



Diet-induced obesity in the short-day-lean Brandt's vole

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ABSTRACT

To test the hypothesis that mammals that show decrease in body mass under short-day condition should be resistant to high-fat induced obesity, we traced the changes of energy balance in a wild rodent, Brandt's voles (*Lasiopodomys brandtii*), which were acclimated to either long day (16 L: 8D, LD) or short day (8 L: 16D, SD) and fed either low-fat diet (LFD) or high-fat diet (HFD) in each photoperiodic manipulation. We found that Brandt's vole was not resistant to high-fat diet-induced obesity and SD, not HFD, induced the elevation in basal metabolic rate, the maximal rate of oxygen consumption after norepinephrine injection, and uncoupling protein 1 content in brown adipose tissue. HFD caused the increase in apparent digestibility and body fat mass, and the decrease in energy intake in both LD and SD voles. The enhancement of energy absorption associated with small intestine tissue recruitment can compensate the lower energy intake, which may contribute to the high-fat diet-induced body fat deposition. Thus, a decrease in body-weight gain but has no resistance to high-fat induced obesity implies an evolutionary and adaptive mechanism which is a benefit for their winter survival.

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1. Introduction

It has been reported that mammalian species displaying different seasonal changes in body mass and body fat mass also show different responses to a high-fat diet [1,2]. Those species that exhibit increases in body mass and body fat mass in response to short day tend to develop the so called high-fat diet-induced obesity (HFD-DIO). So, species showing naturally decrease in body mass and fat mass in the fall and/or in short days are possibly resistant to HFD-DIO [1,2].

Brandt's voles (*Lasiopodomys brandtii*), a typical herbivorous rodent species, mainly live in the Inner Mongolia grasslands of China, Mongolia, and the region of Beikal in Russia [3]. Our previous work has shown that these species display seasonal variations in body mass and body fat mass, energy intake, and thermogenesis [4,5]. Photoperiod has been found to be an important cue to induce the seasonal changes in body mass and energy balance [6,7]. Voles decrease body mass, body fat mass and energy intake, and enhance the thermogenic capacity under short-day conditions [6]. In addition, Brandt's voles fed low quality food show decreases in body mass and energy expenditure associated with suppression in nonshivering thermogenesis (NST) and uncoupling protein 1 (UCP1) level in brown adipose tissue (BAT) [8]. In the present study, we determined the

effect of short day and high-fat diet on body-weight gain, body fat mass, body compositions, energy budgets and BAT UCP1 expression to test the hypothesis that Brandt's voles showing short-day induced decreases in body mass and body fat mass, similar with Siberian hamsters (*Phodopus sungorus*) [9], Shaw's jird (*Meriones shawi*) [1] and meadow voles (*Microtus pennsylvanicus*) [10], should be resistant to the high-fat diet-induced obesity.

2. Materials and methods

2.1. Animals

All experimental procedures were licensed by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Twenty four male Brandt's voles (4.5–5 months of age) were obtained from our breeding colony and maintained under 16 L: 8D (light:dark) photoperiod and 23 ± 1 °C temperature after birth. Food (normal diet: rabbit pellet chow; Beijing KeAo Feed Co.) and water were provided ad libitum. The voles were raised individually in plastic cages (30 × 15 × 20 cm) with sawdust as bedding for 4 weeks prior to experiment beginning and, were then randomly divided into four groups ($n = 6$ in each group). Two groups were maintained in long day (LD), and the other two groups were moved into short day (SD, 8 L: 16D). In each photoperiod, the animals were given free access to either normal diet (hereafter refer to low-fat diet, LFD) or high-fat diet (HFD, it was made by mixing bean oil into the normal diet). The compositions for the two diets were presented in Table 1. The animals in any groups were housed individually throughout the experiment.

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Table 1
Compositions of high- and low-fat diet based on dry mass.

	Low-fat diet (LFD, normal diet)	High-fat diet (HFD)
Crude fat (%)	6.2	21.4
Crude protein (%)	20.8	17.6
NDF (%)	23.1	19.6
ADF (%)	12.5	10.6
Ash (%)	10.0	8.5
Caloric value (kJ/g)	17.5	19.7

Note: NDF, neutral detergent fiber; and ADF, acid detergent fiber.

Body mass was measured before the experiment and every 3 days throughout the acclimation of 7 weeks.

