Effects of fasting and refeeding on body mass, thermogenesis and serum leptin in Brandt's voles (*Lasiopodomys brandtii*)

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ARTICLE INFO

ABSTRACT

(1) To investigate the effect of fasting and refeeding on the body mass, thermogenesis and serum leptin in Brandt's voles, the changes in body and body fat mass, resting metabolic rate (RMR), mitochondrial cytochrome c oxidase (COX) activity in liver and brown adipose tissue (BAT), uncoupling protein 1 (UCP1) content of BAT, serum leptin level and post-fasting food intake were monitored and measured.

(2) Fasting induced significant reduction in body mass and body fat mass. Body mass can be restored to the control level in refeeding voles except for the body fat.

(3) RMR decreased significantly in response to fasting, and can return to the control level after refeeding. Fasting induced significant reduction in total, but not specific, COX activity (nmol O_2/min/total tissue) in liver and BAT, and UCP1 content in BAT, which was reversed after refeeding of 48 h.

(4) Fasting for 12 h induced a rapid reduction in serum leptin content. There were no post-fasting compensatory increases in food intake. Interestingly, Brandt's voles did not recover adipose tissue mass, nor serum leptin levels, on refeeding.

(5) Our data indicate that Brandt's voles can adjust their physiological functions integratively to cope with the starvation by the means of decreasing body mass, adaptive thermogenesis and serum leptin levels. There is no post-fasting hyperphagia in Brandt's voles. The reduction of serum leptin was somewhat earlier than the decline in body fat and body mass.

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1. Introduction

Restricted food intake or even periods of fasting are frequent situations in both humans and animals. During periods of restricted feeding, fasting or starvation, small rodents reduce their body masses and body temperature (Petervari et al., 2002) and energy expenditure (Bézaire et al., 2001; Hayashi and Nagasaka, 1983; Jourdan et al., 1984; Nagashima et al., 2003; Nagy and Pistole, 1988; Samec et al., 1998; Trayhurn and Jennings, 1988), regulating metabolism-related protein expression levels (Ahima et al., 1996; Gianotti et al., 1998; Zhang et al., 2002), and increasing food intake (Samec et al., 1998; Trayhurn and Jennings, 1988) and food hoarding (Buckley and Schneider, 2003; Day et al., 1999; Day and Bartness, 2003).

Brown adipose tissue (BAT) is a heat-producing organ under the control of sympathetic nervous system. It plays an important role in the regulation of the energy balance because it is a major site of both nonshivering and diet-induced theromgenesis in small mammals (Palou et al., 1998), contrary to the white adipose tissue, which serves mainly to store energy as fat. Changes in thermogenic parameters in BAT have been reported in many situations, such as food deprivation, cold acclimation, obesity, aging and exercise (Champigny and Ricquier, 1990; Gianotti et al., 1998; Li et al., 2001; Zhang and Wang, 2006).

Leptin, a relatively new hormone derived primarily from the white adipose tissue (Zhang et al., 1994), plays an important role in controlling food intake, energy expenditure, and energy balance (Rayner and Trayhurn, 2001; Scarpace and Matheny, 1998; Schneider et al., 2000). Exogenous leptin treatments can decrease adiposity by decreasing food intake and increasing energy utilization (Abelenda et al., 2003; Pelleymounter et al., 1995; Scarpace et al., 1997). It is proposed that the sympathetic nervous

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system is the main physiological regulator of leptin production and that it provides a negative feedback loop to adipose tissue in the production of the hormone (Rayner and Trayhurn, 2001; Trayhurn et al., 1995a, 1998; Trayhurn and Beattie, 2001).

Leptin also acts as an afferent satiety hormone by regulating appetite and weight gain (Weigle et al., 1995; Zhang et al., 1994) through a negative feedback loop involving receptors in the hypothalamus (Tartaglia et al., 1995). Fasting leads to a rapid inhibition of *ob* gene expression in white adipose tissue, and there is a concomitant fall in the level of circulating leptin level; these effects can be reversed by refeeding (Hardie et al., 1996; Trayhurn et al., 1995b). Leptin level is regarded as a signal starvation that induced the decrease of body mass and energy expenditure during fasting, increase of food intake and energy expenditure during refeeding (Flier, 1998; Hardie et al., 1996; Scarpace et al., 1997; Zhang et al., 2002). Regulation of the neuroendocrine system during starvation could be the main physiological role of leptin (Ahima et al., 1996).

