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Lipoprotein Lipase in Peripheral Tissues in Rats with Insulin Resistance Induced by Beef Tallow Diet

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Summary The modulation of lipoprotein lipase (LPL) activity and mass was examined in rats with insulin resistance induced by a beef tallow diet. Male sprague-Dawley rats were meal-fed an isoenergetic diet based on either beef tallow or safflower oil for an 8-week period. Insulin resistance brought about by long-term feeding of a beef tallow diet was confirmed by an oral glucose tolerance test. Plasma glucose and insulin levels were higher at almost all times of day in the rats fed the beef tallow diet than in those fed the safflower oil one. LPL activities of the skeletal and cardiac muscles and brown adipose tissue before and after a meal were lower in the beef tallow diet group than in the safflower oil diet group, however those of the perirenal and subcutaneous adipose tissues were not different between the two dietary groups. The amounts of LPL proteins in the soleus muscle and perirenal adipose tissue corresponded to the enzyme activities in these tissues. These results suggest that the effect of dietary saturated fat on LPL activity and mass are different in muscles and adipose tissues and that generalized peripheral insulin resistance, induced by feeding rats a beef tallow diet for a sufficient duration, is not followed by an impairment of the modulation of LPL activities and protein amount.

Key Words: plasma insulin, glucose tolerance test, lipoprotein lipase, beef tallow, safflower oil, rat

Impairment of insulin action on glucose metabolism is accompanied by major changes in lipid metabolism and circulating lipoproteins [1]. One possible pathway by which alterations in insulin sensitivity might affect lipid metabolism

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is through the modulatory action of insulin on lipoprotein lipase (LPL; EC 3.1.1.34). LPL hydrolyzes triacylglycerol carried by very low-density lipoproteins and chylomicrons at the luminal surface of capillaries in most extrahepatic tissues. Fatty acids thus released may then be stored in white adipose tissues and stored or oxidized in brown adipose tissues (BAT) and in skeletal and cardiac muscles [2].

The regulation of LPL is tissue specific. In the postprandial condition, LPL activity increases in white adipose tissues and decreases in skeletal and cardiac muscles, whereas the opposite occurs during fasting [3]. Of the several hormones involved in the modulation of LPL, insulin has been shown to correlate positively with LPL activity in white adipose tissues and negatively in skeletal and cardiac muscles [4-6]. In the former, insulin has been reported to directly increase mRNA levels [6] as well as the activity [7] of LPL. Insulin has also been shown to increase the transcription and activity of LPL postprandially in BAT [8]. The mechanisms whereby administration of insulin [9] or ingestion of meals [4, 10] immediately decreases muscle LPL activity are less clear.

It remains unclear whether LPL becomes resistant to the modulatory effects of insulin in relation to the development of the resistance of glucose metabolism to insulin action. Fasting LPL activity in the adipose tissue has been shown to correlate with serum insulin in obese subjects in some studies [11] but not in others [12]. On the other hand, the inhibitory action of insulin on skeletal muscle LPL activity has been correlated with the serum concentration of insulin in obese subjects with varying degrees of insulin resistance [12].

Insulin resistance has been shown to be promoted by long-term feeding of a high fat diet, especially a diet high in saturated fat [13-15]. We recently reported that serum insulin levels are higher in rats fed a beef tallow (44% oleic, 27% palmitic, and 18% stearic acids) diet than in rats fed a safflower oil (79% linoleic acid) one. These higher levels result in a higher body fat accumulation in the beef tallow fed rats [16, 17]. However, it is unclear whether rats fed the beef tallow diet have developed hyperinsulinemia accompanied with insulin resistance, as serum insulin levels in rats fed the beef tallow diet were measured only at 12:00 or 13:00 h in our previous studies. No study have evaluated the possible alteration in the modulation of LPL activity in the presence of insulin resistance brought about by long-term feeding of a beef tallow diet.

