Journal of Stress Physiology & Biochemistry, Vol. 9 No. 2 2013, pp. 118-130 ISSN 1997-0838

ORIGINAL ARTICLE

Effects of Exogenous Melatonin on Body Mass Regulation and Hormone Concentrations in *Eothenomys miletus*

Zhu Wan-Long[#], Zhang Di[#], Zhang Lin, Wang Zheng-Kun*

Institute of Zoology, School of life Science of Yunnan Normal University, Kunming, 650092, China

[#] W.-L. Zhu and D. Zhang contributed equally to this work

Tel.: +86 0871 5516068

*E-Mail: zwl_8307@163.com

Received November 30, 2012

By regulating the pineal hormone, photoperiods affect many physiological characteristics in small mammals. Thus, melatonin might take part in the thermoregulation of seasonal variations in small mammals. This study determined the influence of melatonin treatment on thermogenic pattern, we measured body mass, thermogenic activities and hormone concentrations of *Eothenomys miletus* were given exogenous melatonin (MLT) for 28 days. The results shown that body mass was reduced significantly, whereas resting metabolic rate (RMR) and nonshivering thermogenesis (NST) increased at 28 days in MLT group compared to control group as well as the oxidative capacities of mitochondria in liver and brown adipose tissue (BAT) were enhanced; the contents of total and mitochodrial protein increased markedly. Melatonin treatment significantly increased the State 3, State 4 respiration of liver mitochondria, and the activity of cytochrome C oxidase (COX) in liver; but the α-glerocephasphate oxidase (α-PGO) capacity showed no differences during the acclimation in liver. Furthermore, the State 4 respiration, the activities of COX and α-PGO in BAT increased, respectively. The activity of thyroxin 5'-deiodinase (T_4 5'-DII) in BAT increased remarkably. The serum content of thyroxine (T_4) decreased, and that of tri-iodothyronine (T_3) increased. Moreover, serum leptin levels showed no significant differences in MLT group compared to control group. Together, these data indicate that melatonin enhances thermogenic capacity in *E. miletus*. Our results suggested that melatonin is potentially involved in the regulation of body mass, adaptive thermogenic capacity and hormone concentrations in *E. miletus*.

Key words: Eothenomys miletus; adaptive thermogenic capacity; exogenous melatonin; hormone concentrations

ORIGINAL ARTICLE

Effects of Exogenous Melatonin on Body Mass Regulation and Hormone Concentrations in *Eothenomys miletus*

Zhu Wan-Long[#], Zhang Di[#], Zhang Lin, Wang Zheng-Kun*

Institute of Zoology, School of life Science of Yunnan Normal University, Kunming, 650092, China
[#] W.-L. Zhu and D. Zhang contributed equally to this work

Tel.: +86 0871 5516068

*E-Mail: zwl_8307@163.com

Received November 30, 2012

By regulating the pineal hormone, photoperiods affect many physiological characteristics in small mammals. Thus, melatonin might take part in the thermoregulation of seasonal variations in small mammals. This study determined the influence of melatonin treatment on thermogenic pattern, we measured body mass, thermogenic activities and hormone concentrations of *Eothenomys miletus* were given exogenous melatonin (MLT) for 28 days. The results shown that body mass was reduced significantly, whereas resting metabolic rate (RMR) and nonshivering thermogenesis (NST) increased at 28 days in MLT group compared to control group as well as the oxidative capacities of mitochondria in liver and brown adipose tissue (BAT) were enhanced; the contents of total and mitochodrial protein increased markedly. Melatonin treatment significantly increased the State 3, State 4 respiration of liver mitochondria, and the activity of cytochrome C oxidase (COX) in liver; but the α-glerocephasphate oxidase (α-PGO) capacity showed no differences during the acclimation in liver. Furthermore, the State 4 respiration, the activities of COX and α-PGO in BAT increased, respectively. The activity of thyroxin 5'-deiodinase (T_4 5'-DII) in BAT increased remarkably. The serum content of thyroxine (T_4) decreased, and that of tri-iodothyronine (T_3) increased. Moreover, serum leptin levels showed no significant differences in MLT group compared to control group. Together, these data indicate that melatonin enhances thermogenic capacity in *E. miletus*. Our results suggested that melatonin is potentially involved in the regulation of body mass, adaptive thermogenic capacity and hormone concentrations in *E. miletus*.

