approximately 2-fold in the lean and obese-INT groups, whereas triglycerides in obese-STZ rats remained unaffected.

The effect of reduced insulinemia for 4 days on the specific activity of LPL in retroperitoneal WAT and interscapular BAT in obese Zucker rats is depicted in Fig 3. In the fasted state, LPL activity in retroperitoneal WAT was 2-fold higher in obese-INT versus lean rats, whereas STZ treatment of obese animals reduced WAT LPL to lean levels (Fig 3A). Acute food intake resulted in a 2-fold increase in WAT LPL of lean rats, whereas LPL activity remained unaffected in obese-INT and obese-STZ animals. The pattern of LPL activity in BAT was comparable in the fasted and fed states (Fig 3B). LPL activity was higher in obese-INT versus lean animals, and STZ treatment decreased

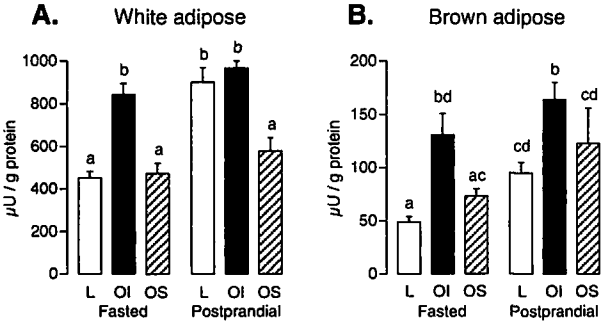

Fig 3. Specific activity of LPL in retroperitoneal WAT (A) and interscapular BAT (B) of lean (L), obese-INT (OI), and obese-STZ (OS) rats in the fasted and postprandial states. Bars represent the mean ± SEM of 7-10 rats. ANOVA (described in Fig 1): LT, $P < .0001$ and .0004; N, $P < .0001$ and .005; and LT × N, $P < .002$ and NS (for WAT and BAT, respectively). Mean values not sharing the same superscript are significantly different ($P < .05$).

the enzyme activity toward lean levels. Food intake resulted in an overall increase in BAT LPL activity, but between-group comparisons showed that the increase was significant in the lean group but not in the obese-INT and obese-STZ groups. In the fasted state, but not in the fed state, LPL activity in both WAT ($r = .71$, $P < .0004$) and BAT ($r = .68$, $P < .003$) was positively correlated with insulinemia.

The long-term condition of the rats exerted a significant effect on LPL specific activity in soleus muscle (Fig 4A). LPL activity was lower in obese-INT compared with lean rats, and STZ treatment of obese animals resulted in a further decline in soleus LPL activity. Food intake did not affect soleus LPL significantly. The long-term condition also affected LPL specific activity of the heart (Fig 4B), as STZ treatment of obese animals caused a reduction in enzyme activity, whereas LPL was comparable in lean and obese-INT animals. Acute food intake tended to reduce heart LPL activity by a mean of 23%, an effect that was independent of the long-term condition of the animals. The relationships between fasting insulinemia and LPL activity in the soleus and heart were poor, whereas fasting, but not postprandial, enzyme activity was negatively correlated with NEFA levels (soleus, $r = -.50$, $P < .009$; heart, $r = -.56$, $P < .006$). Fasting heart ($r = -.56$, $P < .02$), but not soleus ($r = -.40$, $P = .09$), LPL was also negatively correlated with triglyceridemia. No statistical relationship was found between glycemia and LPL activity in any tissue, with the exception of the heart, for which the statistical relationship was explained by the outlying obese-STZ group.

DISCUSSION

The intact obese Zucker *fa/fa* rats in this study were hyperinsulinemic and hypertriglyceridemic, had high fasting NEFA levels, and displayed postprandial glucose intolerance as previously reported.[32-34] These metabolic abnormalities typically accompany the development of a resistance of glucose metabolism to insulin action,[35] including the obese Zucker rat.[36] Treatment of obese animals with STZ caused a large reduction in postprandial insulinemia to levels that were indistinguishable from those of lean animals. In the fasted state, obese-STZ rats remained slightly hyperinsulinemic compared with their lean counterparts. In terms of the effects of STZ-induced modifications in insulinemia on the modulation of LPL, it must be noted that throughout the 4-day period following STZ treatment and preceding food removal, the ad libitum–fed obese Zucker rats were in the postprandial state most of the time. STZ treatment therefore resulted in the maintenance of serum insulin at much lower levels than those of untreated obese animals and close to lean levels during most of this period.

