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Effects of fasting on lipoprotein lipase activity in different depots of white and brown adipose tissues in diet-induced overweight rats

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The aim of the present study was to evaluate the effects of 24 hours of starvation on lipoprotein lipase (LPL) activity in various depots of white and brown adipose tissues in control rats and in rats with two different degrees of overweight, both induced by dietary treatment. In control rats, no changes in LPL immunoreactive mass were observed in either white or brown adipose tissues after fasting, whereas the effects of food deprivation on enzyme activity were opposite in white versus brown adipose tissues. The LPL activity response to fasting was impaired by obesity: White adipose depots of cafeteria obese rats showed a lower ability to downregulate LPL during fasting and the increased LPL activity induced by fasting in brown adipose depots was less intense in the obese rats compared with control animals. When the degree of overweight was reduced, the differences between obese and control rats were also attenuated. (J. Nutr. Biochem. 10:609–614, 1999)

Keywords: lipoprotein lipase; fasting; obesity; white and brown adipose tissues

Introduction

Lipoprotein lipase (LPL; E.C. 3.1.1.34) is the enzyme responsible for the hydrolysis of triglycerides from plasma lipoproteins, mainly chylomicrons and very low density lipoproteins. The released fatty acids are taken up by tissues and either stored, as in the white adipose tissue (WAT), or oxidized, as in heart and brown adipose tissue (BAT). Therefore, LPL controls the lipid deposition in WAT[1] and the supply of fatty acids to BAT for heat production.[2]

The activity of LPL is known to be influenced by nutritional and hormonal status and by environmental conditions,[3–5] the response of the enzyme being tissue specific, a fact that is physiologically important because it directs fatty acid use according to the metabolic demands of individual tissues. Thus, fasting results in a reduction in LPL activity in WAT and an increase in heart.[6] As for BAT, reports show both decreases[7–9] and increases[4,10] in LPL activity with fasting, so the question about the effects of fasting in this tissue remains.

Because of its ability to make free fatty acids available to adipose tissues, LPL has long been thought to play a role in the initiation and development of obesity. LPL activity is markedly elevated in the adipose tissue of genetically obese rodents[11,12] and in obese humans.[13,14] More recently, variations of LPL activity in various adipose depots in humans were shown to correlate with regional differences in the development of obesity.[15]

In the present article, we present the results of a study on the effects of 24 hours of starvation on LPL activity in various depots of WAT and BAT, in control rats and in rats with two different degrees of overweight, both induced by dietary treatment. These two kinds of adipose tissues (white and brown) were chosen because they are differentially involved in energy balance and thus in body weight regulation: WAT takes up and stores fatty acids[14] and BAT, through its thermogenic activity, releases as heat the extra energy that is consumed.[16,17] Different depots of both WAT and BAT have been assessed to account for possible metabolic differences that may mediate the LPL responses to nutritional and physiologic changes.

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Table 1 Caloric and macronurient intake of control (250C and 320C), cafeteria-diet (CAF), and postcafeteria diet (PCF) rats on the day of sacrifice

Intake	Units	250C	CAF	320C	PCF
Energy	KJ/day	204 ± 12	519 ± 38*	203 ± 12	212 ± 21
Protein	g/day	3.65 ± 0.22	4.84 ± 0.34*	3.62 ± 0.38	3.79 ± 0.36
Lipid	g/day	0.673 ± 0.039	7.50 ± 0.62*	0.668 ± 0.071	0.698 ± 0.065
Carbohydrate	g/day	7.76 ± 0.46	6.44 ± 0.50	7.70 ± 0.81	8.06 ± 0.76

Note: Results are the mean ± SEM of six animals per group. Data were analyzed by Student's *t*-test.
Significant differences ($P < 0.05$):
*CAF versus 250C and PCF versus 320C.

We found a differential response of lipoprotein lipase activity in WAT and BAT in response to two different situations: 24 hours of starvation and overweight induced by dietary treatment.

