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The hyperleptinemia and ObRb expression in hyperphagic obese rats

Ying Han [a], Yeonsoo Joe [b], Eunhui Seo [b], Sa Rah Lee [c], Mi-Kyoung Park [c,d], Hye-Jeong Lee [b], Duk Kyu Kim [c,d,*]

[a] Graduate School, Dong-A University College of Medicine, Busan, Republic of Korea
[b] Department of Pharmacology, Dong-A University College of Medicine, Busan, Republic of Korea
[c] Department of Endocrinology, Dong-A University Medical Center, Busan, Republic of Korea
[d] Internal Medicine Department, Dong-A University College of Medicine, Busan, Republic of Korea

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ABSTRACT

Leptin resistance associated with hyperleptinemia in high-fat-diet-induced obese rats and aged obese rats is well established, but it is not clear whether hyperphagia-induced obese rats also develop leptin resistance. We investigated whether Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are a strain of hyperphagia-induced obese rats, develop leptin resistance and whether caloric restriction reversed this leptin resistance-induced leptin receptor (ObRb) deficit. Twenty male OLETF rats, 20 male Long-Evans Tokushima Otsuka (LETO) rats, and 10 male Sprague Dawley (SD) rats were used. All rats were initially studied at 10 weeks of age and were freely fed with standard rat chow and water until they were 38 weeks of age. Daily food intake, body weight, and plasma leptin levels of OLETF rats were remarkably increased compared to LETO or SD rats from 10 to 38 weeks of age. When they were 38 weeks of age, all OLETF rats were randomly divided into two groups. One group was freely fed with standard rat chow (FD, or free diet group), and the other group (RD, or restricted diet group) was fed with only 70% of the amount consumed by the FD group. The LETO and SD rats were dismissed from further study. After 4 weeks of caloric restriction, the average body weight (636 ± 33 g vs. 752 ± 24 g, $P < 0.05$) and abdominal adipose tissue weight (10.6 ± 3.2 g vs. 15.8 ± 1.5 g, $P < 0.05$) of the RD group were decreased compared with those of the FD group. Plasma leptin levels of the RD group were significantly decreased compared with those of the FD group (3.47 ± 1.40 ng/mL vs. 11.55 ± 1.16 ng/mL, $P < 0.05$). The mRNA expression of ObRb and leptin-related suppressor of cytokine signaling 3 (SOCS3) in the hypothalamus, liver, and skeletal muscles of the RD group were significantly decreased compared with those of the FD group. Caloric restriction did not improve leptin receptor (ObRb) deficit or the downstream signaling of leptin in the liver, skeletal muscles, and hypothalamus. Thus, we demonstrated that OLETF rats, which are a strain of hyperphagia-induced obese rats, did not develop central or peripheral leptin resistance. We suggest that hyperleptinemia in OLETF rats is a compensatory mechanism to overcome obesity induced by hyperphagia.

1. Introduction

Obesity is a growing health problem in the world and is a major risk factor for type 2 diabetes and cardiovascular disease. It is well established that obesity is associated with hyperleptinemia and leptin resistance. It has already been demonstrated that leptin resistance exists in various animal models of obesity, including high-fat-diet-induced obese SD rats [1], high-fat-diet-induced obese C57BL/6J mice [2], high-fat/high-sucrose-diet-induced obese F344 × BN rats [3], and age-related obese F344 × BN rats [4–6]. However, the OLETF rat, a rat model of hyperphagia-induced obesity, is known to develop peripheral leptin resistance but not central leptin resistance [7].

In a hyperleptinemic state, the mRNA expression of ObRb in the hypothalamus has been shown to increase at first but then to decrease in the presence of continuous leptin stimulation [8–11]. Consequently, leptin signaling in the hypothalamus is inhibited so that appetite cannot be controlled, although plasma leptin levels are elevated. This leptin insensitivity is called leptin resistance [12]. However, caloric restriction reverses the suppressed expression of ObRb and leptin signaling capacity in the hypothalamus in high-fat-diet-induced obese rats that have developed leptin resistance [10].

OLETF rats, which lack the CCK receptor A due to a spontaneous genetic mutation, are an established model of early-onset hyperphagia-induced obesity [13–15]. OLETF rats are heavier at birth compared with lean LETO control rats [15], and they show hyperphagic characteristics in independent ingestion tests as early as

* Corresponding author. Address: Internal Medicine Department, Dong-A University, College of Medicine, Dongdaeshin-dong, Seo-gu, Busan 602-714, Republic of Korea. Fax: +82 51 242 5852.
E-mail address: dkkim@dau.ac.kr (D.K. Kim).

