Original Article

The effects of high-fat diet feeding over generations on body fat accumulation associated with lipoprotein lipase and leptin in rat adipose tissues

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The effects of high-fat diet feeding over generations on body fat accumulation were studied in rats. Male and female Sprague-Dawley rats fed a high-fat (HF) diet or a low-fat (LF) diet were mated in the same diet group at age 13 weeks, and the male pups (1st generation) obtained were used in this experiment. The 1st generation rats were nurtured by their own mothers (Experiment 1) or F344 foster mother rats (chow-fed) during pregnancy (Experiment 3) and the suckling period (Experiments 2 and 3). After weaning, rats with HF and LF dietary histories were fed a purified diet for 12–17 weeks. Body weights and abdominal adipose tissue weights were greater in rats with HF dietary histories than in those with LF dietary histories, even controlling for environmental backgrounds related to the mother rats during pregnancy and suckling periods. The levels of lipoprotein lipase and leptin mRNA in the perirenal adipose tissue were higher in rats with HF dietary histories. These results suggest that the effects on body fat accumulation of HF diet feeding over generations are not only associated with environmental factors but also with genetic factors. The obesogenous effects of HF diet feeding over generations may be associated with lipoprotein lipase and leptin gene expression on rat adipose tissues.

Key words: dietary history, high-fat diet, body fat, lipoprotein lipase, leptin, inter-generational effects, prenancy, suckling, weaning, rats, obesity.

Introduction

Obesity, or excess body fat accumulation, occurs when energy intake exceeds energy expenditure. Such an imbalance mainly results from overeating induced by a high-energy diet and genetic or acquired defects in metabolism.[1] Many epidemiologic studies suggest that increasing dietary fat content promotes greater body fat accumulation through excess energy intake.[2–4] It has been demonstrated that energy expenditure is lower in infants born to overweight mothers than in infants born to lean mothers, and the former results in obesity.[5] Animal studies have been performed on the effects on offspring bodies of the physiological and nutritional environment of dams during lactation and pregnancy.[6–9] McCance *et al*.[9] suggest that fetuses exposed continuously to high blood glucose have an increased risk of subsequently developing diabetes.

However, we recently reported that in Sprague-Dawley rats fed with an isocaloric diet, lipoprotein lipase (LPL) activity in the perirenal adipose tissue was higher and body fat accumulation was greater in rats with a high-fat (HF) diet (40% of energy as fat) history than in rats with a low-fat (LF) diet (5% of energy as fat) history from the 3rd generation to the 11th generation.[10] These findings suggest that a high-fat dietary history has effects over generations on body fat accumulation in rats. Because LPL is rate-limiting for the uptake of lipoprotein triacylglycerols from circulation,[11–13] a higher LPL activity in the adipose tissues accelerates body fat accumulation. However, it is not clear whether these effects over generations are caused by genetic or environmental factors.

In the present study, we investigated body fat accumulation in 1st generation rats with a HF dietary history or LF dietary history (Experiment 1). In order to clarify the participation of genetic factors on effects over generations, we studied the effects of a HF dietary history on body fat accumulation controlled under conditions for mother rats during pregnancy (Experiment 3) and suckling periods (Experiments 2 and 3). Moreover, in order to clarify the causes of body fat accumulation, we also investigated gene expression of LPL, hormone sensitive lipase (HSL) and leptin in the perirenal adipose tissue.

Materials and methods

All procedures involving animals were approved by the Experimental Animal Care Committee of the University of Tsukuba, Japan.

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Table 1. Composition of the experimental diets

Ingredient	High fat diet	Low fat diet
	g/kg	
Casein	277.6	219.0
L-Methionine	4.3	3.3
Mineral mix*	89.0	70.0
Vitamin mix*	8.9	7.0
Choline bitartrate	3.8	3.0
Cellulose	50.0	50.0
Corn starch	401.0	638.1
Soybean oil	12.2	9.6
Lard	153.3	—
Total energy	16.8 (kJ/g)	13.3 (kJ/g)
	% of energy	
Fat	40.0	5.0
Carbohydrate	35.0	70.0
Protein	25.0	25.0

*Purchased from CLEA Japan Inc. (Tokyo, Japan).

