

Effects of Exogenous Leptin on Body Mass, Thermogenesis Capacity and Hormone Concentrations of Yuman Chinese Vole, *Eothenomys miletus*, Under Varied Photoperiod

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Abstract.- Environmental factors play an important role in the seasonal adaptation of body mass and thermogenesis in wild small mammals. In the present study, we examined the effects of exogenous leptin on the adaptive thermogenesis and hormone concentrations in *Eothenomys miletus* by transferring them from a short (SD, 8h :16h L: D) to long day photoperiod (LD, 16h: 8h L:D). Body mass, body fat mass, rest metabolic rate (RMR), nonshivering thermogenesis (NST), energy intake, body compositions, mitochondrial cytochrome c oxidase (COX) activity and uncoupling protein1 (UCP1) content of brown adipose tissue (BAT), and serum triiodothyronine (T₃), thyroxine (T₄), leptin levels, hypothalamic thyrotropin-releasing hormone (TRH) and corticotrophin-releasing hormone (CRH) levels were measured. The results showed that RMR, NST and energy intake decreased by transferring to LD relative to those for SD, while body mass increased in the first 6 weeks acclimation. It shown that SD voles respond to the hormone by reducing body mass and energy intake by infusing with exogenous leptin. The exogenous leptin treatment induced higher COX activity, UCP1 content, serum T₃, leptin levels, T₃/T₄, TRH and CRH levels in short photoperiod than did the LP at the end of the acclimation. SD voles responded to infusion of leptin by changes in BAT UCP1 content, but LD voles showed no significantly change. Our results indicated that exogenous leptin may induce an increased thermogenesis and hormone concentrations, and decreased body mass and body fat mass. It seemed that *E. miletus* of SD were more sensitive to exogenous leptin.

Keywords: *Eothenomys miletus*, photoperiod, exogenous leptin, UCP1, hormone concentrations.

INTRODUCTION

Seasonal change of photoperiod is an important environmental cue to regulate seasonal responses in small mammals, including variations in body mass, body fat mass, energy intake and thermogenic capacities (Król *et al.*, 2005). Nonshivering thermogenesis (NST) usually occurs in brown adipose tissue (BAT) (Ricquier and Bouillaud, 2000) which is an important mechanism for cold-exposed small mammals to generate heat (Jansky, 1973), and this process is affected by photoperiod (Heldmaier *et al.*, 1981). NST in small mammals is originated principally through activation of uncoupling protein-1 (UCP1) (Wang *et al.*, 2006). UCP1, which is localized in the inner mitochondrial membrane of BAT (Cannon and

Nedergaard, 2004), where cytochrome c oxidase (COX, complex IV) is involved in mitochondrial energy metabolism (Kadenbach *et al.*, 2000). Finally, It has also been demonstrated that thermogenesis is regulated by hormones, such as thyroid hormones (T₃, T₄, hypothalamic TRH and CRH) (Krotkiewski, 2002; Joseph-Bravo *et al.*, 1998). The hormone leptin is considered to be an adipostatic signal regulating food intake, which makes it to be a likely candidate for involvement in regulation of seasonal changes of body mass (Zhang *et al.*, 1994). It also plays a key role in the regulation of energy intake and energy expenditure (Scarpace *et al.*, 1997). The functional relationship between leptin and thermogenic activity of BAT has been demonstrated in the post-cafeteria model of obesity (Rodriguez *et al.*, 2001) and via administration of exogenous leptin (Scarpace *et al.*, 1997; Commins *et al.*, 1999).

Although photoperiodic responses are different in different species, the body mass in *Clethrionomys glareolus* and *C. rutilus* showed no changes under photoperiod acclimation (Feist and