Methods and materials

Animals, feeding schedules, and diets

Female Wistar rats aged 150 days with a starting weight of 268 ± 1 g and housed at 21°C with 12-hour light-dark cycle were used. All animals received drinking water containing a vitamin and mineral supplement (Vitachoc, Neosan Products, Barcelona, Spain) as previously described.[18]

To study the effects of diet-induced overweight on LPL activity, female rats were randomly divided into four dietary groups (ages and weights given are those at the time of sacrifice): **250C**: 250-day-old control rats (294 ± 6 g, $n = 25$); **CAF**: 250-day-old cafeteria-diet-fed obese rats (442 ± 7 g, $n = 22$); **320C**: 320-day-old control rats (300 ± 4 g, $n = 15$); **PCF**: 320-day-old postcafeteria rats (344 ± 6 g, $n = 17$). Until day 150, only standard diet (pelleted standard rate chow type A03 from Panlab, Barcelona, Spain) was provided to all animals. The cafeteria diet was offered to the CAF group from day 150 to day 250. Animals in group PCF were also fed the cafeteria diet from day 150 to day 250, but then were put on standard diet only until day 320. Groups 250C and 320C always received standard diet. In each dietary group, half of the animals were food deprived (leaving only free access to water) for 24 hours before experiments. [The percentage of weight loss was: 250C, 5.61 ± 0.28% ($n = 12$): CAF, 3.73 ± 0.26% ($n = 11$); 320C, 5.27 ± 0.26% ($n = 7$); PCF, 4.60 ± 0.25% (n = 8).]

The foodstuffs of the cafeteria diet commonly used in our laboratory have been previously reported.[18–20] The food intake (cafeteria and/or standard diets) was measured in six animals per experimental group from day 150 to sacrifice. Energy and macronutrient intake of control and overweight rats on the day of sacrifice is shown in *Table 1*. The diet self-selected by CAF rats was hypercaloric, hyperlipidic, isoglucidic, and slightly higher in proteins compared with the control diet.[21] The composition of the cafeteria diet and/or the standard diet taken up by control, PCF, and CAF rats at the day of the sacrifice was: 250C, 320C, and PCF rats, 12.4% lipid, 29.9% protein, and 59.6% carbohydrate; CAF rats, 54.7% lipid, 15.7% protein, and 19.6% carbohydrate.

For LPL immunoreactive mass determination, nine control rats of a mean age of 250 days and weighing approximately 300 g were used. Before sacrifice, five of the animals were fasted for 24 hours with access to water only.

On the day of sacrifice both control and diet-induced overweight rats were anesthetized with pentobarbital (40 mg/kg) and sacrificed by decapitation during the first 3 hours after starting the light cycle. Retroperitoneal (RP-WAT) and ovaric (O-WAT) white adipose tissues and interscapular (I-BAT) and perirenal-periaortic (P-BAT) brown adipose tissues were rapidly removed and measurements were performed on the same day on fresh homogenates as described below.

LPL activity measurement

To assay LPL activity, adipose tissues were homogenized at 4°C with 10 vol of buffer A (1 mM dithiothreitol, 1 mM EDTA, 0.25 M sucrose, 10 mM HEPES, 0.005% heparin, pH 7.5) in a Potter/Elvehjen homogenizer (Anorsa, Barcelona, Spain). The homogenates were centrifuged for 10 minutes at 2,000 rpm at 4°C, and the clear supernatant was used to determine enzyme activity[22,23] and protein content.[24]

One hundred microliters of glycerol tri[9,10(n)-^{3}H]oleate solution in toluene (0.66 μmol; 11 μCi/μmol) was added to a glass tube and dried under a stream of nitrogen-$_2$. One milliliter of buffer B [27.5 mM PIPES, 55.5 mM Cl_2Mg, 0.05% albumin (fatty acid-free), 3.3% (v/v) serum (preheated for 60 min at 50°C), pH 7.5] was added to the tube and sonicated (100W) for 4 or 5 × 30 seconds at 4°C. This was the substrate of the enzyme reaction.

Twenty microliters of the sample was mixed with either 20 μL of buffer A (without heparin) or with 20 μL 5 M NaCl in glass centrifuge tubes. This was performed in triplicate. Rat postheparin plasma (40 μL, (diluted 1:5 in buffer A without heparin) was used as an external standard. As a blank, 20 μL of buffer A was used instead of the supernatant. All tubes were preincubated for 5 minutes at 37°C.