Experiment 1. Effects of dietary history over generations on body fat accumulation

Animals and experimental design. Male and female Sprague-Dawley rats (3 weeks old) were obtained from CLEA Japan (Tokyo, Japan). The rats were randomized and fed either a HF or LF diet. The compositions of both diets are given in Table 1. Both diets contained the same amount of vitamin and mineral mixtures based on energy. The rats were housed at 23°C with light from 0730 to 1930 hours and with free access to water and food. Rats were mated in the same diet group at age 13 weeks, and the male pups obtained were used in this experiment. Litter size for HF dietary history rats was 10 ± 1, while that of LF dietary history rats was 12 ± 1. Litters were adjusted to 7 pups each within 24 h of birth.

After weaning (age 3 weeks), rats with HF and LF dietary histories were fed the high-fat diet (Table 1) *ad libitum* for 17 weeks. The body weight of weaned rats did not differ between the two dietary history groups (mean value: 53 g). After 17 weeks of being fed the experimental diet, the rats were fasted overnight and killed by decapitation. Blood was collected to obtain plasma, and stored at –20°C until analysis. Abdominal adipose tissues (epididymal, perirenal and mesenteric) were removed and weighed, and then stored at –80°C until use.

Determination of gene expression of LPL, HSL and leptin. Total RNA was extracted from the perirenal adipose tissue with a guanidium thiocynate water-saturated phenol extraction method.[14] First-strand cDNA synthesis was performed on 5 µg total RNA using oligo(dT) as described in the manufacturer's instructions (BRL Super Script Kit; Life Technologies, Gaithersburg, MD, USA). The sequences of the primers used for amplification are shown in Table 2. Before polymerase chain reaction (PCR) amplification, the primers were labelled with [γ-^{32}P]ATP (Amersham; Arlington Heights, IL, USA) using T4 polynucleotide kinase (Takara, Tokyo, Japan). The PCR reactions were carried out in a DNA Thermal Cycler (Perkin-Elmer, Branchburg, NJ, USA) using the following cycle conditions: initial denaturation at 94°C for 6 min, denaturation at 94°C for 1 min, annealing at 60°C for 2 min (at 58°C in the case of β-action) and extension at 72°C for 3 min. PCR products (10 µL) were analyzed by electrophoresis in 7.5% polyacrylamide gels. The amounts of mRNA in each sample were quantified by a laser analyzer (Fujix BAS2000; Fuji Film, Tokyo, Japan). The PCR cycles and imaging plate times were kept within their respective exponential and linear range.

Plasma analysis. Plasma insulin and leptin concentrations were determined by enzyme-immunoassay, with kits purchased from Immuno-biological Laboratories (Tokyo, Japan) and Morinaga Life and Science Laboratories (Tokyo, Japan).

Experiment 2. Effects of dietary history over generations on body fat accumulation under controlled dam's conditions during the suckling period

Animals and experimental design. The breeding of successive generations was the same as that described in Experiment 1.

After birth, rats with both HF and LF dietary histories were nursed by prepared inbred F344 [13] foster mother rats (fed CE-2, a commercial rodent diet (CLEA, Japan)) without experimental dietary histories during the suckling period (3 weeks). They were fed the high fat diet (Table 1) *ad libitum* for 17 weeks after weaning. Litter size for HF dietary history rats was 10 ± 1, while for LF dietary history rats it was 12 ± 1. Litters were adjusted to 7 pups for each F344 foster mother within 24 h of birth. The body weight of weaned rats did not differ between the two dietary history groups (mean value: 51 g).

On the final day of the experiment, the rats were fasted overnight and killed by decapitation. Blood and abdominal adipose tissues were collected as described in Expreriment 1.

Determination of gene expression of LPL, HSL and leptin. The method of determination of LPL, HSL and leptin gene expression was the same as that described in Experiment 1.