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Feist, 1986; Heldmaier *et al.*, 1989). These changes have however, been recorded in *Dipodomys ordii* (Gettinger and Ralph, 1985), *Phodopus sungorus* (Wiesinger *et al.*, 1989), *Lasiopodomys brandtii* (Zhao and Wang, 2005), *Meriones unguiculatus* (Zhao and Wang, 2006), and *M. agrestis* (Król *et al.*, 2006). Short photoperiod (SD) increases the NST capacity in many rodents including *Sekeetamys calurus* (Haim, 1996), *Apodemus sylvaticus* (Klaus *et al.*, 1988), and *M. oeconomus* (Wang *et al.*, 1999). It was also found that SD stimulated the increase in NST coupled with elevation in UCP1 content or UCP1 mRNA expression in small mammals, such as *L. brandtii* (Zhao and Wang, 2005), and *P. sungorus* (Demas *et al.*, 2002). It was found that the lower serum leptin level was accompanied with higher energy intake (Li and Wang, 2005). Further, the positive correlation between serum leptin levels and body fat mass has been found in many small mammals including collared lemmings (Johnson *et al.*, 2004), *P. sungorus* (Klingenspor *et al.*, 2000), and *M. unguiculatus* (Li *et al.*, 2004), *E. miletus* (Zhu *et al.*, 2010) and *Apodemus chevrieri* (Zhu *et al.*, 2012a). Exogenous leptin can affect the body mass and thermogenesis in small mammals, such as *P. sungorus* (Klingenspor *et al.*, 2000) and rat (Ahima *et al.*, 1996). Exogenous leptin can make the BAT UCPs mRNA levels increased nearly 30% (Cusin *et al.*, 1998). In ob/ob rats, injection of exogenous leptin resulted in increased BAT UCPs mRNA levels and energy consumption (Scarpace *et al.*, 1997).

The Hengduan Mountains region is located at the boundary between the Palaearctic region and the Oriental region. It is alpine with high mountains and gorges; the diversity and abundance of mammals is high and it is considered to be “the harbor in fourth ice age”. *Eothenomys miletus* commonly known as Yunan Chinese vole is the inherent species in Hengduan mountains region (Zhu *et al.*, 2011a). Environmental factors, such as short photoperiods and cold, are effective cues that influence body mass and thermogenesis separately (Zhu *et al.*, 2008; 2010a,b; 2011a; 2012b). However, we know nothing about the action of exogenous leptin with changes in body mass regulation, thermogenesis and hormone concentrations in *E. miletus* by transferring them from a short (SD, 8h:16h L:D) to long day

photoperiod (LD, 16h: 8h L:D). We compared the responses of LD and SD voles to exogenous leptin treatment by measuring body mass, thermogenesis and hormone concentrations.

MATERIALS AND METHODS

Samples

E. miletus were obtained from a captive population, 150 captured from a farmland (26°15′~26°45′N; 99°40′~99°55′E; altitude 2,590m) in Jianchuan County, Yunnan province, 2010. Mean annual temperature is 9.1°C; average temperatures in January and July are -4.0°C and 24.1°C, respectively. *E. miletus* were bred for two generations in School of Life Science of Yunnan Normal University, and were individually housed in plastic boxes (260mm×160mm×150mm), at the room temperature of 25±1°C, under a photoperiod of 8L:16D (with lights on at 08:00). *E. miletus* were fed standard rabbit pellet chow (crude fat, 6.2%; crude protein, 20.8%; neutral detergent fiber, 21.5%; acid detergent fiber, 12.5%; ash, 10.0%) (Kunming Medical University, Kunming, People’s Republic of China) and water *ad libitum*. Animals were fed daily at 10:00-11:00. The voles were allowed to acclimate to these conditions for 4 weeks. All pregnant, lactating or young individuals were excluded.

Following the acclimation period, animals were weighed and assigned to three groups that were matched for body mass (n=10 in each group, male 16 and female 14). All animals were kept in SD for two weeks to obtain base line measurements of body mass and food intake, after which two groups were exposed to a LD photoperiod (16:8 h L:D, lights on 08:00 h) at 25°C, and two groups remained in SD for 4 weeks. On 43rd day, all animals were implanted subcutaneously with a mini-osmotic pump (Alzet model 2001, capacity 200 µl, release rate 1.0 µl/h, duration of pumping 190 h, Durect Corporation, Cupertino, CA, USA) containing either approximately 200 µg of recombinant murine leptin (Peprtech, London, UK) or phosphate-buffered saline (PBS). The minimal dose of leptin used in this experiment approximately 0.5 µg/g.day (Rousseau *et al.*, 2002). Method of implantation of mini-osmotic pumps is detailed in Król (2005). The experimental design is shown in

Figure 1. At the end, the voles were sacrificed between 09:00 and 11:00 by puncture of the posterior vena cava on 63rd day. Blood samples were collected, clotted for 1 h and centrifuged at 4°C for 30 min at 4,000 rpm. Sera were collected and stored at -80°C until assayed. The interscapular brown adipose tissue (IBAT) was immediately and carefully dissected, weighed and stored at -80°C until assayed.