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postnatal day 2 [16]. The plasma levels of leptin are increased in OLETF rats compared with lean LETO control rats [7]. ObRb is expressed not only in the hypothalamus but also in the liver, skeletal muscles, white adipose tissue, spleen, lung, kidney, heart, and small intestine [17]. The appetite response to exogenous leptin is different according to route of entry in OLETF rats. The central administration of leptin decreases food intake, but peripheral administration of leptin dose not affect food intake [7]. These results suggest that OLETF rats develop peripheral, but not central, leptin resistance.

In this study, we investigated whether OLETF rats exhibit leptin resistance, especially central leptin resistance. We hypothesized that if OLETF rats developed leptin resistance, then suppressed ObRb expression and suppressed leptin signaling may be observed, and caloric restriction may reverse these deficits in ObRb and leptin signaling. We investigated the effect of caloric restriction on ObRb expression and leptin signaling not only in the hypothalamus but also in the liver and skeletal muscles of OLETF rats. We also investigated the variations in plasma leptin levels associated with the age of OLETF rats.

2. Materials and methods

2.1. Animals and treatment

Ten-week-old male OLETF rats ($n = 20$) and male LETO rats ($n = 20$) were kindly donated by Otsuka Pharmaceuticals (Japan). A group of 10-week-old male SD rats ($n = 10$) was purchased from Central Lab Animal Inc. (Korea). All rats were kept in individual cages and fed freely with standard rat chow and water. All rats were cared for during the entire period of experimentation in accordance the Guidelines of Animal Experiments recommended by the Korean Academy of Medical Sciences.

After 28 weeks, all OLETF rats were randomly divided into two groups. One group was fed freely with standard rat chow (FD, or free diet group); while the restricted diet group (RD) was fed only 70% of the amount consumed by the FD group. This caloric restricted diet was maintained for 4 weeks. The LETO and SD rats were dismissed after they had aged 38 weeks.

2.2. Measurement of daily food intake, body weight, and FBS

Daily food intake and body weight were measured every day during the experimental period. The fasting blood sugar (FBS) was measured every 4 weeks using blood drawn from the tail vein after overnight fasting.

2.3. Measurement of abdominal fat weight

After 4 weeks of caloric restriction, all OLETF rats were sacrificed for measurement of abdominal fat weight. Mid-abdominal incisions were created and intra-abdominal organs were exposed. Abdominal adipose tissue was collected from the fat bordering the epididymal adipose tissue. The weight of each dissected fat mass was immediately measured after dissection using a precision digital scale.

2.4. Measurement of plasma adipokines

Plasma was collected by centrifugation of heparinized blood at 2000*g* for 15 min. The plasma insulin level was analyzed with a rat

