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ORIGINAL ARTICLE

Effects of Gradient Photoperiod and Temperature on Energy Metabolism and Body Composition in *Tupaia belangeri chinensis*

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In order to explore the adaptive changes in energy metabolism and body composition in response to a gradual reduction in both ambient temperature and photoperiod, male adult tree shrews *Tupaia belangeri chinensis* were raised under the conditions of 30°C and 12L:12D photoperiod (control group). The treatment group was changed from 25 °C and 16L:8D photoperiod to 5 °C and 8L:16D photoperiod (treatment group) over a period of four weeks and then maintained at those conditions for a further 4 weeks. Changes in body mass, resting metabolic rates (RMR),energy intake, and wet and dry mass of organs and tissues were measured at the end of the acclimation. Body mass in treatment tree shrews was higher than control. RMR showed significant differences between groups. No significant differences were detected in dry matter intake, energy intake, and digestible energy intake in control tree shrews during the whole acclimation period, while these parameters were significantly increased within treatment tree shrews at the end of acclimation, and were significantly higher than those in control tree shrews. Small intestine and stomach dry mass, as well as the wet mass of small intestine, heart, lung, liver and kidney were significantly higher in treatment groups than in controls, but no significant differences were found in the mass of other organs and tissues. These results suggest that increasing body mass and energy intake, together with adjusting the mass of some organs and tissues, are important physiological changes in tree shrews to adapt the changing environmental conditions.

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Shortening of the photoperiod and lowing of ambient temperatures gradually are the main signal for the coming of winter (Heldmaier et al., 1985; Heldmaier et al., 1989). Body mass of some mammals obvious increased under short photoperiod, e.g. *Dicrostonyx groenlandicus* (Nagy et al., 1995). However, *Phodopus sungorus* (Klingenspor et al., 2000), *Eothenomys miletus* (Zhu et al., 2011) and *Lasiopodomys brandtii* (Zhao and Wang, 2005; Li and Wang, 2005) decreased body mass under short photoperiod. There was no effects of photoperiod on resting metabolic rate in *Meriones unguiculatus* (Li et al., 2003), but short photoperiod increased energy intake in *M. Unguiculatus* (Zhao and Wang, 2006a), *L. Brandtii* (Zhao and Wang, 2006b) and *E. miletus* (Zhu et al., 2011). Further, Organ weight and body composition showed adaptive changes under cold acclimation for either in experimental rodents or wild small mammals, such as *L. Brandtii* (Li et al., 1994), *Microtus oeconomus* (Wang et al., 1996), *E. miletus* (Zhu et al., 2010a) and *Apodemus chevrieri* (Zhu et al., 2010b), as well as increasing of energy intake, e.g. rats (Abelenda et al., 2003) and *M. unguiculatus* (Li et al., 2004). Interaction between short photoperiod and low temperature can further improve the mass of liver and BAT, such as *L. Brandtii* (Li et al., 1995), *Sigmodon hispidus* (Tomasi and Michell, 1996), *Ochotona curzoniae* (Wang et al., 1999). Organ weight and body composition also showed changes in *Rattus norvegicus* and *Mesocricetus auratus* under the condition of simulate changes of photoperiod and temperature from summer to winter (Deveci and Egginton, 2002).

The tree shrew (*Tupaia belangeri chinensis*) belongs to Scandebtia, Tupaiidae. They are small mammals of Palaearctic realm and are widely distributed in Southern China, India, and Southeast Asia. The tree shrew has the widest of distribution and occupies the highest of latitude in their family; and they were always habitant in terrestrial, arboreal, mountainous forest and shrub areas (Wang et al. 1991). Our previous studies showed that the resting metabolic rate, nonshivering thermogenesis and the metabolism of energy of *T. belangeri* presented seasonal cycles (Wang et al., 1994). It was showed that injection of exogenous melatonin induced seasonal cycle of the thermogenesis in *T. belangeri* and the thermogenesis in tree shrew were already increased during cold exposure (Li et al., 2001; Zhu et al., 2012a). The present study investigated the role of gradient photoperiod and temperature on energy metabolism and body composition in the tree shrew. We hypothesized that tree shrews change body mass and energy intake, together with adjusting the mass of some organs and tissues under changing environmental conditions.