In the present study, in order to clarify whether LPL under various nutritional conditions (before and after a meal) was modulated by the presence of insulin resistance in rats fed the beef tallow diet, we examined 1) the effect of long-term feeding of a beef tallow diet on a full diurnal cycle of plasma insulin; 2) the resistance of glucose tolerance to insulin; 3) LPL activities and mass before and after a meal in various peripheral tissues of rats.

MATERIALS AND METHODS

All procedures involving animals were approved by the Experimental Animal

Care Committee of the University of Tsukuba.

Animals and diets. Forty male Sprague-Dawley rats (5 weeks old) were obtained from CLEA Japan (Tokyo). One-half of the animals were fed a safflower oil diet; and the other half, a beef tallow diet. The compositions of both diets have been described previously [17]. Both diets provided 45, 35, and 20% of energy as fat, carbohydrate and protein, respectively [17]. The metabolizable energy was 19.7 kJ/g for the safflower oil diet and 18.4 kJ/g for the beef tallow diet.

Experimental design. The animals were individually caged at 22±2°C, with light from 07:00 h to 19:00 h. Each group of rats was meal-fed their designated diet at 08:00–09:00 h and 20:00–21:00 h and was given free access to water for 8 weeks. Both groups of rats were offered the appropriate diet in amounts such that the two groups consumed equal metabolizable energy during the experimental period. The meal-feeding method was used to adjust the energy intake between the two dietary groups. Under meal-feeding conditions, feeding one meal (within 2 h) a day decreases the food intake of the animals, but feeding two meals a day, as was used in this study, minimized the decrease in food intake. The food consumption of rats was approximately the maximal amount of diet that rats could consume under the meal-feeding conditions. On the final day, the rats in each diet group were fed a meal at 08:00–09:00 h. Then, the rats were killed by decapitation at 10:00 h. The adipose tissues (interscapular brown, perirenal, and abdominal subcutaneous), heart and skeletal muscles (soleus, gastrocnemius, tibialis anterior, plantaris, and extensor digitorum longus (EDL)) were quickly removed, weighed and frozen in liquid nitrogen. Samples of epididymal adipose tissue were obtained at surgery from the bilateral epididymides except for blood vessels; and those of the subcutaneous adipose tissue (about 2 g), which were obtained at surgery from bilateral subcutaneous epigastric abdominal depots. All tissue and plasma samples were stored at −80°C, until biochemical analysis could be performed.

Our previous studies reported that intake of a beef tallow diet promotes body fat accumulation (carcass fat and intra-abdominal fat) compared with the intake of a safflower oil diet in rats when the animals were fed under the same conditions as used in the present experiment [16–19]. Therefore, even though carcass fat content was not measured in this experiment.

Diurnal rhythm of plasma insulin, glucose, and triacylglycerol levels. Diurnal variations of plasma insulin, glucose, and triacylglycerol levels were measured in half of the rats in each diet group between week 7 and week 8 of the period of dietary manipulation. Blood samples (150 μl) were obtained from a tail vein at 02:00, 06:00, 10:00, 14:00, 18:00, and 22:00 h and put into the tubes coated with heparin and NaF for the determination of plasma insulin, glucose and triacylglycerol levels.

Oral glucose tolerance test. Before the end of the experiment (1-week period), the remaining 20 rats in each dietary group were orally administered 4 ml/kg body weight of a 50% glucose solution at 08:00 h (fasting condition) [14]. Blood samples were obtained from a tail vein (0 min) and 15, 30, 60, and 120 min

after glucose administration, and transferred to the tubes coated with heparin and NaF before.

Plasma analyses. The plasma insulin concentration was determined by enzyme immunoassay with kits (Insulin EIA kit) purchased from Sanko-Junyaku, Tokyo. Plasma glucose and triacylglycerol concentrations were measured enzymatically with kits (Glucose C-II test, Triglyceride G Test) purchased from Wako Chemical Co., Osaka.