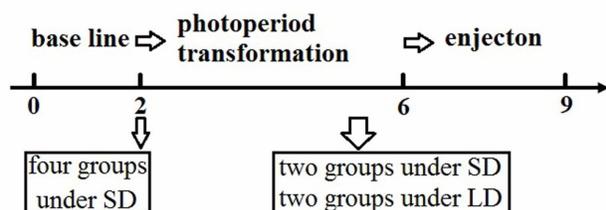


Fig. 1. The experiment started with 40 *E. miletus*, bred at 25±1 °C and under a photoperiod of 8L:16D for the first two weeks. Then two groups were exposed to a LD photoperiod at 25°C, and two groups remained in SD for next 4 weeks. On day 43, all animals were implanted subcutaneously with a mini-osmotic pump containing either recombinant murine leptin or (PBS) for 3 weeks.

Measurement of metabolic rates

Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 25°C within the TNZ (thermal neutral zone). The gas analysis was done by using ML206 gas analysis instrument. The temperature was controlled by SPX-300 artificial climatic engine (±0.5°C), the metabolic chamber had volume of 500ml and flow of 200 ml/min at the end. The voles were stabilized in the metabolic chamber for at least 60 min prior to the RMR measurement. Oxygen consumption was recorded for more than 120 min at 1 min intervals. Ten stable consecutive lowest readings were taken to calculate RMR (Li and Wang, 2005). The metabolic rate was calculated according to Hills (1972). Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) and measured at 25°C. Two consecutive highest recordings of oxygen consumption for more than 60 min at each measurement were taken to calculate the NST (Zhu

et al., 2010b). The doses of NE were approximately 0.8-1.0 mg/kg according to dose-dependent response curves that were carried out before the experiment and the equation described by Heldmaier (1971) as follows:

$$\text{norepinephrine dosage (mg/kg)} = 6.6M^{-0.458}(\text{g}).$$

Energy intake

Energy intake for each experimental group was measured for 7 days (Song and Wang, 2002), and food were provided *ad libitum*. Animals were provided with fixed quantity of food at the same time. The animals were weighed the next day and residual food was collected. Residual food was dried at 65°C for at least 72 h. The caloric contents of food was measured by YX-ZR/Q automatism calorimeter. The calorie of the diet fed to these animals were 18.0±0.8 kJ/g. The energy intake was calculated according to Drozd (1975):

$$\text{Energy intake (KJ/d)} = \text{food (g/d)} \times \text{energy content (KJ/g)}$$

Measurements of COX activity and UCP1 content

Mitochondrial protein concentration was 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).

UCP1 content was measured by Western blotting. Total BAT protein (15 µg per lane) was separated in a discontinuous SDS-polyacrylamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose membrane (Hybond-C, Amersham). To check for the efficiency of protein transfer, gels and nitrocellulose membranes were stained after transferring with Coomassie brilliant blue and Ponceau red, respectively. Unspecific binding sites were saturated with 5% nonfat dry milk in PBS. UCP1 was detected using a polyclonal rabbit UCP1 (1:5000) as a primary antibody (Abcam, Shanghai) and peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno. Inc., USA) as the second antibody. Enhanced chemoluminescence (ECL, Amersham Biosciences, England) was used for the detection of UCP signal.

UCP1 concentration was determined from area readings by using Scion Image Software (Scion Corporation) (Li and Wang, 2005).

Measurement of hormone concentration

Serum leptin levels were determined by radioimmunoassay (RIA) with the 125I Multi-species Kit (Cat. No. XL-85K, Linco Research Inc.). The lowest level of leptin that can be detected by this assay was 1.0 ng/ml when using a 100- μ l sample size. The inter- and intra-assay variability for leptin RIA were <3.6% and 8.7%, respectively.