Plasma analysis. Plasma insulin and leptin concentrations were assayed as in Experiment 1.

Table 2. Sequences of polymerase chain reaction (PCR) primers

Gene	Primer	Nucleolide No.	Size of PCR product (b.p.)	GenBank accession no.
Leptin	5′-CCTATGTTCAAGCTGTGCCT-3′	110-129	449	D45862
	5′-TTCAGGGCTAAGGTCCAACT-3′	539-558		
Lipoprotein lipase	5′-ATGTCCACCTCTTAGGGTAC-3′	629-648	393	L03294
	5′-CTTCATTCAGCAGGGAGTCA-3′	1002-1021		
Hormone sensitive lipase	5′-CAACTCTAGGTCAGCTCTTG-3′	761-780	1244	X51415
	5′-AGCAGCCTTTATGTAGCGTG-3′	1086-2005		

Sequences of each primer pair and their location in sequences cited in the GenBank database are presented.

Experiment 3. Effects of dietary history over generations on body fat accumulation under controlled dam's conditions during pregnancy and suckling periods

Animals and experimental design. The methods were the same as described in Experiment 1 until mating of predecessor rats in both HF and LF dietary groups. On the second day after mating, rats were killed by decapitation. The fertilized eggs were taken from the oviducts and implanted into the oviducts of prepared sham pregnant F344 rats (fed CE-2) without experimental dietary history, and were nurtured by those F344 rats during pregnancy. On day 21 of pregnancy, a cesarean section was performed, and the male pups obtained were used in this experiment. After birth, rats with both HF and LF dietary histories were nursed by prepared F344 foster mothers (fed CE-2) during the suckling period (3 weeks) and were then fed the high-fat diet (Table 1) *ad libitum* for 12 weeks after weaning. Litter size for HF dietary history rats was 7 ± 1, while for LF dietary history rats it was 8 ± 1. The number of pups born to pregnant F344 rats was adjusted to 7 for each F344 foster mother within 24 h of birth. The body weight of weaned rats did not differ between the two dietary history groups (mean value: 62 g).

On the final day of the experiment, the rats were fasted overnight and killed by decapitation. Blood and abdominal adipose tissues were collected as described in Experiment 1.

Determination of gene expression of LPL, HSL and leptin. The method of determination of LPL, HSL and leptin gene expression was the same as that described in Experiment 1.

Plasma analysis. Plasma insulin and leptin concentrations were assayed as in Experiment 1.

Data analysis. Statistical differences were analyzed using Student's *t*-test. Differences in *P* of less than 0.05 were considered significant.

Results

Experiment 1

Body weight, abdominal adipose tissue weights and food intake. Final body weight and abdominal adipose tissue (epididymal, perirenal and mesenteric) weights were significantly greater ($P < 0.05$) in rats with HF dietary histories than in rats with LF dietary histories (Table 3). The rats with HF dietary histories ingested significantly more ($P < 0.05$) food compared with LF dietary history rats (Table 3).

Gene expression of LPL, HSL and leptin. LPL, HSL and leptin mRNA levels in the perirenal adipose tissue were normalized β-action mRNA content, which did not differ between groups (data not shown). The mRNA levels of LPL and leptin were significantly higher ($P < 0.05$) in rats with HF dietary histories than in those with LF dietary histories (Fig. 1). The level of HSL mRNA was not affected by the dietary history (Fig. 1).

Plasma insulin and leptin concentrations. Plasma insulin concentration was significantly higher ($P < 0.05$) in rats with HF dietary histories than in rats with LF dietary histories (0.40 ± 0.09 *vs* 0.21 ± 0.03 nmol/L). Plasma leptin concentration was also significantly higher ($P < 0.01$) in rats with HF dietary histories (0.59 ± 0.07 *vs* 0.13 ± 0.04 nmol/L).