After that, substrate (160 μL) was added to the tubes, and they were incubated for 30 minutes at 37°C. Assay incubations were terminated by the addition of 3 mL of a chloroform:methanol: heptane (1.41:1.25:1) solution, followed by 1 mL of 0.1 M K_2CO_3, 0.1M H_3BO_4 (pH 10.5). The tubes were shaken vigorously and then centrifuged for 10 minutes at 2,000 rpm at 4°C. An aliquot of the upper phase was transferred to a scintillation vial and 5 mL of scintillation cocktail (Optiphase 'Hisafe'II Pharmacia, Barcelona, Spain) was added. The radioactivity was measured in a Beckman (Madrid, Spain) scintillator counter.

LPL activity was defined as the NaCl inhabitable total lipase activity; that is, the difference in activity observed in triplicate samples in the presence or absence of NaCl. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol fatty acid/min.

Quantitative dot-blot immunoassay

Samples of adipose tissues (RP-WAT, O-WAT, I-BAT, and P-BAT) were homogenized and centrifuged the same as for determining LPL activity. To assay the LPL immunoreactive protein, each homogenate supernatant was suitably diluted in TS buffer (20 mM Tris, 500 mM NaCl, pH 7.5) to prepare solutions containing different amounts of protein as follows: RP-WAT and

Table 2 Lipoprotein lipase (LPL) mass and specific activity of retroperitoneal and ovaric white adipose tissues (RP-WAT and O-WAT) and interscapular and perirenal-periaortic brown adipose tissues (I-BAT and P-BAT) of fed and starved control rats

LPL	Units		RP-WAT	O-WAT	*ANOVA*	I-BAT	P-BAT	*ANOVA*
Mass	ng/g tissue	Fed	6.03 ± 1.22	6.00 ± 0.91		557 ± 39	1220 ± 197†	*T*
		Starved	6.81 ± 1.01	7.27 ± 1.84		456 ± 91	890 ± 270	
Specific activity	mU/ng enzyme	Fed	4.50 ± 0.66	4.68 ± 0.64	*S*	0.101 ± 0.012	0.060 ± 0.017	*S*
		Starved	1.80 ± 0.20*	1.56 ± 0.19*		0.236 ± 0.028*	0.183 ± 0.027*	

Note: Results are the mean ± SEM. The number of animals in each group was: fed, $n = 4$; starved, $n = 5$. One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol of fatty acid/min. Data were analyzed by Student's *t*-test and one- and two-way analysis of variance (ANOVA) as described in the Methods and materials section.
Significant differences ($P < 0.05$):
*Starved versus fed.
†O-WAT versus RP-WAT and P-BAT versus I-BAT.
ANOVA significances ($P < 0.05$): S–effect of starvation. T–effect of tissue depot.

O-WAT, 7.5, 12.5, and 17.5 μg/mL, and I-BAT and P-BAT, 2, 5, and 7.5 μg/mL.

The amount of LPL immunoreactive mass was determined using a 48-well slot format Bio-Dot SF microfiltration unit (BioRad Laboratories, Madrid, Spain) in which three sheets of filter paper (BioRad) were used for the membrane support. Before use, the nitrocellulose and paper sheets were soaked in TS buffer, then mounted in the apparatus.