Key words: Eothenomys miletus; adaptive thermogenic capacity; exogenous melatonin; hormone concentrations

Melatonin, is a naturally occurring compound in animals. In animals, circulating levels of the hormone melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions, such as antioxidant defense in unicellular organisms, regulation of

seasonal reproduction in photoperiodic mammals, and free radical scavenging and anti-inflammatory activities (Berlinguer *et al*., 2009). Effects of exogenous melatonin on thermogenesis plays a role in the regulation of circadian rhythm and metabolism of mammals, which provide valuable information for understanding the functions of melatonin (Molik *et al*., 2010). The effects of exogenous melatonin have been investigated in several mammalian species, including body mass of the melatonin groups were lower than those of the control groups in *Phodopus sungorus* (Korhonen *et al*., 2008); melatonin-treated minks had significantly higher leptin levels than the controls, and the melatonin-treated groups had significantly higher thyroxine levels than the controls (Mustonen *et al*., 2000); exogenous administration of melatonin to intact rats resulted in significant decreases in serum leptin levels compared to those of the intact control group (Canpolat *et al*., 2001); it indicateed that melatonin does not affect leptin secretion via mouse adipose tissue (Song and Chen, 2009); and some other animals, e.g. red deer (Asher *et al*., 2011), and rats (Bharti *et al*., 2012).

Change in duration of melatonin secretion thus serves as a biological signal for the organization of day length-dependent seasonal functions such as reproduction and behaviors in seasonal animals (Arendt and Skene, 2005), effect of melatonin on thermogenic characteristics changes with the season in a manner similar to that shown by the action of short photoperiod (Steinlechner and Heldmaier, 1982). In particular, thermogenesis characteristics of BAT are likely also regulated by melatonin. When injection of exogenous melatonin in small rodents, they appeared morphological and physiological characteristics under winter or short photoperiod, and NST increased significantly (Andrews and Belknap, 1993). As the terminal enzyme in oxidative phosphorylation in mitochondria, cytochrome c oxidase (COX, complex IV) is involved in mitochondrial energy metabolism (Kadenbach *et al*., 2000). Triiodothyronine (T_3), is a thyroid hormone, which affects almost every physiological process in mammals, T_3 and its prohormone thyroxine (T_4) is activated by thyroid-stimulating hormone (TSH), which is released from the pituitary gland (Klein, 2006). It has also been demonstrated T_3, T_4 affect adaptive thermogenesis by influencing several aspects of energy metabolism (Krotkiewski, 2002).

Leptin, is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism (Zhang *et al*., 1994), it acts with the OB-Rb receptors in the hypothalamic arcuate nucleus in the regulation of food intake and body mass (Friedman and Halaas, 1998). Leptin has been found to affect food intake, the neuroendocrine axis, metabolism and immunological processes (Hausman and Barb, 2010). Further, the positive correlation between serum leptin levels and body fat mass has been found in many small mammals including *Dicrostonyx groenlandicus* (Johnson *et al*. 2004), *P. sungorus* (Klingenspor *et al*., 2000), and cold acclimated *Meriones unguiculatus* (Li *et al*., 2004), *Eothenomys miletus* (Zhu *et al*., 2010a), *Apodemus chevrieri* (Zhu *et al*., 2011a).

Yunnan red-backed vole, *Eothenomys miletus* (Mammalia: Rodentia: Microtus), is a inherent specie of Hengduan Mountains region (Zheng, 1993; Zhu *et al*., 2011b). The Hengduan Mountains region is located the boundary between the Palaearctic region and the Oriental region. Environmental factors, such as short photoperiods and cold, are effective cues that influence body mass and

thermogenesis, separately (Zhu *et al*., 2008; 2010a; 2010b; 2011b; 2012). However, we know nothing about the action of exogenous melatonin with changes in the regulation of body mass, thermogenesis and hormone concentrations in *E. miletus*. The aim of the present study was to elucidate the role of endogenous melatonin in the regulation of different adaptations using the *E. miletus* as a model. In the present study, by systematically measuring a variety of physiological and hormonal markers indicative of thermogenic capacity, we tested the hypothesis that endogenous melatonin enhances thermogenesis of *E. miletus*. We predicted that *E. miletus* change their body mass and hormone concentrations by injection of exogenous melatonin. Further, melatonin is potentially involved in the regulation of body mass, adaptive thermogenic capacity and hormone concentrations in *E. miletus*.