2.2. Metabolic trial

Oxygen consumption was measured after a 7-week's acclimation using a closed circuit respirometer as described previously [4,11]. The glass chamber size was 3.6 L, and the chamber temperature was controlled within ± 0.5 °C by water bath. Carbon dioxide and water in the metabolic chamber were absorbed with KOH and silica gel. BMR measurement was conducted at the temperature of 30 ± 0.5 °C (the thermoneutral zone for the species is 27.5–32.5 °C) [4]. The animals were made to fast 3 h before being transferred into the chamber. After 1 h stabilization in the chamber, oxygen consumption was recorded for 1 h with 5 min intervals. The two consecutive lowest readings were taken to calculate BMR. Thermogenesis was quantified as the rate of maximum oxygen consumption induced by norepinephrine (NE) that was measured on the next day and stimulated with subcutaneous injection of NE (Shanghai Harvest Pharmaceutical Co. LTD) at 25 ± 1 °C. The mass-dependent dosage of NE was calculated according to the equation: $NE \text{ (mg/kg)} = 6.6 \text{ Mb}^{-0.458} \text{ (g)}$ [12,13]. Oxygen consumption was recorded for 1 h with 5 min intervals. The two consecutive highest readings were taken to calculate NE-induced thermogenesis. Two SD voles and two LD voles were measured at the same time in four metabolic chambers at a time. BMR and NE-induced thermogenesis were corrected to STP conditions and expressed as ml O₂/h [14,15]. All measurements were made between 0900 and 1400.

2.3. Energy intake and digestibility

After short day and high-fat diet acclimation for 7 weeks, energy intake measurement was conducted in metabolism cages as described previously [16–18]. Briefly, food was provided quantitatively and food residues and feces were collected over a 3-day test, and separated manually after they were dried 60 °C to constant mass. Caloric values of diet and feces were measured using a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). Gross energy intake (GEI), digestible energy intake (DEI) and apparent digestibility of energy (hereafter referred to simply as digestibility) were calculated by the following equations [16,17,19]:

$$\text{Dry matter intake (DMI, } g \cdot d^{-1}) = \text{food intake (} g \cdot d^{-1}) \times \text{dry matter content of food (\%);}$$

$$\text{GEI (} kJ \cdot d^{-1}) = \text{dry matter intake (DMI) (} g \cdot d^{-1}) \times \text{gross energy content of food (} kJ \cdot g^{-1});$$

$$\text{DEI (} kJ \cdot d^{-1}) = \text{GEI} - [\text{mass of feces (} g \cdot d^{-1}) \times \text{gross energy content of feces (} kJ \cdot g^{-1})];$$

$$\text{Digestibility (\%)} = \text{DEI / GEI} \times 100\%.$$

2.4. Serum leptin concentration

Trunk blood was collected for leptin measurement when the voles were decapitated at 0900–1100. Serum concentrations of leptin were assayed by RIA using the Linco Research Inc. ¹²⁵I Multi-species Kit (Cat. No. XL-85 K.). This assay has been previously validated for Brandt's voles [5]. The lower and upper limits of the assay were 1 and 50 ng/ml, and the average intra- and inter-assay coefficients variations were less than 8.7% and 3.6%, respectively.

2.4. Measurements of BAT cytochrome c oxidase (COX) activity and UCP1 level

Interscapular BAT was quickly removed and used for preparation of isolated mitochondria, as previous described by Wiesinger et al. [6,20]. The mitochondrial yield per g tissue was determined by the Folin phenol method with bovine serum albumin as standard [21]. COX activity was measured polarographically with oxygen electrode units (Hansatech Instruments LTD., England) [6,22].

BAT UCP1 level measurement was carried out by western blotting as described previously [5,6]. Briefly, 5 μ l BAT mitochondrial proteins (20 μ g per lane) were subjected to SDS-PAGE on 3% stacking gel and 12.5% running gel, and then transferred to a nitrocellulose membrane. After blocking the membrane with a 5% skim milk at 4 °C overnight, UCP1 protein was detected using a rabbit polyclonal anti-hamster UCP1 (1:5000). The membrane was washed with PBS, 0.1% Tween, and then hybridized with a goat anti-rabbit peroxidase-labelled secondary antibody (1:5000) [23]. The signals were detected by chemoluminescence using an enhanced chemoluminescence kit (ECL, Amersham Biosciences, England). UCP1 level was quantified with Scion Image Software (Scion Corporation). UCP1 relative level per mg mitochondrial protein (relative units, RU/mg) multiplied by total mitochondrial protein yield (mg), to make total UCP1 level.

2.5. Body composition and body fat content

Gastrointestinal tract (stomach, small intestine, colon, and cecum) were extracted firstly, weighed with content (to 1 mg) and measured with a ruler (to 0.1 cm), respectively. Then, the contents of the gastrointestinal tract were removed and weighed to get wet mass (to 1 mg). Finally, heart, liver, lung, spleen and kidneys were removed respectively. All tissues and the remaining carcass were weighed (to 1 mg and 0.01 g, respectively) to get wet mass, dried in an oven at 60 °C for at least a week and then reweighed to get the dry mass. Total body fat mass was extracted from the dried carcass by ether extraction in a Soxhlet apparatus.

