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ORIGINAL ARTICLE

Effects of photoperiod on body mass, thermogenesis and body composition in *Eothenomys miletus* during cold exposure

Wan-long Zhu, Shengchang Yang, Jin-hong Cai, Lihua Meng, Zheng-kun Wang*

School of life Science of Yunnan Normal University, Engineering research center of sustainable development and utilization of biomass energy Ministry of Education, The Key Laboratory of Biomass Energy and Environmental Biotechnology in Yunnan Province, Kunming; 650092, China

E-mail:zwl_8307@163.com

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Many small mammals respond to seasonal changes in photoperiod by altering body mass and adiposity. These animals may provide valuable models for understanding the regulation of energy balance. In present study, we examined the effect on body mass, rest metabolic rate, food intake and body composition in cold-acclimated (5 °C) in *Eothenomys miletus* by transferring them from a short (SD, 8h :16h L: D) to long day photoperiod (LD, 16h: 8h L:D). During the first 4 weeks of exposure to SD, *E. miletus* decreased body mass. After the next 4 weeks of exposure to LD, which the average difference between body masses of LD and SD voles was 4.76 g. This 14.74% increase in body mass reflected significant increases in absolute amounts of body components, including wet carcass mass, dry carcass mass and body fat mass. After correcting body composition and organ morphology data for the differences in body mass, only livers, kidney, and small intestine were enlarged due to photoperiod treatment during cold exposure. *E. miletus* increased RMR and energy intake exposure to LD, but maintained a stable level to SD after 28 days. Serum leptin levels were positively correlated with body mass, body fat mass, RMR as well as energy intake. All of the results indicated that *E. miletus* may provide an attractive novel animal model for investigation of the regulation of body mass and energy balance at organism levels. Leptin is potentially involved in the photoperiod induced body mass regulation and thermogenesis in *E. miletus* during cold exposure.

Key words: Eothenomys miletus; thermogenesis; body composition; serum leptin

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Many small mammals respond to seasonal changes in photoperiod by altering body mass and adiposity. These animals may provide valuable models for understanding the regulation of energy balance. In present study, we examined the effect on body mass, rest metabolic rate, food intake and body composition in cold-acclimated (5 °C) in *Eothenomys miletus* by transferring them from a short (SD, 8h :16h L: D) to long day photoperiod (LD, 16h: 8h L:D). During the first 4 weeks of exposure to SD, *E. miletus* decreased body mass. After the next 4 weeks of exposure to LD, which the average difference between body masses of LD and SD voles was 4.76 g. This 14.74% increase in body mass reflected significant increases in absolute amounts of body components, including wet carcass mass, dry carcass mass and body fat mass. After correcting body composition and organ morphology data for the differences in body mass, only livers, kidney, and small intestine were enlarged due to photoperiod treatment during cold exposure. *E. miletus* increased RMR and energy intake exposure to LD, but maintained a stable level to SD after 28 days. Serum leptin levels were positively correlated with body mass, body fat mass, RMR as well as energy intake. All of the results indicated that *E. miletus* may provide an attractive novel animal model for investigation of the regulation of body mass and energy balance at organism levels. Leptin is potentially involved in the photoperiod induced body mass regulation and thermogenesis in *E. miletus* during cold exposure.

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Phenotypic flexibility is the ability of organisms to reversibly modulate traits in response to environmental challenge (Aneta et al., 2009). In recent years, the magnitude of this modulation has been extensively quantified at different levels of biological organization (Sabat and Bozinovic, 2000; Villarin et al., 2003; Kristan and Hammond, 2006). Several studies have demonstrated that processes related to energy acquisition and expenditure is among the most sensitive to changes in environmental conditions (Naya et al., 2008). The responses to changing photoperiod can be readily induced in the laboratory by simulating the natural progression of day lengths or by acutely transferring animals between summer (long) and winter (short) day lengths (Kryl et al., 2005). There were many studies about the transfer of small mammals from long day photoperiod (LD, 16·h:8·h L:D) to short day photoperiod (SD, 8·h:16·h L:D), they showed the changes of body mass, thermogenesis and body composition (Ebling, 1994; Klingenspor et al., 2000; Mercer and Speakman, 2001; Hunter and Nagy, 2002). Leptin is a protein which is synthesized primarily by adipose tissue and is secreted into the bloodstream (Silva, 2006). Leptin is considered to be an adipostatic signal linking energy metabolism, regulating food intake, and providing prompt feedback to brain areas involved in regulation of energy balance (Barb and Kraeling, 2004). It is also hypothesized to contribute to the maintenance of body mass by altering food intake and energy expenditure (Friedman and Hallas, 1998).