MATERIALS AND METHODS

Samples

E. miletus were obtained from a captive population started from approximately 150 captured in farmland (26°15′~26°45′N; 99°40′~99°55′E; altitude 2,590 m) in Jianchuan County, Yunnan province, 2010. Average temperature is 9.1°C each year, minimum average temperature was -4.0°C in January, maximal average temperature was 24.1°C in July. *E. miletus* were breed for two generations (120–150 days of age) in School of life Science of Yunnan Normal University, park in plastic box (260mm×160mm×150mm), housed individually without any bedding material. 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 18; female 12). All three groups were maintained at the room temperature of 25±1°C, under a photoperiod of 12L:12D (with lights on at 08:00). MLT groups: intraperitoneal injection of MLT before the 2-3 hours of the dark period. Melatonin (Sigma, St. Louis, MO, USA) was dissolved in 0.02% ethanol to prepare a final stock solution of 80 mg/mL. It was then stored in darkness at -80°C until use. The stock solution was diluted every 2 days by adding water until reaching the appropriate concentration (20μg/kg BM/day). PBS group: intraperitoneal injection of an equal volume of phosphate-buffered saline (PBS) at the same time of MLT groups. Control group just in this condition for 28 days. At the end, the voles were sacrificed between 09:00 and 11:00 by puncture of the posterior vena cava in 28 days. Mass of liver and BAT were weighted. Blood was centrifuged at 4000 rpm for 30 min, and serum was sampled and stored at −20°C for later measurement.

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: 22.5~30 °C) (Zhu *et al*. 2008), 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 500ml, flow is 200ml/min. The voles were stabilized in the metabolic chamber for at least 60 min prior to the resting metabolic rate (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 (Zhu *et al*., 2010a). All metabolic measurements were performed more than 400h.

The method used for calculating the metabolic rate is detailed in Hill (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 more than 60 min at each measurement were taken to calculate the NST (Zhu *et al*., 2010b). The doses of NE were approximately 0.8-1.0mg/kg according to dose-dependent response curves that were carried out before the experiment and the equation described by Heldmaier (1971): norepinephrine dosage (mg/kg)=$6.6M^{-0.458}$(g).

Measurement of enzyme activity

The protein content of mitochondria was measured by the Folin phenol method (Lowry *et al*., 1951) with bovine serum albumin as standard. State 3 and State 4 of mitochondrial respiration of liver and BAT were measured by the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., Norfolk, England), essentially as described by Estabrook (1967). State 4 respiration was substrate dependent, and substrate and ADP supported state 3 respiration, succinate was used as the substrate in our experiments (Liu *et al*., 2006). Activities of cytochorme C oxidase (COX) were measured by the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., Norfolk, England) (Sundin *et al*., 1987), the α-glycerophosphate oxidase (α-PGO; EC 1.1.3.21) was determined polarographically according to Steffen and Roberts (1977). Thyroxine 5'-deiodinase activity (T_4 5'-DII) in BAT was assayed as previously described (Leonard *et al*., 1983).

Measurement of hormone concentration

Total concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum were determined by radioimmunoassay (RIA) with the 125I Multi-species Kit (GammaCoat, DiaSorin, Stillwater, MN) (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 leptin levels were determined by radioimmunoassay (RIA) with the 125I Multi-species Kit (Millipore), and leptin values were determined in a single RIA. The lowest level of leptin that can be detected by this assay was 1.0 ng/mL when using a 100-μL sample size (instructions for Multi-species Kit). The inter- and intra-assay variabilities for leptin RIA were <3.6% and 8.7%, respectively.

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 and hormone concentrations were analyzed by repeated measures analysis of covariance (ANCOVA) with body mass as a covariate. Results were presented as mean ± SEM, and $p<0.05$ was considered to be statistically significant.

RESULTS

Since no effects were found in any of the measured parameters between control group and PBS group, date were not analyzed between control group and PBS group.