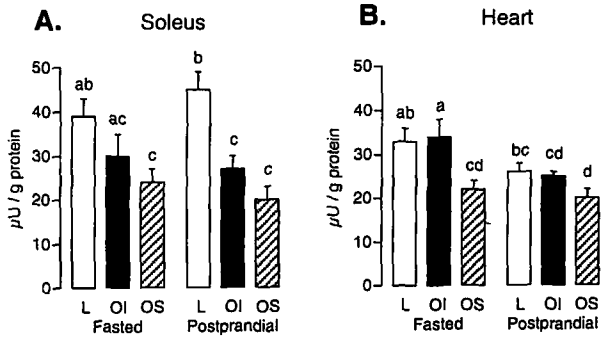

Fig 4. Specific activity of LPL in soleus (A) and heart (B) of lean (L), obese-INT (OI), and obese-STZ (OS) rats in the fasted and postprandial states. Bars represent the mean ± SEM of 7-10 rats. ANOVA (described in Fig 1): LT, $P < .0001$ and .001; N, NS and $P < .01$; and LT × N, NS and NS (for soleus and heart, respectively). Mean values not sharing the same superscript are significantly different ($P < .05$).

In the fasted state, serum NEFAs were higher in obese-INT compared with lean animals, and were further increased in the obese-STZ group. Because of the large fat mass and, at some point, also because of the development of resistance to the antilipolytic action of insulin, obesity results in high circulating levels of NEFA.[37] The maintenance of some sensitivity of adipose lipolysis to insulin in the obese Zucker rats is nevertheless illustrated by the substantial postprandial decrease in serum NEFAs of obese-INT rats, the slightly higher fasting NEFA levels in obese-STZ versus obese-INT rats in the presence of slightly lower insulin levels, and the significant decrease in NEFAs following a minor elevation of insulinemia in obese-STZ rats following food intake. On the other hand, fasting serum triglyceride levels were closely associated with NEFA levels, in accordance with the strong influence of portal NEFA input on liver triglyceride production and secretion[38] and the obesity-associated loss of the inhibitory action of insulin on hepatic very–low-density lipoprotein secretion.[39] The general reduction in tissue LPL activity with STZ treatment of obese rats may have contributed to exacerbate hypertriglyceridemia in this group.

The specific activity of LPL in WAT in the fasted state was more than 2-fold higher in obese-INT versus lean rats, a difference that reached 6-fold on a total tissue basis. The reduction in insulinemia produced by STZ treatment of obese animals decreased the specific activity of WAT LPL to lean levels. This finding demonstrates that in the obese Zucker rat, hyperinsulinemia is responsible for the maintenance of high basal (fasting) adipose LPL activity. This is further supported by the positive statistical relationship between insulin and adipose LPL levels, and is in agreement with the normalization of LPL activity to control levels in adipocytes isolated from obese hyperinsulinemic humans after short-term incubation without insulin.[20] The reduction in LPL specific activity in response to a reduction in insulinemia without any macroscopic alteration in adipose tissue morphology and composition further shows that LPL activity is not simply a function of adipocyte size. In addition, the involvement of endocrine factors other than insulin itself, such as counterregulatory hormones, in the effects of STZ treatment is unlikely because corticosterone levels were unaffected by the insulin status and because changes in catecholamines would have been expected to exert tissue-specific effects on LPL,[40] rather than the congruent decreases in both adipose tissue and muscle that were observed.

As expected, acute food intake resulted in a substantial (100%) elevation in adipose LPL activity in lean animals. However, food intake remained without effect in both obese-INT and obese-STZ groups. Such a lack of response of adipose LPL to food intake has sometimes been reported in obese humans.[20,21] This may be related to the fact that, at least in some adipose depots such as the retroperitoneal depot, sustained

hyperinsulinemia in obese animals may result in the maintenance of a maximal insulin-mediated modulation of LPL in the fasted state, to a level that cannot be exceeded following food intake despite a large elevation in insulinemia. Alternatively, insulin modulates LPL activity at several metabolic steps, including gene expression, mRNA or protein stability, enzyme activation (dimerization), and secretion (for review, see Braun and Severson[41]). There are major differences in the time course of the insulin-mediated modulation of these metabolic steps.[15,21,22,42] It may be that in overt obesity, 1 or more of these metabolic steps, which would be involved in the very early mobilization of LPL following food intake, may become resistant to the action of insulin as suggested by Eckel.[43] This could result in a delayed response of adipose LPL to food intake. Other steps modulating LPL on a longer-term basis would remain insulin-sensitive, thereby accounting for the LPL response to food intake observed later after refeeding[19] and for the reduction in enzyme activity in response to a decrease of insulinemia over longer periods. These scenarios require further investigation, but the present findings clearly show that in the obese Zucker rat, WAT LPL activity does not respond to a 2-hour feeding-induced elevation in insulin in the same manner as in lean animals, whereas it is reduced to lean levels after a few days of exposure to reduced insulinemia. Finally, WAT LPL was not increased by food intake in obese-STZ rats despite low fasting enzyme activity. However, since insulin did not increase significantly in response to food intake in obese-STZ animals, no conclusion can be made as to the postprandial responsiveness of adipose LPL to insulin after exposure to low insulinemia for several days.