LPL activities of various tissues. LPL activities of the muscles and adipose tissues were measured as described in our previous reports [18]. The tissues were prepared by the method of Mori *et al.* [20]. The substrate for LPL was prepared according to the method of Nilsson-Ehle and Schotz [21], but unlabeled triolein was used instead of [^{3}H]triolein. The LPL activity assay was performed by incubation of the extract with the substrate at 37°C for 30 min. The free fatty acids released during the incubation were measured enzymatically with a NEFA C Test purchased from Wako Pure Chemical Co., Osaka. One unit of LPL activity was defined as that catalyzing the release of 1 μmol of free fatty acid per hour.

LPL proteins of the soleus muscle and perirenal adipose tissue. The amount of LPL immunoreactive mass was determined by ELISA, as previously described [22–24]. Briefly, aliquots of tissue extracts in buffer containing 1 M NaCl, 0.1% Triton X-100, 0.1% albumin, protease inhibitors, and Tris-HCl (pH 7.4) were added to 96-well microtiter plates, which had been previously coated overnight with 100 μl of affinity-purified chicken anti-LPL antibody in 0.1 M sodium carbonate, pH 9.3. After incubation for 18 h at 4°C, the wells were washed with phosphate-buffered saline containing 0.1% BSA and 0.1% Triton X-100; and then biotinylated affinity-purified chicken anti-LPL antibody was added and incubation was continued for an additional 18 h at 4°C. After another washing with Tris-HCl buffer, the wells were treated with peroxidase-labeled avidin and the color was developed by the addition of 100 μl of *o*-phenylenediamine (1 mg/ml) and H_2O_2 (0.012%) in 0.1 M sodium citrate, pH 4.5. The colored product was read in an ELISA plate reader at 490 nm. A standard curve was constructed for each plate by use of purified bovine milk LPL. The results were expressed as μg equivalents of purified LPL/g tissue.

Statistical analysis. The statistical differences in body weight and tissue weights were analyzed by Student's *t*-test. Data obtained from the oral glucose tolerance test (OGTT) and the diurnal variations in plasma insulin, glucose, and triacylglycerol levels were analyzed by a factorial analysis of variance (ANOVA) with repeated measures [25]. The statistical differences in the LPL activities and the proteins of peripheral tissues were analyzed by two-way ANOVA [25]. Individual between-group comparisons were carried out by Scheffe's post hoc test [25]. Differences were considered statistically significant at $p<0.05$.

RESULTS

Body tissue weights

Both groups of rats had the same body weight gain during the 8-week experimental period (Table 1). The tissue weights of the heart, skeletal muscles (soleus, gastrocnemius, tibialis anterior, plantaris, EDL), and interscapular BAT were not significantly different between the two dietary groups. However, the perirenal adipose tissue weight was significantly larger in the beef tallow diet group than in the safflower oil diet group (Table 1).

Table 1. Effects of dietary fats on body weight and tissue weights.[1]

		Diet group	
		Safflower oil	Beef tallow
Body weight			
Initial	(g)	139±1	138±1
Final	(g)	423±3	424±4
Gain	(g)	284±3	286±4
Heart	(g)	1.12±0.02	1.10±0.02
Skeletal muscles			
Soleus	(g)	0.32±0.01	0.31±0.01
Gastrocnemius	(g)	4.47±0.07	4.32±0.08
Tibialis anterior	(g)	1.48±0.03	1.43±0.03
Plantaris	(g)	0.79±0.02	0.79±0.02
Extensor digitorum longus	(g)	0.38±0.01	0.36±0.01
Adipose tissues			
Interscapular brown	(g)	0.46±0.02	0.45±0.01
Perirenal	(g)	9.5±0.4	11.9±0.5*

[1]Values are means±SE for 20 rats. *Statistically significant difference ($p<0.05$) from the safflower oil diet group (Student's t-test).

