Lipoprotein Lipase and Hepatic Lipase in Wistar and Sprague-Dawley Rat Tissues. Differences in the Effects of Gender and Fasting

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To evaluate the effects of strain, gender and fasting in the regulation of lipoprotein lipase (LPL) and hepatic lipase (HL) activities were measured in tissues of male and female Wistar and Sprague-Dawley rats after feeding or a 24-h starvation period. It is noteworthy that an effect of gender on LPL activity was observed in Wistar, but not in Sprague-Dawley rats, not only in the basal (fed) activity in several tissues, such as white and brown adipose tissues, heart, and brain, but also in response to fasting which affected LPL activity in brown adipose tissue, heat and lung of female but not of male Wistar rats. By contrast, HL activity in liver, plasma and adrenals of Sprague-Dawley rats was higher in females than in males. No effect of gender on HL activity was observed in Wistar rats. Our results indicate that differences exist between Wistar and Sprague-Dawley rats in the regulation of both LPL and HL. Some of the contradictory results found in the literature may be explained by the differences between rat strains and gender, as well as differences in the nutritional status of the animals.

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Lipoprotein lipase (E.C. 3.1.1.34; LPL) and hepatic lipase (HL) are lipolytic enzymes that are involved in the metabolism of circulating lipoproteins. LPL catalyzes the hydrolysis of circulating triacylglycerols in chylomicra and in very low density lipoproteins (1). Many tissues express the LPL gene, but LPL activity is particularly high in adipose tissue, heart and skeletal muscles, and in lactating mammary gland (2). The liver also expresses high levels of LPL activity during the perinatal period (3). The enzyme is functional at the luminal side of the endothelial cells, but is synthesized inside the parenchymal cells (4–6). HL participates in the clearance of cylomicron remnants by the liver (7) and in the conversion of high density lipoproteins (HDL_2 into HDL_3) thus contributing to the reverse cholesterol transport to the liver (see Ref. 8 for review). It is important that HL is also located at the vasculature (9), although the enzyme is synthesized within the hepatocytes (10). The presence of a lipolytic activity with characteristics similar to those of hepatic lipase was also demonstrated in some steroidogenic tissues, such as adrenals and ovaries in several animal species (11,12), although the enzyme does not appear to be synthesized in these tissues (13,14). It was therefore proposed that the hepatic lipase found in these tissues may originate in the liver (13). Low but significant levels of HL activity were also reported in the plasma of rats (15), as well as of humans (16).

Both LPL and HL are under nutritional control. LPL is increased in white adipose tissue (WAT) but is decreased in heart of fasted rats (see Refs. 17 and 18 for review), although some of the data reported in the literature are in conflict (4,19–22). We have recently observed that the effect of fasting on LPL activity in the liver of neonatal rats depends on the rat strain used as model (23). We have therefore studied the differences between strains in the effect of fasting on both LPL and HL activities in various tissues.

MATERIALS AND METHODS

Animals. Wistar and Sprague-Dawley rats were obtained from Charles River (Barcelona, Spain). Animals were sacrificed by decapitation at the age of 60 d, and tissues [liver, heart, lungs, epididymal or periuterine WAT, interscapular brown adipose tissue (BAT), adrenal glands and brain] were immediately excised, cleaned and frozen in liquid N_2. The blood was collected in heparinized vials, and plasma was obtained by centrifugation (30 min at 1,000 × *g* at 4°C). Plasma was kept at –40°C until used to determine lipolytic activities. Tissue homogenates were prepared in 10 mM Hepes [*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)], pH 7.5, containing 1 mM ethylenediaminetetraacetate and 1 mM dithiothreitol using a Polytron (Kinematica GmbH, Luzern, Switzerland) homogenizer. Homogenates were cleared up by centrifugation (10 min at 10,000 × *g* at 4°C). When necessary, the upper fatty layer was removed, and the supernatant centrifuged again until no lipid layer remained.

