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Abnormal insulin and β-adrenergic modulation of lipoprotein lipase during refeeding after prolonged fasting in the Zucker rat

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Abstract

Aims/hypothesis. To characterise the response of tissue lipoprotein lipase to refeeding after prolonged (24 h) fasting in lean and obese Zucker rats, and to verify whether lipoprotein lipase in obese rats is resistant to the short-term action of insulin and escapes modulation by the β-adrenergic pathway.
Methods. Lean *Fa/?* and obese *fa/fa* male Zucker rats fasted for 24 h and refed at will. Lipoprotein lipase activity in adipose and muscle tissues was assessed in the freely fed and fasted states and at various times during refeeding, with or without β-adrenergic blockade (propranolol).
Results. The 24-h fast erased the phenotype-related differences in insulinaemia and adipose lipoprotein lipase activity present in freely fed rats. Adipose lipoprotein lipase increased twofold in obese rats 1 h after refeeding, whereas no change occurred at that time in lean rats. Activity remained at that level for at least 6 h after refeeding in obese rats, whereas in lean animals it was increased fivefold after 6 h of refeeding. In muscle of obese rats, lipoprotein lipase decreased in response to refeeding, but paradoxically increased twofold in lean animals. Giving propranolol to lean rats before refeeding abolished the atypical response of muscle lipoprotein lipase to food intake and restored the early (1 h after refeeding) increase in adipose lipoprotein lipase but had no effect in obese rats.
Conclusion/interpretation. Refeeding after prolonged fasting activates the β-adrenergic pathway in lean rats, which transiently counteracts insulin-mediated modulation of lipoprotein lipase. The β-adrenergic pathway is not activated by refeeding in adipose tissue and muscle of the obese Zucker rat. In the obese Zucker rat, the early modulation of adipose lipoprotein lipase activity is abnormal upon refeeding after prolonged fasting, suggesting short-term resistance to the action of insulin. [Diabetologia (2000) 43: 866–874]

Keywords Lipoprotein lipase, obesity, Zucker rat, food deprivation, food intake, insulin, insulin resistance, β–adrenergic pathway, adipose tissue, skeletal muscle.

Lipoprotein lipase (LPL, EC 3.1.1.34), the enzyme that hydrolyses triglycerides carried within the vascular compartment by triglyceride-rich lipoproteins, is modulated by several hormones in a tissue-specific manner. In food intake-induced increase in adipose tissue and decrease in muscle LPL activity [1–4], insulin has recently been shown to constitute a necessary and sufficient modulator [5]. When solicited, other endocrine factors such as the β-adrenergic component of the sympathetic efferent pathways (SEP) also modulate LPL in a tissue-specific fashion, in exact opposition to insulin action [6–8]. By virtue of its function and modulation, LPL plays an important part in the determination of triglyceridaemia, partic-

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Abbreviations: LPL, Lipoprotein lipase; SEP, sympathetic efferent pathways; WAT, white adipose tissue; BAT, brown adipose tissue.

ularly in the postprandial state, as well as in the partitioning of triglyceride-derived fatty acids among storage and oxidative tissues.

Obesity is typically associated with resistance of glucose metabolism to the action of insulin [9–11]. Other insulin-sensitive metabolic events such as vasodilation [12, 13] and adipose lipolysis [14] can become progressively insulin resistant as well. Whether this is the case for LPL remains a matter of debate. On the one hand that adipose LPL is high and muscle LPL is low in obesity-associated insulin resistance with compensatory hyperinsulinaemia [15–17] argues in favour of an intact sensitivity of LPL to insulin. On the other hand, several lines of evidence suggest that, under short-term modulation, LPL seems resistant to insulin in concomitance with glucose resistance to the hormone. In fasted obese subjects, the dose-response curve of adipose LPL to insulin infusion was reported to be shifted towards higher insulin concentrations [18]. The relative increase in adipose LPL activity above fasting levels 4 h after the beginning of a high-carbohydrate, insulinogenic meal was also found to be lower in obese than in lean subjects [19]. Taken together, the above observations suggest that chronic hyperinsulinaemia does bring about high adipose and low muscle LPL, as assessed in free feeding or fasting conditions but that there exists some degree of resistance of LPL to short-term changes in insulin, such as those elicited by meal intake or insulin infusion.

