ORIGINAL PAPER

Leptin resistance does not induce hyperphagia in the rat

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Abstract Leptin has been thought to work as a mediator for body weight control by inhibiting food intake. Leptin, however, cannot prevent obesity induced by a high-fat diet (HFD) probably because of leptin resistance. We investigated daily feeding and weight gain when ordinary chow (OC) was changed to a HFD in male rats. Food intake, by weight, significantly increased the next day, but gradually decreased until at 20 days the HFD intake contained the same calories as consumed by the OC-fed control rats. The reduction in food intake occurred only during the night without change of preference for the HFD, even after leptin resistance had developed. Nonetheless, the HFD-fed rats gained more weight than the controls. From the present experiment, it is concluded that leptin resistance does not induce hyperphagia, and suggested that body weight is not regulated to be constant.

Keywords Leptin resistance · High fat diet · Food intake · Body weight

Introduction

Control of body weight in mature animals has been demonstrated by studies in which humans or animals are made to either gain or lose weight by externally controlled changes in food intake. When the humans or the other animals are subsequently allowed to feed ad libitum, they return to control body weight by adjusting voluntary food intake to compensate for the previously enforced intake [1–4]. Since the discovery of leptin [5], it has been expected to work as a mediator for body weight control by inhibiting food intake. However, in spite of the presence of a homeostatic mechanism for maintaining body weight constant, animals fed on food with higher fat contents readily become obese [6–8]. This is generally explained to be attributed to leptin resistance; a state in which circulating leptin levels are elevated coincident with ongoing hyperphagia and obesity, or a state in which exogenously administered leptin fails to inhibit food intake [3, 9]. The mechanisms underlying the normalization of body weight following increased body weight resulting from augmented calorie intake are not well understood [3]. There is an argument that energy homeostasis operates primarily to defend against weight loss and that, over the course evolution, biological defense against weight gain was not selected for [9].

If the anorexigenic effect of leptin is physiological, then food intake must be increased when leptin resistance is established, but there have been few reports of whether this is the case. Here, we tested this proposition. We examined if food intake is indeed increased when the rat acquires leptin resistance. We found that daily food intake was actually decreased in leptin-resistant rats fed on a high-fat diet (HFD) compared with leptin-responsive rats fed on ordinary chow (OC), and that both groups took a similar amount of calories.

Materials and methods

Animals and surgical description

Sixty-four male Wistar rats were obtained from Charles River Japan (Yokohama, Japan) and were caged singly and

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kept in an environmentally controlled room ($23 \pm 2°C$, lights on 0600–1800 hours) with free access to tap water and ordinary pellet food (NMF; Oriental Yeast, Tokyo, Japan) containing 3.49 kcal/g, or a HFD (D12492; Research Diets, New Brunswick, NJ, USA) containing 5.2 kcal/g. Food intake and body weight were measured at 0930–1000 hours every morning, except for the experiment on circadian variation where food intake and body weight were measured at 0600 and 1800 hours.

In order to inject leptin into a lateral cerebral ventricle, 29 male rats were implanted with a guide cannula. A stainless steel guide cannula (1 mm outer diameter) was implanted into the right lateral ventricle (posterior −0.8 mm, medial lateral +1.5 mm, and ventral −4 mm, relative to bregma according to a rat brain atlas [10]), using a stereotaxic frame (Narishige SR-6N, Tokyo) under anesthesia with pentobarbital sodium. All experiments were conducted in the home cage, after at least 10 days of recovery period. Leptin (Peptide Institute, Osaka, Japan) dissolved in 0.9% saline was administered in a volume of 5 µl using a 10-µl microsyringe attached to polyethylene tubing and a stainless steel injection cannula (0.6 mm outer diameter) extending 0.5 mm beyond the tip of the guide cannula.

For estimating when the rats became leptin resistant, 10 and 50 days after the change of the food from OC to the HFD, 13 rats were injected with leptin (20 µg in 5 µl of saline) or 5 µl of saline at 1600 hours into the lateral ventricle. Food intake was measured 24 h later. Blood samples (0.5 ml) were taken from tail veins of another 12 rats under ether anesthesia on days −1, 10 and 50 after the food change. Serum leptin levels were determined using a rat leptin RIA kit (Linco; Millipore Japan, Tokyo).