LPL assay. LPL activity was determined as described (24). The assay mixture contained 0.6 mM glycerol tri[9,10(n)-^{3}H]oleate (12 Ci/mol), 50 mM $MgCl_2$, 0.05% albumin (fatty acid-free), 3% serum (preheated for 60 min at 50°C), 25 mM Pipes [piperazine-*N,N'-bis*(2-ethanesulfonic acid)], pH 7.5, and 0.02 mL of sample in a final volume of 0.2 mL. The incubation was carried out for 30 min at 25°C. The reaction was terminated, and the released [^{3}H]oleate was quantified, as previously described (25). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 μmol of oleate per min.

HL assay. HL activity was determined as previously described (26). The assay mixture contained 2.5 mM glycerol tri[9,10(n)-^{3}H]oleate (0.3 Ci/mol), 0.75 M NaCl, 3% albumin (fatty acid-free), 50 mM Tris [tris(hydroxymethyl)-aminomethane], pH 8.5, and 0.05 mL of sample in a final volume of 0.2 mL. The incubation was carried out for 30 min at 25°C. The reaction was terminated, and the released [^{3}H]oleate was quantified as described above for LPL.

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Abbreviations: BAT, brown adipose tissue; HDL, high density lipoproteins; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); HL, hepatic lipase; LPL, lipoprotein lipase; Pipes, piperazine-*N,N'-bis*(2-ethanesulfonic acid); Tris, tris(hydroxymethyl)aminomethane; WAT, white adipose tissue.

RESULTS

Effect of gender. At the age of 60 d, the body weight was lower in Wistar than in Sprague-Dawley rats (Table 1). Body weights of females were about 40% lower than those of males in both strains. Similar differences were also observed in several tissues, notably in liver, weights, but also in heart and lung weights.

In both Wistar and Sprague-Dawley rats, the distribution of LPL activity between tissues was similar (Table 2). However, gender affected LPL activity in tissues of Wistar but not in tissues of Sprague-Dawley rats. Thus, in Wistar rats LPL activity in WAT and brain was lower in females than in males, but the activity in heart and BAT was higher in females than in males. LPL activity was not detectable in liver and plasma of adult animals (data not shown). The lack of an effect of gender on LPL activity in Sprague-Dawley rats has already been reported (27).

We studied HL activity in liver, plasma and adrenals (Table 3). As previously described (11,12), we found that HL activity was higher in liver than in adrenals and that a substantial amount could be detected in plasma. No effect of gender was seen in Wistar rats (this agrees with previous results from our laboratory; see Ref. 28), but in Sprague-Dawley rats HL activity was 35–55% higher in females than in males, not only in the liver but also in plasma and adrenals.

As shown in Table 3, fasting caused a decrease in the HL activity not only in liver but also in plasma of Wistar and Sprague-Dawley rats. No significant differences were found between female and male animals in their response to fasting. In adrenals, fasting also produced a decrease in HL activity, but the differences were only significant in male Wistar and in female Sprague-Dawley rats.

DISCUSSION

Several studies have demonstrated differences in both adipose tissue (29–31) and postheparin plasma (32) LPL activity between men and women. For rodents, scarce and conflicting results have been reported (27,33,34). Our data indicate that the effect of gender on both tissue LPL and HL very much depends on the rat strain used.

The effect of gender on LPL activity is likely to be related to the role of estrogens. It is well established that, in female rats, ovariectomy increases LPL activity in WAT (33), and estrogen treatment restores the lower LPL activity in this tissue (33,5). In the heart, LPL activity is increased by estrogen treatment (27). Androgens may not be directly involved in the effect of gender on LPL, as castration of male rats did not produce any alteration in adipose tissue LPL activity (27). In humans, where LPL activity in WAT is higher in women than in men, estrogen treatment increases LPL activity (see Ref. 17 for review). The role of estrogen in the effect of gender on HL is not clear for the rat. Malendowicz and Paluszak (36) found that ovariectomy had no effect on HL-like activity in adrenals, and Staels *et al.* (37) described that the increase in both hepatic lipase activity and *m*RNA relative content in the liver of ovariectomized rats was not observed in pair-fed animals. They also found no differences between female and male Wistar rats in HL *m*RNA relative content in the liver (37). Androgens are more likely involved in the effect of gender on hepatic lipase, as it was reported that orchiectomy resulted in an increase in the HL-like activity in adrenals, and that testosterone-replacement therapy restored normal values (36). In humans, the relationship between estrogens and hepatic lipase is well established (38). Estrogen administration to postmenopausal women decreased postheparin plasma HL activity (39), even in diet-controlled trials (40). Furthermore, changes in postheparin plasma HL activity were countercurrent to those of 15-β-estradiol in the course of the estral cycle (41). In addition, HL activity in postheparin plasma was shown to be higher in men than in women (42). Therefore, profound differences between rats and humans appear to exist in the effect of sex hormones on both LPL and HL. Our results further suggest that differences between rat strains may exist in the effects of these hormones.