In the present series of studies, the responsiveness of LPL to food intake was explored in the Zucker rat, a genetic model of obesity with severe resistance of glucose metabolism to insulin and compensatory hyperinsulinaemia [20, 21]. The fasting-refeeding protocol aimed to deprive the animals of food for sufficient time for insulinaemia and adipose LPL of obese animals to be lowered to lean levels, such that similar basal conditions would be present upon refeeding. In a preliminary study carried out to determine such a time point, insulinaemia and adipose tissue LPL in obese Zucker rats were found to reach lean levels after 24 h of fasting. In lean rats refed after the 24-h fast, a lack of increase in adipose tissue LPL, which occurs within 1 h of refeeding after a short-term (8–10 h) fast [5], was noted. Long-term food deprivation can result in the activation of the SEP upon refeeding [22], which could counteract the insulin-mediated modulation of LPL by decreasing white adipose tissue (WAT) and increasing muscle LPL activity [6]. Sympathetic activity is, however, impaired in obese rodent models of obesity, at least in brown adipose tissue [23–26]. Given the above findings, the first aim of the present studies was to determine in lean and obese Zucker rats the time course of the tissue-specific changes in LPL activity in response to food intake, after the establishment of similar fasting insulinaemia and LPL activity in both phenotypes. The second aim was to verify the hypothesis that the β-adrenergic component of the SEP contributes to the tissue-specific modulation of LPL upon refeeding after long-term fasting. Finally, the third aim was to test the hypothesis that such postprandial modulation of LPL by the SEP does not occur in the obese Zucker rat.

Materials and methods

Animals and feeding protocol. We purchased two cohorts of lean (*Fa/?*) and obese (*fa/fa*) male Zucker rats, aged 5–6 weeks, from Charles River Canada (St-Constant, Québec, Canada). Rats were cared for and handled in accordance with the Canadian Guide for the Care and Use of Laboratory Animals, and protocols were approved by the Laval University Animal Care Committee. Rats were housed individually in stainless steel cages under a controlled temperature (23 ± 1 °C) and lighting (14 h dark: 10 h light). Rats had free access to tap water and a stock diet (Charles River Rodent Diet No 5075, Ralston Products, Woodstock, Canada). A week after their arrival, rats of the first cohort were killed either after free overnight access to food, after a 24-h period of food deprivation, or after 1, 3, 6, 12, and 24 h of free refeeding following the 24-h fast (four animals per time point). The onset of refeeding was adjusted so that all rats were killed 1 h after the beginning of the lighted period to allow comparison of groups at the same period of the circadian glucocorticoid rhythm.

Involvement of the β-adrenergic component of the SEP. A nutritional protocol similar to that described above was used to assess the involvement of the β-adrenergic component of the SEP in the second cohort of rats. In this case, rats were killed either after a 24-h period of food deprivation, or after 1 h of free refeeding following the 24-h fast. Refed rats were injected i.p. with either DL-propranolol (25 mg/kg) or saline 1 h before refeeding (five animals per group). The β-adrenergic pathway is the predominant component of the SEP that modulates LPL in WAT [7], brown adipose tissue (BAT) [27] and muscle [28]. Propranolol was selected as the β-adrenergic antagonist because it has the widest affinity towards β-adrenergic receptor subtypes [29]. In the rat, three receptor subtypes (β1, β2, and β3) are found in WAT and BAT, β2 receptors and possibly an additional, atypical β receptor predominate in skeletal muscle [30], whereas β1 and β2 receptors are found in the heart. The respective involvement of various subtypes of β-adrenergic receptors in the modulation of LPL in specific tissues has not been clearly established. Hence the selection of the non-specific β-adrenergic antagonist propranolol, which is able to antagonise actions mediated by β1, β2, as well as β3 [29, 30]. Propranolol at the dose used here has been shown to prevent in vivo the exercise-induced increase in plasma NEFA [31] and to antagonise the alterations in tissue LPL brought by short-term cold exposure [32].

Plasma and tissue sampling. In both protocols, rats were anaesthetized with an i.p. injection of 4 ml/kg of a ketamine (20 mg/ml) – xylazine (2.5 mg/ml) solution and blood and tissues were harvested immediately thereafter. Immediately after the opening of the thoracic cage, blood was collected by cardiac puncture, centrifuged (1500 *g*, 15 min at 4 °C), and the separated plasma was strored at –70 °C until later biochemical measurements. Inguinal, epididymal, retroperitoneal WAT, interscapular BAT, soleus, vastus lateralis, gastrocnemius (in the second

protocol) and heart muscles were excised, approximately 50 mg from each tissue were homogenised and processed exactly as described earlier [3, 6] and stored at –70 °C until LPL activity measurement.

Plasma measurements. Plasma glucose concentrations were measured with an automated glucose oxidase method using a Beckman glucose analyser. Insulin was measured by RIA using a reagent kit from Linco Research (St. Charles, Mo., USA) with rat insulin as standard. Plasma triglycerides were measured by an enzymatic method using a reagent kit from Boehringer Mannheim (Montréal, Québec, Canada) which allowed correction for free glycerol. Plasma NEFA were also measured by an enzymatic colorimetric technique (Wako Pure Chemical Industries, Richmond, Va., USA).