One hundred and four days after the exchange in the food supplied, half of the rats ($n = 4$) of the HFD group were injected with leptin (20 µg in 5 µl of saline) or 5 µl of saline at 1600 hours into the lateral ventricle, respectively. Food intake and body weight was measured 24 h later. Then, the reverse treatment was performed; the saline-treated rats were injected with leptin and leptin-treated rats were administered saline. OC control rats were treated similarly. The HFD rats were tested again after being returned to OC food for 10 days (HFD + OC group). The rats were euthanatized by anesthetic (pentobarbital sodium) overdose at the end of the experiment. Cannula placement was confirmed by visual inspection of the cannula tip location within the lateral ventricle following 2% dye solution (Pontamine Sky Blue 6B; Tokyo Kasei Kogyo, Tokyo)

Another group of seven male rats was used to examine preference for the HFD. They were allowed to access freely both the OC and HFD using a container with a partition to separate the two foods.

The experimental protocol was approved by the Animal Care Committee of University of Fukui.

Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM). The data were statistically evaluated with one-way or two-way analysis of variance followed by Tukey test or Student's t test.

Results

Effects of changing food from OC to HFD on food intake and body weight

The daily food intake of the male rats ($n = 8$) expressed as weight significantly ($P < 0.01$) increased 1 day after changing the food from OC (27.5 ± 1.0 g) to the HFD (31.6 ± 1.4 g). Then, it gradually decreased to the weight similar to that of OC-fed rats in 5–7 days, and further decreased until at 20 days the intake contained the same amount of calories consumed by the control rats fed on OC (Fig. 1a, b). At that time, the HFD rats ate 19.8 ± 0.8 g/day (103.0 ± 4.2 kcal/day) and OC rats ate 28.9 ± 0.6 g/day (100.9 ± 2.1 kcal/day). The decreased food intake lasted until the end of this study, 200 days after the change in diet. When the energy intake became not significantly different between OC rats and HFD rats, food intake during the day was similar in both groups; for example, mean values from 100 and 110 days: 6.8 ± 0.7 g for OC rats and 6.6 ± 0.7 g for HFD rats. The reduction in daily food intake in the HFD rats appeared only during the night time (24.5 ± 1.9 g for OC rats and 14.8 ± 0.8 g for HFD rats) as shown in Fig. 2. Nonetheless, HFD rats became significantly (at least $P < 0.05$) heavier than CO controls 43 days after the diet exchange.

Effects of changing food from OC to HFD on food intake in HFD-induced leptin resistant rats

The injection of leptin (20 µg) into the lateral ventricle of HFD rats ($n = 6$) significantly ($P < 0.05$) decreased food intake the next day (98.9 ± 17.1 kcal/day) 10 days after the initiation of the HFD compared with that in the HFD rats ($n = 7$) injected with saline (135.9 ± 5.7 kcal/day). However, the same leptin treatment did not reduce food intake 50 days after the introduction of the HFD (117.0 ± 5.6 kcal/day for leptin injection, $n = 7$ and 124.4 ± 57.2 kcal/day for saline injection, $n = 6$).

Serum leptin concentrations were 0.96 ± 0.30, 2.36 ± 0.29, 6.26 ± 0.86 ng/ml on days −1, 10 and 50 after the start of HFD feeding, respectively, in OC rats ($n = 6$), and 1.20 ± 0.33, 4.54 ± 0.58, and 18.37 ± 2.31 ng/ml in HFD rats ($n = 6$), respectively. Serum leptin levels were significantly ($P < 0.05$) elevated in HFD rats compared with OC rats only on day 50.