The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum were determined using RIA kits (China Institute of Atomic Energy). These kits were validated for all species tested by cross-activity. The intra- and inter-assay coefficients of variation were 2.4% and 8.8% for the T_3 , 4.3% and 7.6% for T_4 , respectively. Serum melatonin concentrations were measured by ELISA method (Bioteke Co. Beijing, China). Hypothalamic thyrotropin-releasing hormone (TRH) and corticotrophin-releasing hormone (CRH) levels were determined by TRH radioimmunoassay kit and CRH radioimmunoassay kit (Beijing North Institute of Biotechnology) (Du and You, 1992).

Morphology

At the end, after collecting trunk blood, the visceral organs, including liver, BAT, heart, lung, kidneys, spleen and gastrointestinal tract (stomach, small intestine, cecum, large intestine), were extracted and weighed (± 1 mg). The stomach and intestines were rinsed with saline to eliminate all the gut contents, before being dried and weighed. The remaining carcass and all the organs were dried to constant mass in an oven at 60 °C (at least 72 h), and then weighed again to obtain the dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Zhao and Wang, 2006).

Statistical analysis

Data were analyzed using SPSS 15.0 software package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. Throughout

the acclimation, changes in body mass, RMR, NST, energy intake were analyzed by repeated measures analysis of covariance (ANCOVA) with body mass as a covariate. The data were separated and analyzed independently for each day using two-sample *t* tests. Body compositions, COX activity and UCP1, and T_3 , T_4 , leptin levels, TRH and CRH levels were analyzed by one-way analysis of covariance (ANCOVA) with body mass as a covariate. Results were presented as mean \pm SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Since no gender effects were found in any of the measured parameters, data from females and males were combined thereafter.

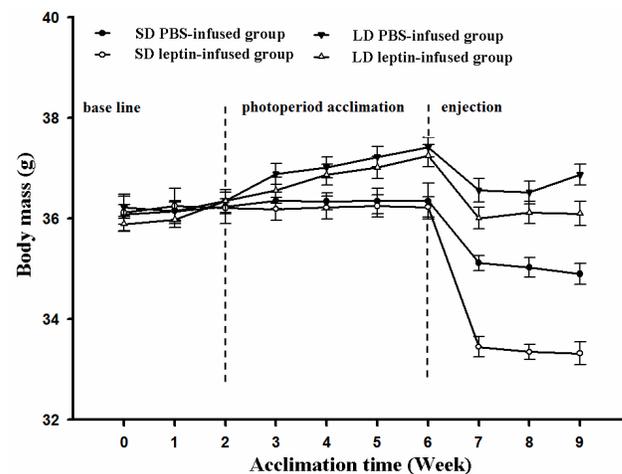


Fig. 2. Effects of exogenous leptin on body mass in photoperiod acclimated voles, *Eothenomys miletus*.

Body mass and body composition

Body mass of *E. miletus* showed no significant difference in the 0-2 weeks ($P > 0.05$). During the 2-6 weeks of LD exposure, the LD voles exhibited substantially greater body mass increase ($F_{3,36} = 2.322$, $n = 40$, $P < 0.05$) compared to SD voles that kept relatively constant body mass (Table I). Body mass exposed to LD increased significantly at the end of 6 weeks compared to SD voles ($t = -1.685$, $n = 20$, $P < 0.05$, Fig. 2). The decrease of body mass observed in all voles between days 43 and 49 was related to the implantation of mini-osmotic pumps.

Table I.- Effects of exogenous leptin on body mass and body compositions in photoperiod acclimation voles (*Eothenomys miletus*)