Table 2. Diurnal variation in plasma glucose, insulin, and triacylglycerol concentrations of rats fed the beef tallow diet or the safflower oil diet.[1]

	Time of day					
	02:00	06:00	10:00	14:00	18:00	22:00
Glucose (mmol/liter)						
Safflower oil	8.33±0.17	7.42±0.09	8.03±0.34	7.92±0.17	7.79±0.14	8.04±0.09
Beef tallow	8.86±0.29	7.49±0.23	8.81±0.19	9.30±0.26*	8.64±0.28*	8.73±0.19*
Insulin (μU/ml)						
Safflower oil	22.7±1.3	14.8±1.1	20.7±1.3	17.8±1.3	18.3±1.7	19.9±0.8
Beef tallow	24.9±1.8	18.8±0.7*	27.3±1.1*	22.6±1.9	23.0±1.7	27.6±2.3*
Triacylglycerol (mg/dl)						
Safflower oil	136.3±9.2	116.9±9.4	141.5±28.0	212.3±15.9	154.4±13.3	129.2±9.4
Beef tallow	345.0±48.2*	343.6±68.9*	444.2±55.5*	662.5±72.2*	441.2±66.7*	379.5±38.2*

[1]Values are means±SEM for 10 rats. *Statistically significant difference ($p<0.05$) from the safflower oil diet (ANOVA with repeated measures and Scheffe's test).

Table 3. Time course of plasma glucose and insulin concentrations during oral glucose tolerance test in rats fed the beef tallow diet or the safflower oil diet.[1]

	Time after administration (min)				
	0	15	30	60	120
Glucose (mmol/liter)					
Safflower oil	7.33±0.18	10.7±0.28	9.30±0.29	9.23±0.31	8.07±0.19
Beef talow	7.48±0.25	10.7±0.24	9.67±0.30	9.76±0.24	7.74±0.16
Insulin (μU/ml)					
Safflower oil	10.6±1.4	33.0±3.4	19.4±2.0	16.5±1.9	10.4±1.2
Beef tallow	16.2±0.6*	48.9±4.1*	26.8±2.7*	23.3±1.9*	14.8±1.5*

[1]Values are means±SEM for 10 rats. *Statistically significant difference ($p<0.05$) from the safflower oil diet (ANOVA with repeated measures and Scheffe's test).

Table 4. Lipoprotein lipase activities of muscles in rats fed the beef tallow diet or the safflower oil diet before and after a meal.[1]

	Diet group	
	Safflower oil	Beef tallow
	(U/g tissue)	
Soleus		
Preprandial	23.1±0.6	15.4±0.7*
Postprandial	15.0±0.8§	12.3±1.1
Gastrocnemius		
Preprandial	12.5±0.5	10.1±0.5*
Postprandial	11.5±0.6	8.1±0.4*§
Tibialis anterior		
Preprandial	12.7±1.5	8.1±1.0*
Postprandial	9.6±0.5	6.0±0.6*
Plantaris		
Preprandial	10.6±0.5	9.6±0.5
Postprandial	9.5±0.7	7.7±0.9§
Extensor digitorum longus		
Preprandial	10.4±0.5	8.4±0.7*
Postprandial	9.8±0.5	6.2±0.7*§
Cardiac		
Preprandial	72.5±2.0	48.4±1.8*
Postprandial	66.2±1.4	41.2±1.4*§

[1]Values are means±SE for 10 rats. *Statistically significant difference ($p<0.05$) from the safflower oil diet group. §Statistically significant difference ($p<0.05$) from the preprandial value (two-way ANOVA and Scheffe's test).

Diurnal rhythm of plasma insulin, glucose, and triacylglycerol levels

Plasma glucose levels and insulin levels were higher at all times of day tested in rats fed the beef tallow diet than in those fed the safflower oil diet. Compared with the levels in rats fed a safflower oil diet, glucose levels in rats fed a beef tallow diet increased significantly after 14:00 h (Table 2). Plasma triacylglycerol concentrations were significantly higher at all times in the beef tallow diet group (Table 2).

Table 5. Lipoprotein lipase activities of adipose tissues in rats fed the beef tallow diet or the safflower oil diet before and after a meal.[1]

	Diet group	
	Safflower oil	Beef tallow
	(U/g tissue)	
Interscapular brown		
Preprandial	53.5±1.9	40.3±0.5*
Postprandial	88.3±1.6§	69.5±1.6*§
Perirenal		
Preprandial	7.9±0.6	8.9±0.7
Postprandial	8.1±0.7	9.2±0.4
Subcutaneous		
Preprandial	7.4±0.5	7.4±0.5
Postprandial	8.3±0.6	9.1±0.7

[1]Values are means±SE for 10 rats. *Statistically significant difference ($p<0.05$) from the safflower oil diet group. §Statistically significant difference ($p<0.05$) from the preprandial value (two-way ANOVA and Scheffe's test).