Fig. 1 Daily food intake (expressed by weight (**a**) or energy (**b**)) and body weight (**c**) of the male rats fed on ordinary chow (*OC*) or a high-fat diet (*HFD*). The food for half of the rats (*n* = 8) was changed from OC to the HFD on day −1; other rats (*n* = 8) were continued to be fed OC. Food intake increased temporally after the food change from OC to HFD, but gradually decreased to the amount which contained the same amount of calories and remained stable even after their body weight was significantly heavier than that of OC rats. Values are group mean ± SEM. *$P < 0.05$, **$P < 001$ between OC and HFD rats
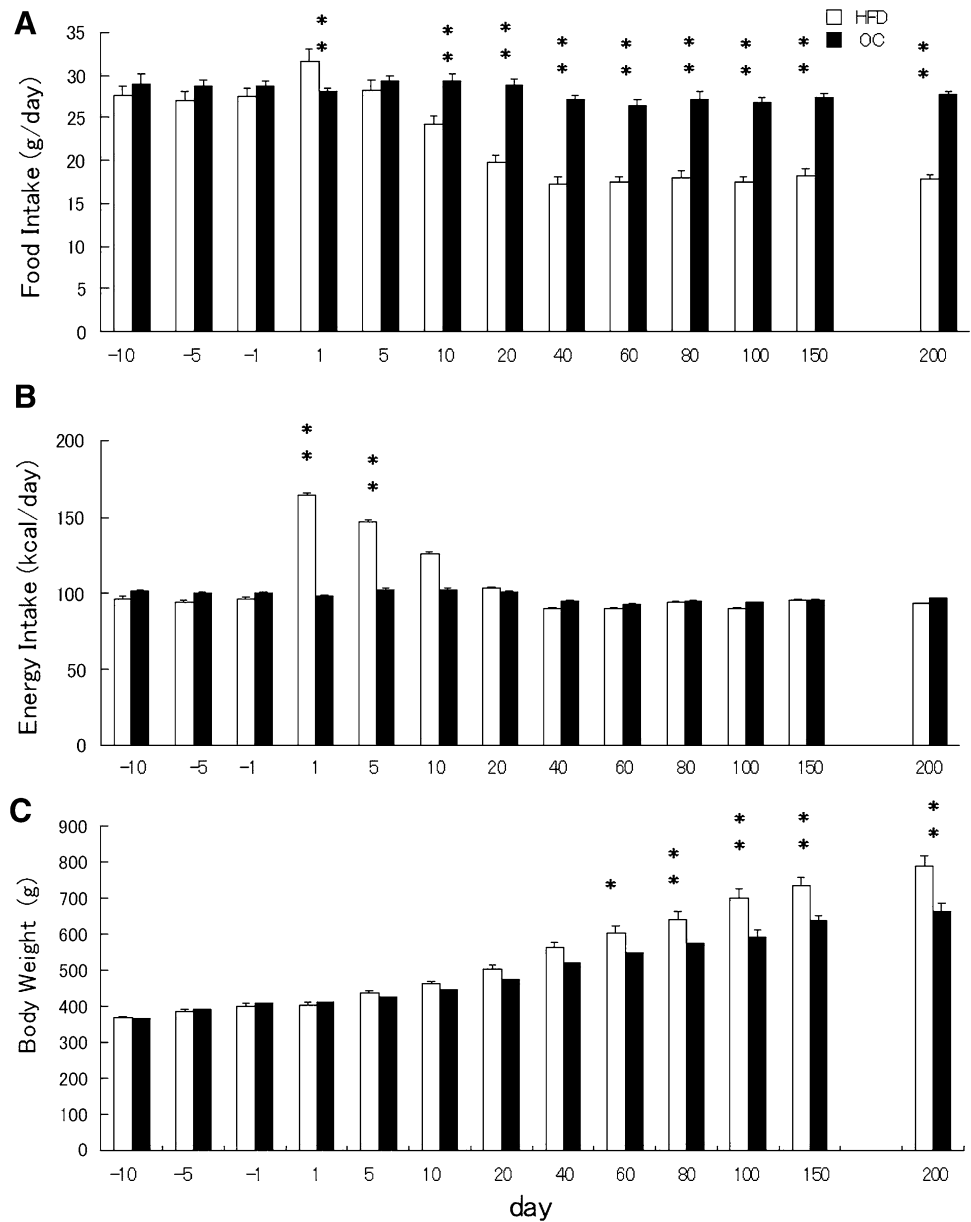
Another 16 male rats were reared on the OC diet, and, for 8 rats of this cohort, the food was changed to the HFD on the same day as the rats described above. After 104 days, the leptin (20 μg) injection into the lateral cerebral ventricle of the OC rats significantly ($P < 0.05$) decreased food intake and body weight gain next day, as shown in Fig. 3a, b, compared with controls injected with intracerebroventricular (i.c.v.) saline. This anorexigenic effect of leptin was not observed in the rats fed the HFD for 104 days, indicating they were in a state of leptin resistance. Two weeks later, the HFD was returned to the OC in the HFD rats for 10 days, during which food intake was first decreased and then gradually increased. The leptin resistance was not recovered by the 10-day absence of HFD, since i.c.v. injection of 20 μg of leptin did not reduce