Parameters	SD PBS-infused group	SD leptin-infused group	LD PBS-infused group	LD leptin-infused group	P value
Body mass (g)	34.90±0.21	33.32±0.23	36.87±0.21	36.10±0.24	<0.05
Body fat mass (g)	3.95±0.06	3.21±0.09	4.54±0.06	4.42±0.08	<0.05
Carcass dry mass (g)	7.12±0.11	7.01±0.04	7.54±0.10	7.35±0.07	ns
Wet mass (g)					
Heart (g)	0.198±0.007	0.202±0.006	0.212±0.005	0.214±0.009	ns
Lungs (g)	0.288±0.035	0.279±0.041	0.301±0.032	0.295±0.026	ns
Kidney (g)	0.186±0.009	0.192±0.008	0.196±0.007	0.187±0.011	ns
Spleen (g)	0.018±0.003	0.022±0.006	0.019±0.005	0.019±0.007	ns
Stomach (g)	0.386±0.025	0.385±0.031	0.411±0.036	0.415±0.042	ns
Small intestine (g)	0.702±0.047	0.790±0.052	0.675±0.036	0.688±0.046	<0.05
Cecum (g)	0.396±0.022	0.421±0.036	0.378±0.026	0.395±0.024	ns
Large intestine (g)	0.342±0.015	0.312±0.018	0.348±0.021	0.334±0.018	ns
Dry mass (g)					
Heart (g)	0.039±0.004	0.038±0.003	0.042±0.005	0.041±0.006	ns
Lungs (g)	0.065±0.008	0.063±0.006	0.068±0.011	0.065±0.007	ns
Kidney (g)	0.042±0.002	0.040±0.006	0.042±0.006	0.044±0.005	ns
Spleen (g)	0.003±0.001	0.003±0.001	0.004±0.001	0.004±0.001	ns
Stomach (g)	0.099±0.009	0.095±0.006	0.089±0.008	0.092±0.006	ns
Small intestine (g)	0.026±0.005	0.032±0.009	0.028±0.004	0.028±0.008	ns
Cecum (g)	0.032±0.002	0.041±0.001	0.030±0.004	0.031±0.005	<0.05
Large intestine (g)	0.041±0.002	0.039±0.005	0.041±0.006	0.039±0.004	ns

Over this period of time, the LD and SD voles infused with PBS lost on average 0.86±0.18 g and 1.23±0.14 g, respectively. Between weeks 7 and 9 of SD and LD exposure, changes of body mass in LD voles were not significantly different from those in PBS-infused LD controls. In contrast, changes of body mass in SD leptin-infused voles before and after surgery was significantly different from that of SD PBS-infused animals ($F_{3,36}=6.598$, $N=40$, $P<0.01$) (Fig. 2). At the end, body fat mass showed significant differences between SD leptin-infused group and SD PBS-infused group ($t=-1.965$, $N=20$, $P<0.05$), but there was no changes of body fat mass between LD leptin-infused group and LD PBS-infused group. For the body compositions, wet mass of small intestine and dry mass of cecum were higher in SD leptin-infused group than that of the other three groups ($F_{3,36}=2.012$, $N=40$, $P<0.05$; $F_{3,36}=1.567$, $N=40$, $P<0.05$).

RMR, NST and energy intake

At the end of acclimation, RMR and NST

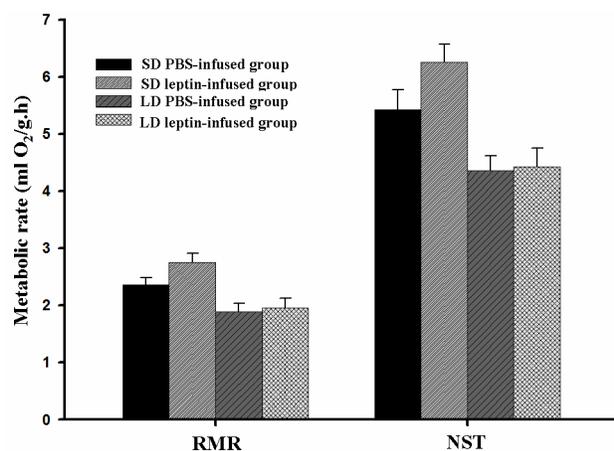
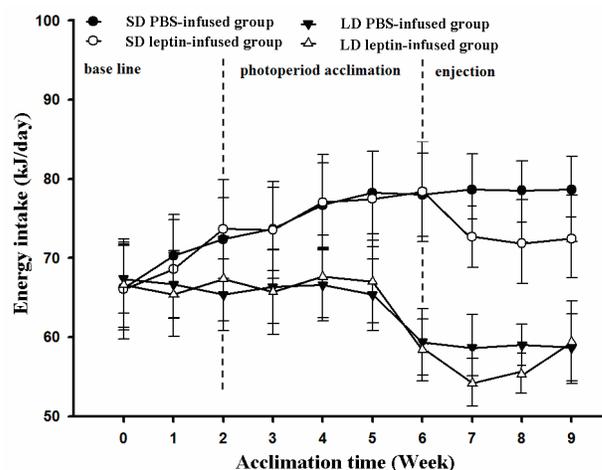
showed significant differences between SD leptin-infused group and SD PBS-infused group, but there were no changes of RMR and NST between LD leptin-infused group and LD PBS-infused group (Fig. 3). Prior to leptin treatment (weeks 0-2), the LD and SD voles did not differ in their energy intake ($P>0.05$) (Fig. 4). All voles for both SD and LD of leptin-infused group increased energy intake on week 7 as compared to PBS-infused group. At the end of acclimation, energy intake of LD and SD voles infused with PBS returned to the level observed prior to surgery ($P>0.05$). In SD photoperiod, however, the response of leptin-infused voles was significantly different to PBS-infused animals between week 7 to 9.