Lipoprotein lipase activity. Enzyme activity was quantified in tissue homogenates exactly as described earlier [3, 6]. 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, which contained 10 MBq/l [carboxyl-^{14}C]triolein (Amersham, Oakville, 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 (NEN, Montréal, Canada) and sample radioactivity was quantified in a scintillation counter. Lipoprotein lipase activity was calculated by subtracting lipolytic activity measured 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. The activity of LPL was expressed as microunits (1 µU = 1 µmol NEFA released per hour of incubation at 28 °C). The interassay coefficient of variation was 3.2 % and was measured using bovine skim milk as a standard source of LPL. Protein content of the tissue extracts was measured by the method described previously [33]. To account for large lean-obese differences in triglyceride content per unit weight of adipose and other tissues, LPL activity was expressed as specific activity (µU/ g tissue protein).

Statistical analysis. Data are presented as means ± SEM. Differences between individual group means were analysed by Fisher's protected least squares difference test. Differences were considered statistically significant at p less than 0.05.

Results

Response of LPL activity and plasma variables to refeeding after 24 h of food deprivation. Cumulative food intake of lean and obese Zucker rats upon refeeding after a 24-h fast is summarised in Table 1. Lean and obese animals ingested food to the same extent during the first 12 h of refeeding. Thereafter, obese animals ingested more food than the lean animals. In the freely fed state, obese Zucker rats were hyperinsulinaemic compared with their lean counterparts (Fig. 1). Food deprivation for 24 h induced a pronounced reduction in insulimaenia in obese rats, to levels that were not statistically different from those of fasted lean animals. In lean rats, refeeding induced a rise in plasma insulin concentrations within the first h, which was maintained until the sixth h. In obese animals, refeeding also led to a rapid increase in plasma insulin concentrations within 1 h, which then decreased to stable concentrations that were similar to those measured in the freely fed state. Glycaemia was similar in lean and obese rats both in the freely fed and the fasted states (Fig. 1). Glycaemia was not significantly affected by refeeding in lean rats, whereas in the obese strain glycaemia increased progressively until the sixth h of refeeding. Obese rats remained hyperglycaemic compared with their lean counterparts throughout the 24-h refeeding period. In the freely fed state, plasma NEFA were similar in both strains (Fig. 1). The 24-h fast induced a sixfold increase in plasma NEFA in lean and obese rats, the latter showing NEFA concentrations that were twofold higher than those of their lean counterparts. In both lean and obese animals, refeeding abolished the fasting-induced increase in plasma NEFA concentrations, which remained stable and similar in both strains throughout the refeeding period. Obese Zucker rats were hypertriglyceridaemic compared with their lean counterparts at all times studied (Fig. 1). Triglyceridaemia was reduced by fasting and increased progressively with refeeding in both strains. By 24 h after the beginning of refeeding, all plasma variables had returned to levels observed in the freely fed state in both lean and obese rats.

Table 1. Cumulative food consumption (g) during the last 24 h before death in lean and obese Zucker rats

	Lean *(Fa/?)*	Obese *(fa/fa)*
Freely fed	18.5 ± 0.3	32.0 ± 3.1[a]
Food deprived 24 h (FD 24)	–	–
FD 24 + 1 h refeeding	3.2 ± 0.6	3.8 ± 0.6
FD 24 + 3 h refeeding	5.2 ± 0.2	5.5 ± 0.7
FD 24 + 6 h refeeding	9.2 ± 0.2	11.3 ± 1.0
FD 24 + 12 h refeeding	14.3 ± 0.7	16.7 ± 1.6
FD 24 + 24 h refeeding	19.7 ± 0.6	27.7 ± 1.4[a]

The freely fed group was not food-deprived.
[a] Different from lean animals, $p < 0.05$. Value are means ± SEM ($n = 4$)

In all three WAT depots harvested in the freely fed state, LPL activity of obese rats was at least twice as high as that of lean rats (Fig. 2). At 24 h after food removal, LPL activity was similar in both strains in the retroperitoneal depot (Fig. 2) whereas in the epididymal and inguinal depots, LPL was modestly but significantly higher in obese than in lean animals (Fig. 2). After the 24-h fasting period, refeeding resulted in a rapid (within 1 h) increase in LPL activity in all adipose depots of obese rats ($p < 0.0001$ compared with fasting values in all depots) and enzyme activity remained relatively constant until 6 h after the onset of refeeding. After 12 h of refeeding, LPL activity in WAT of obese rats had returned to that of the freely fed state in the inguinal and retroperitoneal depots, whereas longer than 12 h of refeeding was