2.6. Statistical analysis

Data were analyzed using the SPSS software package. Body-weight gain was analyzed by a two-way analysis of variance (ANOVA) (photoperiod \times diet). The body-weight gain over the whole acclimation was measured with repeated measurements, followed by Tukey's HSD post-hoc to determine the daily variation. BMR, NE-induced thermogenesis, energy parameters, serum leptin concentrations, BAT COX activity and UCP1 level, and body composition were analyzed by a two-way ANOVA or two-way ANCOVA and, body mass or body carcass mass used as a co-variate where appropriate. Post-hoc comparisons were conducted using the Tukey's HSD test when required. Statistical significance was determined at $P < 0.05$. All data were expressed as means \pm s.e.m.

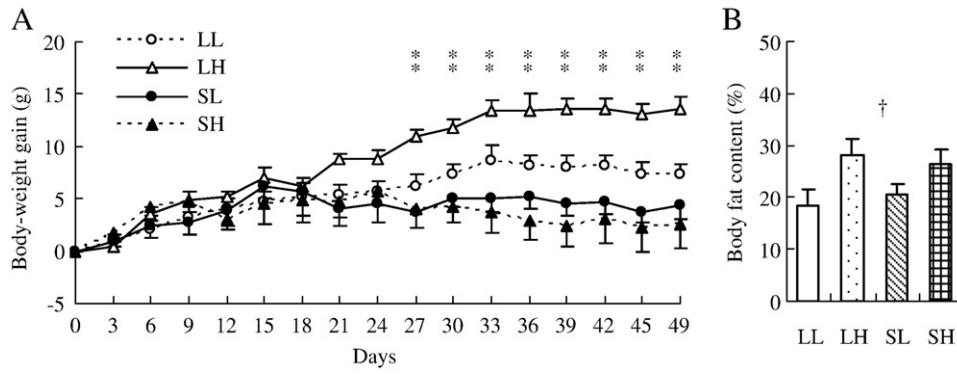


Fig. 1. Body-weight gain (A) and total body fat content (B) in Brandt's voles acclimated to short-day and high-fat diet. LL, long-day and low-fat diet; LH, long-day and high-fat diet; SL, short-day and low-fat diet; SH, short-day and high-fat diet. The body-weight gain did not differ between low- and high-fat diet group, but did between short- and long-day voles (diet, $P > 0.05$; photoperiod, $P < 0.01$). In contrast, diet, but not photoperiod, has significant effects on body fat content (diet, $P < 0.05$; photoperiod, $P > 0.05$). Data are mean \pm s.e.m. ****Indicated that weight gain of long-day voles became higher than short-day voles on day 27 and thereafter (day 27, $P < 0.01$). †Main effect of diet ($P < 0.05$).

3. Results

3.1. Body-weight gain

The body-weight gain of long-day voles was significantly higher than that of short-day voles on day 27 and thereafter (Fig. 1A). The changes of weight gain in short-day voles showed clearly increasing phase in the first two weeks and stable phase on day 15 till the end of acclimation. Body-weight gain was not affected by diet alone, but only by high-fat diet in combination with long photoperiod.

3.2. BMR and NE-induced thermogenesis

Short-day acclimation significantly increased BMR (Fig. 2). Consistent with the changes of BMR, the maximal rate of oxygen consumption after NE injection became higher in short-day voles. Neither BMR nor the maximal rate of oxygen consumption after NE injection was affected by high-fat feeding or high-fat diet in combination with long day. However, in the group fed high-fat diet or exposed to short day, no significant increase in the rate of oxygen consumption of NE-induced minus BMR was observed. In addition, BMR was significantly positively correlated with body mass.