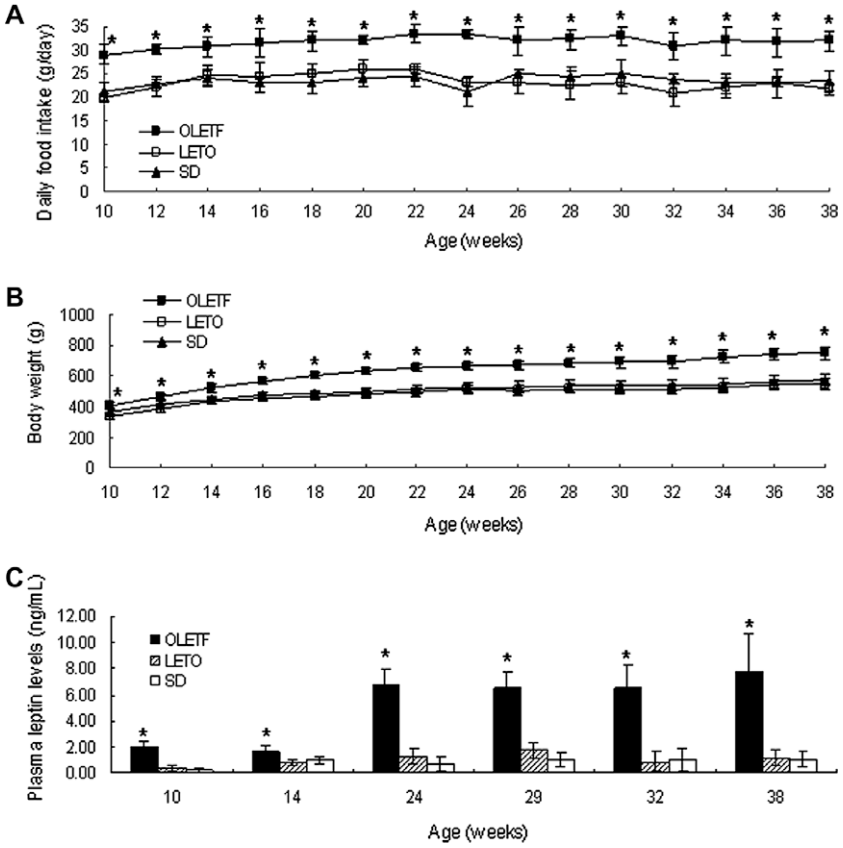

Fig. 1. (A) Comparisons of daily food intake among OLETF, LETO, and SD rats. OLETF rats consumed significantly more food than LETO or SD rats from 10 to 38 weeks of age. (B) Comparisons of body weights among OLETF, LETO, and SD rats. Average body weight of OLETF rats was significantly higher than that of LETO or SD rats from 10 to 38 weeks of age. (C) Comparison of plasma leptin levels among OLETF, LETO, and SD rats. Plasma leptin levels of OLETF rats were significantly higher than those of LETO or SD rats from 10 to 38 weeks of age. Values represent means ± SEM of OLETF ($n = 20$), LETO ($n = 20$), and SD ($n = 10$) rats. $^*P < 0.05$ vs. LETO or SD rats.

insulin ELISA kit (Central Lab. Animal Inc., Korea). The plasma leptin level was analyzed with a rat leptin ELISA kit (Linco Research, Inc., USA). Plasma adiponectin, TNF-α, and IL-6 levels were analyzed with rat adiponectin, TNF-α, and IL-6 ELISA kits (R&D Systems, Inc., USA).

2.5. Reverse transcription polymerase chain reaction analysis

Total RNA was isolated with Trizol reagent (Invitrogen Inc., USA), and single-stranded cDNA was synthesized from 5 μg of total RNA with oligo(dT)15 primers, M-MLV reverse transcriptase, M-MLV 5× reaction buffer, dNTPs, and ribonuclease inhibitor (Promega Corporation Inc., USA). The sequences of the sense and antisense primers used for amplification were as follows: ObRb, 5′-TCC ACCCAAAATTCTGACGA-3′, and 5′-AATTCAGCGTAGCGGTGATG-3′; SOCS3, 5′-TGGTCACCCACAGCAAGTTT-3′, and 5′-TGTCGCGGATAAG AAAGGTG-3′; 18S rRNA, 5′-GATGGTAGTCGCCGTGCCT-3′, and 5′-CCTTCCTTGGATGTGGTAGCC-3′. PCR analyses were performed using a GeneAmp PCR System 2400 (Perkin Elmer, USA). Each reaction was carried out with 10 μL of 2× Prime Taq Premix, 1 μL of cDNA, 1 μL of forward primer, 1 μL of reverse primer, and 7 μL of distilled water. The invariant control used for all studies was 18S.

2.6. Statistics

The significance of differences between groups was analyzed by an unpaired two-tailed Student's *t*-test. All results are expressed as mean values ± SEM and were analyzed using SPSS software for Windows (version 12.0, SPSS Inc., USA). Differences were considered significant when their *p*-values were less than 0.05.

3. Results

3.1. Comparison of daily food intake, body weight, and leptin between the OLETF rats and their lean controls

The OLETF rats consumed significantly more food than the LETO or SD rats from 10 to 38 weeks of age (Fig. 1A). This hyperphagia led to obesity in the OLETF rats. The average body weight of OLETF rats was significantly higher than that of the LETO or SD rats from 10 to 38 weeks of age (Fig. 1B). Plasma leptin levels of the OLETF rats were significantly higher than those of the LETO or SD rats from 10 to 38 weeks of age (Fig. 1C).

3.2. Change of body weight and abdominal adiposity after caloric restriction

There was no difference in average body weight between the FD and RD groups before caloric restriction. After 4 weeks of caloric restriction, the average body weight of the RD group was significantly decreased compared with that of the FD group (644 ± 31 g vs. 753 ± 27 g, $P < 0.05$) (Fig. 2A).