food intake. Next day, the food was changed to the HFD again in the 8 rats and 8 OC control rats. The response to this food change was similar in both the leptin-resistant and leptin-sensitive groups (Fig. 3c): there was an initial increase, with a following gradual decrease, resulting in taking energy intake similar to that in the OC controls.

Change in preference for the HFD after food change from OC to HFD

In order to determine whether the decrease of food intake following temporary hyperphagia with the HFD can be attributed to a change of preference for the HFD, a food preference test was performed using another 7 male rats. They were presented with both CO and the HFD. As shown
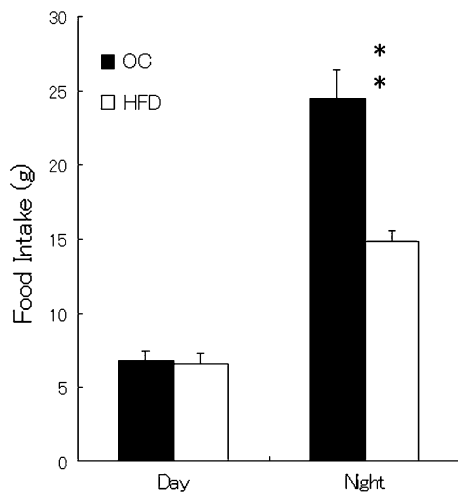
Fig. 2 Mean food intake of *OC* rats ($n = 8$) and *HFD* rats ($n = 8$) during light and dark period between 100 and 110 days after the initiation of HFD feeding. Values are group mean ± SEM. **$P < 001$ between *OC* and *HFD* rats

in Fig. 4, the rats consumed the HFD as $96.3 \pm 2.9\%$ of total daily food intake the first day, and this slightly decreased to $87.9 \pm 5.3\%$ from the 4th day and remained between 87.4 and 95.4% afterwards until the 20th day. Total food intake was highest on the first day of the test and decreased gradually as seen in the experiment when food was changed from OC to the HFD.

Discussion

The present study clearly demonstrated that leptin resistance does not bring about hyperphagia. The rats fed the HFD became leptin resistant between 10 and 50 days after starting the HFD. HFD rats did not decrease food intake in response to leptin administration and showed significantly higher leptin concentrations in blood than OC rats on day 50. The leptin resistance remained on day 104. However, during this period, food intake decreased gradually and got stabilized at a level of energy intake similar to that in OC-fed controls. They never exhibited hyperphagia except for the initial 4 days, even when they were significantly heavier than the control rats. This result confirmed the previous report that HFD-induced hyperphagia does not last for long [11]. We further demonstrated that a similar response was observed after the HFD rats became leptin resistant, indicating that the rat does not overeat even after the leptin resistance is established; leptin resistance never induced hyperphagia. Indeed, HFD consumption decreased below OC intake in control rats, indicating that leptin seems not to be involved in this decrease in HFD intake. The finding differs from the previous report that leptin-invoked leptin resistance perturbs the regulation of energy homeostasis in