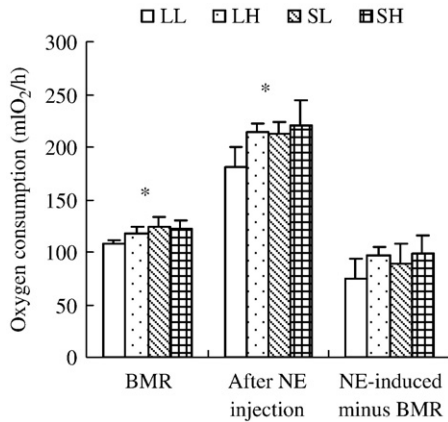


Fig. 2. Basal metabolic rate (BMR), the maximal rate of oxygen consumption after NE injection and NE-induced minus BMR in Brandt's voles acclimated to short day and high-fat diet. LL, long-day and low-fat diet; LH, long-day and high-fat diet; SL, short-day and low-fat diet; SH, short-day and high-fat diet. Both BMR and oxygen consumption after NE injection became higher in short-day voles (photoperiod, $P < 0.05$), while they were not affected by diet (diet, $P > 0.05$). Additionally, neither short-day nor high-fat diet imposed effect on NE-induced minus BMR (photoperiod, $P > 0.05$; diet, $P > 0.05$). Data are mean \pm s.e.m. *Main effect of short day ($P < 0.05$).

3.3. Energy intake and digestibility

The dry matter intake (DMI) and gross energy intake (GEI) of groups fed high-fat diet became lower than that of low-fat diet groups (Fig. 3A,B). Consistent with DMI and GEI, high-fat diet feeding significantly decreased the digestive energy intake (Fig. 3C). In contrast, digestibility of animals consuming high-fat diet became higher compared with that of their low-fat diet counterpart (Fig. 3D). Further, high-fat diet feeding caused 20% increase in digestibility in long-day voles and 16% in short-day voles in comparison with low-fat

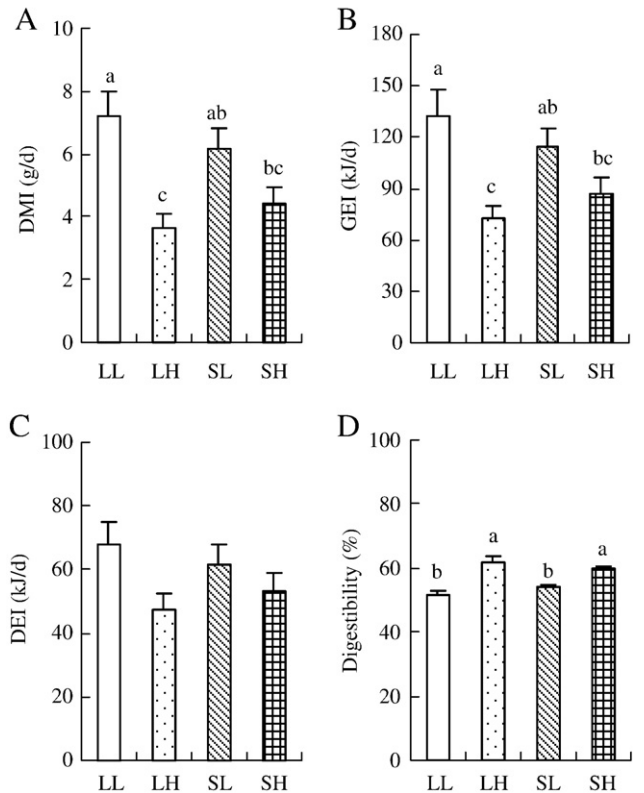


Fig. 3. Dry matter intake (DMI, A), gross energy intake (GEI, B), digestible energy intake (DEI, C) and digestibility (D) in Brandt's voles acclimated to short-day and high-fat diet. LL, long-day and low-fat diet; LH, long-day and high-fat diet; SL, short-day and low-fat diet; SH, short-day and high-fat diet. High-fat diet, not short day, imposed significantly effect on DMI, GEI, DEI and digestibility. Different letters identify statistically significant difference ($P < 0.05$). Data are mean \pm s.e.m.

Table 2
Mitochondrial protein yield, COX activity and UCP1 level in Brandt's voles acclimated to short day and high-fat diet.

	Long day (LD)		Short day (SD)		P
	Low-fat diet (LFD)	High-fat diet (HFD)	Low-fat diet (LFD)	High-fat diet (HFD)	
BAT mass (mg)	174 ± 21	192 ± 26	148 ± 16	165 ± 10	ns
Mitochondrial protein yield (mg/g tissue)	7.7 ± 0.6 ^b	8.3 ± 0.4 ^b	10.4 ± 0.3 ^a	8.3 ± 0.5 ^b	P**; PxD**
(mg in whole tissue)	1.33 ± 0.20	1.56 ± 0.17	1.53 ± 0.15	1.38 ± 0.12	ns
COX activity (nmol O ₂ /min/mg Mt protein)	57 ± 5	65 ± 6	74 ± 1	68 ± 3	P*
(nmol O ₂ /min/g tissue)	428 ± 15 ^b	545 ± 60 ^{ab}	770 ± 27 ^a	582 ± 34 ^{ab}	P**
(nmol O ₂ /min in whole tissue)	75 ± 11	100 ± 11	113 ± 11	116 ± 21	ns
BAT UCP1 level (relative units per mg protein)	1.00 ± 0.13	1.39 ± 0.25	1.93 ± 0.44	1.88 ± 0.35	P*
(relative units with total protein)	1.30 ± 0.21	2.06 ± 0.28	2.90 ± 0.65	2.51 ± 0.41	P*