After 4 weeks of caloric restriction, the abdominal adipose tissue weight of the RD group was significantly decreased compared with that of the FD group (10.6 ± 1.6 g vs. 15.8 ± 1.5 g, $P < 0.05$) (Fig. 2B).

3.3. Changes in plasma leptin levels after caloric restriction

There was no difference in plasma leptin levels between the FD and RD groups before caloric restriction. After 4 weeks of caloric restriction, plasma leptin levels of the RD group were significantly decreased compared with those of the FD group (3.47 ± 1.40 ng/mL vs. 11.55 ± 1.16 ng/mL, $P < 0.05$) (Table 1).

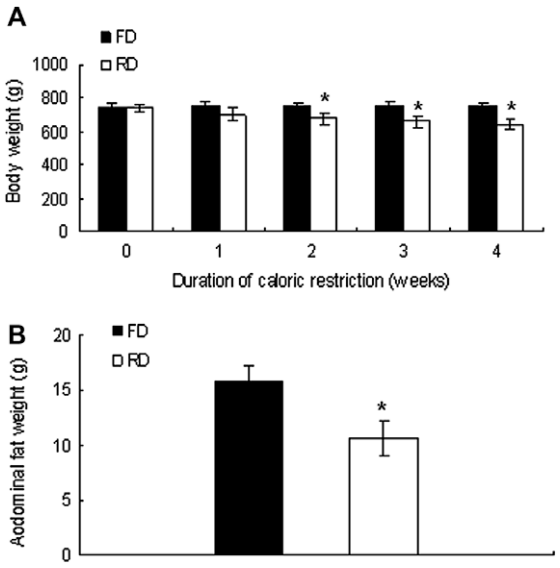

Fig. 2. (A) Changes in body weights for the FD and RD groups. There was no difference in the average body weight between the FD and RD groups before caloric restriction. After 1 week of caloric restriction, the average body weight of the RD group seemed to be decreased compared to that of the FD group, but this difference was not statistically significant (705 ± 42 g vs. 759 ± 24 g, $P = 0.05$). The average body weight of the RD group was significantly decreased compared to that of the FD group after 2 weeks (680 ± 34 g vs. 754 ± 26 g, $P < 0.05$), 3 weeks (659 ± 32 g vs. 753 ± 31 g, $P < 0.05$), and 4 weeks of caloric restriction (644 ± 31 g vs. 753 ± 27 g, $P < 0.05$). (B) Change in abdominal adipose tissue weight for the FD and RD groups. After 4 weeks of caloric restriction, the abdominal adipose tissue weight of the RD group was significantly decreased compared to that of the FD group (10.6 ± 1.6 g vs. 15.8 ± 1.5 g, $P < 0.05$). Values represent means ± SEM of the FD ($n = 10$) and RD ($n = 10$) groups. *$P < 0.05$ vs. FD group. FD, free diet; RD, restricted diet.

3.4. Changes in other metabolic parameters after caloric restriction

There was no difference in FBS and plasma insulin levels between the FD and RD groups before caloric restriction. After 4 weeks of caloric restriction, there was no difference in FBS and plasma insulin levels between the FD and RD groups (Table 1).

There was no difference in other adipokines such as adiponectin, TNF-α, or IL-6 between the FD and RD groups before caloric restriction. In addition, there was no difference between the FD and RD groups after caloric restriction. Only leptin levels in the RD group were significantly decreased compared with those of the FD group after caloric restriction (Table 1).

3.5. Effect of caloric restriction on ObRb and SOCS 3 expression

The mRNA expression of ObRb in the hypothalamus of the RD group was significantly decreased compared with that of the FD group. The mRNA expression of SOCS 3 in the hypothalamus of the RD group was significantly decreased compared with that of the FD group (Fig. 3).

The mRNA expression of ObRb in both the liver and skeletal muscles of the RD group was significantly decreased compared with that of the FD group. The mRNA expression of SOCS 3 in both the liver and skeletal muscles of the RD group was significantly decreased compared with that of the FD group (Fig. 3).

4. Discussion

In this study, we observed that caloric restriction decreased hypothalamic ObRb expression and leptin-related SOCS 3 expression in OLETF rats. In addition, caloric restriction decreased ObRb expression and leptin-related SOCS 3 expression in the liver and

Table 1
FBS, plasma levels of insulin and adipokines of the FD and RD groups of OLETF rats before and after caloric restriction.