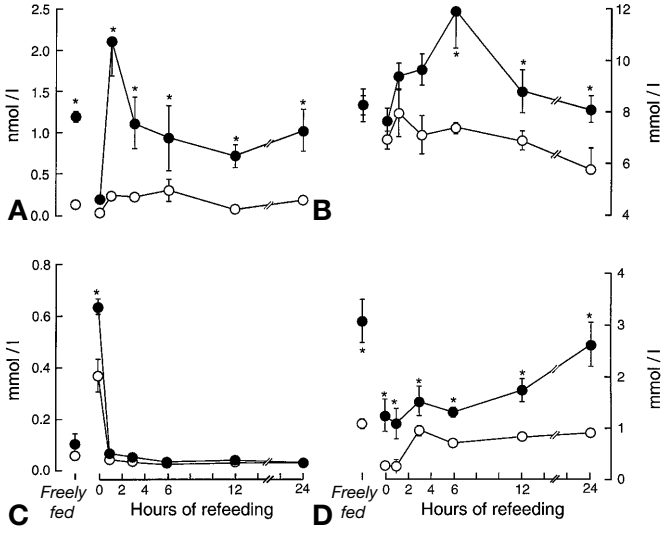

Fig. 1 A–D. Plasma insulin (**A**), glucose (**B**), nonesterified fatty acids (**C**) and triglycerides (**D**) in lean and obese Zucker rats killed either before (freely fed) or after a 24-h fast (0 h of refeeding) or at different times after subsequent refeeding. Symbols represent means ± SEM of four animals. * Different from lean rats ($p < 0.05$). ○ Lean *Fa/?*, ● obese *fa/fa*

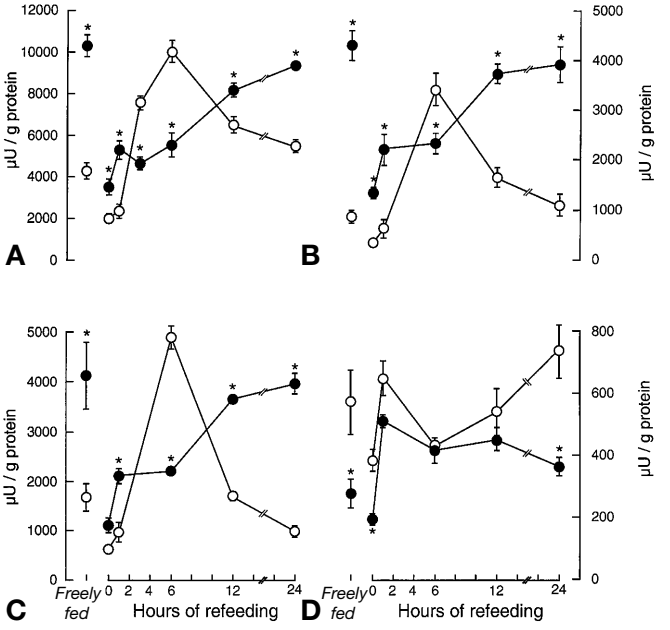

Fig. 2 A–D. Specific activity of lipoprotein lipase in epididymal (**A**), inguinal (**B**) and retroperitoneal (**C**) white adipose depots and in interscapular brown (**D**) adipose tissue of lean and obese Zucker rats killed either before (freely fed) or after a 24-h fast (0 h of refeeding) or at different times after subsequent refeeding. Symbols represent means ± SEM of four animals. * Different from lean rats ($p < 0.05$). ○ Lean *Fa/?*, ● obese *fa/fa*

necessary for epididymal LPL to reach values measured in the freely fed state. In lean animals, WAT LPL activity after 1 h of refeeding was similar to fasting values. By 3 h after the beginning of refeeding, a large increase was observed in the epididymal depot and LPL activity peaked at 6 h in all three depots. At this time point, LPL activity was at least 500% higher than that observed after 24 h of food deprivation and at least twofold larger than that measured in the freely fed state. In WAT, LPL activity decreased progressively after the sixth h of refeeding, to reach freely fed values after 24 h of refeeding. In BAT, LPL activity responded to the feeding status similarly in both lean and obese animals, in the face of an overall lower enzyme activity in obese than in lean rats (Fig. 2). The BAT LPL was twofold higher in lean than in obese rats in the freely fed and fasted states as well as after 24 h of refeeding. Within the first h of the refeeding period, BAT LPL increased by 60% in lean and 160% in obese animals. During the course of the refeeding period, no difference between strains was observed in BAT LPL activity until the 24th h of refeeding.