Experiment 2

Body weight, abdominal adipose tissue weights and food intake. Total body weight gain and abdominal adipose tissue

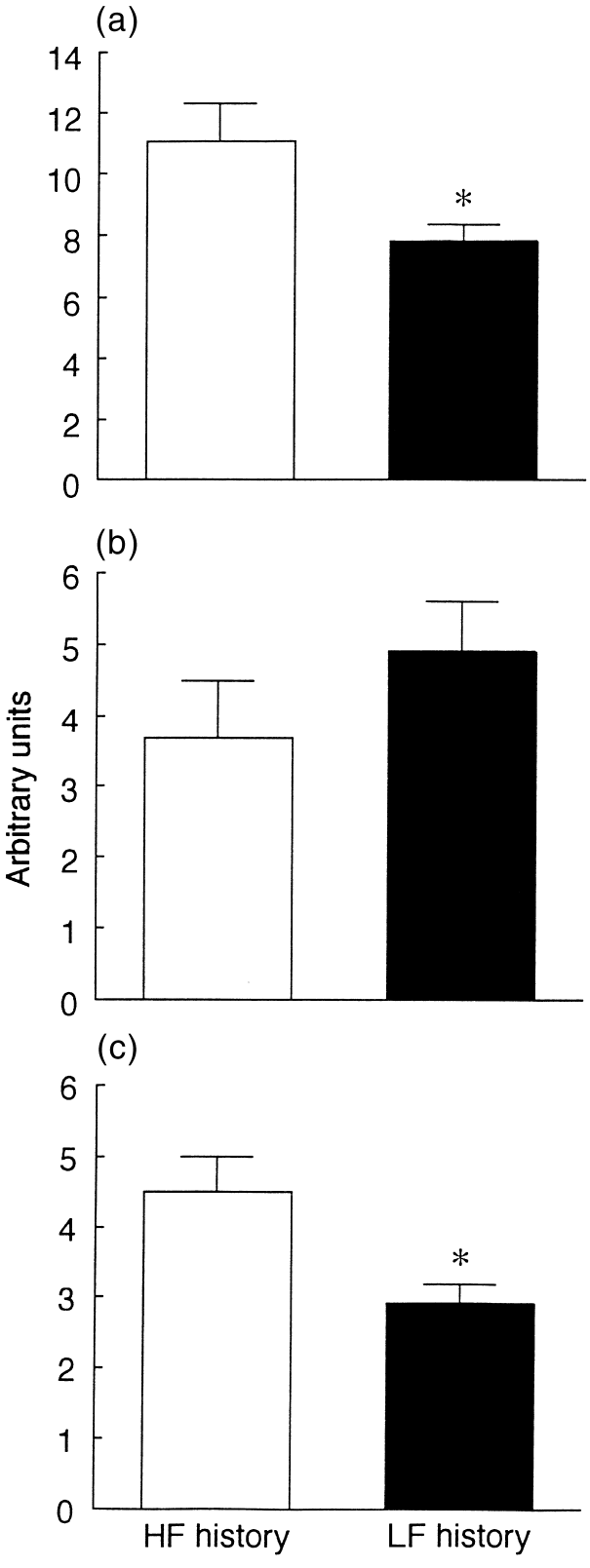

Figure 1. (a) Lipoprotein lipase, (b) hormone sensitive lipase, and (c) leptin mRNA levels normalized to β-actin mRNA content in the perirenal adipose tissue from rats with high-fat dietary histories and low-fat dietary histories (Experiment 1). HF, high-fat; LF, low-fat. Values are means and SE for 6–9 rats. *Statistically significant difference ($P < 0.05$) between two groups (Student's *t*-test).

weights were significantly greater ($P < 0.05$) in rats with HF dietary histories than in rats with LF dietary histories (Table 4). The rats with HF dietary histories ingested significantly more ($P < 0.05$) food compared with LF dietary history rats (Table 4).

Gene expression of LPL, HSL and leptin. The mRNA levels of LPL and leptin were significantly higher ($P < 0.05$) in rats with HF dietary histories than in those with LF dietary histories (Fig. 2). The level of HSL mRNA was not affected by dietary history (Fig. 2).