Body mass, RMR and NST

Prior to acclimation, body mass of three groups showed no significant differences ($F=0.523$, $p>0.05$). After 4-weeks acclimation, body mass of MLT group

decreased significantly by injection of exogenous melatonin (F=2.365, p<0.05) (Fig.1). Body mass of MLT group decreased 3.84±0.88g compared to control group (42.71±1.13g). RMR and NST in MLT group showed significant differences compared to control group and PBS group, respectively (RMR, F=1.985, p<0.05; NST, F=2.322, p<0.05) (Fig. 2 and 3).

Thermogenic capacity of liver and BAT

For *E. miletus*, there was a significant effect of exogenous melatonin on liver mass among the three groups (F=2.586, p<0.05, Table 1). MLT group induced a increase in total protein, mitochondrial protein, State 3 respiration, State 4 respiration and COX capacity in liver compared with control group or PBS group (total protein: F=2.214, p<0.05; mitochondrial protein: F=2.865, p<0.05; State 3 respiration: F=1.895, p<0.05; State 4 respiration: F=5.462, p<0.01; COX capacity: F=7.121, p<0.01), but the α-PGO capacity showed no differences during the acclimation (F=0.542, p>0.05).

For *E. miletus*, there was no significant effect of exogenous melatonin on BAT mass among the three groups (F=0.152, p>0.05, Table 1), but total protein, mitochondrial protein of BAT in MLT group differed compared with control group or PBS group (total protein: F=4.325, p<0.01; mitochondrial protein: F=5.698, p<0.01). MLT group displayed higher State 4 respiration than that of other two group (F=2.412, p<0.05), and also had a significant difference in COX capacity and α-PGO capacity (COX capacity: F=6.254, p<0.01; α-PGO capacity: F=2.854, p<0.05). Similarly, MLT group had higher T_4 5'-DII concentrations compared to other groups (F=8.324, p<0.01).

Hormone concentration

MLT group displayed higher serum T_3 (F=9.358, p<0.01) and lower serum T_4 concentrations (F=4.362, p<0.01) (Table 3). Further, it found no difference in serum leptin levels in three groups (p>0.05).

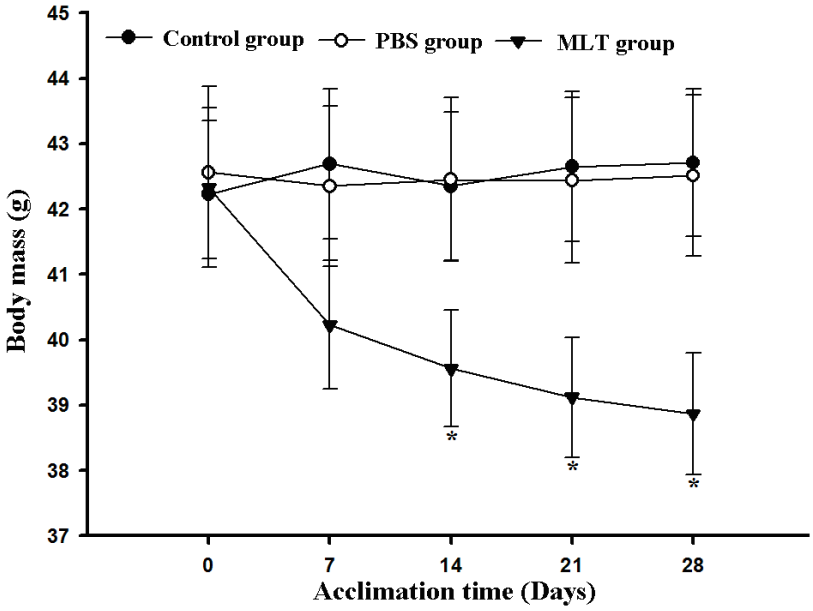

Figure 1: Effect of injecting melatonin (20μg/kg BM/day) on body mass in *E. miletus* (n=30). Data are mean±SE. *: p<0.05; compare with control group. Note: MLT: melatonin; PBS: phosphate-buffered saline.