COX activity, hormones, UCP1 content and serum leptin level

At the end of the acclimation, there were no significant difference in mass of liver and BAT in the four groups (Table II). The exogenous leptin treatment induced higher COX activity, UCP1

Table II.- Effects of exogenous leptin on enzyme activity and hormone concentration in photoperiod acclimation voles (*Eothenomys miletus*)

Parameters	SD PBS-infused group	SD leptin-infused group	LD PBS-infused group	LD leptin-infused group	P value
Liver					
Mass (g)	2.12±0.11	2.16±0.08	2.18±0.12	2.14±0.07	ns
Mt protein content (mg/g tissue)	25.32±1.52	29.24±1.24	22.21±1.54	23.21±1.25	<0.05
COX activity (nmol/min g tissue)	52.21±2.23	63.21±2.01	41.20±3.05	42.36±2.36	<0.01
BAT					
Mass (g)	0.25±0.02	0.28±0.01	0.26±0.02	0.24±0.03	ns
Mt protein content (mg/g tissue)	10.62±0.62	12.63±0.56	8.56±0.44	8.65±0.36	<0.01
COX activity (nmol/min g tissue)	589±23	632±35	545±25	562±33	<0.05
UCP1 content (pmol/mg mitochondrial protein)	512±32	562±23	482±15	503±32	<0.05
Hormones					
T ₄ (ng/ml)	52.36±3.01	48.25±2.23	51.23±2.14	49.36±1.88	ns
T ₃ (ng/ml)	1.65±0.16	1.89±0.14	1.35±0.26	1.44±0.24	<0.05
T ₃ / T ₄	0.032±0.005	0.039±0.002	0.026±0.003	0.029±0.003	<0.05
Concentration of TRH in hypothalamus (ng/mg protein)	3.36±0.15	3.56±0.21	2.68±0.19	2.82±0.14	<0.01
Concentration of CRF in hypothalamus (ng/mg protein)	3.15±0.24	3.45±0.23	2.38±0.35	2.48±0.26	<0.05
Serum leptin level (ng/ml)	1.61±0.02	9.62±0.72	1.65±0.03	8.36±0.56	<0.01

Fig. 3. Effects of exogenous leptin on RMR and NST in photoperiod acclimation voles (*Eothenomys miletus*)Fig. 4. Effects of exogenous leptin on energy intake in photoperiod acclimation voles (*Eothenomys miletus*)

content, T₃, leptin levels, T₃/T₄, hypothalamic TRH and CRH levels in short photoperiod than did the LD at the end of the acclimation. SD voles responded to infusion of leptin by changes in BAT UCP1 content, but LD voles showed no significant change. The levels of circulating leptin

averaged 1.61±0.02, 9.62±0.72, 1.65±0.03 and 8.36±0.56 ng/ml in SD PBS-infused, SD leptin-infused, LD PBS-infused and LD leptin-infused voles, respectively. The leptin levels in voles infused with exogenous leptin were significantly higher than

in PBS-infused individuals (Table II). The levels of circulating leptin were positively correlated with UCP1 content in the leptin-infused voles ($r=0.652$, $N=20$, $P<0.01$).