MATERIALS AND METHODS

Animals

Tree shrew (*T. belangeri chinese*) were captured in farmland (26°15´-26°45´N; 99°40´-99°55´E; altitude 2,590 m) in Jianchuan County, Yunnan province, 2011. After being captured, the tree shrew was transported to the School of Life Sciences of Yunnan Normal University, Kunming, China (1910 m in altitude). Animals were kept individually in wire cages (35×25×20 cm), with the cooked feed (approximate composition of 69.1% young chickenfeed feed, 2.7% Whole Milk Powder, 6.9% sugar and 21.3% flour). After 1 month stabilization, male adult tree shrews *T. belangeri chinensis* were assigned to two experimental groups, samples of each group were 10. The control group were maintained under 12L: 12D (light:dark, lights on 08:00) photoperiod and room temperature was 25°C. The treatment group was changed from 25°C and 16L:8D photoperiod to 5°C and 8L:16D photoperiod (treatment group) over a period of four weeks and then maintained at those conditions for a further 4 weeks. The experimental design is shown in Figure 1. All pregnant, lactating or young individuals were excluded.

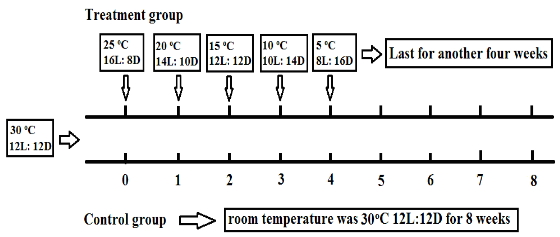

Figure 1 Reducing ambient temperature (from 25°C to 5°C) and photoperiod (from 16L: 8D to 8L: 16D) during experiment for 8 weeks.

Measurement of metabolic rates

Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 30°C within the TNZ (thermal neutral zone), gas analysis were using ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5 °C), the metabolic chamber volume is 500 ml, flow is 200 ml/min at the end. Tree shrews 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). Calculate method of metabolic rate is descript in detailed by Hills (Hills, 1972).

Energy budget

Energy budgets were measured from the balance of food intake and fecal output (Song and Wang 2001). Animals were individually housed in metabolic cages (20 cm × 15 cm × 15 cm) without nest materials for one week. They were fed a standard quantity of food each day between 10:00 and 11:00 am. On the following day they were weighed and the remaining food, feces and urine were collected. Residual food and feces were dried to constant weight and dry mass determined to the nearest 0.1 g. The energy contents of the samples were measured using an automatic bomb calorimeter (model YX-ZR/Q, Changsha, China). Gross energy intake(GEI), digestible energy intake (DEI) and digestibility of energy were calculated according to the literature (Liu et al., 2003).

GEI (kJ/day) = Dry food intake (g/day) × caloric value (kJ/g) of dry food;

DEI (kJ/day) = GEI-[mass of feces (g/d) × gross energy content of feces (kJ/day)];

Digestibility(%) = DEI/GEI.

Morphology

The visceral organs, including heart, lung, liver, kidneys, spleen, testes, brown adipose tissue (BAT), white adipose tissue(WAT) around kidneys, WAT around testes and gastrointestinal tract (stomach, small intestine, caecum, large intestine), were extracted and weighed from animals in the control group and the treat group. The stomach and intestines were then rinsed with saline to eliminate all gut contents before being dried and reweighed. The remaining carcass and all of 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.

Statistical analyses

Statistical analyses were performed using SPSS 15.0 for Windows statistical package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. Differences in body mass, RMR, and energy intake during the course of acclimation were assessed by repeated measures analysis of variance (ANOVA), and significant group differences were further evaluated by Duncan test. Group differences of data between treat group and control group were analyzed by covariance analysis. Results are presented as mean ± SEM and P<0.05 was considered to be statistically significant.

RESULTS

Body mass and RMR

Prior to acclimation, there was no significant difference in body mass between the control and acclimation group (P>0.05). Body mass in treat group gradually increased during the 8-weeks acclimation (Fig. 2). During the course of acclimation, there were significant differences in body mass within the treated group (F=5.361, P<0.01), but not within the control group (F=0.425, P>0.05). Prior to acclimation, BMR in tree shrews showed no significant difference between groups (P>0.05). BMR in treat group gradually increased during the acclimation, but there were no significant differences in RMR within the control group (Fig. 3).