Plasma insulin and leptin concentrations. Plasma leptin concentrations were significantly higher ($P < 0.05$) in rats with HF dietary histories than in rats with LF dietary histories (0.40 ± 0.05 *vs* 0.10 ± 0.02 nmol/L). Plasma insulin concentrations were also higher in rats with HF dietary histories but the difference was not significant (0.34 ± 0.05 *vs* 0.29 ± 0.07 nmol/L).

Experiment 3

Body weight, abdominal adipose tissue weights and food intake. Total body weight gain was significantly greater ($P < 0.05$) in rats with HF dietary histories than in rats with LF dietary histories, and abdominal adipose tissue weights were also greater ($P < 0.05$) in rats with HF dietary histories (Table 5). The rats with HF dietary histories ingested significantly more ($P < 0.05$) food compared with the LF dietary history rats (Table 5).

Gene expression of LPL, HSL and leptin. The mRNA levels of LPL and leptin were significantly higher ($P < 0.05$) in rats with HF dietary histories than in those with LF dietary histories (Fig. 3). The level of HSL mRNA was not affected by the dietary history (Fig. 3).

Table 3. Effects of dietary history on final body weight, abdominal adipose tissue weights and food intake (Experiment 1)

	HF	LF
Final body weight (g)	610 ± 17	458 ± 20*
Abdominal adipose tissue		
Epididymal (g)	16.8 ± 2.1	11.0 ± 1.3*
Perirenal (g)	22.2 ± 2.3	11.6 ± 1.3*
Mesenteric (g)	10.5 ± 1.9	5.1 ± 0.5*
Food intake (kJ/day)	273 ± 4	252 ± 8*

Values are means ± SE for 6–9 rats. HF, high-fat diet history; LF, low-fat diet history. *Statistically significant difference ($P < 0.05$) versus HF group (Student's *t*-test).

Table 4. Effects of dietary history on final body weight, abdominal adipose tissue weights and food intake in rats suckled by F344 foster mothers (Experiment 2)

	HF	LF
Final body weight (g)	541 ± 16	414 ± 16*
Abdominal adipose tissue		
Epididymal (g)	13.3 ± 0.8	8.7 ± 0.6*
Perirenal (g)	16.7 ± 1.3	9.0 ± 1.0*
Mesenteric (g)	7.2 ± 0.5	4.7 ± 0.3*
Food intake (kJ/day)	266 ± 6	236 ± 4*

Values are means ± SE for 6–7 rats. HF, high-fat diet history; LF, low-fat diet history. *Statistically significant difference ($P < 0.05$) versus HF group (Student's *t*-test).

Plasma insulin and leptin concentrations. Plasma insulin concentration was significantly higher ($P < 0.01$) in rats with HF dietary histories than in rats with LF dietary histories (0.11 ± 0.01 *vs* 0.06 ± 0.01 nmol/L). Plasma leptin