The Hengduan Mountains region is located 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". Therefore small mammals may differ from those from other regions. *Eothenomys* were the proper genus in China, were a special group in *Microtus*, and were the typical animals in Hengduan Mountains region. *E. miletus* is the inherent species in Hengduan mountains region (Zhu et al., 2011a), has special status in *Microtus*. Now few studies about *E. miletus* were reported (Zhu et al., 2008; 2010a; 2010b; 2011b). In the present study, we examined the effect on body mass, thermogenesis and body composition in cold-acclimated *E. miletus* by transferring them from short to long day photoperiod. We predicted that *E. miletus* change their body mass, thermogenesis and body composition by transferring them from short to long day photoperiod, and leptin would be involved in the regulation of body mass and thermogenesis.

MATERIALS AND METHODS

Samples

E. miletus were captured in farmland (26°15'~26°45'N; 99°40'~99°55'E; altitude 2,590m) in Jianchuan County, Yunnan province, 2011. Average temperature is 9.1ºC each year, minimum average temperature was -4.0ºC in January, maximal average temperature was 24.1ºC in July.

E. miletus were breed for two generations in School of life Science of Yunnan Normal University, park in plastic box (260mmx160mmx150mm), one in a box without any bedding material, and were maintained at the room temperature of 25±1°C, under a photoperiod of 12L:12D (with lights on at 08:00). After 1 month stabilization, animas were randomly divided into two groups (n=7 each group). Following the acclimation period, all animals were kept in SD for a further 24days to obtain baseline measurements of body mass and food intake, after which 7 voles were exposed to a long day photoperiod (LD, 16h: 8h L: D, lights on 04:00h) at 5°C, whereas 7 control voles remained in original SD conditions. The voles were sacrificed between 09:00 and 11:00 by puncture of the posterior vena cava. Blood was centrifuged at 4000 rpm for 30 min, and serum was sampled and stored at −20°C for later measurement. All voles were dissected to evaluate organ morphology. All pregnant, lactating or young individuals were excluded.

Measurement of metabolic rates

Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 25ºC within the TNZ (thermal neutral zone), gas analysis were using ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5ºC), the metabolic chamber volume is 500ml, flow is 200 ml/min. The tree shrew were stabilized in the metabolic chamber for at least 60 min prior to the BMR measurement, oxygen consumption was recorded for more than 60·min at 5·min intervals. Two stable consecutive lowest readings were taken to calculate BMR (Li and Wang, 2005). Calculate method of metabolic rate is detailed by Hills (Hills, 1972).

Energy budget

Energy budget were measured by food equity (Rosenmann and Morrison, 1974), one animal in a metabolic cage ($20\times15\times15cm^3$) , had no nest materials. After adaptation one week, energy budget were measured. Animals were feeding fix quantify and time, next day weighted animal body mass, collected residual food, faeces. Residual food and faeces were dryness in vacuum airer until the mass were invariable, got sample (about 1g, precision: 0.0001g), the samples were measured by YX-ZR/Q automatism calorimeters instrument. The calorie of the diet fed to these animals were 18.0±0.8 kJ/g. Calculate the energy intake. The calculation was according to corresponding reports (Drozdz, 1975):

Energy intake (KJ/d) =food (g/d)* energy content (KJ/g)

Measurement of serum leptin level and thyroid hormones

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-μl sample size. And the inter- and intra-assay variability for leptin RIA were <3.6% and 8.7%, respectively.

Carcass and body fat content

Gastrointestinal tract (stomach, small intestine, cecum, large intestine) and the heart, lungs, pancreas, spleen, kidneys 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 in an oven at 60 °C to constant mass (at least 72 h), and then weighed again to obtain the dry mass. The remaining carcass and all the organs were dried in an oven at 60°C to constant mass (at least 72 h), and then weighed again to obtain the dry mass. The difference between the wet carcass mass and dry carcass mass was the water mass of carcass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Zhang and Wang, 2007).