In the soleus, vastus lateralis and heart muscles, LPL activity was 1.5 to 2-fold lower in obese than in lean rats as measured in the freely fed state (Fig. 3). Fasting for 24 h increased LPL activity in the soleus and the heart of both strains ($p < 0.03$), whereas a similar but non-statistically significant tendency was noted in the vastus lateralis muscle. Fasting LPL activity remained generally higher in muscles of lean animals than in those of obese rats. In soleus and vastus lateralis muscles, 1 h of refeeding resulted in rapid, opposite changes according to phenotype. Lipoprotein lipase activity of lean animals increased more than 50% in both skeletal muscles ($p < 0.0001$) whereas that of obese rats decreased by 50% ($p < 0.02$), to levels that were not different from those observed in the freely fed state. In lean rats, LPL activity decreased progressively from 3 h after the beginning of refeeding but did not reach free feeding levels until the 12th h of refeeding. Skeletal muscle LPL activity in obese animals remained at low levels throughout the refeeding period. In the heart, food intake decreased LPL activity of both lean and obese rats in a similar pattern, although slightly more so in lean than in obese animals (Fig. 3).

Effects of the β-adrenergic antagonist propranolol on the LPL response to refeeding after 24 h of food deprivation. During the 1-h refeeding period, lean and obese rats spontaneously ingested approximately 5 g of food and before treatment with propranolol had no effect on food intake in either strain (data not shown). As in the first study described above, 1 h of refeeding resulted in a larger increase in insulinaemia in obese than in lean rats and in a pronounced decrease in plasma NEFA concentrations in both lean and obese animals (Table 2). Glucose concentrations of lean and obese rats were unchanged after 1 h of refeeding. In lean rats, refeeding had no effect on trigly-

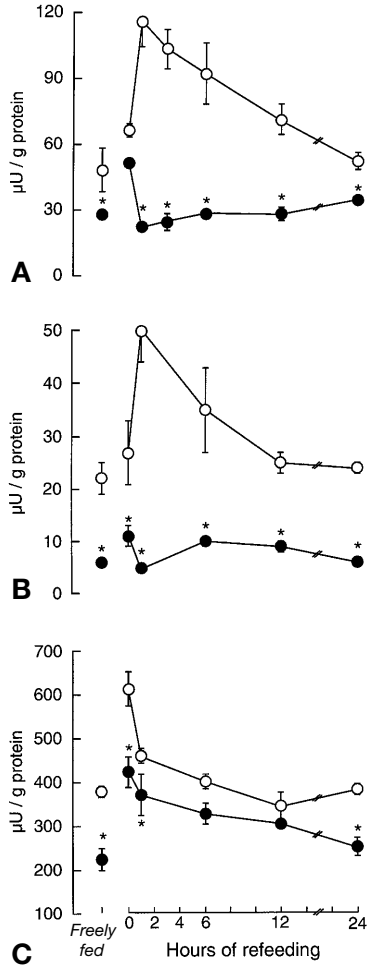

Fig. 3 A–C. Specific activity of lipoprotein lipase in soleus (**A**) and vastus lateralis (**B**) skeletal muscles and in the heart (**C**) of lean and obese Zucker rats killed either before (freely fed) or after a 24-h fast (0 h of refeeding) or at different times after subsequent refeeding. Symbols represent means ± SEM of four animals. * Different from lean rats ($p < 0.05$). ○ Lean *Fa/?*, ● obese *fa/fa*

ceridaemia whereas the latter was decreased in obese animals. Glycaemia was the only plasma variable to be statistically significantly altered by propranolol treatment, which increased blood glucose in both lean and obese animals.

After 24 h of fasting, WAT LPL activity of lean and obese rats was not significantly different from each other in the epididymal and the retroperitoneal depots (Fig. 4). In the subcutaneous inguinal depot, LPL activity of obese rats remained higher ($p < 0.003$) than that of lean animals (Fig. 4). As in the time-course study described above, 1 h of refeeding increased WAT LPL in obese rats in the three depots studied ($p < 0.0001$) whereas it had no effect on WAT LPL of lean animals. In the latter group, propranolol injection resulted in a pronounced increase in LPL activity in the epididymal (+ 140 %), retroperitoneal (+ 80 %) and inguinal (+ 106 %) depots, whereas WAT LPL of obese rats was not altered by the treatment. After 24 h of food deprivation, BAT LPL activity was lower ($p < 0.05$) in obese than in lean rats (Fig. 4). Refeeding increased LPL activity of BAT in both lean and obese animals, although slightly more so in the latter group. Propranolol abolished the food intake-induced increase in BAT LPL in lean rats but had no effect in the obese animals.

LPL activity in soleus, vastus lateralis and gastrocnemius was similar in lean and obese rats after food deprivation (Fig. 5). As seen in the time-course study described above, 1 h of refeeding lean rats resulted in a strong increase in LPL specific activity in the soleus (+ 31 %, $p < 0.03$), vastus lateralis (+ 123 %, $p < 0.0001$) and gastrocnemius (+ 44 %, $p < 0.001$). In contrast, 1 h of refeeding obese rats induced a reduction in LPL activity in the three skeletal muscles harvested (–30 to –50 %, $p < 0.01$ in soleus and gastrocnemius, $p < 0.04$ in vastus lateralis). Giving propranolol to lean rats totally prevented the food intake-mediated increase in LPL activity in all skeletal muscles whereas it had no effect in obese animals. In the heart, LPL specific activity of lean rats was higher ($p < 0.003$) than that of obese animals after 24 h of fasting (Fig. 5). Refeeding decreased heart LPL activity in lean rats only and this reduction was not affected by propranolol. Neither did the drug exert any notable effect on heart LPL in obese animals.