LPL samples (200 μL) were applied in duplicate to individual wells and light pressure was applied to pull the liquid through the membrane. Then, the wells were washed with 200 μL of TS buffer. The apparatus was disassembled and the nitrocellulose sheet was incubated overnight with a blocking solution [TS/Tween buffer (50 mM Tris, 150 mM NaCl, 0.5% Tween 20, pH 7.5) containing 1% bovine serum albumin (BSA)] and washed three times in TS/Tween buffer (5 min). The nitrocellulose was then treated with a chicken polyclonal antibody raised against bovine LPL as previously described,[25] diluted 1,000-fold in TS/Tween buffer containing 0.1% BSA for 2 hours at room temperature. It was then washed three times in TS/Tween buffer (5 min). The nitrocellulose sheet was then treated for 1 hour with alkaline phosphatase-conjugated rabbit anti-chicken immunoglobulin G (IgG) diluted 5,000-fold in TS/Tween buffer containing 0.1% BSA. After washing three times as above, the final development was then carried out with an alkaline phosphatase conjugate substrate kit (BioRad) according to the instructions of the manufacturer. After that the membrane was rinsed with distilled water and dried at room temperature. For quantitative analysis, the membrane was scanned with a BioImage computing densitometer (Millipore, Bedford, MA USA). A standard curve was constructed for each membrane using purified bovine milk LPL (0–40 ng/well). The results were expressed as nanogram equivalent of purified bovine LPL per gram of tissue.

Statistical analysis

All data are reported as group mean values ± standard error. Differences between groups were assessed by one-way and two-way analysis of variance and Student's *t* test performed with DBASE IV and SPSS-X packages on a VAX8820 computer. A *P*-value of less than 0.05 was considered statistically significant.

Results

Effects of 24 hours of starvation on LPL immunoreactive protein

The amount of LPL protein per gram of tissue was markedly greater in brown fat than in white fat (*Table 2*) and the effects of fasting on LPL specific activity were opposite in both white and brown adipose tissues. However, because the LPL mass determination method should be considered semi-quantitative when the results in BAT versus WAT are compared—because interference due to the different composition of both tissues cannot be ruled out—comparisons are made between depots of the same tissue.

LPL immunoreactive protein was similar in both white adipose depots (RP-WAT and O-WAT) and was not affected by 24 hours of starvation. LPL mass was significantly increased in P-BAT compared with I-BAT and in both depots no changes were observed in response to starvation. LPL specific activity was significantly decreased by fasting in both WATs and significantly increased in both BATs.

Effects of dietary obesity on LPL activity in various depots of WAT and BAT

Both groups of overweight rats used here showed two different degrees of obesity attained by dietary treatment. The rats of the CAF group became obese (50.3% excess of body weight) by eating a cafeteria diet for 100 days (from day 150 to day 250 of age) whereas rats of the PCF group ate the cafeteria diet for the same 100 days but then changed to standard diet only for 70 additional days. At the end of the treatment these rats had 14.7% excess body weight and a food intake in the same amount of the standard diet as their control group (320C, 203 ± 21 KJ/day; PCF, 212 ± 21 KJ/day). In each dietary group, LPL activity was compared in two different depots of both WAT and BAT and the effect of 24 hours of starvation was also analyzed.

It can be seen in *Table 3* that both types of WAT (RP-WAT and O-WAT) were threefold greater in CAF rats than in their controls. The reversion to standard diet from cafeteria diet caused a decrease in the mass of both adipose depots that was more marked in the ovaric one (RP-WAT and O-WAT were 1.7 and 1.3 times heavier in PCF animals than in controls, respectively). No effects of 24 hours of starvation on the weight of both white adipose depots were observed.

In control groups (250C and 320C) starvation decreased LPL activity of both WAT depots. The response to starvation was different in each depot of overweight rats: In RT-WAT enzyme activity was not affected by starvation whereas it was increased in O-WAT.

Tissue weight and LPL activity of BAT depots are shown in *Table 4*. I-BAT and P-BAT were significantly heavier in both CAF and PCF rats than in their respective controls (250C and 320C groups). With respect to lean animals, I-BAT and P-BAT were 2

Table 3 Tissue weight and lipoprotein lipase (LPL) activity of retroperitoneal and ovaric white adipose tissues (RP-WAT and O-WAT) of control (250C and 320C), cafeteria-diet (CAF), and postcafeteria diet (PCF) rats in fed and starved situations