DISCUSSION

Body mass, body compositions

Photoperiod is an important cue triggering seasonal responses in small mammals (Genin and Perret, 2000). Some small mammals such as *M. pennsylvanicus* (Dark and Zucker, 1986), Siberian hamsters (Demas *et al.*, 2002) reduced body mass while increased thermogenesis in SD conditions (Geiser *et al.*, 2007). Injection of exogenous leptin in *P. sungorus* reduced body mass both in LD and SD. The reduction in body fat mass was more pronounced under SD conditions. This shows that *P. sungorus* was more sensitive to SD conditions (Klingenspor *et al.*, 2000). In the present study, *E. miletus* gained body mass in LD group, which is similar to that reported in Syrian hamsters (Campbell and Tabor, 1983) and collared lemmings (Nagy, 1993). In contrast to LD leptin-infused group, *E. miletus* decreased body mass and body fat mass in SD leptin-infused group. And the wet mass of small intestine and dry mass of cecum were higher in SD leptin-infused group than that the other three group, these data suggested *E. miletus* employ a strategy of maximizing body growth in response to injection of exogenous leptin associated with short photoperiod.

RMR, NST, energy intake, COX and hormone concentration

Changes of photoperiod are considered to be important cues which affect RMR in many small mammals, such as *Acomys russatus* (Haim and Zisapel, 1995), *Mus macedonicus* (Haim *et al.*, 1999). The body mass, body fat mass and food intake decrease, whereas metabolic rate and body temperature increase after injection with exogenous leptin in C57BL/6J mice (Pelleymounter *et al.*, 1995). In our studies, RMR in SD leptin-infused voles after 9 weeks photoperiodic acclimation was higher than that of other groups. Further, liver, as an important energy-expending organ, is considered to make a large contribution to RMR (Cannon and

Nedergaard, 2004). In the present study, mitochondrial protein content and COX activity in SD leptin-infused voles were significantly higher than that of other groups, which was consistent with the changes in RMR. BAT is the main site of NST production in small mammals (Klingenspor *et al.*, 2000). In our present study, although no differences in BAT mass were found between SD and LD voles, NST was significantly elevated in SD leptin-infused voles. Further, UCP1 content in BAT was higher in SD leptin-infused than that in LD voles. Our data also showed that BAT mitochondrial protein content and COX activity were affected by exogenous leptin in *E. miletus*. The function of thyroid hormone is to stimulate cell to produce thermogenesis. The change of the level of thyroid hormones can reflect the thermogenesis characters in cold conditions (Tomasi and Michell, 1994). T_3 increased during cold acclimation (Gordon, 1993). Similar studies were found in *Scalopus aquaticus* (Leach *et al.* 1962), rat (Balsam and Leppo, 1974) and mouse (Wills and Schindler, 1970). The T_4 level decreased or remained constant (McNabb, 1992). In the current study, serum T_3 concentration was significantly higher in SD leptin-infused voles, and the serum T_4 concentration showed no changes. Hypothalamic TRH and CRH levels were also significantly higher in SD leptin-infused voles, which was consistent with the increase in NST. Exogenous leptin induced a significant loss of energy intake in SD voles. By contrast, energy intake of LD voles was not affected by the leptin infusion at the end of acclimation (Król *et al.*, 2006).

Serum leptin level and UCP1 content

Leptin administration is thought to increase energy expenditure as indicated by elevated expression of UCP1 or UCP1 mRNA level (Zhao and Wang, 2005). Indeed, a positive relationship between BAT UCP1 gene expression and serum leptin level has been demonstrated in many rodents (Schwartz, 2000). But for *M. agrestis*, neither photoperiod nor treatment had a significant effect on BAT UCP1 gene expression (Król *et al.*, 2006). Interestingly, it showed a negative relationship between BAT UCP1 contents and serum leptin level in *A. chevrieri* (Zhu *et al.*, 2011b). In the present study, the levels of circulating leptin were positively

correlated with UCP1 content in the leptin-infused voles.

CONCLUSIONS

In conclusion, by infusing voles with exogenous leptin, it has been demonstrated that SD voles respond to the hormone by reducing body mass and energy intake, whereas LD voles increased body mass. The exogenous leptin treatment induced higher COX activity, UCP1 content, serum T₃, leptin levels, T₃/T₄, hypothalamic TRH and CRH levels in short photoperiod than did the LD at the end of the acclimation. There was a positive relationship between BAT UCP1 contents and serum leptin level in SD leptin-infused voles. It seemed that *E. miletus* of SD were more sensitive to exogenous leptin.

ACKNOWLEDGMENTS

The project was financially supported by National Science Foundation of China (No.31260097); Project of Basic research for application in Yunnan Province (No. 2011FZ082).

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(Received 6 February 2013; revised 25 June 2013)