LPL activity responded to the decrease of insulinemia caused by STZ treatment in obese rats comparably in BAT and WAT. Indeed, BAT LPL specific activity in the fasted state, which was nearly 3-fold higher in obese-INT versus lean rats, was reduced to lean levels after 4 days of exposure to low insulinemia. Although the modulation of LPL is distinct in BAT versus WAT, at least with regard to the opposite action of the sympathoadrenal system on enzyme activity,[40] the present findings are in accordance with the positive modulatory effect of insulin on BAT LPL activity, which parallels that in WAT.[44] Acute food intake resulted in an elevation of BAT LPL activity in lean animals, whereas the enzyme activity remained unchanged in obese-INT rats, as was the case for WAT.

Fasting soleus LPL activity was slightly reduced in obese-INT rats compared with lean controls, but a significant decrease in both skeletal muscle and heart LPL activity in obese rats was found after a reduction of serum insulin with STZ treatment. These findings do not support a direct role for insulinemia per se in the long-term modulation of muscle LPL activity. Instead, fasting LPL activity in both the soleus and the heart gradually decreased in parallel with the elevation in NEFA and triglyceride levels in obese-INT and obese-STZ rats compared with lean controls. This relationship is underlined by the negative correlations between circulating NEFAs and triglycerides and soleus and heart LPL activity under fasting conditions. As previously suggested by Ferraro et al,[27] the degree of dependence of muscle energy metabolism on circulating lipids may determine muscle LPL activity in abnormal metabolic conditions. The proposition that the abundance of NEFAs and triglycerides in the circulation may chronically modulate muscle LPL is supported by several lines of evidence. Muscle LPL is generally decreased in obese and non–insulin-dependent diabetic humans who concomitantly display elevated circulating NEFA levels.[23,45] NEFAs and triglyceride-rich lipoproteins were shown to reduce LPL activity in the perfused heart,[46] and STZ treatment of obese Zucker rats reportedly decreased heart LPL only when circulating lipids were increased.[46] Finally, feeding a high-fat diet to lean rats is associated with lower serum NEFA and triglyceride levels and higher muscle LPL activity compared with feeding a high-carbohydrate diet.[16] Although these lines of evidence and the present findings suggest the existence of a relationship between the availability or utilization of lipid substrates and the modulation of muscle LPL, the putative underlying mechanisms remain elusive. Finally, muscle LPL has been shown to be inversely related to the rate of muscle glucose transport after a euglycemic clamp[25,26] or food intake[22] in normal and obese humans. Although the efficiency of insulin action on glucose metabolism was not directly addressed here, STZ is known to cause a certain degree of insulin resistance,[47] which may have been additive to that already present in the obese rats. Therefore, a relationship between muscle LPL activity and glucose metabolism, rather than that of lipids is a possibility that remains to be further explored.

A long-term negative effect of elevated circulating lipids on muscle LPL activity is not incompatible with the fact that insulin itself may acutely modulate the enzyme in muscle tissue, as previously reported.[1,16,22-25] In the present study, the acute intake of food did not alter soleus LPL activity, consistent with our previous study in which diet composition was shown to be an important determinant of the postprandial response of muscle LPL.[16] However, food intake acutely reduced enzyme activity in the heart of lean and obese-INT rats despite a large decrease in circulating NEFA levels. Therefore, the postprandial elevation in insulinemia may have acutely reduced heart LPL independently of its action on lipemia. Such a direct rapid action of insulin is supported by the lack of a postprandial decrease of heart LPL in obese-STZ animals, in which the food intake–induced increase in insulin was minimal. Finally, as in the case of adipose tissue, the apparent differences between the acute and longer-term modulation of muscle LPL may be related to the sensitivity and time course of the response of various regulatory steps either to insulin or to energy substrates.

In summary, the present study shows that a reduction of hyperinsulinemia via administration of low-dose STZ completely normalized LPL activity in WAT and BAT of the obese Zucker rat without a change in the fat mass. In contrast, the already low muscle LPL activity was further decreased in concomitance with increased plasma lipid levels. Therefore, in the obese Zucker rat, hyperinsulinemia is responsible for the maintenance of elevated basal LPL activity in adipose tissue independently of the fat mass, whereas muscle LPL appears to be more strongly and inversely related to the availability or tissue utilization of lipid substrates.

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