Energy intake

There was no difference in gross energy intake between treated and control tree shrews prior to acclimation (P>0.05). Dry matter intake, energy intake and dry matter intake in treated group of tree shrews increased during acclimation, but digestibility showed no significant difference (Fig 4A, B, C, D). There were no marked differences in dry matter intake, energy intake, dry matter intake and digestibility within the control group during the acclimation course.

Morphology

Small intestine and stomach dry mass, as well as the wet mass of small intestine, heart, lung, liver and kidney were significantly higher in treatment groups than in controls, but no significant differences were found in the mass of other organs and tissues (Table 1).

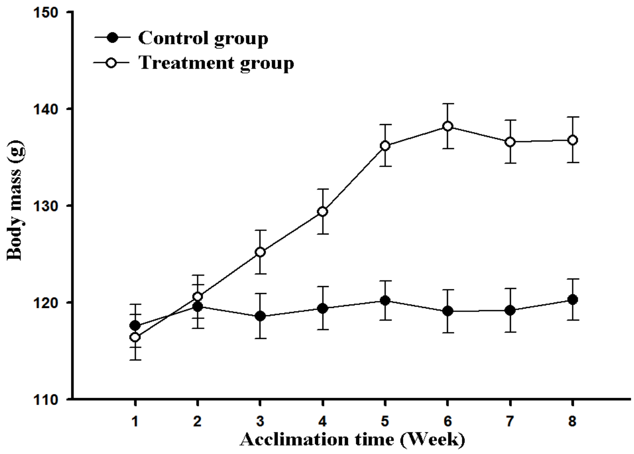

Figure 2 Effects of reducing photoperiod and ambient temperature on body mass in *Tupaia belangeri chinensis*

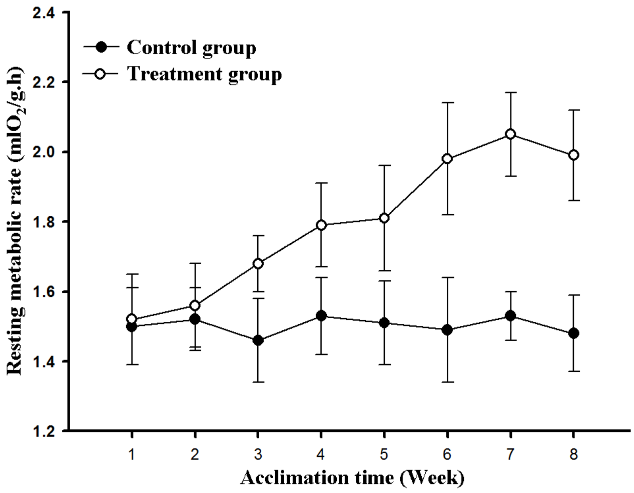

Figure 3 Effects of reducing photoperiod and ambient temperature on resting metabolic rate (RMR) in *Tupaia belangeri chinensis*