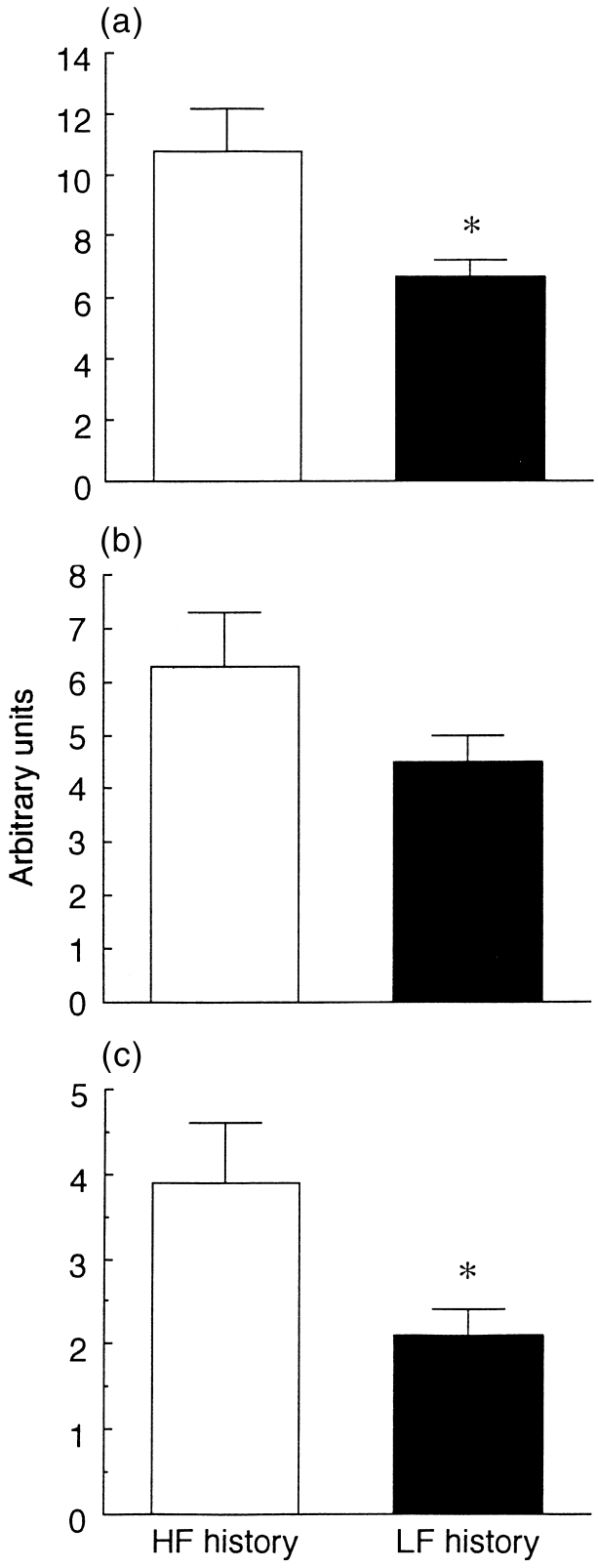

Figure 2. (a) Lipoprotein lipase, (b) hormone sensitive lipase, and (c) leptin mRNA levels normalized to β-actin mRNA content in the perirenal adipose tissue from rats with high-fat dietary histories and low-fat dietary histories (Experiment 2). HF, high-fat; LF, low-fat. Values are means and SE for 6–7 rats. *Statistically significant difference ($P < 0.05$) between two groups (Student's *t*-test).

concentrations were also significantly higher ($P < 0.05$) in rats with HF dietary histories (0.34 ± 0.07 *vs* 0.16 ± 0.02 nmol/L).

Discussion

Our previous study showed that HF dietary history had effects over generations on body fat accumulation in rats.[10] The present study examined whether or not those effects over generations were caused by genetic factors. It is clearly shown here that body weight gain and abdominal adipose tissue weights (an index of body fat accumulation[15,16]) were greater in rats with HF dietary histories than in those with LF dietary histories even when controlling for environmental background with respect to mother rats during pregnancy and suckling periods. These results suggest that the effects of a HF diet over generations are associated with genetic factors.

Lipoprotein lipase has its physiological site of action at the luminal surface of capillary endothelial cells where the enzyme hydrolyzes the triacylglycerol component of circulating lipoprotein particles, chylomicrons and very low density lipoproteins, to provide free fatty acids for tissue utilization in the heart and skeletal muscles or for fat accumulation in the adipose tissues.[11–13] Lipoprotein lipase plays a primary role in triacylglycerol metabolism as well as an important role in certain metabolic disorders, including obesity.[17,18] Genetic and diet-induced obesity are each clearly associated with increases in LPL protein levels in the adipose tissue of humans[19] and rodents.[20] Lipoprotein lipase mRNA was well correlated with LPL protein and LPL activity in the adipose tissues.[11] The higher levels of adipose tissue LPL mRNA in the rats with HF dietary histories suggest that blood triacylglycerol was taken into adipose tissue at a higher rate in the HF dietary history rats. We previously reported that the LPL activity in the perirenal adipose tissue was higher in the rats with HF dietary histories than in those with LF dietary histories.[10] Our present results support these previous findings.