Table 6. Lipoprotein lipase contents in the soleus muscle and perirenal adipose tissue in rats fed the beef tallow diet or the safflower oil diet before and after a meal.[1]

	Diet group	
	Safflower oil	Beef tallow
	(μg/g tissue)	
Soleus muscle		
Preprandial	6.8±0.8	5.0±0.2
Postprandial	5.0±0.3	4.5±0.4
Perirenal adipose tissue		
Preprandial	2.3±0.5	2.4±0.4
Postprandial	2.7±0.3	2.5±0.3

[1]Values are means±SE for 10 rats.

Oral glucose tolerance test

Table 3 shows the time course of plasma glucose and insulin concentrations during the OGTT. Plasma glucose levels were not different between the two dietary groups, although plasma insulin levels were higher at all times in the beef tallow diet group.

LPL activities of various tissues

LPL activities of the skeletal and cardiac muscles before and after a meal were significantly lower in the beef tallow diet group than in the safflower oil diet group except that the differences were not significant in the plantaris and soleus muscles after a meal (Table 4). Meal ingestion decreased LPL activities of various muscles, and the differences were significant in the soleus muscle of the safflower oil diet group and in the gastrocnemius, plantaris, EDL, and cardiac muscles of the beef

tallow diet group (Table 4). The LPL activity in the soleus muscle was higher than that in any other skeletal muscles, but the LPL activity in the cardiac muscle was 314–697% that of skeletal muscles (Table 4).

LPL activities of the interscapular BAT before and after a meal were significantly lower in the beef tallow than in the safflower oil diet group, but they increased significantly during meal ingestion (Table 5). LPL activities of the perirenal and subcutaneous adipose tissues were not different between the two dietary groups, and they increased, but not significantly, with meal ingestion (Table 5).

LPL proteins of the soleus muscle and perirenal adipose tissue

LPL protein content in the soleus muscle was lower in the beef tallow diet group than in the safflower oil diet group and it decreased with meal ingestion in both groups, although the differences in values were not significant (Table 6). Neither dietary fats nor meal ingestion affected significantly the LPL protein content in the perirenal adipose tissue (Table 6).

DISCUSSION

During the experimental period of meal-feeding, rats of both dietary groups ingested isocaloric amounts of nutrients and had similar final body weights, although the beef tallow diet group had larger perirenal adipose tissue weight compared with the safflower oil diet group. These results confirm our previous findings that body fat accumulation is greater in the beef tallow diet group [16–19].

In the present experiment, we found that plasma insulin levels were higher at all times of day in the rats fed the beef tallow diet than in those fed the safflower oil one. We previously suggested that sympathetic activity in the pancreas was lower in the beef tallow diet group than in the safflower oil diet group, resulting in higher serum insulin level in the former [18]. Our present findings confirmed these previous results. However, we recognized in the present study that plasma glucose levels were significantly higher after 14:00 h in the beef tallow diet group compared with the safflower oil diet group, which result suggests that the higher plasma insulin concentration in the beef tallow diet group is not sufficient to reduce the plasma glucose to the levels of the safflower oil diet group. On the other hand, the plasma insulin concentration during the OGTT was higher in the rats fed the beef tallow diet, although plasma glucose levels were not significantly different between the two dietary groups. The higher plasma insulin concentration during OGTT was necessary for the beef tallow-fed rats to carry plasma glucose to the levels of the safflower oil-fed rats. There results suggest that insulin resistance was present in the rats fed the beef tallow diet. However, the reason why plasma glucose levels were not significantly different during OGTT between the two dietary groups is unclear in spite of the hyperglycemia in the beef tallow diet group

after 14:00 h in the experiment on diurnal variation.