Table 2. Plasma glucose, insulin, non-esterified fatty acids (NEFA) and triglycerides in lean and obese Zucker rats killed either after a 24-h fast or 1 h of subsequent refeeding with or without prior i.p. injection of propranolol (Prop)

	Lean *Fa/?*			Obese *fa/fa*		
	Fasted	Refed + Saline	Refed + Prop	Fasted	Refed + Saline	Refed + Prop
Glucose (mmol/l)	6.6 ± 0.9^a	7.0 ± 0.2^a	8.2 ± 0.4^a	10.9 ± 0.8^b	12.1 ± 1.1^b	15.7 ± 1.0^c
Insulin (nmol/l)	0.07 ± 0.02^a	0.41 ± 0.10^b	0.57 ± 0.13^b	0.33 ± 0.02^b	2.61 ± 0.57^c	2.75 ± 0.60^c
NEFA (mmol/l)	0.37 ± 0.02^a	0.06 ± 0.01^b	0.10 ± 0.02^b	0.67 ± 0.03^c	0.10 ± 0.01^b	0.11 ± 0.01^b
Triglycerides (mmol/l)	0.48 ± 0.06^a	0.47 ± 0.08^a	0.78 ± 0.25^a	1.83 ± 0.34^b	0.87 ± 0.07^c	1.10 ± 0.17^c

Values are means ± SEM of five animals. Means not sharing a common superscript are different from each other ($p < 0.05$)

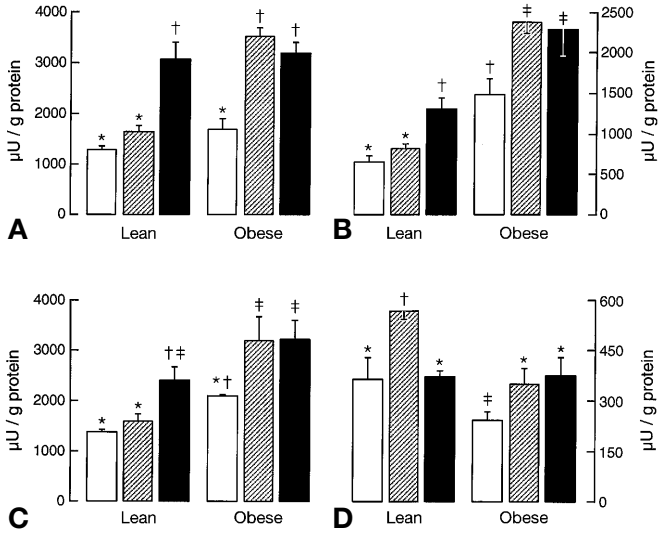

Fig. 4 A–D. Specific activity of lipoprotein lipase in epididymal (**A**), inguinal (**B**) and retroperitoneal (**C**) white adipose depots and in interscapular brown (**D**) adipose tissue of lean and obese Zucker rats killed either after a 24-h fast or 1 h of subsequent refeeding with or without prior i.p. injection of propranolol (25 mg/kg). Bars represent means ± SEM of five animals. Bars not sharing a common symbol are significantly different from each other at $p < 0.05$. □ fasted, ▨ 1 h refed + saline, ■ 1 h refed + propranolol