	Units		250C	CAF	*ANOVA*	320C	PCF	*ANOVA*
RP-WAT								
Weight	g	Fed	4.37 ± 0.43	13.6 ± 1.1*	*D*	4.90 ± 0.37	8.24 ± 0.69*	*D*
		Starved	4.13 ± 0.32	13.7 ± 1.3*		4.69 ± 0.41	8.38 ± 0.94*	
LPL activity	mU/g tissue	Fed	27.1 ± 4.0	19.7 ± 2.8	*S*	29.2 ± 6.9	22.7 ± 3.6	*S*
		Starved	12.2 ± 1.3†	18.0 ± 3.9		12.4 ± 1.6†	17.8 ± 1.8	
	mU/mg prot	Fed	2.60 ± 0.51	2.01 ± 0.28	*S*	3.19 ± 0.77	2.30 ± 0.37	*S*
		Starved	1.07 ± 0.13†	1.78 ± 0.38		1.22 ± 0.16†	1.65 ± 0.18	
O-WAT								
Weight	g	Fed	8.71 ± 0.71	23.0 ± 1.3*	*D*	9.77 ± 0.55	13.6 ± 0.8*	*D*
		Starved	7.65 ± 0.43	22.6 ± 1.5*		9.51 ± 0.64	12.5 ± 0.5*	
LPL activity	mU/g tissue	Fed	28.1 ± 3.8	19.1 ± 1.1	*DxS, S*	30.1 ± 5.6	33.3 ± 4.6	*S*
		Starved	11.4 ± 1.4†	30.2 ± 4.1†*		10.9 ± 1.5†	18.4 ± 0.9†*	
	mU/mg prot	Fed	2.65 ± 0.43	2.09 ± 0.21	*DxS, D*	2.61 ± 0.43	2.90 ± 0.35	*S*
		Starved	1.03 ± 0.13†	3.15 ± 0.49*		1.02 ± 0.12†	1.70 ± 0.11†*	

Note: Results are the mean ± SEM. The number of animals in each group was: 250C fed, $n = 13$; 250C starved, $n = 12$; CAF fed, $n = 11$; CAF starved, $n = 11$; 320C fed, $n = 8$; 320C starved, $n = 7$; PCF fed, $n = 9$; PCF starved, $n = 8$. One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol of fatty acid/min. Data were analyzed by Student's *t*-test and one- and two-way analysis of variance (ANOVA) as described in the Methods and materials section.
Significant differences ($P < 0.05$):
*CAF versus 250C and PCF versus 320C.
†Starved versus fed.
ANOVA significances ($P < 0.05$): *S*–effect of starvation. *D*–effect of dietary treatment. *DxS*–interaction of dietary treatment and starvation.

and 1.5 times heavier in CAF and PCF rats, respectively. A period of 24 hours of starvation induced a significant decrease of the size of both depots in control groups whereas no effect of fasting was seen in overweight rats.

LPL activity in BAT was not affected by excess weight, although in control animals with ageing (from day 250 to day 320), a significant increase could be observed. Enzyme activity was higher in all starved animals, but the magnitude of the increase was different when control and overweight rats were compared. Thus, in both lean groups and in the CAF group, the LPL activity increased approximately twice whereas in PCF rats the activity was three times higher in the starved animals than in the fed animals.

An additional observation is that of the lower LPL activity in both BAT depots of 320C rats compared with 250C rats. In the absence of dietary differences, this observation could be attributed to an effect of ageing. Thus, the decrease in LPL activity could be associated with both a decrease in thermogenic activity and thermoregulatory needs that have been found to occur with ageing.[19]

Table 4 Tissue weight and lipoprotein lipase (LPL) activity of interscapular and perirenal-periaortic brown adipose tissues (I-BAT and P-BAT) of control (250C and 320C), cafeteria-diet fed (CAF), and postcafeteria fed (PCF) rats in fed and starved situations