	Before caloric restriction (n = 20)			After caloric restriction (n = 20)		
	FD	RD	P-value	FD	RD	P-value
FBS (mg/dL)	170 ± 31	166 ± 16	0.40	165 ± 33	161 ± 11	0.37
Insulin (pg/mL)	1.86 ± 0.84	1.93 ± 1.22	0.47	2.15 ± 1.20	1.36 ± 1.11	0.18
Leptin (ng/mL)	10.23 ± 1.53	10.10 ± 5.24	0.48	11.55 ± 1.16	3.47 ± 1.40	0.002
Adiponectin (ng/mL)	0.24 ± 0.08	0.23 ± 0.04	0.40	0.23 ± 0.03	0.22 ± 0.04	0.33
TNF-α (ng/mL)	0.13 ± 0.04	0.12 ± 0.05	0.38	0.44 ± 0.04	0.60 ± 0.19	0.10
IL-6 (ng/mL)	0.90 ± 0.05	0.90 ± 0.02	0.38	2.10 ± 0.10	2.93 ± 1.12	0.14

FBS, fasting blood sugar; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; FD, free diet; RD, restricted diet.

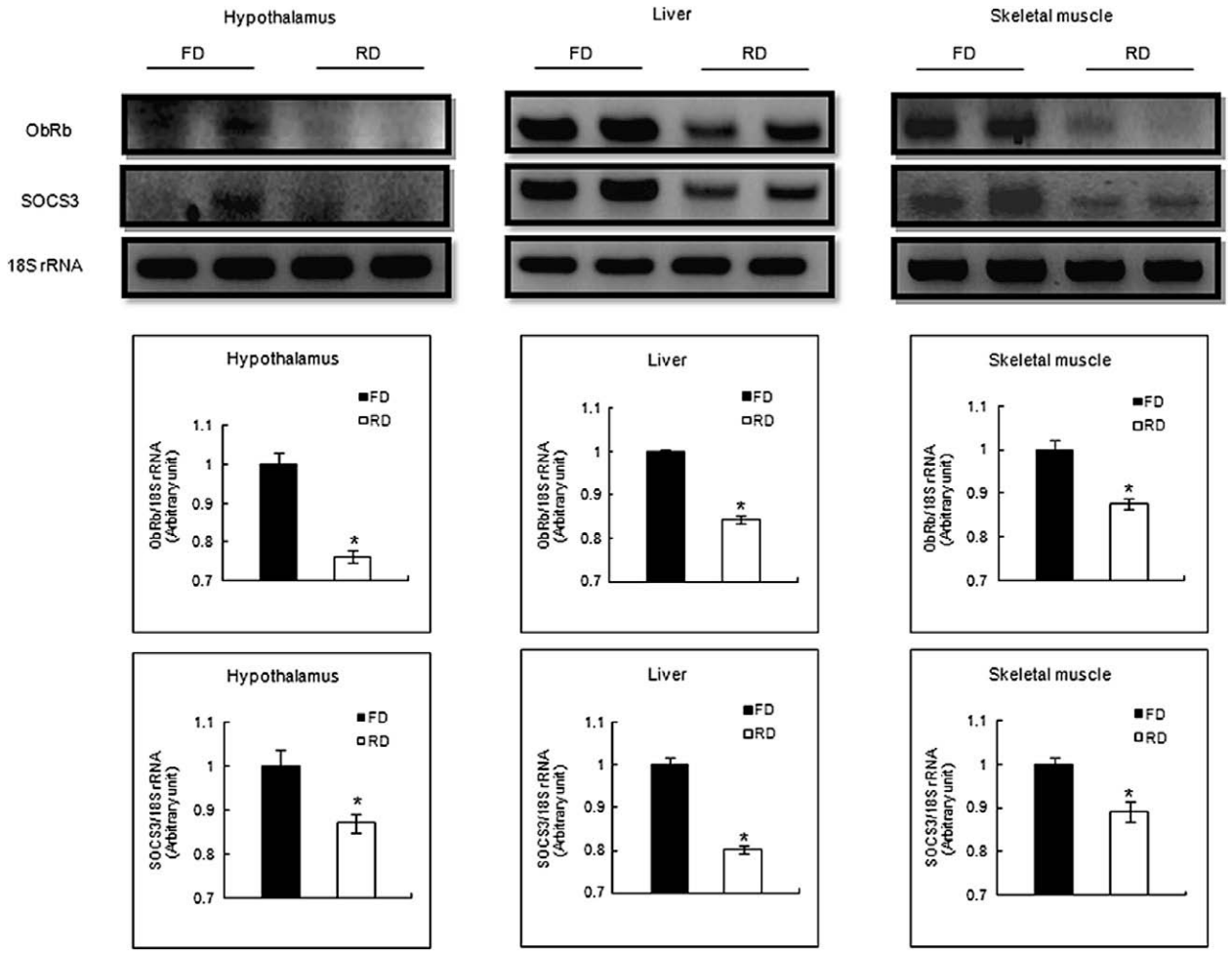

Fig. 3. ObRb and SOCS 3 expression in the hypothalamus, liver, and skeletal muscles of the FD and RD groups in OLETF rats after caloric restriction. After 4 weeks of caloric restriction, both ObRb and SOCS 3 expression levels in the RD group were significantly decreased compared to the FD group. Values represent means ± SEM of the FD (n = 10) and RD (n = 10) groups. $^{*}P < 0.05$ vs. FD group. FD, free diet; RD, restricted diet.

skeletal muscles. These findings suggest that OLETF rats do not develop central or peripheral leptin resistance even though they present hyperleptinemia. We did not compare the differences in ObRb expression between OLETF rats and their lean controls. However, the lack of such a comparison dose not affect our results because caloric restriction decreased ObRb expression in this study. The comparison study would be needed only if caloric restriction increased ObRb expression.

There is a contradictory report that showed that OLETF rats had peripheral leptin resistance. The appetite was not affected when a dose of leptin (2 mg/kg) was administered via intra-peritoneal (i.p.) injection, whereas appetite was decreased when leptin was administrated via intra-cerebroventricular (i.c.v.) injection [7]. This peripheral leptin resistance also presented in high-fat-diet-induced obese C57BL/6 and AKR mice with a different dose of leptin (10 mg/kg, i.p.) [18], but did not present in high-fat-induced obese mice with a higher dose (12.5 mg/kg, i.p., twice daily) [19]. Though the leptin infusion study is a very useful method to document the existence of leptin resistance in animal models of obesity, results might be different according to the amount of infused leptin, as we discussed above. Thus, we should be cautious when we interpret the results of leptin infusion studies until the ideal infusion dose of leptin is established for the protocol.