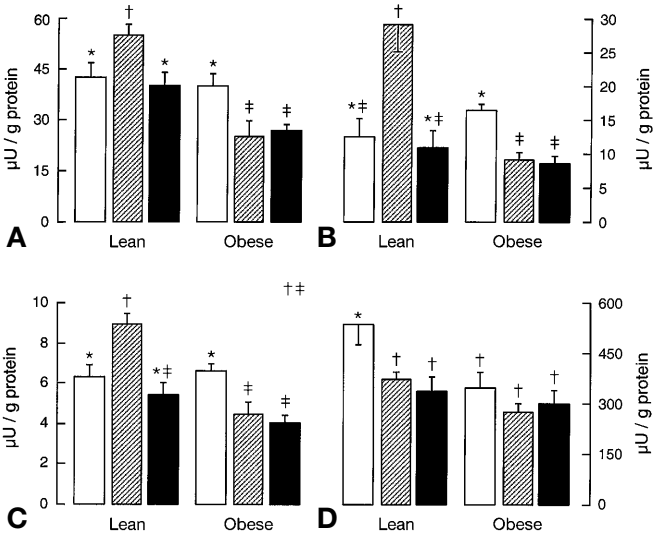

Fig. 5 A–D. Specific activity of lipoprotein lipase in soleus (**A**) vastus lateralis (**B**) skeletal muscles gastrocnemius (**C**) and in the heart (**D**) of lean and obese Zucker rats killed either after a 24-h fast or 1 h subsequent to refeeding with or without prior i.p. injection of propranolol (25 mg/kg). Bars represent means ± SEM of five animals. Bars not sharing a common symbol are significantly different from each other at $p < 0.05$. □ fasted, ▨ 1 h refed + saline, ■ 1 h refed + propranolol

Discussion

Our studies led to the identification in the Zucker rat of three characteristics of the response of LPL to food intake after prolonged fasting, which differ from the classic postprandial behaviour of the enzyme. Firstly, the early postprandial modulation of adipose and muscle LPL by insulin is transiently counteracted in lean rats by the activation of the SEP, the magnitude of which could be related to the duration of food deprivation that precedes refeeding. Secondly, in obese rats, the β-adrenergic pathway is defective in WAT as well as in muscle, allowing the typical, tissue-specific postprandial modulation of LPL by insulin. Thirdly, the early modulation upon refeeding of LPL activity in WAT of the obese Zucker rat is transiently resistant to the action of insulin.

Food deprivation and refeeding induce large tissue-specific changes in the availability of active LPL. Studies in rodents have shown that, in WAT, fasting reduces the active portion of LPL without affecting the synthesis or total mass of the protein and that feeding is associated with the activation of an inactive LPL pool [34]. In congruence with the effects of fasting and feeding, insulin increases WAT LPL through post-translational mechanisms that are incompletely defined but which are independent of transcriptional events [19, 35] for at least 8 h after exposure to insulin [36–38]. In WAT, it is thought that insulin participates in the release of active LPL, the activation of an inactive pool of enzyme present in the fasted state and the prevention of LPL degradation [34, 39, 40]. The activity of LPL assessed here therefore constitutes the major modulated variable in the present conditions. The similarity between the effects of the nutritional status and insulin on LPL modulation is not coincidental because we have shown that the tissue-specific changes in LPL activity brought by acute food intake after a 12-h fast are entirely dependent upon the postprandial excursions of insulinaemia [5]. In muscle tissues, fasting results in increased LPL activity [4, 41], as confirmed here, through mechanisms that are still not clear [42, 43]. Because insulin plays a critical part in the decrease in muscle LPL in response to food intake after a short-term fast [5], it can be suggested that increased muscle LPL activity in the fasted state is a consequence of the low prevailing insulinaemia.

In lean rats subjected to a mild fasting protocol such as food deprivation for 12 h during the lighted period, refeeding induces within 1 h an insulin-dependent increase in WAT and BAT LPL activity and a decrease in muscle LPL [5]. In the present study, despite an increase in insulinaemia that was similar to that in other studies conducted in the fasting-feeding transition [3, 5], WAT LPL in lean rats subjected to a 24-h fast remained unaltered after 1 h of refeeding. Such a lack of an early response under similar conditions has been reported previously [44], and it contrasts with the very early stimulation of WAT LPL after shorter-term fasting [5]. Propranolol, a non-specific β-adrenergic antagonist, restored the ha-

bitual increase in WAT LPL activity 1 h after refeeding. It is well established that in lean rats long-term treatment with β-adrenergic agonists, such as isoproterenol, lowers adipose and increases muscle LPL activity [6, 8]. The present findings extend the physiological significance of these effects by showing the existence of a short-term role of the SEP in the tissue-specific postprandial response of LPL to food intake after a relatively long period of food deprivation. The effect of the SEP on adipose LPL in lean rats was probably short-lived, as it seemed to be no longer present after 3 h of refeeding and beyond, at least in WAT. By 6 h into refeeding, WAT LPL activity increased beyond levels measured in free feeding conditions, perhaps because of the recruitment of a larger inactive enzyme pool that had accumulated during long-term fasting.