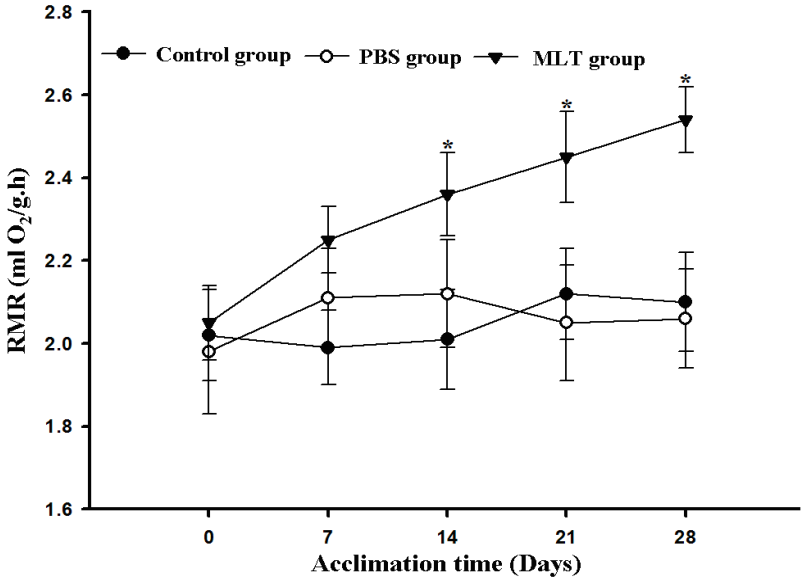

Figure 2: Effect of injecting melatonin (20μg/kg BM/day) on resting metabolic rate (RMR) in *E. miletus* (n=30). Data are mean±SE. *: p<0.05; compare with control group. Note: MLT: melatonin; PBS: phosphate-buffered saline.

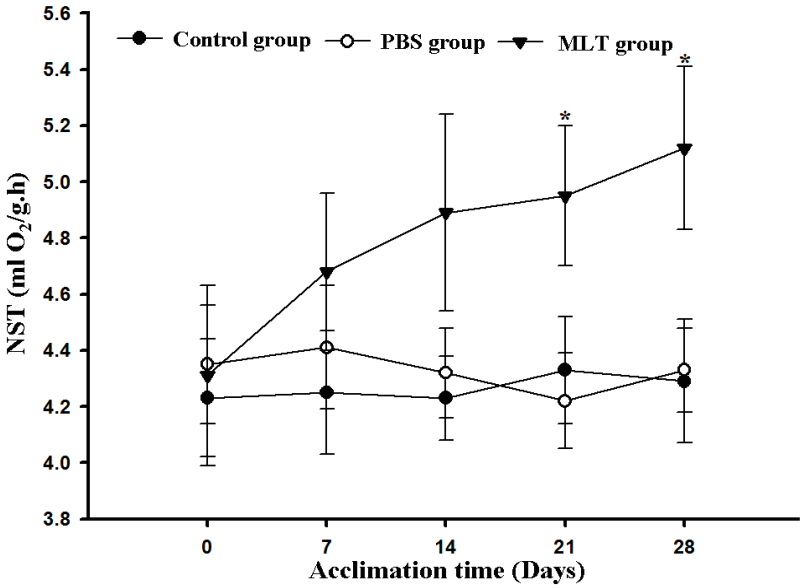

Figure 3: Effect of injecting melatonin (20μg/kg BM/day) on nonshivering thermogenesis (NST) in *E. miletus* (n=30). Data are mean±SE. *: p<0.05; compare with control group. Note: MLT: melatonin; PBS: phosphate-buffered saline.

Table 1. Effects of exogenous melatonin on mitochondrial (Mt) protein, cytochrome c oxidase (COX) activity and α-glycerophophate oxidase(α-PGO) in liver in *Eothenomys miletus.*

Parameters	Control (n=10)	PBS (n=10)	MLT (n=10)
Body mass (g)	42.71±1.13 [a]	42.51±1.23 [a]	38.87±0.93 [b]
Liver mass (g)	2.12±0.25 [b]	2.15±0.16 [b]	2.23±0.19 [a]
Total protein (mg/g)	51.32±3.21 [b]	48.36±2.65 [b]	60.25±3.58 [a]
Mitochondrial protein (mg/g)	16.58±2.31 [b]	16.42±0.98 [b]	22.36±2.03 [a]
State 3 respiration (nmol O_2/min.mg)	30.36±1.95 [b]	31.26±2.08 [b]	37.48±1.62 [a]
State 4 respiration (nmol O_2/min.mg)	53.62±3.24 [b]	50.36±2.96 [b]	68.51±3.26 [a]
COX (nmol O_2/min.mg)	45.62±5.65 [b]	46.32±3.26 [b]	69.32±4.61 [a]
α-PGO (nmol O_2/min.mg)	38.25±2.01 [a]	37.23±2.36 [a]	40.21±2.95 [a]

Different superscripts in each row indicate significant difference (P < 0.05).