In many species, food deprivation results in decreased plasma concentrations of leptin, increased central release of neuropeptide Y (NPY) and food intake relative to that of *ad libitum*-fed controls (Friedman and Halaas, 1998; Woods et al., 1998). This phenomenon is termed post-fasting (postfast) hyperphagia and is common in laboratory animals, such as rats and mice. However, not all species show compensatory postfast hyperphagia, although plasma leptin concentrations fall rapidly after the start of food deprivation, such as Syrian hamsters (*Mesocricetus auratus*) (Bartness, 1997; Schneider et al., 2000) and Siberian hamsters (*Phodopus sungorus sungorus*) (Day et al., 1999; Day and Bartness, 2003).

Brandt's vole (*Lasiopodomys brandtii*) is a small mammalian herbivore and is primarily distributed in the Inner Mongolia grasslands of China, Mongolia and the Baikal region of Russia. It has been reported that Brandt's voles increased energy intake and thermogenesis in association with decreases in body weight, body fat mass, and serum leptin levels in winter conditions (Li and Wang, 2005). Leptin may act as a starvation signal to permit the increase in energy intake for energy exhaust mainly as thermogenesis for winter adaptation (Flier, 1998; Li and Wang, 2005).

However, we know nothing about fasting cues with changes in serum leptin levels and its role in body mass regulation and thermogenesis in Brandt's voles. In the present study, we examined the effect of fasting and refeeding on body mass, metabolic thermogenesis and serum leptin levels in Brandt's voles. We hypothesized that Brandt's voles can decrease body mass, body fat mass, and thermogenesis in association with the decreases in serum leptin levels in response to the fasting stress. We also want to know if Brandt's voles will show the postfast hyperphagia during refeeding, which has been observed in some rodent species.

2. Meterials and methods

2.1. Experimental animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Brandt's voles were live-trapped in Inner Mongolian grasslands and raised in the laboratory at the Institute of Zoology, the Chinese Academy of Sciences, under a light cycle of 12L:12D and a temperature of $23 \pm 1\,^{\circ}C$. Voles were fed standard rabbit pellet chow (crude fat, 6.2%; crude protein, 20.8%) (Beijing Ke Ao Feed Co., Beijing, People's Republic of China) and water *ad libitum*. Subjects were housed singly in plastic cages ($30\,cm \times 15\,cm \times 20\,cm$) that contained sawdust bedding.

56 adult males with similar body mass were used in our experiments which were divided into seven groups of eight individuals as follows: group 1 (control): fed control voles; group 2 (12 hF): fasted for 12 h; group 3 (24 hF): fasted for 24 h; group 4 (36 hF): fasted for 36 h; group 5 (12 hR): refed for 12 h following fasted for 36 h; group 6 (48 hR): refed for 48 h following fasted for 36 h; group 7 (7 dR): refed for 7 d following fasted for 36 h.

2.2. Body composition measurement

At the end of experiment all animals were killed and the digestive tract, heart, lung, liver, spleen, kidney, interscapular BAT, gonads, white adipose tissue around epididymis (EWAT) and white adipose tissue around the kidney (PWAT) were removed from each carcass. We weighed these eviscerated carcasses (wet carcass mass), oven-dried each to constant mass at $60\,^{\circ}C$, and reweighed them (dry carcass mass). Body fat content was measured by ether extraction in a Sohxlet apparatus. Hind-limb muscles were also removed and weighed.

2.3. Food intake

The voles of group 5–7 were refed with enough food. They had foods and water *ad libitum* and were killed by decapitation at the end of experiment. The uneaten foods were collected, oven-dried at $60\,^{\circ}C$ to constant mass.

2.4. Metabolic trials

Resting metabolic rate (RMR, is the amount of energy expended while at rest in a neutrally temperate environment) was measured by using an established closed-circuit respirometer at $29 \pm 0.5\,^{\circ}C$, which is within the thermoneutral zone for this species (Li and Wang, 2005; Zhao and Wang, 2007). Each subject was weighed and put into a metabolic chamber (3.6 liters) and adapted for 60 min. Temperature inside the chamber was maintained by a water bath ($\pm 0.5\,^{\circ}C$). KOH and silica gel were used to absorb carbon dioxide and water in the metabolic chamber. Oxygen consumption was recorded for 60 min (with 5 min intervals). The two stable consecutive lowest readings were taken to calculate RMR and corrected to standard temperature and pressure condition (STP).

2.5. Measurement of serum leptin

All subjects were sacrificed by decapitation between 0900 and 1100. Trunk blood was collected in a glass centrifuge tube for leptin measurement. Blood was centrifuged at 4000 rpm for 30 min, and serum was sampled and stored at $-75\,^{\circ}C$. Serum leptin concentrations were measured by radioimmunoassay (RIA) using the Linco 125I Multi-species Kit (Cat. no. XL-85 K, Linco Research Inc.), which has been validated previously (Li and Wang, 2005). The lowest level of leptin that can be detected by this assay is 1.0 ng/ml when using a 100 μl sample size (Instructions for Multi-species leptin RIA Kit) (Li and Wang, 2005). Inter- and intra-assay variabilities for leptin RIA were 3.6% and 8.7%, respectively.