LPL activities in the soleus, gastrocnemius, tibialis anterior, plantaris, EDL and cardiac muscles responded to food intake similarly, as was expected from previous findings [26]. LPL activity in the cardiac muscle was 3–6 times greater than that in the skeletal muscles, as is greater the heart contains a larger number of mitochondria, in which the fat oxidation is greater than that in skeletal muscles [27]. Enzyme activities in the skeletal and cardiac muscles before and after a meal were lower in the rats fed the beef tallow diet than in those fed the safflower oil one, although skeletal and cardiac muscle LPL activities in both dietary groups were modified at almost the same rate by the meal ingestion, which was concomitant with the elevation in postprandial insulin in both dietary groups. These results suggest that in the rats fed the beef tallow diet, LPL clearly retained the ability to be stimulated postprandially by insulin secretion in the presence of insulin resistance. In the soleus muscle, the amount of LPL protein responded in much the same way as the enzyme activity, although the differences between the two dietary groups, and between pre- and postprandial amounts, were not significant. It is thought that LPL in skeletal muscles may be affected post-translationally by insulin or by other hormonal and/or nutritional factors [28].

In both dietary groups, BAT LPL activity was lower during fasting compared with the postprandial state, in accordance with findings in previous studies [29]. These observations suggest that the postprandial increase in BAT LPL of the beef tallow-fed rats with insulin resistance after meal ingestion may have been a consequence of an elevation in insulinemia. Moreover, it is suggested that the increase in LPL activity in the BAT after a meal supplies triacylglycerol fatty acids to the BAT from the bloodstream in order to cause diet-induced thermogenesis [30, 31]. Enzyme activity in the BAT before and after a meal was lower in the rats fed the beef tallow diet. BAT LPL is under β-adrenergic modulation [8]. We previously reported that norepinephrine turnover [16] rate and β-adrenergic receptor binding [17] (indexes of sympathetic nervous system involvement) in the BAT were lower in rats fed a beef tallow diet than in safflower oil-fed one. These results suggest that BAT LPL is modulated by other factors as well as by insulin.

Many studies have reported that LPL in white adipose tissues was affected by meal ingestion and insulin infusion [15, 28]. However, in the present study, LPL activities in the perirenal and subcutaneous adipose tissues were scarcely altered by meal ingestion in either dietary group. Moreover, enzyme activities in the adipose tissues before and after a meal were not different between rats of the beef tallow diet group and those of the safflower oil diet group. The amount of LPL mass in the perirenal adipose tissue also was not affected by meal ingestion and dietary fat. We previously reported that sympathetic activities in the epididymal and subcutaneous adipose tissues were lower in a beef tallow fed than in a safflower oil-fed rats [17]. Therefore, LPL in the white adipose tissues of rats fed a beef tallow diet may not be related to hyperinsulinemia, a decline of sympathetic activity, or the

presence of insulin resistance, although these mechanisms are unclear in this experiment.

Finally, we found higher levels of hyperlipidemia in rats fed the beef tallow diet compared with rats fed the safflower oil diet at all times of day tested in this experiment. The BAT and muscles of rats fed the beef tallow diet appeared to have lower LPL activities before and after a meal than those of rats fed the safflower oil diet, suggesting that blood triacylglycerol was absorbed into these tissues at a lower rate in the beef tallow diet group than in the safflower oil diet group. On the other hand, higher triacylglycerol levels in the beef tallow diet group may increase the fat uptake to adipose tissues from the bloodstream, even though LPL activities are not different between two dietary groups. These results may explain the mechanism by which larger body fat accumulation occurred in the rats fed the beef tallow diet.

In conclusion, the present study demonstrates that dietary saturated fat decreases LPL activities and mass in the muscles and BAT, whereas it does not affect those in white adipose tissues. In addition, the study shows that generalized peripheral insulin resistance, induced by feeding rats a beef tallow diet for a sufficient duration, is not followed by an impairment of the modulation LPL activities and protein amounts by food intake or in all likelihood by changes in insulinemia induced by food intake.

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