response to high fat exposure, as the energy consumption is maintained at an elevated level above that of high-fat-fed animals 18 days after a HFD [12]. This group further demonstrated that a leptin antagonist infusion into a lateral ventricle induced similar abnormality in homeostatic normalization of elevated energy intake after high-fat feeding for 7 days, while controls rats showed nearly normalized caloric intake [13]. It is difficult to explain the difference between these results and the present findings that the response to the intake of high-fat food was not different between before and after leptin resistance had developed. However, it is noteworthy that the previous data demonstrated that food intake expressed by weight decreased below that of chow-fed animals on day 18, suggesting food weight intake was actually decreased in leptin-resistant rats even though they took more energy than OC control rats [12]. In the experiment using a leptin antagonist, it is not possible to know the change in food intake later than 7 days after being fed a HFD [13], but it seems probable that the rats that were given a leptin antagonist infusion also continued to reduce their food intake until their energy intake became similar to that in the control rats. We must be cautious that the rats used in their experiment seem to be extremely efficient in accumulating energy in terms of the calories taken. The rats gained body weight significantly more than OC controls in only 2 days. In our experiments, HFD rats became heavier than OC rats 43 days after the start of HFD feeding. Recently, using leptin receptor-deficient obese Zucker (fa/fa) rats, White et al. [14] demonstrated that intact leptin signaling is not required for the decrease in food intake that occurs during overfeeding. Evidently, more studies are needed to determine whether leptin is involved in the mechanism for food intake reduction during HFD feeding.

When food was changed from OC to the HFD, the next day the rats ate 20–30% more HFD (30–45% in terms of calories) than OC, probably because they prefer the palatable taste of fat. Leptin receptors are expressed on the midbrain dopamine neurons [15], and are involved in the motivated behavior of feeding, probably through altering neural activity of the nucleus accumbens [16, 17]. In addition, overfeeding induces rapid leptin release from adipose tissue as early as 2 or 3 days after the start of overfeeding [13, 18]. Thus, we examined the possibility that the reduction in food intake during HFD feeding is due to an alteration in preference for the HFD. As shown in the preference experiment (Fig. 4), the rats took more than 87% of total daily food as the HFD when exposed to both OC and the HFD. Daily total food intake was increased at first when the preference test was started, but gradually decreased as similarly seen when the rats were exposed to only the HFD. However, the preference for the HFD remained stable for at least 20 days, thus a change in taste preference for or habituation to the HFD was not an