2.6. Measurements of cytochrome c oxidase (COX) activity and UCP1 content

Liver and interscapular BAT were removed and weighed after trunk blood was collected. Mitochondrial protein was prepared as described in our previous study (Zhao and Wang, 2006). Mitochondrial protein concentration were determined by the Folin phenol method (Lowry et al., 1951) with bovine serum

album as standards. The COX activity was measured by the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., Norfolk, England) (Sundin et al., 1987).

BAT mitochondrial protein (20 µg per lane) was separated in a discontinuous SDS-polyacyalamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose member (Hybond-C, Amersham Biosciences, Buckinghamshire, UK). UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5000) (supplied by Dr. M. Klingenspor, Department of Biology, University of Marburg, Marburg, Germany) as a primary antibody and sheep

anti-rabbit (1:5000) (Jackson, USA, Baltimore, PA, USA) as the secondly antibody (Klingenspor et al., 1996). We used enhanced chemiluminescence kit (ECL, Amersham Biosciences, England) as detection system and unspecific binding sites were saturated with 5% degrease milk in PBS. UCP1 concentration was expressed as relative units (RU), as determined from area readings by using Scion Image (Li and Wang, 2005; Zhang and Wang, 2006).

2.7. Data analysis

Data were analyzed using SPSS version 10.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. The differences in parameters during the experimental course were analyzed by repeated measures, followed by least-significant difference (LSD) post-hoc tests. If there was no homogeneity of variance, the Tamhane post-hoc tests were used. Group differences in RMR and food intake were analyzed by one-way analysis of covariance (ANCOVA) with body mass as a covariate. One-way ANOVA for repeated measures was applied to evaluate the effect of the fasting/refeeding protocol on body mass, body composition, BAT and liver thermogenesis, UCP1, and leptin (Pilegaard et al., 2003). To detect possible associations of serum leptin with body fat mass, we used Pearson-correlation analysis. All values are presented as means $\pm$ SE. For all statistical tests, differences with $P < 0.05$ were considered to be statistically significant.
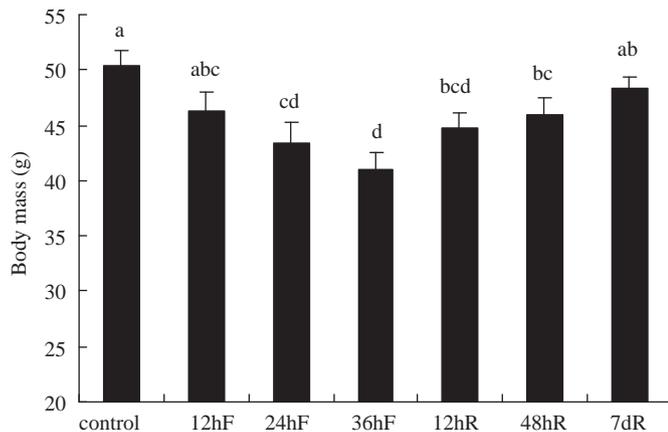

Fig. 1. Effects of fasting and refeeding on body mass in Brandt's voles. Values that share different superscripts are significantly different at $P < 0.05$ among the groups. Body mass decreased significantly in response to fasting of 24–36 h, which was returned to the control level after 7 d refeeding.
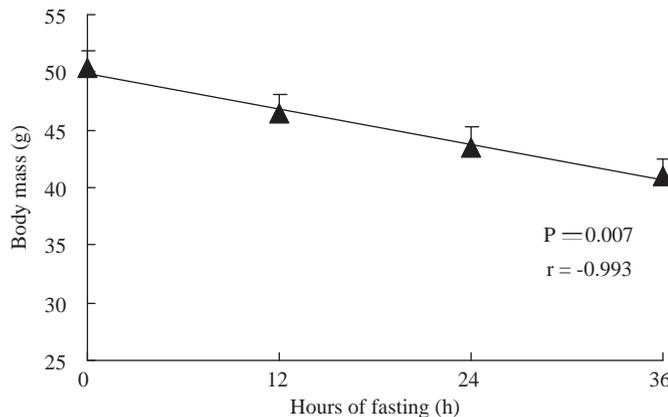
3. Results

3.1. Body mass and body composition

There was a significant change in body mass of Brandt's voles in response to fasting and refeeding ($F_{(6,49)} = 4.084$, $P = 0.002$). Fasting of 24 h ($P = 0.002$) led to a significant reduction in body mass that was restored to the control level after 7 d of refeeding (Fig. 1). There is a linear regression relationship between body mass and hours of fasting ($P = 0.007$, $r = -0.993$) (Fig. 2).