TABLE 1

Body and Tissue Weight of Wistar and Sprague-Dawley Rats[a]

	Wistar			Sprague-Dawley		
	Male	Female	*P* vs. male	Male	Female	*P* vs. male
Body weight (g)	318 ± 4	188 ± 1	<0.001	370 ± 4	238 ± 1	<0.001
Tissue weight (g)						
WAT	1.85 ± 0.17	2.06 ± 0.10	NS	3.22 ± 0.41	2.89 ± 0.14	NS
BAT	0.18 ± 0.02	0.14 ± 0.03	NS	0.39 ± 0.02	0.29 ± 0.03	NS
Heart	0.85 ± 0.01	0.59 ± 0.02	<0.001	0.99 ± 0.03	0.76 ± 0.01	<0.01
Lung	1.40 ± 0.04	1.01 ± 0.01	<0.001	1.37 ± 0.03	1.17 ± 0.03	<0.01
Brain	1.38 ± 0.03	1.31 ± 0.03	NS	1.41 ± 0.02	1.33 ± 0.04	NS
Liver	13.96 ± 0.09	7.78 ± 0.44	<0.001	15.59 ± 0.42	9.55 ± 0.19	<0.001
Adrenals	0.058 ± 0.008	0.068 ± 0.004	NS	0.062 ± 0.003	0.064 ± 0.007	NS

[a]Results are the means ± SE of 5–6 animals per group. Statistical comparisons were made by Student's *t*-test. NS, nonsignificant differences; WAT, white adipose tissue (gonadal); BAT, brown adipose tissue (interscapular).

TABLE 2

Lipoprotein Lipase Activity in Tissues of Wistar and Sprague-Dawley Rats[a]

	Wistar			Sprague-Dawley		
	Fed	Fasted	*P* vs. fed	Fed	Fasted	*P* vs. fed
White adipose tissue						
Male	78 ± 5	15 ± 5	<0.001	69 ± 10	14 ± 1	<0.001
Female	53 ± 5	11 ± 1	<0.001	69 ± 11	15 ± 1	<0.001
P vs. male	<0.05			NS		
Brown adipose tissue						
Male	42 ± 7	31 ± 6	NS	30 ± 3	26 ± 3	NS
Female	68 ± 5	19 ± 4	<0.001	27 ± 8	26 ± 8	NS
P vs. male	<0.05			NS		
Heart						
Male	137 ± 9	140 ± 11	NS	129 ± 8	334 ± 34	<0.01
Female	162 ± 6	189 ± 5	<0.05	130 ± 14	200 ± 13	<0.01
P vs. male	<0.05			NS		
Lung						
Male	44 ± 8	59 ± 10	NS	55 ± 4	63 ± 6	NS
Female	51 ± 4	69 ± 2	<0.01	66 ± 4	63 ± 12	NS
P vs. male	NS			NS		
Brain						
Male	4.3 ± 0.6	3.6 ± 0.4	NS	3.3 ± 0.3	2.5 ± 0.2	NS
Female	2.9 ± 0.1	3.1 ± 0.3	NS	3.1 ± 0.2	2.4 ± 0.3	NS
P vs. male	<0.05			NS		

[a]Tissues were obtained from 60-day-old rats either fed or fasted and processed to determine lipoprotein lipase activity. Results are expressed in mU/g tissue and are means ± SE of 5–6 animals per group. Statistical comparisons were made by Student's *t*-test. NS, nonsignificant differences.