Muscle LPL activity in lean rats rapidly increased with refeeding after 24 h of food deprivation, in contrast with the reduction in LPL activity brought by food intake after a shorter-term fast [5, 41]. A similar observation has been reported previously [43] but the mechanisms of this response were not explored. Disappearance of the feeding-induced rise in muscle LPL by propranolol observed here shows that activation of the SEP was responsible for the increase in skeletal muscle LPL of lean rats to refeeding after prolonged food deprivation. This finding is consistent with food intake increasing sympathetic activity in muscle [45] and treatment of lean fed rats with β-adrenergic agonists increasing muscle LPL [6, 8]. It can therefore be concluded that, as in WAT, refeeding after prolonged fasting is associated with a strong activation of the sympathetic drive to skeletal muscle, which transiently overwhelms the action of insulin on muscle LPL. As to BAT, giving propranolol to lean rats showed that the early increase in LPL was partly mediated by the β-adrenergic component of the SEP. Because both the SEP and insulin increase BAT LPL activity [46, 47], the LPL increase upon refeeding in lean rats was the result of the combined effects of β-adrenergic stimulation and high insulinaemia. Also of note is that heart LPL seemed not to be modulated short-term by the SEP in either lean or obese rats, in agreement with earlier findings in lean rats [6] and its decrease following refeeding was probably due to the concomitant rise in insulinaemia [5].

The response of tissue LPL to refeeding in obese rats differed from that of their lean counterparts in two major ways. Firstly, adipose and muscle LPL of obese animals did not respond to β-adrenergic blockade. The SEP has been reported to be defective in obese humans [48] and in genetic models of obesity [26, 49]. Given that obese Zucker rats produce less catecholamines in response to stress [23, 50], whereas their adipose tissues are able to respond to β-adrenergic agonists [51], it is likely that the absence of effect of propranolol in the obese group was due to an impaired activation of the SEP upon refeeding. Subnormal sympathetic activity has been described in BAT of obese animals [26], which is supported by the lack of effect of propranolol on BAT LPL in our study. Our findings constitute direct evidence that such a defect extends to WAT and skeletal muscle. In the absence of the influence of the SEP in obese animals, the early (1 h) increase in WAT and BAT LPL and the decrease in muscle LPL activity in response to refeeding was probably the consequence of insulin action, as is the case in lean animals refed after short-term fasting [5].

The presence of a transient resistance to the short-term action of insulin constitutes the second germane characteristic of the adipose LPL response to refeeding that distinguishes obese Zucker rats from their lean counterparts. Our findings suggest that the early postprandial modulation of adipose tissue LPL activity by insulin occurs in sequential steps, of which some appear to be insulin resistant in obese rats. An initial, rapid (within 1 h) increase in adipose LPL activity was observed with refeeding in all three WAT depots of obese rats. Adipose LPL activity in obese rats then remained at that level over 6 h after the onset of food intake and reached pre-fasting levels not earlier than 12 h after the onset of refeeding. This contrasts with the considerable increase in WAT LPL observed during this period in lean rats (500 % in lean vs 200 % in obese above fasting at 6 h). Although the exact nature of the post-translational events that lead to such an LPL activity profile are still to be established, it is clear that some insulin-modulated process of recruitment of active LPL in response to food intake is defective in obese Zucker rats. Worthy of note is that a similarly abnormal postprandial modulation of tissue LPL has been observed in preliminary studies with Sprague Dawley rats with diet-induced insulin resistance (Picard F, Deshaies Y, unpublished observations). Therefore, the abnormalities of LPL modulation observed here are not specific to the Zucker rat but appear instead to be more generally characteristic of an insulin-resistant state. Two studies in obese humans have reported such an altered modulation of adipose LPL in response to insulin infusion [18] or to a high-carbohydrate, insulinogenic meal [19]. In the obese Zucker rat, other adipose enzymes such as hormone-sensitive lipase seem to remain sensitive to the postprandial rise in insulin, as witnessed by the large postprandial decrease in plasma NEFA. It is also important to note that adipose LPL of obese Zucker rats had nearly returned to the freely fed levels after 12 h of refeeding, which confirms that the enzyme retains the capacity to respond to long-term hyperinsulinaemia [52], albeit in a delayed fashion.

Muscle LPL in the obese rats seemed to remain sensitive to insulin because in absence of activation

of the SEP it decreased in response to refeeding. Whether full sensitivity to insulin was maintained could not be established in the present conditions. In the heart, in which the β-component of the SEP did not modulate LPL activity, the initial decrease in LPL upon refeeding was, however, somewhat less pronounced in obese than in lean animals, suggesting a less efficient response to increased insulinaemia. Finally, in BAT, given the lack of effect of propranolol in obese rats, postprandial hyperinsulinaemia was probably the sole modulator of the increase in LPL activity.

The abnormalities in the modulation of LPL observed in the obese, insulin-resistant Zucker rats have potential implications in lipid metabolism. Transient resistance to the insulin-mediated postprandial activation of adipose LPL would tend to impair the clearance of plasma triglycerides [53, 54] and to contribute to the atherogenic potential of postprandial lipaemia [55], particularly in the face of the persistence of a postprandial decrease in muscle LPL activity. In addition, an insulin resistance-related defect in the activation of the β-adrenergic component of the SEP in adipose tissue and muscle could profoundly affect lipid trafficking and metabolism in conditions such as stress and exercise, which normally solicit the SEP for extended periods of time.

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