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, BMR and energy intake were analyzed by a two-way analysis of covariance (ANCOVA) with body mass as a covariate. Body composition between SD and LD were analyzed by independent t-test. Pearson's correlation was performed to detect possible correlations among serum leptin and body mass, body fat mass, RMR and energy intake. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass and serum leptin

No group differences were found between acclimation voles (F=0.972, P>0.05, Fig 1) before acclimation. During the acclimation, both acclimation voles decreased body mass in the first 4 weeks, SD voles showed relatively stable body mass after 4 weeks(F=0.073, P>0.05, Fig 1), whereas the LD voles showed a gradual increase over the next 4-week acclimation (F=2.449, P<0.05, Fig 1). Correlation analysis indicated that body mass was positively correlated with body fat mass (r = 0.836, P < 0.01; Fig 2A). There had positive correlation between serum leptin level and body mass (r = 0.960, P < 0.01; Fig 2B), between serum leptin level and body fat mass during the acclimation(r = 0.784, P < 0.01; Fig 2C).

RMR and energy intake

Prior to acclimation, no group differences in RMR between acclimation voles (t=0.318, P>0.05). During the acclimation, both acclimation voles increased RMR in the first 4 weeks, SD voles showed relatively stable RMR after 4 weeks (F=0.125, P>0.05, Fig 3), whereas the LD voles showed a gradual increase over the next 4-week acclimation (F=3.245, P<0.05, Fig 3). Prior to acclimation, no group differences in energy intake between acclimation voles (t=0.364, P>0.05). During the acclimation, both acclimation voles increased energy intake in the first 4 weeks, SD voles showed relatively stable energy intake after 4 weeks(F=0.296, P>0.05, Fig 4), whereas the LD voles showed a gradual increase over the next 4-week acclimation (F=2.985, P<0.05, Fig 4). There had positive correlation between serum leptin level and RMR (r = 0.956, P < 0.01; Fig 5A), between serum leptin level and energy intake during the acclimation(r = 0.676, P < 0.01; Fig 5B).

Body composition

At the end of the acclimation, wet carcass mass, dry carcass mass and body fat mass showed a significant differences between SD voles and LD voles (t=2.242, P<0.01, Table 1), (t=2.717, P<0.05, Table 1), (t=2.035, P<0.05, Table 1), respectively. No differences were found in water of carcass between the two groups (t=1.603; P>0.05, Table 1). By contrast, the wet organ masses of liver, kidney and small intestine showed significant between the LD and SD voles (Table 2), the dry organ masses of liver and kidney showed significant between the LD and SD voles (Table 3).

Table 1. Changes of body compositions exposed to short and long day photoperiods during cold exposure *E. miletus*

Parameters	SD	LD	P value
Body mass(g)	22.52±0.51	26.12±0.98	0.042
Wet carcass mass(g)	32.29±0.86	37.05±0.95	0.001
Dry carcass mass(g)	11.27±0.34	13.44±0.72	0.019
Water of carcass(g)	11.26±0.70	12.64±0.49	0.135
Body fat mass(g)	4.62±0.14	5.50±0.29	0.025

Table 2. Changes of mean wet organ masses exposed to short and long day photoperiods during cold exposure *E. miletus*

Parameters	SD	LD	P value
Heart(g)	0.233±0.019	0.251±0.013	0.271
Lungs(g)	0.308±0.015	0.324±0.008	0.401
Liver(g)	1.525±0.071	1.848±0.078	0.012
Kidney(g)	0.179±0.010	0.218±0.014	0.049
Spleen(g)	0.020±0.002	0.020±0.003	0.896
Stomach(g)	0.405±0.031	0.414±0.013	0.071
Small intestine(g)	0.674±0.024	0.751±0.015	0.023
Cecum(g)	0.420±0.015	0.438±0.014	0.443
Large intestine(g)	0.325±0.013	0.335±0.013	0.608

Table 3. Changes of mean dry organ masses exposed to short and long day photoperiods during cold exposure *E. miletus*

Parameters	SD	LD	P value
Heart(g)	0.043±0.002	0.045±0.002	0.610
Lungs(g)	0.066±0.002	0.070±0.001	0.131
Liver(g)	0.384±0.015	0.497±0.022	0.001
Kidney(g)	0.040±0.001	0.043±0.001	0.020
Spleen(g)	0.004±0.001	0.004±0.001	0.763
Stomach(g)	0.093±0.008	0.093±0.009	0.324
Small intestine(g)	0.029±0.001	0.027±0.001	0.676
Cecum(g)	0.041±0.003	0.041±0.002	0.981
Large intestine(g)	0.044±0.003	0.044±0.004	0.906