Fasting and refeeding had significant effects on the body fat mass of the voles ($F_{(6,49)} = 3.003$, $P = 0.014$). Body fat reduced significantly after 24 and 36 h fasting, however, it was not returned to the control level after 7 d of refeeding (Table 1).

Fasting 24 h ($P = 0.04$) and 36 h ($P < 0.001$) induced significant decreases in BAT mass, which restored to the control level after 7 d refeeding. 12 h fasting produced a significant loss in liver mass ($P < 0.001$), which was returned to the control level after 48 h refeeding (Table 1). It showed that liver mass decreased and

Fig. 2. The relationship between the body mass and fasting time in Brandt's voles.

Table 1
Effects of fasting and refeeding on the mass of body fat, BAT, liver, and other tissues in Brandt's voles.

Groups	Control	12 hF	24 hF	36 hF	12 hR	48 hR	7 dR
BAT (mg)	181 $\pm$ 13[a]	171 $\pm$ 13[ab]	143 $\pm$ 21[bc]	108 $\pm$ 13[cd]	88 $\pm$ 9[d]	108 $\pm$ 7[cd]	163 $\pm$ 6[ab]
Liver (mg)	1940 $\pm$ 99[a]	300 $\pm$ 39[e]	1480 $\pm$ 69[de]	1501 $\pm$ 58[cde]	1652 $\pm$ 75[bcd]	2004 $\pm$ 143[a]	1874 $\pm$ 162[ab]
Heart (mg)	216 $\pm$ 9	211 $\pm$ 10	218 $\pm$ 14	203 $\pm$ 9	193 $\pm$ 4	185 $\pm$ 9	214 $\pm$ 9
Kidney (mg)	487 $\pm$ 30	455 $\pm$ 18	467 $\pm$ 26	466 $\pm$ 27	466 $\pm$ 6	496 $\pm$ 11	512 $\pm$ 13
EWAT (mg)	570 $\pm$ 48	602 $\pm$ 108	418 $\pm$ 92	394 $\pm$ 63	550 $\pm$ 51	479 $\pm$ 65	561 $\pm$ 54
PWAT (mg)	446 $\pm$ 45	456 $\pm$ 99	308 $\pm$ 89	224 $\pm$ 75	312 $\pm$ 78	191 $\pm$ 51	353 $\pm$ 61
muscle (mg)	734 $\pm$ 46	769 $\pm$ 37	671 $\pm$ 46	729 $\pm$ 38	675 $\pm$ 16	718 $\pm$ 40	715 $\pm$ 34
Wet carcass mass (g)	33.0 $\pm$ 1.1[a]	32.7 $\pm$ 1.2[ab]	29.4 $\pm$ 1.7[bc]	28.4 $\pm$ 1.2[c]	29.4 $\pm$ 1.1[bc]	29.3 $\pm$ 1.3[c]	31.2 $\pm$ 0.9[abc]
Dry carcass mass (g)	14.6 $\pm$ 0.5[a]	14.4 $\pm$ 0.7[ab]	12.3 $\pm$ 1.0[bc]	11.3 $\pm$ 0.7[c]	13.0 $\pm$ 0.9[abc]	12.0 $\pm$ 0.9[c]	12.9 $\pm$ 0.7[abc]
Body fat (g)	7.3 $\pm$ 0.5[a]	6.7 $\pm$ 0.6[ab]	5.1 $\pm$ 0.8[bc]	4.2 $\pm$ 0.6[c]	5.3 $\pm$ 0.8[bc]	4.4 $\pm$ 0.8[c]	5.2 $\pm$ 0.5[bc]
Body water (g)	18.4 $\pm$ 0.8	18.3 $\pm$ 0.6	17.1 $\pm$ 0.9	17.1 $\pm$ 0.6	16.4 $\pm$ 0.2	17.2 $\pm$ 0.8	18.3 $\pm$ 0.4
Sample size	8	8	8	8	8	8	8

Note: Significant differences between groups are indicated by different superscript letters in the same line ($P < 0.05$).

recovered much more rapidly on fasting and refeeding than did the mass of BAT.

3.2. RMR, COX activity and UCP1

Fasting and refeeding had significant effects on RMR ($F_{(6,48)} = 8.455$, $P < 0.001$). Fasting of 24 h ($P = 0.004$) and 36 h ($P = 0.002$) induced the significant decrease in RMR, and the change was reversed after refeeding for 7 d (Fig. 3).

Fasting induced marked reductions in total mitochondrial (Mt) protein content (mg/total tissue) and COX activity (nmol O_2/min/total tissue) in liver and BAT, and they both restored to the control after refeeding 48 h (Table 2 and Table 3).