Table 2 Effects of exogenous melatonin on mitochondrial (Mt) protein, cytochrome c oxidase (COX) activity and α-glycerophophate oxidase(α-PGO) in BAT in *Eothenomys miletus.*

Parameters	Control (n=10)	PBS (n=10)	MLT (n=10)
BAT mass (g)	0.259±0.06 [a]	0.245±0.08 [a]	0.261±0.09 [a]
Total protein (mg/g)	6.25±0.98 [b]	5.86±0.66 [b]	11.36±1.21 [a]
Mitochondrial protein (mg/g)	9.63±0.45 [b]	9.86±0.64 [b]	15.25±1.03 [a]
State 4 respiration (nmol O_2/min.mg)	25.63±1.84 [b]	23.21±1.23 [b]	31.58±2.06 [a]
COX (nmol O_2/min.mg)	1456±87 [b]	1423±68 [b]	1644±72 [a]
α-PGO (nmol O_2/min.mg)	125.63±5.31 [b]	121.03±6.58 [b]	144.69±5.98 [a]
T_4 5'-DII (pmol O_2/ min.mg)	12.36±0.56 [b]	13.25±0.86 [b]	22.32±1.12 [a]

Different superscripts in each row indicate significant difference (P < 0.05).

Table 3 Effects of exogenous melatonin on serum T_3, serum T_4 concentrations and serum leptin levels in *Eothenomys miletus.*

Parameters	Control (n=10)	PBS (n=10)	MLT (n=10)
T_3 (ng/ml)	0.59±0.05 [b]	0.61±0.04 [b]	1.18±0.05 [a]
T_4 (ng/ ml)	35.36±2.36 [a]	34.26±2.21 [a]	28.25±2.58 [b]
serum leptin levels (mg/ ml)	1.56±0.08 [a]	1.51±0.04 [a]	1.45±0.06 [a]

Different superscripts in each row indicate significant difference (P < 0.05).

DISCUSSION

Body mass

Melatonin, a naturally occurring indoleamine molecule, involved in the regulation of many physiological processes in many mammals (Moore and Menaker, 2011), it is a highly conserved molecule, present in organism from unicellular organisms to all vertebrates (Paradies *et al*., 2010). Melatonin is involved in energy metabolism and body weight control in small animals. Many studies show that chronic melatonin supplementation in drinking water reduces body weight and abdominal fat in experimental animals, especially in the middle-aged rats (Wolden-Hanson *et al*., 2000). Either by subcutaneous implanted or intramuscular injection of melatonin in some small mammals, which lead to some of the adaptive increase in thermogenic capacity including mass of BAT increased, enhancement of oxidative capacity, or decreased body temperature, all these adaptive traits may responsible to the regulation of energy consumption resisted in winter or low temperature (Korhonen *et al*., 2008). In previous studies, it showed that young adult mice (1 month old) given oral melatonin supplementation with a relatively high dose (35 μg/d/mouse) caused no significant changes in body weight gain and body fat content (Song and Chen, 2009). The control and melatonin groups gained 5.5% and 12.5% in body mass of Siberian hamster, respectively (Korhonen *et al*., 2008). But in the present study, body mass decreased significantly in MLT group, similar studies were found in cold exposure or SD condition in *E. miletus* (Zhu *et al*., 2010a; 2011b), and consistent with sheep (Zhu *et al*., 2011), it indicated that melatonin could serve as a factor to alter animal's thermogenic adaptation (Hall and Lynoh, 1985).

Thermogenic capacity

Liver function shows high mass-specific energy metabolism and plays a major role in RMR (Couture and Hulbert, 1995). Exogenous melatonin increase mitochondrial proteins, mitochondrial COX activity, and activation of liver mitochondrial respiration in Brandt's voles (Hou *et al*., 1998). In the present

study, increase of RMR in the MLT group of *E. miletus* was according to the increase of liver mass, which was further supported by other biochemical markers examined in liver, including higher total protein, mitochondrial protein content, State 3 respiration, State 4 respiration and COX activity.