Fig. 3 Response to leptin and to the change of food from OC to the HFD in the rats fed *OC* or *HFD*. After 104 days fed on the HFD, the anorexigenic effect of leptin was tested by injecting 20 μg of leptin or 5 μl of saline into a lateral cerebral ventricle of in the OC (*n* = 8, respectively) and the HFD (*n* = 8, respectively) rats (**a** food intake, **b** body weight change). The HFD rats were tested again after their food was returned to the OC for 10 days (*HFD + OC* group). Only OC rats showed leptin sensitivity and HFD rats and HDF + OC rats were insensitive to leptin. Values are mean ± SEM. *P* < 0.05, between leptin and saline injection. Changes in food intake after food was switched from ordinary chow (OC) to the high-fat diet (HFD) in leptin-resistant and leptin-responsive rats (**c**). Rats were made leptin-resistant by HFD feeding for 104 days; these rats were then fed OC for 10 days, then HFD was returned on day −1 (*n* = 8). The leptin-resistant and leptin-sensitive rats showed similar patterns of food intake responses to the HFD. Values are mean ± SEM. *P* < 0.05, between −1 and 1 day values in both leptin-resistant and leptin-sensitive rats
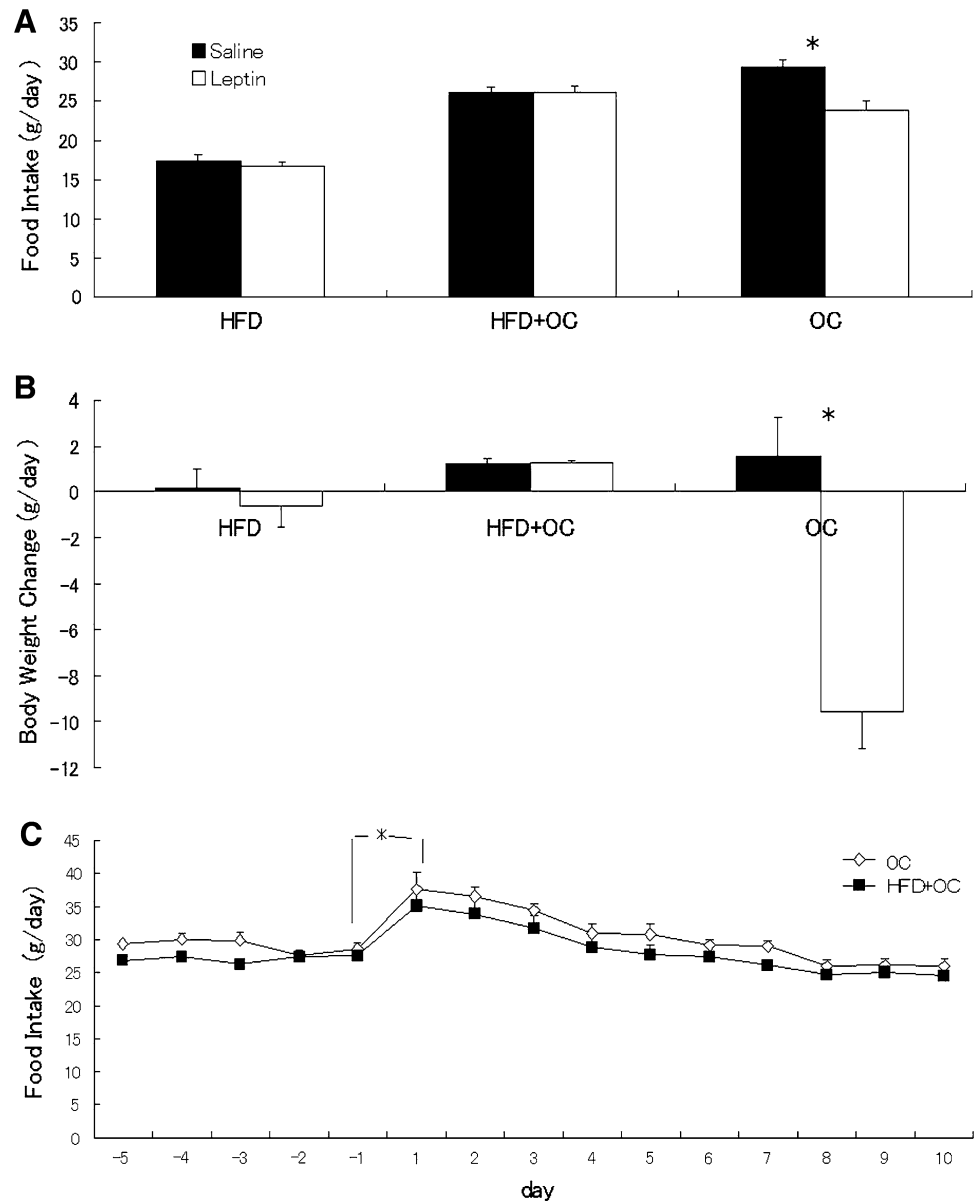

explanation for the decline in HFD intake after the initial increase.

A prolonged alteration in body weight without change in energy intake similar to our finding has been reported in other situations. Exposure to a single social defeat [19] or 3-h restraint stress for 3 consecutive days [20] caused a body weight loss that failed to return long after stress-induced hypophagia returned to the control levels. Estrogen injection into ovariectomized rats induces temporal decrease in food intake with permanent body weight reduction [21]. In addition, an inhibitor of fatty acid synthase reduces food intake for 1 day but weight loss lasts for at least 5 days after its injection [22]. It is possible to speculate that long-term HFD feeding, stress, estrogen, and change in fat metabolism alter the set point around which

body weight is regulated, but is such a set point altered so easily? It seems more plausible to hypothesize that body weight is not regulated to be constant and that the food intake is regulated to keep the energy intake constant. This conclusion supports the theory of energostatic control of feeding proposed by Booth [23, 24], which says that primary metabolic control of food intake is an adjustment of the meal pattern that brings the current energy balance towards the null point. In supporting the energostatic hypothesis, an intermediate in the fatty acid biosynthesis pathway malonyl-coenzyme A, has emerged as a major regulator of energy homeostasis in the hypothalamus [25, 26]. In addition to a HFD feeding, leptin [27, 28] and estrogen [29, 30] have profound effects on fat metabolism. Furthermore, our finding that reduction of food intake with