Hormone sensitive lipase is an important regulator for keeping fat content in adipose tissue constant when greater amounts of triacylglycerol are delivered and hydrolyzed efficiently. The present study showed that the level of HSL mRNA was not affected by dietary history. However, Shimada *et al*. reported that the overexpression of LPL increases the HSL mRNA level and activity in the adipose tissue of mice and does not induce obesity by enhancing the hydrolysis of triacylglycerol in the adipose tissue.[21] The reasons for the discrepancies between Shimada's study and ours are not known, although the enhancement of gene expression of LPL induced by HF diet feeding over generations may be mainly due to the molecular mechanism of body fat accumulation.

Table 5. Effects of dietary history on final body weight, abdominal adipose tissue weights and food intake in rats born and suckled by F344 foster mothers (Experiment 3)

	HF	LF
Final body weight (g)	384 ± 19	338 ± 6*
Abdominal adipose tissue		
Epididymal (g)	8.6 ± 0.6	7.2 ± 0.3*
Perirenal (g)	9.4 ± 1.2	6.6 ± 0.3*
Mesenteric (g)	7.0 ± 0.5	5.7 ± 0.2*
Food intake (kJ/day)	204 ± 8	184 ± 2*

Values are means ± SE for 8 rats. HF, high fat diet history; LF, low fat diet history. *Statistically significant difference ($P < 0.05$) versus HF group (Student's *t*-test).

