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Increased lipoprotein lipase content in the adipose tissue of suckling and weaning obese Zucker rats

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The aim of this study was to determine whether the increase in lipoprotein lipase activity displayed by the adipose tissue of obese (*fa/fa*) rats as compared with that of lean (*Fa/fa*) rats could be ascribed to a change in the content or in the catalytic properties of the enzyme. The question was addressed in rats of two ages: in 7-day-old suckling and in 30-day-old post-weaning pups. Inguinal fat-pads were removed surgically (7 days of age) or after killing (30 days of age), and acetone-extract powders were prepared. The relative quantity of enzyme was assessed by immunotitration using an antiserum raised in goat against purified lipoprotein lipase from rat adipose tissue. The results indicate that increases in enzyme activity in obese animals were strictly paralleled by increases in the amount of enzyme in suckling as well as in post-weaning pups. Moreover, the apparent K_m values of lipoprotein lipase for its substrate triacylglycerol were identical in the two genotypes. In conclusion, the genotype-mediated increase in lipoprotein lipase activity in adipose tissue of obese Zucker rats was fully accounted for by an increase in the content of the enzyme. In addition, this work documents the mechanism of the increase in lipoprotein lipase activity during weaning, which is mediated mainly through changes in the adipose-tissue enzyme content.

INTRODUCTION

Adipose-tissue lipoprotein lipase (LPL), acting at the surface of the capillary endothelium, catalyses the hydrolysis of triacylglycerol from chylomicrons and very-low-density lipoproteins to non-esterified fatty acids which can then enter the adipocytes. This enzyme, whose activity is controlled by nutritional and hormonal (mainly insulin) factors, plays a critical role in the regulation of adipose-tissue mass. It has been well documented that the grossly overdeveloped adipose tissue of the adult obese Zucker rat exhibits a several-fold increase in lipoprotein lipase activity as compared with that of lean control rats [1,2]. It might be postulated that the increased LPL activity is simply brought about by the hyperinsulinaemia that develops in those rats after weaning [3], as there is much evidence that, in adipose tissue from normal rats, LPL activity is directly related to the plasma insulin concentrations [4,5]. However, work in our and other laboratories has shown that a significantly increased LPL activity was already present in the adipose tissue of obese pups in mid-suckling [2], and even as early as 7 days of age [6]. At this stage, obese pups are strictly normoinsulinaemic [7,8]. These two sets of observations suggested that differential mechanisms might underlie the increases in LPL activity in the mutant rat before and after the emergence of hyperinsulinaemia. Therefore, taking advantage of a recently developed antibody to purified LPL from rat adipose tissue [9], we have examined the changes in LPL activity and content in adipose tissue from obese pups under suckling (7 days of age) and post-weaning (30 days of age) conditions.

MATERIALS AND METHODS

Obese (*fa/fa*) Zucker rats and their lean (*Fa/fa*) littermates were bred in our laboratory from pairs originally provided by the Harriet G. Bird Memorial Laboratory, Stowe, MA, U.S.A. They were kept at 23 ± 1 °C in rooms exposed to light between 07:00 and 19:00 h and fed on a stock diet, containing (by wt.) 26.7% protein, 5.6% fat, 56.6% carbohydrate, 4.5% cellulose and 6.5% minerals (UAR, Epinay sur Orge, France). Known heterozygous (*Fa/fa*) lean females and obese (*fa/fa*) males were mated. From this mating 50% of the litter is expected to be obese and 50% lean and of the heterozygous genotype. We selected litters of 10–12 pups. Subcutaneous inguinal adipose tissue was removed from fed pups at 7 or 30 days of age. Since there is no rapid way to identify pup genotypes at 7 days of age, we surgically removed the whole right fat-pad under light diethyl ether anaesthesia, in order to keep the pups alive for later (6 weeks of age) genotype identification. The 30-day-old rats, weaned 2 days earlier, were killed by decapitation and identified on the basis of their inguinal fat-pad weight.

Tissues were weighed and homogenized (1 ml/50 mg) in ice-cold 0.15 M-NaCl containing 0.4% (w/v) bovine serum albumin as carrier protein and 2 i.u. of heparin/ml, and acetone/diethyl ether-dried powders were prepared. Tissues from 7-day-old rats were processed as described by Nilsson-Ehle *et al.* [10] for small tissue samples to avoid losses of enzyme protein. Acetone-dried powders were suspended in 5 mM-sodium veronal buffer (1.7 ml/10 mg) containing 1 M-glycerol and 0.15 M-NaCl, pH 7.4. A clear supernatant was obtained after centri-

Abbreviation used: LPL, lipoprotein lipase (EC 3.1.1.34).

fugation (3000 *g* for 15 min at 4 °C) and used without delay for LPL studies.