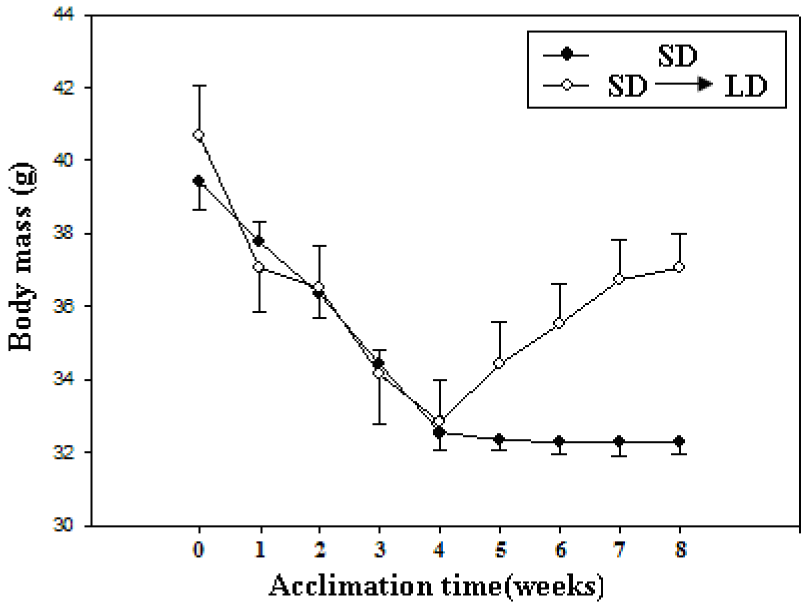

Figure 1 Effect of body mass exposed to short and long day photoperiods during cold exposure in *E. miletus*

DISCUSSION

Many small mammals respond to winter-associated environmental cues by reducing body or enhancing thermogenesis (Heldmaier et al., 1982; 1989), such as *Microus brandti* (Li and Wang, 2005), *Djungarian hamster* (Steinlechner et al., 1983). Moreover, photoperiod is one of the environmental cues triggering seasonal responses in small mammals. Some small mammals reduced body mass while increased thermogenesis in SD conditions (Geiser et al., 2007; Arnold et al., 2011), such as Siberian hamsters (Demas et al., 2002), Brandt's voles (Zhao and Wang, 2005). In the present study, it was clear that *E. miletus* showed decreasing of body mass in short day photoperiod during cold exposure in the first 4 weeks. But when *E. miletus* transferred from a short (SD, 8h: 16h L: D) to long day photoperiod (LD, 16h: 8h L: D). Body mass of *E. miletus* increased exposure to long day photoperiod. After the next 4 weeks of exposure to LD, which the average difference between body masses of LD and SD voles was 4.76 g. This 14.74% increase in body mass reflected significant increases in absolute amounts of body components, including wet carcass mass, dry carcass mass and body fat mass. At the end of the acclimation, our present study showed that SD voles had lower body mass and body fat mass compared with LD voles. As dry carcass mass in SD voles was also lower than in LD while no differences were found in water of carcass mass between the two groups.