Fig. 4 A change of preference for the HFD. A food preference test was performed using 7 male rats. They were presented with both CO and the HFD. HDF preference was expressed as percentage of the HFD of total daily food intake. The rats constantly preferred the HFD to OC throughout the experimental period
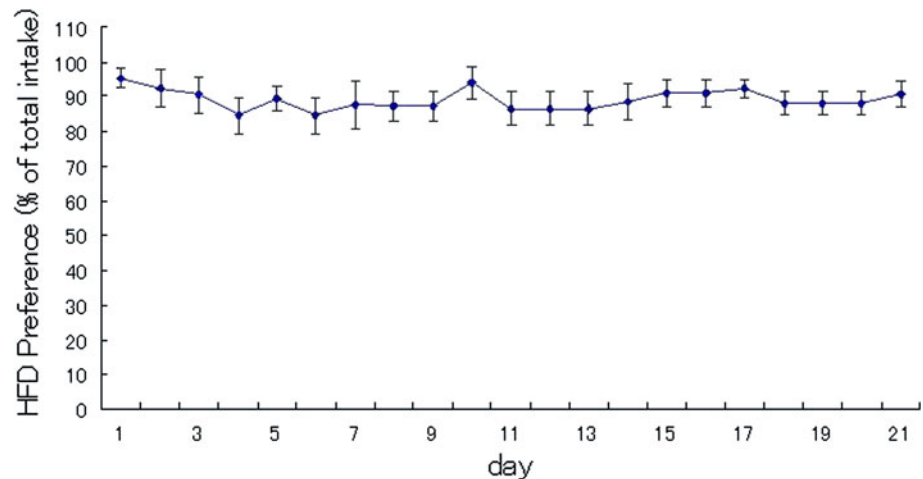
continuous exposure to the HFD occurred only during the night time (Fig. 2) may be due to the association of circadian alteration between fat synthesis at night and fat mobilization during the day with a cycle of nocturnal hyperphagia [31, 32]. It is an interesting question to be clarified whether the altered malonyl-coenzyme A content in the hypothalamus, which changes quite quickly [33], can explain the alterations in food intake that occurs in the long term, as observed in the present experiments. It is important to elucidate the mechanism for the reduction of food intake after voluntary overfeeding, as disturbance of this homeostatic function may induce overfeeding and obesity.