LPL activity was assayed as described by Nilsson-Ehle & Schotz [11], with as substrate serum-activated [^{14}C]oleic acid-labelled trioleylglycerol emulsified with distearoyl phosphatidylcholine. Incubations were carried out at 37 °C for 30 min. LPL activity was calculated as the difference between total and remaining lipolytic activity after the addition of 1 M-NaCl in the assay mixture (inhibition > 80 %).

The immunotitration studies were carried out by using an antiserum against purified LPL from rat adipose tissue, raised in goat as previously described [9]. This polyclonal antibody has been shown to recognize both the inactive precursor and the active forms of the enzyme [12]. A fixed amount of enzyme suspension (50 μl) was mixed with increasing amounts of soluble antiserum diluted in non-immune goat serum/NaCl (1:19, v/v) to a final volume of 50 μl and incubated for 1 h at 4 °C. The residual LPL activity was then assayed directly in the incubation tube by adding 100 μl of the substrate emulsion. In studies using non-soluble anti-LPL antibody, conditions under which there is competition between active and inactive forms of the enzyme [12], the IgG fraction, prepared by double precipitation with $(NH_4)_2SO_4$ and ion-exchange chromatography (on DEAE-cellulose DE52; Whatman) was coupled to agarose (Affi-gel 10; Bio-Rad). Immunotitration was then performed by incubating a fixed amount of enzyme with increasing amounts of immobilized antibody in final volume of 0.4 ml. After 1 h at 4 °C, the beads were sedimented by centrifugation (5000 *g* for 15 min at 4 °C), and the supernatants were used for the determinations of residual LPL activity.

For kinetic studies the substrate emulsions were serially diluted with 0.13 M-Tris/HCl buffer, pH 8.2, containing 2% (w/v) albumin, 16.6% (v/v) heat-inactivated rat serum and 20% (v/v) glycerol to give triacylglycerol concentrations in the range 5.6–0.5 mM. Apparent Michaelis constants were calculated from Lineweaver – Burk plots by using the method of least squares.

Statistical differences between the two genotypes were assessed by Student's *t* test.

RESULTS

Table 1 shows body weights, inguinal-fat-pad weights and adipose-tissue LPL activities in lean and obese Zucker pups at 7 days of age. There is clearly a great variability in the development of pups, as assessed by body and adipose-tissue weights, from one litter to another, even between litters identical in pup number. This is likely to be explained in a large part by lactating-performance differences between mothers. In the smallest pups (litters A and B), no difference between lean and obese littermates could be detected in either pad weight or LPL activity. In contrast, in litters C, D, E and F, where pups weighed 25–50% more than in litters A and B, both fat-pad weight and LPL activity were significantly increased in obese as compared with lean pups. It was not possible to measure the protein content of the tissue extracts used here for LPL determination, since serum albumin was included in their preparations. However, we have previously observed that the increase in fat-pad weight in 7-day-old obese pups was mainly due to fat accretion. At this age, the lipid content of inguinal fat-pads was shown to vary from 33% in lean pups to 50% in obese pups [6], with no significant change in fat-free tissue weight in the two genotypes. Accordingly, the protein content measured in the post-mitochondrial supernatants of inguinal-fat-pad homogenates has been consistently observed to be similar in lean and obese pups at 7 days of age: 0.90 mg/pad in lean and 1.04 mg in obese ($p > 0.10$) for pads weighing 36 mg and 49 mg in lean and obese pups respectively [13]. Therefore it can be estimated that the increase in LPL activity per mg of protein in obese compared with lean pups is equivalent to the increase in LPL per total tissue.

Table 1. Body weight, inguinal-adipose-tissue weight and LPL activity in 7-day-old Zucker rats

Values for individual litters (A–F) are given as means ± S.E.M., with the numbers of littermates for each genotype in parentheses: $*P < 0.05$, $**P < 0.01$ (Student's *t* test).