Fasting and refeeding significantly affected UCP1 content of BAT ($F_{(6,49)} = 2.485$, $P = 0.035$) (Fig. 4). UCP1 content reduced
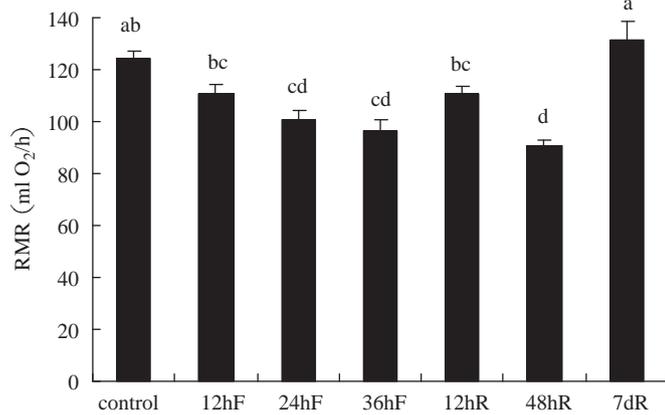
significantly after fasting for 36 h ($P = 0.028$), and it was restored to the control level after refeeding for 48 h.

3.3. Food intake and serum leptin level

No significant differences in food intake were found between refed and control groups during the course of refeeding (Fig. 5). It indicated that after being fasted for 36 h Brandt's voles did not show hyperphagia during the refeeding of 7 d.

Significant responses to fasting and refeeding in serum leptin level was observed (Fig. 6; $F_{(6,49)} = 11.515$, $P < 0.001$). 12 h fasting induced a rapid reduction in serum leptin content ($P < 0.001$).

Fig. 3. Effects of fasting and refeeding on RMR in Brandt's voles. RMR (ml O_2/h) was expressed per animal. Values that share different superscripts are significantly different at $P < 0.05$ among the groups. Fasting of 24–36 h induced the significant decrease of RMR, which was returned to the control level after refeeding.
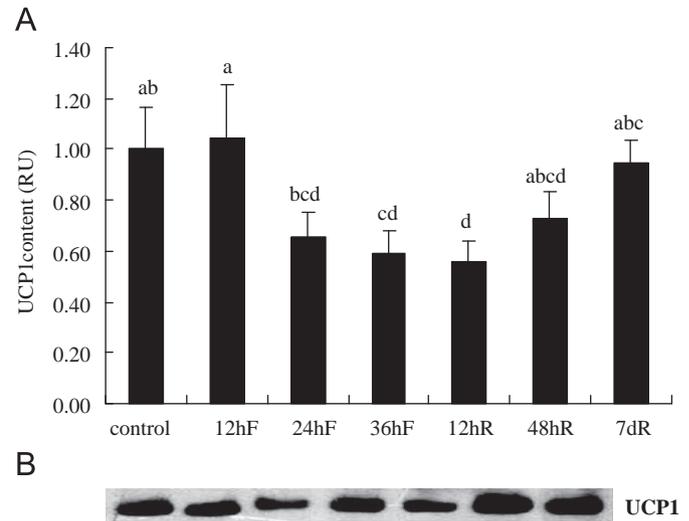

Fig. 4. Effects of fasting and refeeding on UCP1 content in BAT in Brandt's voles. Means with the different letters within the seven groups are significantly different at $P < 0.05$. (A) UCP1 content reduced significantly after fasting for 36 h compared to the control. (B) Western blotting detection of UCP1 content for control, fasting and refeeding groups. The blots from the left to right matched those in A.

Table 2
Effects of fasting and refeeding on liver mitochondrial (Mt) protein content and cytochrome c oxidase (COX) activity in Brandt's voles.

Groups	Mt protein (mg/g tissue)	Total Mt protein (mg/total tissue)	COX-specific activity (nmol O_2/min/mg Mt protein)	COX activity (nmol O_2/min/total tissue)	Sample size
Control	22.73 ± 1.31^{ab}	43.57 ± 2.20^a	45.47 ± 3.66	1956.86 ± 144.87^b	8
12 hF	20.73 ± 1.25^{ab}	27.01 ± 1.91^c	56.87 ± 5.99	1515.76 ± 159.75^{cd}	8
24 hF	19.80 ± 0.90^b	28.97 ± 0.92^{bc}	49.55 ± 2.26	1431.82 ± 68.67^d	8
36 hF	19.38 ± 0.62^b	28.96 ± 1.00^{bc}	48.56 ± 2.83	1412.80 ± 113.85^d	8
12 hR	19.50 ± 1.69^{ab}	31.82 ± 2.36^{abc}	48.95 ± 3.69	1511.29 ± 89.56^{cd}	8
48 hR	20.55 ± 1.81^{ab}	39.88 ± 2.09^a	47.19 ± 2.35	1861.36 ± 95.12^{bc}	8
7 dR	26.90 ± 1.27^a	50.31 ± 4.75^{ab}	54.50 ± 2.73	2692.53 ± 202.43^a	8

Note: Significant differences between groups are indicated by different superscript letters in the same row ($P < 0.05$).