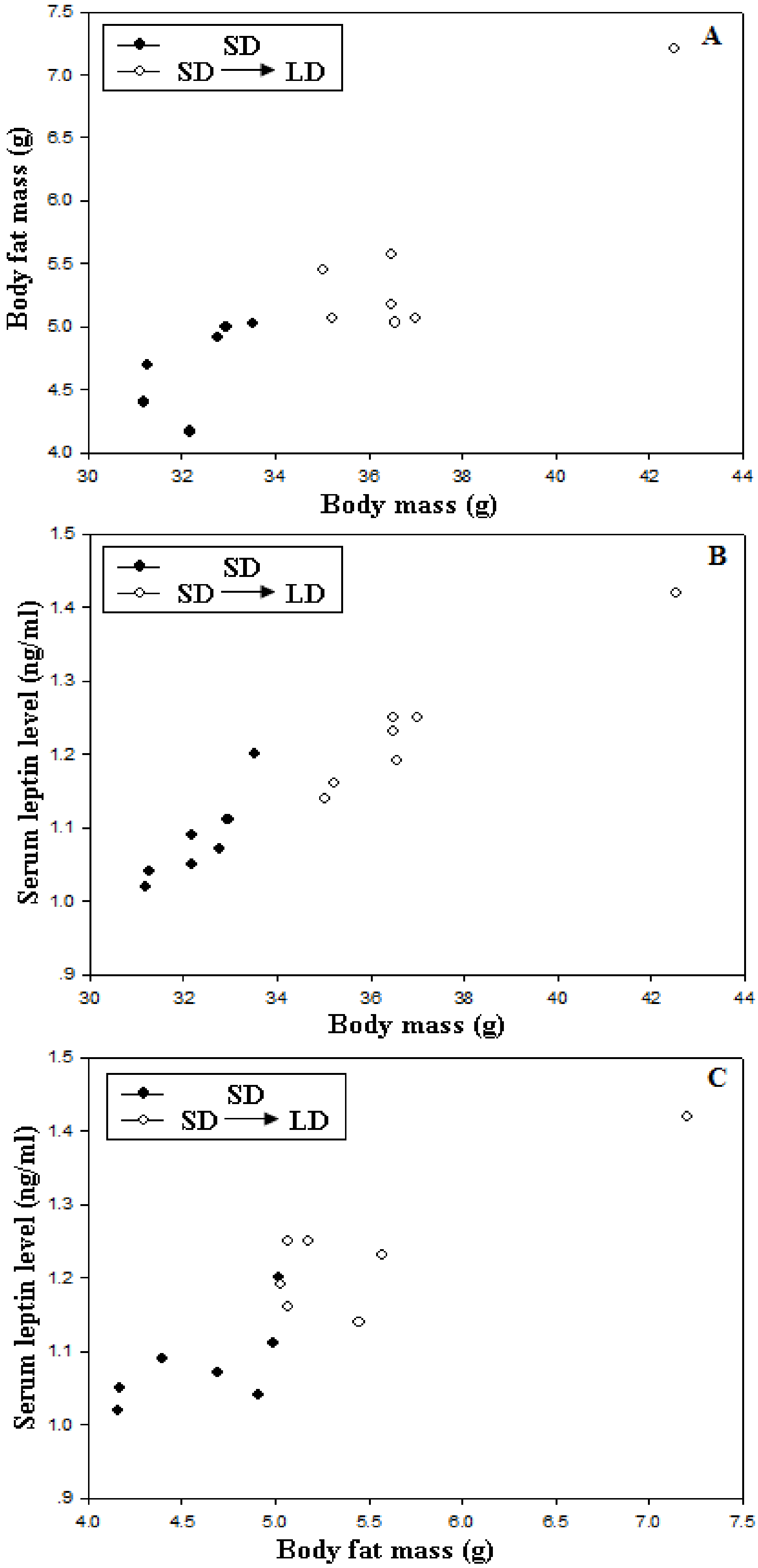

Figure 2 Correlation of body mass with body fat mass (A), serum leptin levels with body mass (B), body fat mass (C) in *E. miletus*

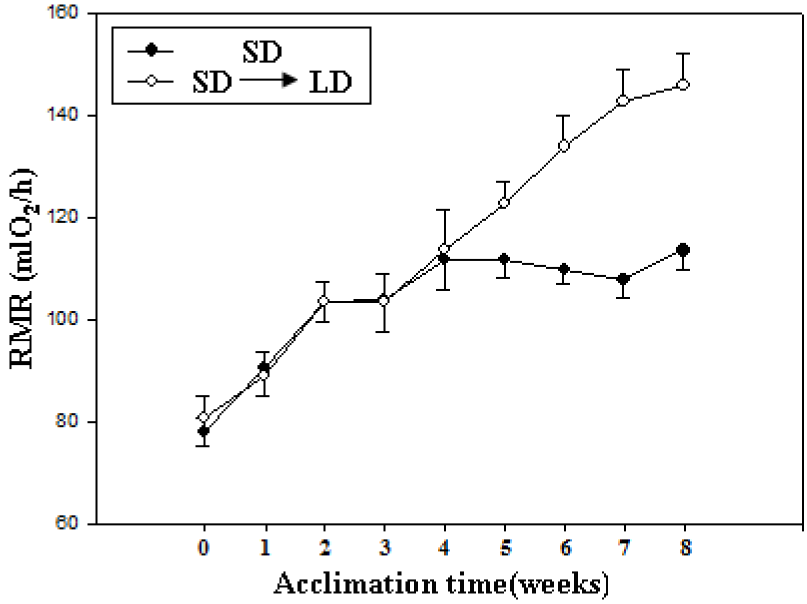

Figure 3 Effect of RMR exposed to short and long day photoperiods during cold exposure in *E. miletus*