	Litters		Body wt. (g)	Fat-pad wt. (mg)	LPL activity (μmol/h per pad)
A	Lean	(4)	10.1±0.17	21±1.8	0.51±0.085
	Obese	(8)	10.1±0.13	21±1.8	0.47±0.078
B	Lean	(4)	9.6±0.37	15±1.2	0.83±0.029
	Obese	(5)	9.5±0.21	21±1.7	0.76±0.246
C	Lean	(5)	13.9±0.57	25±1.0	0.74±0.082
	Obese	(7)	14.3±0.35	37±3.3*	1.59±0.088**
D	Lean	(6)	12.5±0.28	34±2.4	0.71±0.066
	Obese	(5)	12.5±0.09	42±1.2*	1.00±0.070*
E	Lean	(3)	14.6±0.72	36±5.3	0.35±0.028
	Obese	(4)	14.7±0.19	52±4.8*	0.87±0.071**
F	Lean	(6)	12.4±0.29	25±1.8	0.45±0.075
	Obese	(3)	13.0±0.65	40±5.2*	0.88±0.162*

Although the nutritional status of pups was not assessed in the present study, a difference between the two genotypes is unlikely, since previous work from our laboratory has pointed out that no change in feeding habits could be detected between lean and obese pups at 7 days of age. In that work [6], stomach contents, measured in pups which had been kept away from their mothers for 3 h and then re-fed for 3 h, weighed 290 ± 23 mg in lean and 316 ± 44 mg in obese pups ($P > 0.1$). These data were obtained from 13 lean and 8 obese rats, whose fat-pads weighed 29 ± 1.4 mg and 59 ± 6.1 mg respectively. In addition, the same study showed that milk intake, as estimated by an isotope-dilution technique, was identical in 5-day-old lean and obese pups.

Table 2 shows that the *fa/fa* genotype had a dramatic effect on both adipose-tissue weight and LPL activity in post-weaning rats, where 7- and 11-fold increases over the lean values were observed respectively. At 30 days of age, inguinal fat-pads from obese rats contain twice as much protein as those from lean rats [7]. Therefore, it can be estimated that adipose-tissue LPL specific activity in obese rats is increased 5-fold over the lean values.

Table 2. Adipose-tissue weight and LPL activity in 30-day-old Zucker rats

Results are means ± S.E.M., with the numbers of animals in parentheses: ***$P < 0.001$ (Student's t test).

Genotype		Fat-pad wt. (mg)	LPL activity (μmol/h per pad)
Lean	(7)	200 ± 27	4.24 ± 1.78
Obese	(8)	1344 ± 193***	46.48 ± 10.04***

From Tables 1 and 2 it is clear that developmental and dietary changes that occur between 7 and 30 days of age are associated with an increase in adipose-tissue LPL activity, largely genotype-dependent: whereas the enzyme activity per tissue (average of 28 lean and 32 obese pups in Table 1) was multiplied 7-fold in lean rats, it was multiplied 50-fold in obese rats.

To gain insight into the mechanism underlying the increase in LPL activity in the adipose tissue of the mutant rats, we carried out immunotitration of the enzyme in both lean and obese pups under suckling (pups of litter C) and post-weaning conditions. The immunotitration curves, illustrated in Fig. 1, show that the amount of enzyme activity neutralized was proportional to the amount of soluble antibody added, in all groups of rats. By extrapolation of the plots to zero enzyme activity, the amount of anti-LPL serum required to inactivate 1 unit of enzyme activity could be calculated. The results summarized in Table 3 demonstrate that there was no significant difference between lean and obese pups under both suckling and post-weaning conditions, indicating that the catalytic efficiency per active LPL molecule was similar irrespective of the genotype of the pups. Therefore the differences between lean and obese animals in lipoprotein lipase content per inguinal tissue, as estimated by the amount of antibody required to inactivate the total tissue activity, were strictly parallel to the differences in enzyme activity. Previous studies have shown that LPL is synthesized in an inactive form that is processed into the catalytically active form [12]. This inactive form cannot be detected with the soluble antibody, but competes with the active form when immobilized antibody is used [12]. Immunotitration experiments performed with immobilized anti-