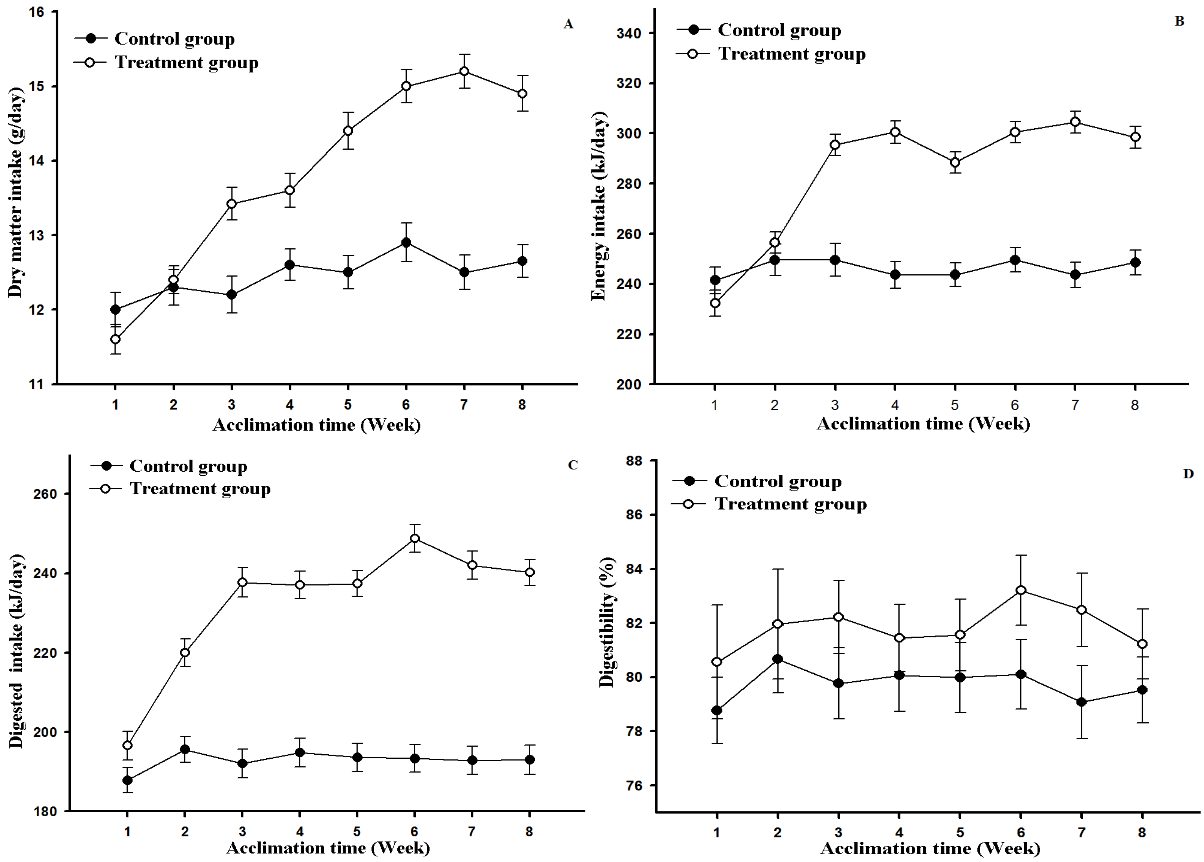

Figure 4 Effects of reducing photoperiod and ambient temperature on dry matter intake (A),energy intake (B), digested energy (C) and digestibility(D) in *Tupaia belangeri chinensis*

Table 1. Effects of reducing photoperiod and temperature on the organ and tissue masses in *Tupaia belangeri chinensis*

Parameters	Control group	Treatment group	P value
Heart wet mass (g)	0.590±0.013	0.684±0.015	<0.05
Heart dry mass (g)	0.162±0.009	0.164±0.008	>0.05
Lung wet mass (g)	0.602±0.035	0.0596±0.024	>0.05
Lung dry mass (g)	0.136±0.005	0.0140±0.005	>0.05
Liver wet mass	4.83±0.085	5.306±0.094	<0.01
Liver dry mass (g)	1.690±0.033	1.704±0.035	>0.05
BAT wet mass (g)	0.570±0.007	0.572±0.008	>0.05
BAT dry mass (g)	0.322±0.003	0.0348±0.002	>0.05
Kidney wet mass (g)	1.342±0.046	1.570±0.032	<0.05
Kidney dry mass (g)	0.370±0.004	0.378±0.006	>0.05
WAT around kidneys wet mass (g)	0.582±0.006	0.572±0.005	>0.05
WAT around kidneys dry mass (g)	0.508±0.005	0.492±0.005	>0.05
Testes wet mass (g)	1.912±0.095	1.892±0.078	>0.05
Testes dry mass (g)	0.292±0.004	0.302±0.005	>0.05
WAT around testes wet mass (g)	1.624±0.069	1.578±0.088	>0.05
WAT around testes dry mass (g)	1.370±0.015	1.376±0.021	>0.05
Stomach wet mass (g)	0.832±0.012	0.850±0.009	>0.05
Stomach dry mass (g)	0.164±0.001	0.212±0.002	<0.05
Small intestine wet mass (g)	2.072±0.086	2.570±0.091	<0.01
Small intestine dry mass (g)	0.402±0.006	0.482±0.005	<0.05
Caecum wet mass (g)	1.712±0.089	1.662±0.079	>0.05
Caecum dry mass (g)	0.242±0.005	0.246±0.007	>0.05
Large intestine wet mass (g)	1.162±0.053	1.168±0.063	>0.05
Large intestine dry mass (g)	0.204±0.003	0.218±0.008	>0.05