Injection or implanted of exogenous melatonin lead to increasing of NST significantly in some rodents, such as NST increased 42.1% in *Phodopus sungorus* were given exogenous melatonin, *Peromyscus leucopus* increased by 26% (Hall and Lynoh, 1985). A potential mechanism is that melatonin promotes the recruitment of BAT as well as enhances its activity. This effect would raise the metabolic rate by stimulating thermogenesis, heat generation through uncoupling oxidative phosphorylation in mitochondria (Tan *et al*., 2011). In our study, although BAT mass in MLT group did not change significantly compared with the control group of *E. miletus*, but the BAT total protein increased, especially to increasing of mitochondrial protein content, which demonstrating that melatonin acting as a key signaling agent to regulate protein synthesis, and mitochondrial protein elevated respiratory protein and respiratory enzyme concentrations, thereby increasing the thermogenesis. Melatonin treatment also increased the mean rate of protein synthesis in rats of different age (Brodsky *et al*., 2008). It is also known that melatonin interferes with glucose utilization and protein synthesis in *Suprachiasmatic Nucleus* (Cassone, 1991). COX activity in the BAT has been shown significantly enhanced by injection of exogenous melatonin, all of these results indicated that BAT heat production capacity is believed to be increasing as a result of exogenous melatonin, which showed an important biological mechanism for increasing of NST in *E. miletus.* There is an evidence that T_4 5'-DII activity in BAT was mediated by melatonin, which regulating adaptive thermogenesis for mammals (Puig-Domingo *et al*., 1988). Similar to our results in the present study, T_4 5'-DII activity increased in MLT group after injection of exogenous melatonin, which lead to increasing of adaptive thermogenesis in *E. miletus*.

Hormone concentration

The physiological roles of melatonin involve regulation of thermogenesis, which was modulate by the pineal gland-hypothalamus-pituitary axis (Haim, 1982). It had been reported that to melatonin treatment can modify the circulating levels of a number of hormones related to the metabolic regulation (Rios-Lugo *et al*., 2010). Previously published studies were confirmed that melatonin can regulate T_4 5'-DII activity in BAT, and T_4 can be converted to T_3 by Type II iodothyronine 5P-deiodinase (DII) in BAT (Lanni *et al*., 2003), Which plays an important role on the regulation of serum T_3 concentrations (Brzezinska-Slebodzinska and Slebodzinska, 1993). In our study, serum content of T_3 increased, and T_4 decreased in MLT group in *E. miletus*, it also indicated that T_4 can be converted to T_3 in peripheral tissues in BAT and liver, which lead to the increasing of T_3 concentration, suggesting that melatonin treatment plays an important role on regulation of serum T_3 and T_4 concentrations. And thyroid hormone are considered to regulate heat production increased under melatonin treatment. It found a significant increase in plasma leptin levels after oral melatonin supplementation (Song and Chen, 2009). Further, this observation is consonant with a previous study (Baltaci and Mogulkoc, 2007). Interestingly, it showed no difference in serum leptin levels in three groups in our study, similar to the results of *E. miletus* under different photoperiods (Zhu *et al*.,

2011b), it indicated that melatonin alone does not have a direct effect on adiposity's leptin secretion in *E. miletus*. The specific difference need further studies.

In conclusion, it showed that body mass was reduced significantly and RMR and NST increased observably at 28 days in melatonin treatment group. Melatonin induced an increased in the contents of total protein and mitochondrial protein of liver and BAT, a rise of State 3 respiration, State 4 respiration and COX activity in liver and BAT, but did not significantly stimulate α-PGO activity in liver. Serum T_3 content was enhanced significantly by injection of exogenous melatonin. These results indicated that thermogenic capacities of *E. miletus* are markedly stimulated by exogenous melatonin. Further, melatonin is potentially involved in the regulation of body mass, adaptive thermogenic capacity and hormone concentrations in *E. miletus*.

ACKNOWLEDGMENTS

This research was financially supported by National Science Foundation of China (No. 31260097), Basilic Project of Yunnan Province (No. 2011FZ082).

REFERENCES

Andrews, R.A., Belknap, R.W. (1993) Seasonal acclimation of prairie deer mice. *Int. J. Biometeorol.*, **37**: 190-193.