Values are expressed as absolute mean ± s.e.m. P, photoperiod; P × D, the interaction of photoperiod and diet. Different superscripts in each row means significant difference (P < 0.05). ns, non-significant (P > 0.05); *P < 0.05; **P < 0.01.

diet group. Neither energy intake nor digestibility was affected by photoperiod.

3.4. BAT cytochrome c oxidase (COX) activity and UCP1 level

Mitochondrial yield per g tissue was significantly increased by giving high-fat diet to the short-day voles. Total mitochondrial yield was not affected by the experimental conditions, and nor was total BAT COX activity (Table 2). BAT UCP1 level was significantly affected by photoperiod, 93% higher in short-day voles than that in long-day voles (Table 2, Fig. 4).

3.5. Body compositions, body fat mass and serum leptin concentration

There was significant effect of diet on body fat mass and body fat content, in which body fat content became 52% higher in long-day voles fed high-fat diet than the individuals fed low-fat diet (Fig. 1B; Table 3). Similar to body fat mass, the voles fed high-fat diet intended to show higher serum leptin level compared with the voles fed low-fat diet, but this trend was not significant. Serum leptin level tended to be positively correlated with body fat mass (Fig. 5A), and negatively correlated with gross energy intake (Fig. 5B).

The wet and dry mass of stomach in voles fed high-fat diet became lighter than that of voles fed low-fat diet, while they were not affected by short-day acclimation (Table 4). The patterns of variations in the sizes, mass with content, wet and dry mass of colon and caecum were similar to that observed in the stomach, i.e. they were lower in the

voles fed high-fat diet. In contrast, the voles fed high-fat diet exhibited higher dry mass of small intestine compared with that of the animals fed low-fat diet. For the total digestive tract, the size, mass with content and wet mass became lower in the animals fed high-fat diet. Relative to long-day voles, size of small intestine, caecum and total digestive tract became higher in short-day voles (Table 4).

4. Discussion

Our data showed that short day, not high-fat diet stimulated the increases in metabolic rate and thermogenesis. However, energetic parameters were affected by high-fat diet alone. Further, DMI and GEI were higher in the voles fed low-fat diet than the animals fed high-fat diet. High-fat diet stimulated the increases in digestibility and body fat mass in Brandt's voles.

4.1. Body mass and body fat mass

Similar to Siberian hamsters [24,25] and meadow voles [10], Brandt's voles showed a decrease in body mass and body fat mass in short-day conditions [26]. The present data also showed that weight gain became higher in long-day voles than short-day voles. Lu et al. (2007) found that Brandt's vole experiencing short day history

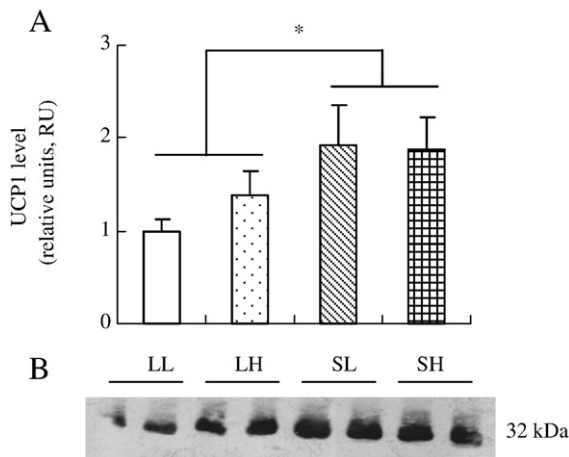


Fig. 4. Uncoupling protein 1 level (UCP1, A) and the immunogel for UCP1 (B) in Brandt's voles acclimated to short day and high-fat diet. LL, long-day and low-fat diet; LH, long-day and high-fat diet; SL, short-day and low-fat diet; SH, short-day and high-fat diet. *Main effect of photoperiod (P < 0.05). Data are mean ± s.e.m.

Table 3
Body compositions, body fat content, serum leptin levels in Brandt's voles acclimated to short day and high-fat diet.