Short- and long-term caloric restrictions have been shown to dramatically reduce plasma leptin levels in obese humans and rats [20–21]. In this study, we reported significant reductions in plasma leptin levels following 4 weeks of caloric restriction. Because body weight and abdominal adipose tissue weight for the RD group were also decreased compared to those of the FD group, it is impossible to distinguish whether the reduction of plasma leptin levels was primarily caused by caloric restriction or by loss of body fat. However, the plasma levels of other adipokines did not show differences between the RD and FD group, only leptin. We cautiously suggest that reductions of plasma leptin levels are primarily related to caloric restriction rather than loss of body fat.

In addition, we observed that there is a great difference in plasma leptin levels between 14 and 24 weeks of age, after which the plasma leptin levels showed a plateau. This profile of leptin levels is similar in LETO and SD rats (statistical data were not presented). These phenomena are another clue that leptin resistance is not presented in OLETF rats as well as LETO and SD rats. If there would be leptin resistance in OLETF rats, the plasma leptin levels would not be sustained, and would increase with age [8].

As we already demonstrated, the plasma levels of leptin in OLETF rats were higher than those of controls. The body weights of OLETF rats were also heavier than those of controls. We presume that the hyperleptinemia of OLETF rats is a compensatory mechanism against weight gain induced by hyperphagia. Hyperphagia is induced by the state of CCK receptor A deficit [13–14]. CCK is a hormone related to anorexia [21–22].

These results suggest that hyperphagic drive due to CCK deficit overwhelmed the anorectic drive induced by leptin stimulation. If this explanation is true, we can make another hypothesis that there might be CCK deficiency or CCK resistance in obese patients who show hyperleptinemia. There are few studies concerning plasma or tissue CCK levels in human obesity. Future research in this field might discover several interesting findings that will improve our understanding of human obesity.

In conclusion, hyperleptinemia in OLETF rats was not associated with leptin resistance. We suggest that hyperleptinemia in OLETF rats is a compensatory mechanism to overcome obesity induced by hyperphagia.

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