DISCUSSION

Body weight is an important index to reflect the nutritional status for small mammals, its stability depends on balance between energy intake and energy expenditure. Most small mammals declined body mass to reduce the total energy consumption in the winter, e.g. *Clethrionomys glareolus*, *C. gapperi* and *M. auratus* (Klaus et al., 1988; Merritt and Zegers, 1991; Concannon et al., 2001). When the long photoperiod transform to short photoperiod, body mass in *D. groenlandicus* increased significantly (Nagy et al., 1995), while *P. sungorus* were significantly decreased (Klingenspor et al., 2000), which mainly related to reducing of energy intake (Knopper and Boily, 2000). Body mass in rats was lower in a short photoperiod than that under a long photoperiod, which indicted that this is due to redistribution of energy intake in a long photoperiod, Irrelevant to energy intake (Larkin et al., 1991). In the present study, With photoperiod and temperature gradient decreases, body mass increased in the treatment group of tree shrews, which was significantly higher than that of control group, and energy intake also increased significantly, which may be used primarily to maintain body weight homeostasis and resist cold stress, similar to the results in the seasonal changes in tree shrews (Zhu et al., 2012b).

During 8 weeks acclimation, RMR in the treatment group of tree shrews increased significantly, which consistent to the results in seasonal acclimatization research(Zhu et al., 2012b). Many of small mammals had the higher RMR in autumn and winter than that in the summer (Lovegrove, 2005), but there are also some animal' RMR remains unchanged, such as the plateau pika (Wang et al., 1999). Low temperature and short photoperiod can significantly influence of Brandt's vole RMR, the interaction between the two factors can further enhance this effect (Li et al., 1995).Short photoperiod also had obvious effect on RMR in tree shrews (Zhang et al., 2012a). In our study, long time in low temperature and short photoperiod conditions, RMR in tree shrews increased significantly, which mainly by improving its own metabolism level to adapt to environmental changes.

In the present study, small intestine and stomach, heart, liver, lung and kidney and other organ weight in the treatment group of tree shrews were higher than that in control group, but the testis, adrenal and thymus weight showed no significant difference. When the temperature transform from 30 ^{o}C to 6 ^{o}C, liver, intestines, stomach and heart weight in rat was also increased significantly (Heroux and Gridgeman, 1958). For *R. norvegicus* and *M. auratus*, it showed that heart and lung weight increased significantly under the gradient of the photoperiod and temperature (Deveci and Egginton, 2002). *T. belangeri chinese* is a hibernating animal, increased heart weight was advanced to resist cold stress.

Digestive tract morphology related to the quality of the food and ingredients, food quality and energy stress in *Microtus ochrogaster* showed a significant effect on the digestive tract morphology, When faced with low temperature stress, the small intestinal length and weight increased, when feeding with high fiber foods, caecum of length and weight increased (Gross et al., 1985). In our study, dry weight of small intestine and stomach increased as well as energy intake and digest energy were also greatly improved, which indicated that increased the volume of the digestive tract to improving the ability in digest food in *T. belangeri chinese*.

Moreover, BAT weight were higher in treatment group than that in control group, but the difference was not significant, while the liver wet weight was significantly higher than that of control group. BAT weight increased in the first 4 weeks and declined in the next 4 weeks in M. auratus under a gradual change in photoperiod and temperature conditions (Deveci and Egginton, 2002). Compared with other tissues, increased BAT weight was most obvious during cold acclimation, rat BAT weight increased by 30%, with other parts of the BAT increased by 14%, M. auratus were increased by 35% and 120% (Deveci and Egginton, 2002). BAT weight increases significantly in tree shrews during cold exposure (Zhang et al., 2012b; 2012c). Liver heat production can be accounted for about 25% of total heat production (Schmidt-Nielsen, 1997). Liver mass increased significantly under a gradual change in photoperiod and temperature conditions, which indicated that liver had contribution to the production of heat regulation.

In conclusion, all results suggested that increasing body mass and energy intake, together with adjusting the mass of some organs and tissues, are important physiological changes in tree shrews to adapt the changing environmental conditions.

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