	Long day (LD)		Short day (SD)		P
	Low-fat diet (LFD)	High-fat diet (HFD)	Low-fat diet (LFD)	High-fat diet (HFD)	
Wet carcass mass (g)	48 ± 3	53 ± 3	43 ± 3	47 ± 4	ns
Dry carcass mass (g)	19 ± 3	25 ± 3	19 ± 2	22 ± 2	ns
Body fat mass (g)	9.1 ± 1.9	15.3 ± 2.4	9.1 ± 1.4	12.4 ± 1.8	D*
Serum leptin levels (ng/ml)	5.3 ± 0.3	6.1 ± 0.4	5.4 ± 0.3	6.33 ± 0.71	ns
Wet mass (mg)					
Liver	2427 ± 204	2711 ± 217	2155 ± 153	2593 ± 253	ns
Heart	237 ± 17	271 ± 11	234 ± 12	260 ± 17	ns
Lung	644 ± 59 ^a	596 ± 36 ^{ab}	445 ± 35 ^b	498 ± 36 ^{ab}	P*
Spleen	34 ± 3	33 ± 5	45 ± 2	37 ± 4	P**
Kidneys	573 ± 24	532 ± 37	519 ± 12	501 ± 33	ns
Dry mass (mg)					
Liver	674 ± 58	716 ± 40	603 ± 38	667 ± 60	ns
Heart	56 ± 5	66 ± 4	59 ± 4	63 ± 4	ns
Lung	169 ± 28	151 ± 16	106 ± 8	136 ± 7	ns
Spleen	7.7 ± 1.7	9.5 ± 1.5	10.3 ± 3.0	10.8 ± 2.2	ns
Kidneys	139 ± 6	141 ± 12	137 ± 7	127 ± 30	ns

Values are expressed as absolute mean ± s.e.m. P, photoperiod; D, diet; P × D, the interaction of photoperiod and diet. Different superscripts in each row means significant difference (P < 0.05). *P < 0.05; **P < 0.01.

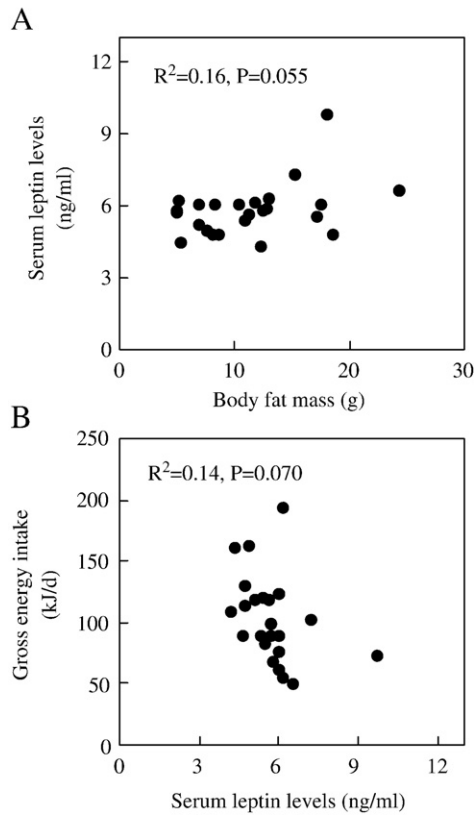


Fig. 5. Correlations between serum leptin levels and total body fat (A) and gross energy intake (B) in Brandt's voles acclimated to short-day and high-fat diet. Data are plotted.

increased their body mass and body fat mass after being transferred to long-day condition [27], suggesting not only short day, but also long day imposed the great effect on body mass. It was reported that the species showing decreases in body mass or body fat mass in fall for

seasonal acclimatization or short day for acclimation tended to resist to high-fat diet-induced obesity [1,2,10,28,29]. In contrast to Siberian hamsters [24,25], meadow voles [10], Shaw's jirds [1] and bank voles (*Clethrionomys glareolus*) [2], Brandt's voles that were exposed to high-fat diet increased their body fat mass in either long or short day, and showed high-fat diet-induced obesity [5,6,30]. Thus Brandt's voles, the photoperiodic species, are not resistant to high-fat diet-induced obesity.

4.2. Energy intake and digestibility

It has been found that there is a positive relationship between dietary fat content and prevalence of obesity [31]. Hyperphagia associated with high-fat diet is suggested to play a role in the development of obesity in some species, including human [2,31–33]. The present data also showed that increases in body fat mass and body fat content were accompanied by hyperphagia in the voles fed high-fat diet. In contrast, other rodent species, such as Shaw's jirds [1], were reported that high-fat diet did not induce hyperphagia, which was not necessary for high-fat diet-induced obesity [1,34,35]. These studies might suggest that there was species specific response to high-fat diet acclimation. For Brandt's voles, the higher digestibility in high-fat diet group was likely to compensate for the decrease in gross energy intake, which was similar to the study on Bank vole [2,36]. Together, these results indicated that high quality food associated with high digestibility might contribute to high-fat diet-induced obesity.