Table 3
Effects of fasting and refeeding on brown adipose tissue (BAT) mitochondrial (Mt) protein and cytochrome c oxidase (COX) activity in Brandt's voles.

Groups	Mt protein (mg/g tissue)	Total Mt protein (mg/total tissue)	COX-specific activity (nmol O_2/min/mg Mt protein)	COX activity (nmol O_2/min/total tissue)	Sample size
Control	8.06 ± 0.34	1.46 ± 0.12^a	412.86 ± 17.97	595.89 ± 45.29^{ab}	8
12 hF	9.12 ± 0.70	1.59 ± 0.21^{ab}	416.40 ± 18.08	671.34 ± 102.74^{abc}	8
24 hF	7.56 ± 0.73	0.99 ± 0.06^{ab}	428.26 ± 30.70	418.17 ± 28.01^{bc}	8
36 hF	8.36 ± 0.31	0.80 ± 0.07^b	439.09 ± 35.05	359.05 ± 44.89^c	8
12 hR	8.25 ± 0.51	0.75 ± 0.11^b	438.78 ± 11.79	323.03 ± 42.31^c	8
48 hR	10.28 ± 0.65	1.13 ± 0.12^{ab}	431.94 ± 12.38	486.55 ± 54.35^{abc}	8
7 dR	8.22 ± 0.71	1.32 ± 0.11^a	465.76 ± 17.06	608.19 ± 41.05^a	8

Note: Significant differences between groups are indicated by different superscript letters in the same row ($P < 0.05$).
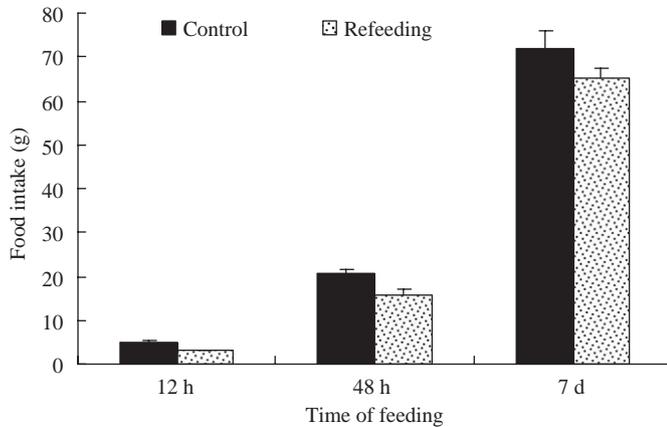
Fig. 5. Comparison of food intake between refed and control groups in Brandt's voles. No significant differences were found between refed and control groups.
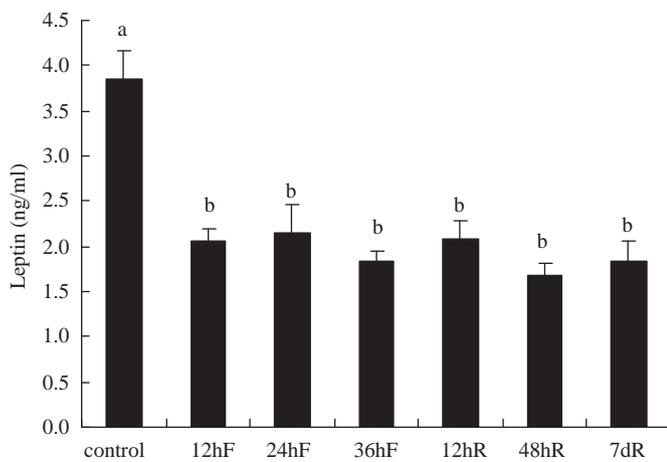

Fig. 6. Effects of fasting and refeeding on serum leptin levels in Brandt's voles. Values that do not share a letter are significantly different at $P < 0.001$. Fasting induced a rapid decrease in serum leptin, and there was no recovery during refeeding.
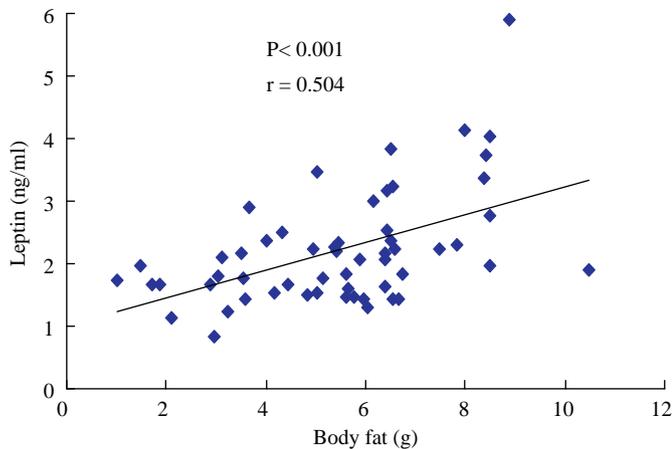

Fig. 7. The relationship between serum leptin content and body fat. Serum leptin content was positively correlated with body fat mass in Brandt's voles ($P < 0.001$).