	Units		250C	CAF	*ANOVA*	320C	PCF	*ANOVA*
I-BAT								
Weight	mg	Fed	432 ± 25	1040 ± 80*	*D,S*	498 ± 28	666 ± 48*	*D,S*
		Starved	380 ± 26†	829 ± 63*		388 ± 25†	580 ± 69*	
LPL activity	mU/g tissue	Fed	56.4 ± 6.4	47.4 ± 5.7	*D,S*	34.0 ± 4.6	24.9 ± 2.7	*S*
		Starved	108 ± 13†	80.6 ± 7.0†		66.5 ± 21.2	71.6 ± 11.7†	
	mU/mg prot	Fed	1.74 ± 0.22	2.23 ± 0.35	*S*	1.04 ± 0.14	0.791 ± 0.112	*S*
		Starved	2.94 ± 0.36†	3.27 ± 0.28†		1.71 ± 0.44	2.30 ± 0.42†	
P-BAT								
Weight	g	Fed	461 ± 44	980 ± 86*	*D*	474 ± 34	646 ± 61*	*D,S*
		Starved	414 ± 43	843 ± 70*		363 ± 39†	538 ± 65*	
LPL activity	mU/g tissue	Fed	73.6 ± 21.0	48.2 ± 7.4	*D,S*	35.9 ± 4.7	29.0 ± 2.7	*S*
		Starved	163 ± 24†	93.1 ± 12.3†*		91.8 ± 15.0†	89.5 ± 20.0†	
	mU/mg prot	Fed	1.94 ± 0.60	1.66 ± 0.33	*S*	1.07 ± 0.14	0.808 ± 0.072	*S*
		Starved	4.18 ± 0.71†	2.67 ± 0.31†		2.33 ± 0.27†	2.12 ± 0.47†	

Note: Results are the mean ± SEM. The number of animals in each group was: 250C fed, $n = 13$; 250C starved, $n = 12$; CAF fed, $n = 11$; CAF starved, $n = 11$; 320C fed, $n = 8$; 320C starved, $n = 7$; PCF fed, $n = 9$; PCF starved, $n = 8$. One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol of fatty acid/min. Data were analyzed by Student's *t*-test and one- and two-way analysis of variance (ANOVA) as described in the Methods and materials section.
Significant differences ($P < 0.05$):
*CAF versus 250C and PCF versus 320C.
†Starved versus fed.
ANOVA significances ($P < 0.05$): *S*–effect of starvation. *D*–effect of dietary treatment. *DxS*–interaction of dietary treatment and starvation.

Discussion

The effects of 24 hours of fasting on LPL activity are opposite in WAT versus BAT

The effects of the nutritional state on the ratio of LPL catalytic activity and LPL mass in WAT have been extensively stud ied.[3,13,26] The lack of effect of 24 hours of starvation on LPL immunoreactive mass in white fat found in our study is in agreement with previously published reports,[27] and the fasting-induced decrease in the LPL activity has been reported to be due to a decrease of the relative amount of the active form.[3,27]

Many studies have focused on the effects of cold exposure on the regulation of LPL activity in BAT.[5,28,29] However, the effects of fasting on the regulation of enzyme activity in this tissue have received minor attention and there have been no studies comparing different depots of brown fat. Here we show that the P-BAT depot presents higher amounts of LPL than the interscapular fat, although no significant differences on enzyme activity could be observed when both depots of BAT were compared.

On the other hand, our results indicate that the increased enzyme activity observed in both depots of brown fat of starved rats is not accompanied by an increased amount of the enzyme. This indicates the existence of posttranslational regulatory mechanisms of LPL activity in brown fat, as has been suggested in other physiologic conditions, such as pregnancy and lactation,[29] and cold exposure.[28] In BAT in contrast to WAT, the increased LPL activity observed in starved rats appears to be an increase of the relative amount of the active form.

Impairment by obesity of the LPL activity adaptative response to food deprivation

The excess of body and tissue weights attained by feeding rats the cafeteria diet is in accordance with that obtained in previous studies.[18,19,30] The PCF rats have been considered to be metabolically highly efficient because although they maintain higher body and tissue weights than the controls, their food intake is similar to the lean animals.[18–20]

It could be expected that LPL plays a role in the abnormal overdevelopment of adipose tissue that occurs in the obese condition, because LPL activity is markedly elevated in the adipose tissue of genetically obese rodents[11,12] and in obese humans.[13] However, our results show that, in the fed state, the activity of LPL in the different WAT depots of both overweight groups (CAF and PCF) of animals was similar to those of the control rats. An explanation for these results could be based on results obtained by de Gasquet et al.,[31] who reported a significantly higher LPL activity in adipose tissue of obese rats compared with controls but only after having been fed a high fat diet for a very long time period (more than 180 days), whereas after 3 months of feeding the high fat diet, the adipose enzyme activity was at the control level, which would agree with our results.