There is a general agreement in the literature concerning the effect of fasting on PL activity in WAT (see Ref. 17 for review), and we have found that LPL activity in this tissue was decreased by fasting in all animals studied. For the heart, there exists some consensus that fasting increases LPL activity, but some studies failed to show such an effect (see Ref. 18 for review). Our results indicate that this apparent contradiction may be due, at least in part, to the gender or the strain of rats used in these studies, as we found that fasting produced a stronger effect in the heart of Sprague-Dawley rats (about a 2-fold increase) than in the heart of Wistar rats (1.2-fold increase in females and no effect in males).

LPL activity in rat brain is known to be decreased by extended (three days) fasting (43,44). Our results, indicating no effect of 24-h fasting, are in agreement with those reported by Gavin *et al.* (44). Little is known about the effect of the nutritional status on LPL activity in BAT and lung. Very early studies by Hamosh and Hamosh (45) on the effect of fasting in Sprague-Dawley rats showed no effect on

TABLE 3

Hepatic Lipase Activity in Tissues of Wistar and Sprague-Dawley Rats[a]

	Wistar			Sprague-Dawley		
	Fed	Fasted	*P* vs. fed	Fed	Fasted	*P* vs. fed
Liver (mU/g)						
Male	633 ± 33	433 ± 21	<0.001	546 ± 18	372 ± 22	<0.001
Female	627 ± 31	494 ± 36	<0.05	739 ± 29	473 ± 32	<0.001
P vs. male	NS			<0.05		
Plasma (mU/mL)						
Male	6.6 ± 0.4	3.9 ± 0.2	<0.001	5.9 ± 0.4	3.9 ± 0.2	<0.01
Female	6.2 ± 0.2	4.9 ± 0.5	<0.05	8.1 ± 0.5	4.7 ± 0.4	<0.001
P vs. male	NS			<0.05		
Adrenals (mU/g)						
Male	119 ± 5	77 ± 5	<0.01	233 ± 18	196 ± 13	NS
Female	97 ± 11	79 ± 5	NS	360 ± 37	209 ± 50	<0.05
P vs. male	NS			<0.05		

[a]Hepatic lipase activity was determined in the liver, plasma and adrenals of Wistar and Sprague-Dawley rats, fed or fasted (24 h). Results are means ± SE of five animals per group. Statistical differences were determined by Student's *t*-test. NS, nonsignificant differences.

LPL activity in lungs. In BAT of male Wistar rats, it was shown that after three days of fasting, LPL activity in this tissue was decreased (46). Our results showing that fasting affected LPL activity in both BAT and lung only in female Wistar rats are consistent with these reports.

The results indicate that in Wistar rats differences exist between females and males not only in the LPL activity in several tissues, but also in their response to fasting. This suggests that sex hormones may affect the action of hormones directly responsible for the effect of fasting. Differences between female and male rats in their response to hormones have been described. For example, the sensitivity of hepatocytes to the stimulation of glycogen phosphorylase by adrenaline is an order of magnitude higher in male than in female rats (47). This is known to be due to the different mechanism of action of catecholamines in the liver, i.e., the α_1-receptor-mediated calcium mobilization in males and the β-receptor-mediated cAMP increase in females (48). Also, the sensitivity of adipocytes to stimulation of glucose metabolism by insulin is greater in female than in male rats (49). Furthermore, while in hepatocytes from male rats prostaglandins of the E series inhibit hormone-stimulated glycogenolysis, in hepatocytes from female rats they do not (50). The mechanisms by which sex hormones produce these differences are largely unknown. The relationship between sex and other hormones in the regulation of LPL in rat tissues has not yet been explored. To address this question and to elucidate the reasons for the differences in the response of LPL to fasting between rat strains will give new insights into how this important enzyme of lipoprotein metabolism is regulated.

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