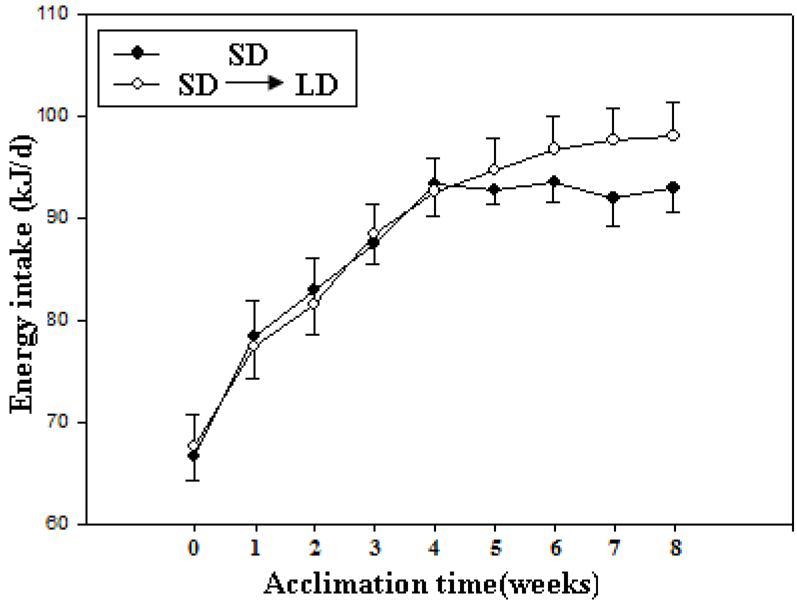

Figure 4 Effect of energy intake exposed to short and long day photoperiods during cold exposure in *E. miletus*

Changes of photoperiod are considered to be an important cue affecting RMR in many small mammals (Haim et al., 1999). In the present study, LD voles after 4 weeks photoperiodic acclimation showed higher RMR than that in SD voles during the cold exposure. These data clearly indicated that RMR was affected by changes of photoperiod in *E. miletus*. Energy intake also showed the similar trend. The increases of RMR and energy intake were the cause of increased in body mass in *E. miletus*. In addition, themorphology of the organs was found to be altered to meet the increase in energy intake. Livers, kidney, and small intestine were enlarged due to photoperiod treatment during cold exposure. Liver, as an important energy-expending organ, is considered to make a large contribution to RMR. In our study, kidney and small intestine in LD voles was significantly higher than that in SD voles, which was consistent with the changes in BMR and energy intake.