In agreement with previously published results,[3,26,32] 24 hours of starvation induced an approximately 60% decrease in the LPL activity of WAT of lean control rats compared with the value in fed animals. However, there are important differences between the control and dietary obese rats. Neither WAT depots of CAF rats showed a downregulation of LPL activity during starvation: The enzyme activity was similar to fed animals in retroperitoneal depot and significantly increased in the ovaric one.

The lower ability of WAT depots to downregulate LPL during fasting has been suggested to play a possible role in the development of obesity[27] and a previous report showed abnormally elevated LPL activity during fasting in Zucker obese rats.[10] The lack of fasting-induced downregulation in WAT can be due to a combination of hyperinsulinemia and a resistance to insulin, as is suggested by the fact that insulin is known to stimulate LPL in WAT[33] and that a certain insulin resistance is characteristic of obesity.[34]

In obese rats, this lack of a response to food deprivation could result in a continuous lipid deposition in adipose tissue throughout the day, because it has been reported that LPL activity displays a diurnal rhythm that corresponds to the feeding pattern.[27] Thus, between meals obese animals may be more capable of retaining fatty acids in their storage tissues than are control rats.

In PCF rats, which showed a moderate excess of body weight, retroperitoneal depot LPL activity was similar in both fed and starved conditions, whereas in the ovarian tissue, which behaved like controls, there was a significant decrease of LPL activity in response to fasting (55% of the fed value in overweight rats compared with 36% of the fed value in control rats). This could be related to a recovery of the sensitivity to insulin with weight loss, as is observed in human patients.[34] However, this recovery would occur with a different degree in the different adipose depots, as can be noted here in the ovarian tissue, which had a higher decrease in mass compared with the retroperitoneal depot.

BAT plays a major role in nonshivering thermogenesis and in diet-induced thermogenesis because of its ability to generate heat by fatty acid oxidation.[16] Thus, it is involved in the regulation of body weight in a different way than is WAT. Because the amount of stored triglyceride in BAT is small, a good supply of fatty acids to the tissue is required to supply thermogenic machinery. Thus, in this sense, a diminution of LPL activity in brown fat could be involved in the development of obesity. However, in the fed state, LPL activity of both BAT depots studied was similar in both overweight groups compared with control rats, although when food was deprived LPL activity was significantly decreased in the P-BAT depot of CAF rats with respect to their respective controls.

It was interesting to note the attenuating effect of fasting on the LPL activity in the CAF group compared with their controls. The reduced response to the shift from the fed to the food-deprived situation has been previously described for thermogenic parameters in brown adipose mitochondria,[19] such as the UCP1 content, which suggests some loss of sensitivity of the BAT to food intake.

The thermogenic activity in BAT decreases with fasting[19] whereas it is interesting to point out the significantly increased LPL activity that was observed here in both BAT depots in all starved rats. The fasting-induced increase in BAT LPL activity can be related to two main physiologic functions. First, heat production is needed to maintain body temperature. Interestingly, in this sense, in control rats the increase of the LPL activity was higher in the P-BAT depot, probably because this inner location could be more involved in thermoreregulation. On the other hand, it has been reported that the fatty acids that result from the action of LPL can be metabolized in subjacent tissue cells or transported in the blood in complex with albumin to other sites for metabolism.[35–37] What is more, a role for LPL in brown adipose fat supplying free fatty acids to other tissues cannot be ruled out.

In conclusion, the present paper shows that dietary obesity results in important impairments of WAT and BAT LPL activity. The downregulatory response induced by food deprivation that occurred in WAT of control rats was not seen in obese animals. In addition, BAT responded to fasting by increasing the LPL activity, which is also obesity impaired because it was less intense in the obese rats than in controls. These differences in the LPL activity were reduced in the PCF rats when the extent of obesity was also reduced.

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