Regulation in morpho-physiology of digestive tract is an important way to cope with different kinds of diets [19]. The present data showed that voles fed high-fat diet increased the dry mass of small intestine. The increase in the tissue recruitment of small intestine associated with upregulated protein synthesis could result in the enhancement of nutrient absorption, which might be related to the elevated digestibility for the voles fed high-fat diet. Adaptive adjustments in morphology in colon and caecum also play important roles in small mammals exposed to different quality diets, such as Brandt's voles [8,37]. For Brandt's voles, high-fat diet decreased colon and caecum in size and mass. It seemed that small intestine recruitment might be an

Table 4
Digestive tract in Brandt's voles acclimated to short day and high-fat diet.

	Long day (LD)		Short day (SD)		P
	Low-fat diet (LFD)	High-fat diet (HFD)	Low-fat diet (LFD)	High-fat diet (HFD)	
Stomach					
Size (cm)	2.0 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	2.1 ± 0.2	ns
Mass with content (mg)	1295 ± 161	1258 ± 42	1136 ± 283	1831 ± 254	ns
Wet mass (mg)	450 ± 37 ^a	350 ± 19 ^{ab}	422 ± 19 ^{ab}	339 ± 30 ^b	D**
Dry mass (mg)	108 ± 7 ^a	91 ± 6 ^{ab}	97 ± 5 ^{ab}	79 ± 4 ^b	D**
Small intestine					
Size (cm)	27.1 ± 0.8	28.0 ± 0.7	29.1 ± 0.8	28.6 ± 1.1	P*
Mass with content (mg)	2135 ± 149	2158 ± 232	1959 ± 160	2199 ± 228	ns
Wet mass (mg)	607 ± 31	613 ± 47	564 ± 29	605 ± 42	ns
Dry mass (mg)	110 ± 8 ^b	141 ± 10 ^{ab}	108 ± 8 ^b	145 ± 9 ^a	D*
Colon					
Size (cm)	25.6 ± 0.6	23.2 ± 0.7	25.9 ± 0.6	24.4 ± 0.7	D**
Mass with content (mg)	1419 ± 203 ^a	751 ± 74 ^b	1090 ± 66 ^{ab}	915 ± 64 ^b	D**; Px D*
Wet mass (mg)	430 ± 23 ^a	302 ± 18 ^{bc}	376 ± 15 ^{ab}	271 ± 19 ^c	D**
Dry mass (mg)	77 ± 10	70 ± 7	77 ± 11	64 ± 4	D*
Caecum					
Size (cm)	14.4 ± 0.7	13.2 ± 0.8	15.8 ± 1.0	13.4 ± 0.4	P*; D**
Mass with content (mg)	4108 ± 604 ^a	2369 ± 230 ^b	3853 ± 230 ^{ab}	2730 ± 204 ^{ab}	D**
Wet mass (mg)	487 ± 27 ^a	347 ± 24 ^b	455 ± 30 ^a	346 ± 27 ^b	D**
Dry mass (mg)	77 ± 6	60 ± 9	83 ± 8	70 ± 13	D*
Total digestive tract					
Size (cm)	69 ± 1	67 ± 2	73 ± 2	68 ± 2	P**; D**
Mass with content (mg)	8956 ± 1021	6536 ± 528	8037 ± 670	7675 ± 578	D**
Wet mass (mg)	1973 ± 101 ^a	1611 ± 102 ^{ab}	1816 ± 84 ^{ab}	1562 ± 112 ^b	D**
Dry mass (mg)	355 ± 18	377 ± 22	354 ± 19	370 ± 23	ns

Values are expressed as absolute mean ± s.e.m. P, photoperiod; D, diet; P × D, the interaction of photoperiod and diet. Different superscripts in each row means significant difference (P < 0.05). *P < 0.05; **P < 0.01.

adaptive regulation in enhancing the absorption in Brandt's voles fed high-fat diet. In addition, the bean oil is easier to digest, which is likely to contribute the increased efficiency of digestion and absorption.