Leptin did not restore to the control level during 7 d of refeeding (Fig. 6). There was a positive correlation between serum leptin content and body fat mass ($P < 0.001$, $r = 0.504$) (Fig. 7).

4. Discussion

4.1. The effect of fasting and refeeding on body mass and body composition

Fasting induced a significant lineally decrease in body mass and can be restored to the control level after 7 d refeeding. Fuglei and Oritsland (1999) found that starvation induced the decline in body mass for adult male Wistar rats. Our finding of fasting–refeeding-associated change in body mass of Brandt's voles is consistent with the previous findings in other rodents (Bézaire et al., 2001; Freminet, 1981; Kouda et al., 2004; Trayhurn and Jennings, 1988). The decrease of body mass was related to the reduce of body fat, carcass and gut content (Mustonen et al., 2005). Fasting led to a substantial fall in the body fat mass in Brandt's voles, similar results have also been found in mice (Trayhurn and Jennings, 1988). It suggested that Brandt's voles probably mobilized fat stores as their main energy source during the fasting (Bézaire et al., 2001). However, other voles, for example, *Microtus pennsylavnicus* (Nagy and Pistole, 1988), *Clethrionomys rutilus* and *Clethrionomys rufocanus* (Mosin, 1984) utilized carbohydrates as the major fuel substrate in both the fed and fasted states. Since body fat mass cannot return to the control level after 7 d refeeding (see Table 1), it may be speculated that the recovery of body mass was not only due to the increase of body fat, but also related to the recovery of carcass and visceral organs (Freminet, 1981).

Fasting of 12 h can lead a significant reduction in liver mass, suggesting that voles could mobilize the liver glycogen quickly (Freminet, 1981; Nagy and Pistole, 1988), in respect that fasting caused a rapid decline in the plasma glucose levels (Mustonen et al., 2008). Liver mass restored to the control level after 48 h refeeding, and this would be related to the quick recovery of liver glycogen content (Freminet, 1981; Ji and Friedman, 1999; McGarry et al., 1973), which was due to immediate increase of blood glucose during the first hours of refeeding (Holness et al., 1988; McGarry et al., 1973).

4.2. The effect of fasting and refeeding on RMR, COX activity and UCP1

Our result showed that fasting can lead to a substantial fall in RMR and can be restored on refeeding, which is in consistence with the previous reports on meadow voles (Nagy and Pistole, 1988), rats (Nagashima et al., 2003), and mice (Bézaire et al., 2001; Trayhurn and Jennings, 1988). During fasting it is of critical importance to maintain blood glucose levels for the proper functioning of the central nervous system. The reduction in metabolic rate will lead to a decrease in the utilization of substrates by peripheral tissues and in this way more glucose would be made available to the central nervous system (Nagy and Pistole, 1988). Thyroid hormonal changes during fasting and refeeding would play an important role in the reduction and recovery of RMR, which has been found that serum levels of thyroxine (T4) and triiodothyronine (T3) decreased upon fasting, then they increased during refeeding (Kmieć et al., 1998). It was also indicated that fasting induced the decrease of plasma T4 concentrations in the common vole (*Microtus arvalis*) (Mustonen et al., 2008). RMR restored to the control level after 7 d refeeding in Brandt's voles, during which food intake itself induced an increase in metabolism, known as postprandial-deprived thermogenesis and/or diet-induced thermogenesis (Nagashima et al., 2003).

The result that fasting of 36 h induced a significant fall in total mitochondrial concentration of BAT in Brandt's voles is in

consistent with the previous findings in the meadow voles (Nagy and Pistole, 1988) and mice (Trayhurn and Jennings, 1988). In our study 36 h fasting led UCP1 content in BAT to decline significantly. It seemed that the effect of fasting on UCP1 was through the decrease of mitochondrial protein quantity in BAT, in keeping with the previous study in gold hamsters (Levin and Trayhurn, 1987).