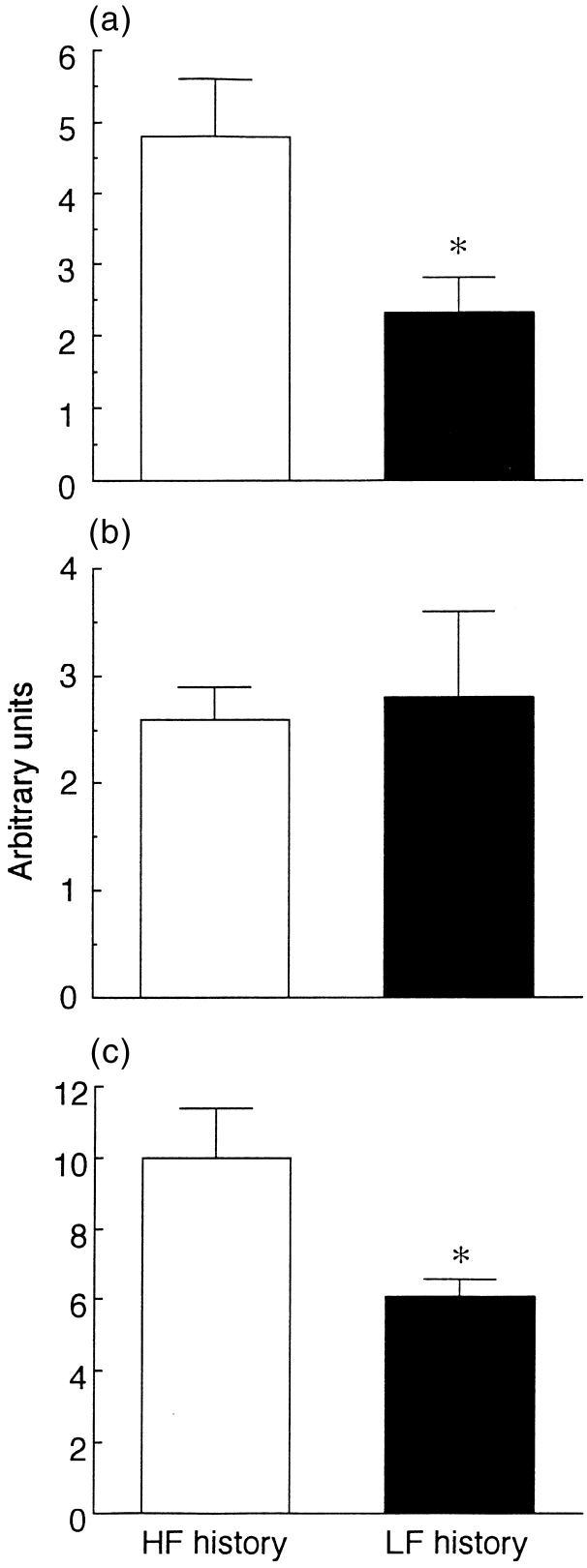

Figure 3. (a) Lipoprotein lipase, (b) hormone sensitive lipase, and (c) leptin mRNA levels normalized to β-actin mRNA content in the perirenal adipose tissue from rats with high-fat dietary histories and low-fat dietary histories (Experiment 3). HF, high-fat; LF, low-fat. Values are means and SE for eight rats. *Statistically significant difference ($P < 0.05$) between two groups (Student's *t*-test).

In the present study, the rats with HF dietary histories had higher fasting plasma leptin concentrations and higher gene expressions of leptin in the perirenal adipose tissue compared with the rats with LF dietary histories. Leptin, a hormone signal produced exclusively by the adipose tissues, conveys to the brain information on the size of energy stores and activates the hypothalamic centres that regulate energy intake and expenditure.[22] It may be assumed that most obese individuals with a higher set point for body weight are resistant to leptin.[23] From this point of view, the rats with HF dietary histories would have a higher set point for body weight. The mechanism that regulates leptin expression and secretion has not yet been clarified.[24,25] However, the present findings demonstrate that the effects over generations of HF diets might be associated with the persistent development of leptin resistance.

The higher plasma insulin concentrations in rats with HF dietary histories might also play a role in the accumulation of body fat. The predominant (if not the only) effect of insulin appears to be to increase the synthesis of LPL.[26] In this study, LPL mRNA levels in adipose tissues were significantly higher in rats with HF dietary histories. It has been reported that plasma insulin correlated positively with plasma leptin in mice and humans.[27–29] We have shown here that plasma insulin levels were similar to plasma leptin levels in rats with both HF and LF dietary histories. Kieffer *et al*.[30] recently reported the expression of leptin receptor mRNA on pancreatic β-cells. In this experiment, the higher plasma leptin in rats with HF dietary histories might have stimulated insulin secretion from pancreatic β-cells.

In the present study, all rats were fed experimental diets *ad libitum*. The method of isocaloric feeding has been used in several experiments to prevent hyperphagia. However, this method often disrupted the normal circadian patterns of feeding in the animals.[31] On the other hand, hyperphagia is considered to be an important factor in obesity. The rats with HF dietary histories did appear hyperphagic in *ad libitum* conditions compared with those with LF dietary histories.

The rats with both dietary histories were fed only HF diets (obesogenic environment) in all experiments. Because diet-induced obesity is caused by genetic factors under the obesogenic environment,[32,33] we experimented on rats with HF and LF dietary histories only under this obesogenic condition in this study.

In Experiment 3, the rats were killed at 12 weeks, while in the other experiments, the animals were killed at 17 weeks. Prior to Experiment 3, we found that body weight, plasma leptin and insulin concentrations were higher in rats with HF dietary histories by 12 weeks of age (data not shown). In order to clarify the fat deposition and mRNA levels in the adipose tissue at an earlier age, we killed the animals at 12 weeks of age.

Body weight and abdominal adipose tissue weights were greater in rats with HF dietary histories than in those with LF dietary histories even if controlling for environmental conditions related to mother rats during pregnancy and suckling periods. These results suggest that the effects of HF diet feeding over generations are related to genetic factors. The obesogenic effects of HF diet feeding over generations may be associated with lipoprotein lipase and leptin gene expression in the adipose tissue. The present findings do not explain the mechanism by which HF diets impair genetic programming. More detailed study is required to clarify this mechanism.

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