4.3. Serum leptin concentration, body fat mass and energy balance

Leptin plays an important role in energy metabolism, in which it has stimulated considerable interest in the neural and genetic mechanisms underpinning body-weight regulation [38,39]. Photoperiod is suggested to be an effective factor in the leptin-mediated regulations of energy balance and body weight [26,40,41], for instance, Brandt's voles differed in leptin levels after they were exposed to the increasing and decreasing photoperiod [26]. However, we did not observe significant effect of short day alone on serum leptin levels and, the correlation between leptin levels and body fat content was not significant. It was previously reported that leptin sensitivity was affected by photoperiod, and sensitivity to leptin in short-day hamsters was higher than that of long-day hamsters [40]. But for Brandt's voles, the sensitivity to leptin might be associated with decreasing photoperiod as well as increasing photoperiod [26]. We previously found leptin level was positively correlated with body fat and negatively with energy intake in this vole strain fed high-fiber or low-fiber diet [8], whereas we did not in the voles acclimated to high-fat diet in the present study. High-fiber diet-induced an increase in food intake, which seemed to be the result of decreasing sensitivity to leptin [8], but it might not be the case in the voles fed high-fat diet. However, without infusing leptin in voles, it is not possible to determine whether there are any differences in sensitivity to leptin between the voles exposed to high-fat diet and short day.

4.4. BMR, NE-induced thermogenesis and UCP1 level

It has been known that changes of BMR and NST, the major components of total energy expenditure, are important in the regulation of energy budgets and body mass in many small mammals in response to environmental fluctuations including temperature, photoperiod or food [2,42–44]. Short-day voles increased BMR and the maximal rate of oxygen consumption after NE injection, but did not increase thermogenesis indicative of NE-induced minus BMR, which was inconsistent with the previous study [6]. This inconsistency was likely due to the different NST calculation, in the study by Zhao and Wang (2005), NST was quantified as the maximal rate of oxygen consumption after NE injection, whereas, it should be defined NST_{max} as Nespolo et al. (2001) suggested [45] and, the maximum VO_2 after NE injection minus BMR was therefore termed NST [45–47]. In this case, short-day did not increase NST, but did increase BMR and NST_{max} , which was accompanied by the enhancement of BAT COX activity and upregulated BAT UCP1 levels [6,48–50]. We also did not observe significant effect of high-fat diet on BMR and thermogenesis, although it was previously reported that poor quality food decreased BMR and thermogenesis in other rodents [8,51–53] and high-fat diet-induced BAT hypertrophy [41,54–57]. For photoperiodic voles, these thermogenic parameters were not affected by high-fat diet, but were influenced by short-day acclimation [6,26,41].

Brandt's voles show significant changes in weight gain, body fat mass, energy intake and thermogenesis in the wild or outdoor enclosure [4,5]. Consistent with meadow voles [10], Shaw's jirds [1], Siberian hamsters [29], Brandt's voles show lower body mass and body fat mass, and higher energy intake and thermogenesis in fall and/or under short-day condition [5,6,26]. It was proposed that the species showing the decreased body mass in response to fall or short day would resist high-fat diet-induced obesity [1,10,29]. However, Brandt's vole fed high-fat diet showed higher body fat mass in either long or short days, indicating that this was inconsistent with the above hypothesis. Body-weight gain did not become higher in short-day voles, but did in long-day animals, which might be due to the

higher energy expenditure associated with the increased BMR and thermogenesis in short-day voles. Again, these results suggested that photoperiod, instead of food, played an important role in seasonal body-weight regulation for the photoperiodic Brandt's voles.

A high-fat diet failed to induce the increases in food intake and higher energy expenditure associated with BMR and thermogenesis, indicating that behavioral patterns might play a potential role in high-fat diet-induced obesity. It is proposed that there is a dynamic trade-off of fat storage between the risks of starvation and predation. Carrying around a larger fat storage may enhance the capacity to survive a food shortage, but simultaneously increases the probability of being killed by a predator [58,59]. Thus the relatively higher body fat content of the voles exposed to high-fat diet might be due to a low foraging activity, but the behavioral patterns were unfortunately unmeasured in this study. Additionally, there might be different response to high-fat diet for the animals exhibiting resistance to diet-induced obesity [60–63]. For example, high-fat diet increased activity in Bank voles [2,60] and meadow voles [64,65], and thermogenic capacity in Siberian hamsters [9]. These results suggest that the resistance to diet-induced obesity observed in photoperiodic animals is likely due to the increases in energy expended on metabolism and/or activity. However, this may be not the case for Brandt's voles.

In summary, Brandt's vole showed physiological regulations in metabolic rate, thermogenesis and energy budgets under different photoperiod and food quality. Short day stimulated the enhancement of thermogenesis. High-fat diet significantly increased the digestibility and body fat mass for the voles exposed to either long or short day. Brandt's vole, showing lower body mass and body fat mass in response to short day, did not show the resistance to high-fat diet-induced obesity, suggesting a good model for studying the physiological process and mechanism of high-fat diet-induced obesity.

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