4.3. The effect of fasting and refeeding on food intake and serum leptin

In our present study Brandt's voles were not hyperphagia during the period of refeeding. Compensatory increases in food intake are commonly observed after a period of food deprivation in many species, including jirds (*Meriones shawi*) (Demas and Bartness, 1999), laboratory mice and rats (Ji and Friedman, 1999; Samec et al., 1998; Trayhurn and Jennings, 1988). Hamsters, for example, Syrian hamsters (*Mesocricetus auratus*) (Buckley and Schneider, 2003; Levin and Trayhurn, 1987; Schneider et al., 2000) and Siberian hamsters (*Phodopus sungorus*) (Day et al., 1999; Day and Bartness, 2003), in contrast, show a unique response to fasting—a lack of postfast increase in food intake. No hyperphagia in Brandt's voles presumably accounted for the observation that the voles did not recover body fat mass after refeeding.

Both food deprivation and chronic food restriction increase food hoarding in laboratory rats (Cabanac and Swiergiel, 1989), Syrian hamsters (Schneider et al., 2000), and Siberian hamsters (Bartness and Clein, 1994; Wood and Bartness, 1996). Overton and Williams (2004) reviewed that fasting and daily caloric restriction induce torpor in mice (Overton and Williams, 2004). The lack of postfast hyperphagia might also be linked to the tendency to engage in hibernation (Syrian hamsters) or daily torpor (Siberian hamsters), as first suggested by Bartness (1990). However, Brandt's voles do not enter torpor or hibernation in winter. Instead, they constructed complex burrows, gathered and stored food for wintering (Zhong et al., 2007). We hypothesized that Brandt's voles would increase the food hoarding behavior after postfast refeeding, similar to Syrian and Siberian hamsters. This hypothesis need to be affirmed by further research. There is caecotrophic behavior in Brandt's voles. The contribution of caecotrophy to the dietary intake of crude protein is about 9% on a high-protein, easily digested commercial rabbit pellet diet (Liu et al., 2007). It suggested that caecotrophy would also be related to no hyperphagia in Brandt's voles.

Our results showed that serum leptin concentrations fall rapidly after the start of food deprivation. Many studies reported that fasting process resulted in the declination in serum leptin level (Hardie et al., 1996; MacDougald et al., 1995; Tauson and Forsberg, 2002; Trayhurn et al., 1995a; Zhang et al., 2002) and leptin mRNA in white adipose tissue (Hardie et al., 1996; Zhang et al., 2002). It is widely believed that falling leptin concentrations are a trigger for increased food intake, and yet food-deprived voles do not exhibit postfast hyperphagia. It is not known why increased leptin can decrease food intake, whereas the falling concentrations of serum leptin fail to increase the food intake in hamsters. For example, in Syrian hamsters, food deprivation leads to significant decreases in serum leptin concentrations (Schneider et al., 2000), without concomitant increases in daily food intake (Bartness, 1997). Fasting-induced reduction in serum leptin failed to increase postfast food intake in Brandt's voles, although decreased serum leptin in winter was found to be associated with increased energy intake in Brandt's voles in our previous study (Li and Wang, 2005).

In our study, there were no compensatory increases in food intake and serum leptin level, in contrast, leptin level cannot be recovered to the normal level during the refeeding period. No

postfast hyperphagia in Brandt's voles possibly is related to other factors for the regulation of controlling energy homeostasis in the neuroendocrine system (Swart et al., 2002; Zhang et al., 2002). Fasting and refeeding can induce the complicated reaction in hormone, nerve system and metabolism. Leptin level was always lower than that of control during the refeeding, at the same time, body fat mass did not recover to the control level after 7 d refeeding as well. It has been suggested that plasma leptin levels can reflect the body fat mass under steady state (Sinha and Caro, 1998) and correlate strongly with body weight (Zhang et al., 1994). Serum leptin levels displayed diurnal rhythms in rodents (Ahren, 2000; Sanchez et al., 2004), so serum leptin levels of Brandt's voles were measured between 0900 and 1100 in this study. Lower body fat level is regarded as a condition of hunger (Flier, 1998). Since the body fat mass and serum leptin level did not restore to the normal after refeeding, it looked that Brandt's voles were still at the condition of undernutrition. The reduction of serum leptin was somewhat earlier than the decline in body fat and body mass, suggesting that leptin as a starvation signal mediated predominantly the fall in body mass and energy expenditure (Flier, 1998; Li and Wang, 2005).

In summary, in responding to fasting Brandt's voles decreased body mass, metabolism thermogenesis, and serum leptin concentration, and can be restored to the control level except for serum leptin and body fat mass. No postfast hyperphagia in Brandt's voles was observed in this study. These results suggest that Brandt's voles can adjust the status of physiology integratively to cope with the lack of food, and serum leptin level acted as a starvation signal to mediate predominantly the reduction in body mass and energy expenditure.

Acknowledgements

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