References

1. Friedman MI, Stricker EM (1976) The physiological psychology of hunger: a physiological perspective. Psychol Rev 83:409–431
2. Stricker EM (1978) Hyperphagia. N Engl J Med 298:1010–1013
3. Morrison CD (2008) Leptin resistance and the response to positive energy balance. Physiol Behav 94:660–663
4. Shin AC, Zheng H, Berthoud HR (2009) An expanded view of energy homeostasis: neural integration of metabolic, cognitive, and emotional drives to eat. Physiol Behav 97:572–580
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
6. Sclafani A, Springer D (1976) Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol Behav 17:461–471
7. Rolls BJ, Rowe EA, Turner RC (1980) Persistent obesity in rats following a period of consumption of a mixed, high energy diet. J Physiol 298:415–427
8. Popkin BM, Doak CM (1998) The obesity epidemic is a worldwide phenomenon. Nutr Rev 56:106–114
9. Schwartz MW, Niswender KD (2004) Adiposity signaling and biological defense against weight gain: absence of protection or central hormone resistance? J Clin Endocrinol Metab 89: 5889–5897
10. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, San Diego
11. Wilsey J, Zolotukhin S, Prima V, Scarpace PJ (2003) Central leptin gene therapy fails to overcome leptin resistance associated with diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 285:R1011–R1020
12. Scarpace PJ, Matheny M, Tumer N, Cheng KY, Zhang Y (2005) Leptin resistance exacerbates diet-induced obesity and is associated with diminished maximal leptinsignalling capacity in rats. Diabetologia 48:1075–1083
13. Zhang J, Matheny MK, Tumer N, Mitchell MK, Scarpace PJ (2007) Leptin antagonist reveals that the normalization of caloric intake and the thermic effect of food after high-fat feeding are leptin dependent. Am J Physiol Regul Integr Comp Physiol l292:R868–R874
14. White CL, Purpera MN, Ballard K, Morrison CD (2010) Decreased food intake following overfeeding involves leptin-dependent and leptin-independent mechanisms. Physiol Behav 100:408–416
15. Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG (2003) Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. Brain Res 964:107–115
16. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, DiLeone RJ (2006) Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 51:801–810
17. Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS (2006) Leptin regulation of the meso-accumbens dopamine pathway. Neuron 51:811–822
18. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L (2001) Overfeeding rapidly induces leptin and insulin resistance. Diabetes 50:2786–2791
19. Meerlo P, Overkamp GJ, Koolhaas JM (1997) Behavioural and physiological consequences of a single social defeat in Roman high- and low-avoidance rats. Psychoneuroendocrinology 22:155–168
20. Harris RB, Zhou J, Youngblood BD, Rybkin II, Smagin GN, Ryan DH (1998) Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. Am J Physiol 275:R1928–R1938
21. Wade GN (1975) Some effects of ovarian hormones on food intake and body weight in female rats. J Comp Physiol Psychol 88:183–193

22. Aja S, Bi S, Knipp SB, McFadden JM, Ronnett GV, Kuhajda FP, Moran TH (2006) Intracerebroventricular C75 decreases meal frequency and reduces AgRP gene expression in rats. Am J Physiol Regul Integr Comp Physiol 291:R148–R154

23. Booth DA (1972) Postabsorptively induced suppression of appetite and the energostatic control of feeding. Physiol Behav 9:199–202

24. Toates FM, Booth DA (1974) Control of food intake by energy supply. Nature 251:710–711

25. Hu Z, Cha SH, Chohnan S, Lane MD (2003) Hypothalamic malonyl-CoA as a mediator of feeding behavior. Proc Natl Acad Sci USA 100:12624–12629

26. Wolfgang MJ, Lane MD (2008) Hypothalamic malonyl-coenzyme A and the control of energy balance. Mol Endocrinol 22:2012–2020

27. Lee WN, Bassilian S, Lim S, Boros LG (2000) Loss of regulation of lipogenesis in the Zucker diabetic (ZDF) rat. Am J Physiol Endocrinol Metab 279:E425–E432

28. Unger RH, Zhou YT, Orci L (1999) Regulation of fatty acid homeostasis in cells: novel role of leptin. Proc Natl Acad Sci USA 96:2327–2332

29. Hansen FM, Fahmy N, Nielsen JH (1980) The influence of sexual hormones on lipogenesis and lipolysis in rat fat cells. Acta Endocrinol (Copenhagen) 95:566–570

30. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER (2000) Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA 97:12735–12740

31. Larue-Achagiotis C, Le Magnen J (1982) Effects of short-term nocturnal and diurnal food deprivation on subsequent feeding in intact and VMH lesioned rats: relation to blood glucose level. Physiol Behav 28:245–248

32. Penicaud L, Larue-Achagiotis C, Le Magnen J (1983) Endocrine basis for weight gain after fasting or VMH lesion in rats. Am J Physiol 245:E246–E252

33. Sucajtys-Szulc E, Turyn J, Goyke E, Korczynska J, Stelmanska E, Slominska E, Smolenski RT, Rutkowski B, Swierczynski J (2010) Differential effect of prolonged food restriction and fasting on hypothalamic malonyl-CoA concentration and expression of orexigenic and anorexigenicneuropeptides genes in rats. Neuropeptides 44:17–23