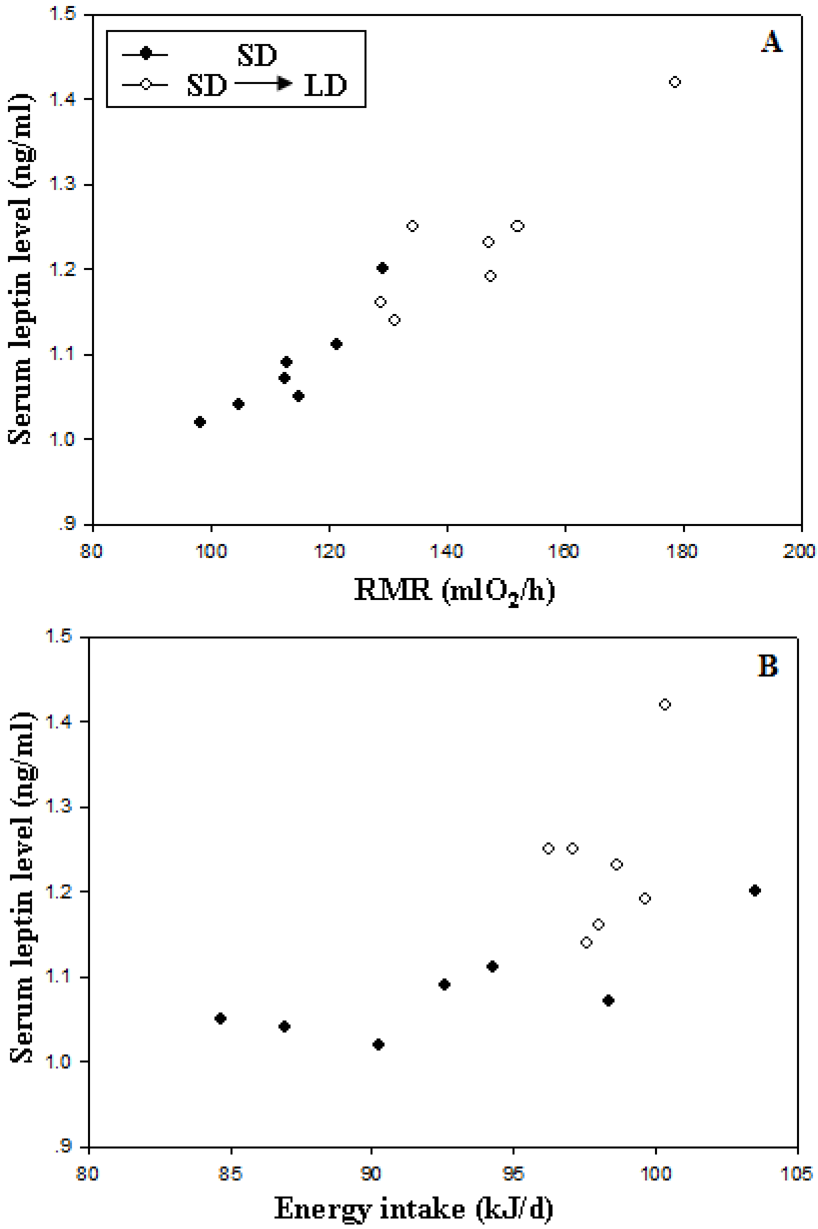

Figure 5 Correlation of serum leptin levels with RMR (A), energy intake (B) in *E. miletus*

Leptin level may reflect the contents of fattiness, could serve as a signal to the brain, to regulate food intake and energy expenditure and resistance to obesity (Michael et al., 2000). There was a positive correlation between leptin level and body mass (Friedman and Hallas, 1998). Subsequent parabiosis experiments in rodents supported the tenets of the lipostasis theory in that the serum "satiety signal" was at a higher concentration in obese animals than in lean animals (Concannon et al., 2001). In our result, although SD voles showed relatively lower serum leptin levels compared with LD voles. Further correlation analysis showed that serum leptin levels were positively correlated with body mass and body fat mass, indicating that leptin may act as "adiposity indicator" (Zhao and Wang, 2005). To regulate body mass, leptin influences energy balance via its effects on both food intake and energy expenditure (Concannon et al., 2001). Leptin administration is thought to increase energy expenditure, it has been reported that lower serum leptin levels were accompanied with higher energy intake in Brandt's voles, indicating that leptin acted as a starvation signal (Liu et al., 2007). Furthermore, correlation analysis showed that serum leptin levels were positively correlated with RMR and energy intake, it was opposite to our previous studies (Zhu et al., 2010a; 2011b). It may indicated that the anorectic and thermogenic effects of leptin may be dissociated in cold-acclimated (5 °C) in *Eothenomys miletus* by transferring them from a short (SD, 8h :16h L: D) to long day photoperiod (LD, 16h: 8h L:D). It need further study.

In conclusion, *E. miletus* decreased body mass during the first 4 weeks of exposure to SD. After the next 4 weeks of exposure to LD, which the average difference between body masses of LD and SD voles was 4.76 g. This increase in body mass reflected significant increases in absolute amounts of body components, including wet carcass mass, dry carcass mass and body fat mass. Livers, kidney, and small intestine were enlarged due to photoperiod treatment during cold exposure. *E. miletus* increased RMR and energy intake exposure to LD, but maintained a stable level to SD after 28 days. Serum leptin levels were positively correlated with body mass and body fat mass. Leptin is potentially involved in the photoperiod induced body mass regulation and thermogenesis in *E. miletus* during cold exposure.

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