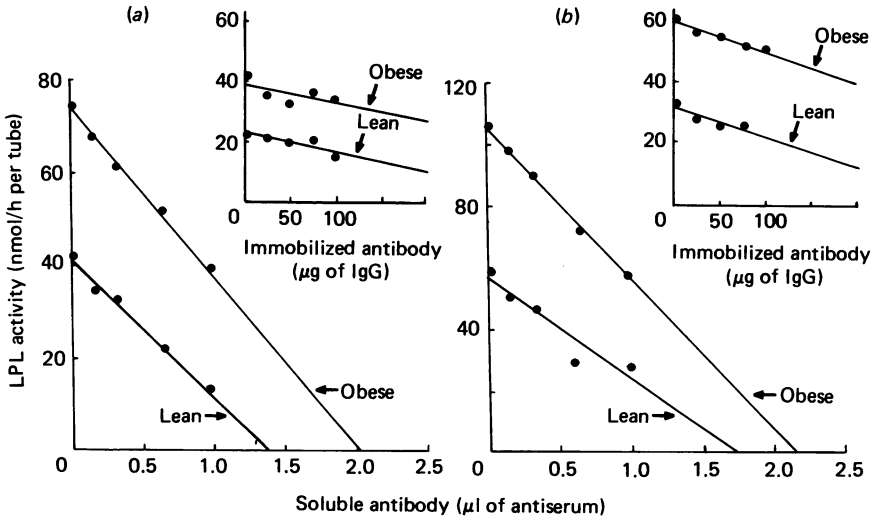

Fig. 1. Immunotitration curves of LPL in acetone/ether extracts of inguinal adipose tissue (*a*) from 7-day-old and (*b*) from 30-day-old lean and obese littermates

Immunotitration assays were performed as described in the Materials and methods section. Each plot is representative of one rat in each group. Linear extrapolation of the plot to the abscissa (soluble antibody) gives the end point of the titration used to calculate the equivalence point, in μl/unit (see Table 3). The insets show immunotitration curves obtained with immobilized antibody as described in the Materials and methods section. Panel (*a*), pups were those of litter F (Table 1). Panel (*b*), plots are representative of one from two rats in each group.

Table 3. Amount of antiserum required to inactivate LPL activity in lean and obese rats

Results are means ± S.E.M., with the numbers of pups in parentheses (1 unit of LPL activity represents 1 nmol of non-esterified fatty acids released/h): $*P < 0.05$, $***P < 0.001$, lean versus obese; $\dagger P < 0.05$, 30-day-old versus 7-day-old rats (Student's *t* test). The 7-day-old pups were those of litter C, described in Table 1. The 30-day-old pups were the same as in Table 2.

		Rats	
Age	Antiserum	Lean	Obese
7 days	μl/unit	0.043 ± 0.007 (5)	0.034 ± 0.004 (7)
	μl/fat-pad	30.1 ± 4.8 (5)*	53.0 ± 5.6 (7)
30 days	μl/unit	0.029 ± 0.004 (7)	0.023 ± 0.002 (8)†
	μl/fat-pad	113 ± 23 (7)†***	992 ± 172 (8)†

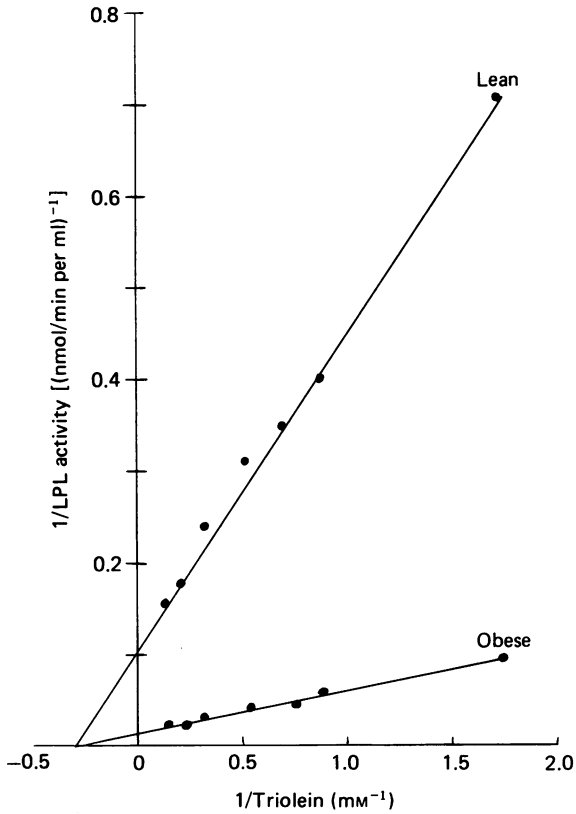

Fig. 2. Lineweaver–Burk plots of LPL activity in adipose tissue from 30-day-old lean and obese Zucker rats

Triacylglycerol emulsions were serially diluted as described in the Materials and methods section. Each point represents an average value calculated from duplicate assays. The plots are representative of one rat in each group.

LPL (Fig. 1 insets) in adipose-tissue extracts from 7-day-old rats (pups of litter F) and 30-day-old animals show that immunotitration lines were parallel in lean and obese rats. This indicates that the ratio of enzymically active to immunologically active but enzymically inactive LPL molecules was not changed in the obese rats. Taken together, these results strongly suggested that the genotype effect on adipose-tissue LPL activity was due to an effect on the amount of enzyme protein, and not to activation of a constant amount of the enzyme or to a change in the catalytic properties of the enzyme. Further verification of this latter conclusion was obtained by the measurement of Michaelis constants for the enzyme substrate triolein, in adipose-tissue acetone/ether-dried powders from lean and obese post-weaning pups, as shown in Fig. 2. No difference in apparent K_m values was observed between the two genotypes: 2.7 nM- and 2.9 nM-triolein for lean and obese respectively ($P > 0.1$; four experiments).

This work provides information also on the mechanism responsible for the striking increases in LPL activity observed in adipose tissue after weaning. The 6-fold and 29-fold rises in enzyme activity that occurred in lean and obese pups respectively from 7 days of age (pups of litter C) to 30 days of age were associated with about 4-fold and 20-fold rises in enzyme content. Table 3 shows that there was a non-negligible decrease in equivalence points for the enzyme between 7 and 30 days of age, although the difference reached statistical significance only in obese rats. That indicates that the large increase in enzyme activity between suckling and weaning might be contributed to, in addition to the change in the amount of enzyme playing the main role, by an increased catalytic efficiency of the enzyme.

DISCUSSION

It is now well established that LPL plays an important role in the regulation of adipose-tissue mass and is tightly controlled by nutritional conditions. The present work shows, in agreement with previous studies [14,15], that adipose-tissue LPL activity increases manyfold from suckling to weaning. The combined increases in carbohydrate intake and blood insulin concentrations that occur at weaning are likely to be the triggering factors of the increase in LPL activity, since the presence of both insulin and glucose has been shown to be crucial to achieve maximal induction of LPL activity in cultured 3T3-L1 cells [16]. By using an antiserum to LPL, we were able here to document for the first time the mechanism of the effect of weaning on this enzyme. Our data provide evidence that the increase in LPL activity is mediated mainly by an increase in enzyme content. The same mechanism has been reported for the nutritional regulation of LPL activity during the fasting/re-feeding paradigm in adipose tissue [17].

In agreement with previous reports [1,2,6], our present data show that adipose-tissue LPL activity is also under the control of genetic factors, as mutant *fa/fa* rats exhibit a high activity as compared with their lean littermates. The elucidation of the mechanism underlying

this increased LPL activity in the adipose tissue of obese pups is of critical importance to an understanding of the biochemical lesion of this genetic disorder. The immunotitration studies reported here disclosed that the tissue content of LPL increased strictly in parallel with augmentation of enzyme activity. Altered rates of conversion of inactive into active form(s) of the enzyme, or altered catalytic properties of the enzyme, could not be detected in the mutant rat. Therefore the present work supports the conclusion that the rise in LPL activity in the obese rat is due to an elevated quantity of LPL.

At 30 days of age, obese rats are frankly hyperinsulinaemic. Therefore, in these animals an increased LPL content could be expected, from the previously documented regulatory role of insulin on LPL synthesis in adipose tissue of normal rats [18,19]. In contrast, in 7-day-old pups, insulin cannot be invoked to explain the increased LPL content, since it has never been possible to observe any change in blood insulin concentrations in suckling obese pups before 17 days of age [7]. Thus Zucker & Antoniades [3] in 2-week-old rats, Bazin & Lavau [7] in 7-, 9-, 14- and 16-day-old rats, and Turkenkopf *et al.* [8] in 5-, 7-, 10- and 15-day-old rats, could not detect any difference in insulinaemia between lean and obese pups. That suggests that the increase in LPL content is one of the primary expressions of the *fa* gene not depending on hyperinsulinaemia to develop. However, the observation made in the present study, that increased LPL activity was not consistently present in obese pups by 7 days of age, especially in those pups whose growth was delayed, presumably by inadequate milk supply, points out the role of nutrient factors in the timing of the expression of this phenotypic trait. The same phenomenon has been shown to occur for adipose-tissue fatty acid synthetase, whose activity was also increased by 7 days of age in obese pups, characterized by enlarged fat-pads, as compared with their lean littermates [13]. Previously we showed a markedly increased efficiency of insulin to stimulate glucose utilization at very low insulin concentrations [20], as well as a markedly increased pool of intracellular glucose transporters [21] in adipocytes of 30-day-old obese rats. These abnormalities, if already present in 7-day-old obese pups, could provide an explanation for the higher LPL content in adipose tissue through the stimulatory effect of glycolytic metabolites on LPL synthesis [16]. The roles of enhanced synthesis rates and/or decreased catabolic rates in the increase in LPL content in the adipose tissue of the obese rat remain to be established.

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