Arendt, J., Skene, D.J. (2005) Melatonin as a chronobiotic. *Sleep Med Rev.*, **9(1):** 25-39.

Asher, G.W., Archer, J.A., Ward, J.F., Scott, I.C., Littlejohn, R.P. (2011) Effect of melatonin implants on the incidence and timing of puberty in female red deer (*Cervus elaphus*). *Anim. Reprod. Sci.,* **123**: 202-209.

Baltaci, A.K., Mogulkoc, R. (2007) Pinealectomy and melatonin administration in rats: their effects on plasma leptin levels and relationship with zinc. *Acta Biol. Hung.*, **58**: 335-343.

Berlinguer, F., Leoni, G.G., Succu, S., Spezzigu, A., Madeddu, M., Satta, V. (2009) Exogenous melatonin positively influences follicular dynamics, oocyte developmental competence and blast cyst output in a goat model. *J. Pineal Res.*, **46**: 383-391.

Bharti, V.K., Srivastava, R.S., Malik, J.K., Spence, D.W., Pandi, S.R., Brown, G.M. (2012) Evaluation of blood antioxidant defense and apoptosis in peripheral lymphocytes on exogenous administration of pineal proteins and melatonin in rats. *J. Physiol. Biochem.*, **68**: 237-245.

Brodsky, V.Y., Golichenkov, V.A., Zvezdina, N.D., Dubovaya, T.K., Fateeva, V.I., Malchenko, L.A., Burlakova, O.V., Bespyatykh, A.Y. (2010) Melatonin promotes and synchronizes protein synthesis in hepatocyte culture from old rats. *Russian J. Devel. Biol.*, **39(6):** 357-361.

Brzezinska-Slebodzinska, E., Slebodzinski, A.B. (1993) Cold induced changes of thyroxin 5'- and 5'-monodeiodinase activity in brown adipose tissue of neonatal rabbits implications for thermogenesis. *J. Therm. Biol.*, **18**: 189-195.

Canpolat, S., Sandal, S., Yilmaz, B., Yasar, A., Kutlu, S., Baydas, G., Kelestimur, H. (2001) Effects of pinealectomy and exogenous melatonin on serum leptin levels in male rat. *Eur. J. Pharmacol.,* **428**: 145-148.

Cassone, V.M. (1991) Melatonin and suprachiasmatic nucleus functions. In DC Klein, RY Moore, SM Reppert, eds. Suprachiasmatic Nucleus. New York: Oxford University Press, pp. 309-323.

Couture, P., Hulbert, A.J. (1995) Relationship between body mass, tissue metabolic rate, and sodium pump activity in mammalian liver and kidney. *Am. J. Physiol.,* **268**: 641-650.

Estabrook, R.W. (1967) Mitochondrial respiratory control and the polarographic measurement of ADP: O ratios. *Meths. Enzymol.*, **5:** 41-47.

Friedman, J.M., Halaas, J.L. (1998) Leptin and the regulation of body weight in mammals. *Nature,* **395**: 763-770.

Haim, A., Fourie, F.R. (1982) Effects of melatonin on heat production and enzymatic activity in diurnal and in nocturnal rodents. *Comp. Biochem. Physiol.,* **71**: 473-475.

Hall, E.S., Lynch, G.R. (1985) Two daily melatonin injections differentially induce nonshivering thermogenesis and gonadalregression in the mouse (*Peromyscus leucopus*). *Life Sci.,* **37**: 783-788.

Hausman, G.J., Barb, C.R. (2010) Adipose tissue and the reproductive axis: biological aspects. In C Levy-Marchal, L Pénicaud. eds. Adipose tissue development: from animal models to clinical conditions: Endocr Dev. Basel press, Karger, pp. 31-44.

Heldmaier, G. (1971) Nonshivering thermogenesis and body size in 567 mammals. *J. Comp. Physiol. B*, **73**: 222-248.

Hill, R.W. (1972) Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. App. Physiol.*, **33**: 261-263.

Hou, J.J., Huang, C.X., Li, Q.F. (1998) Melatonin induced thermogenesis in Brandt's voles (*Microtus brandti*). *Acta. Zool. Sin*. **44**: 20-26.

Johnson, M.S., Onorato, D.P., Gower, B.A. (2004) Weight change affects serum leptin and corticosterone in the collared lemming. *Gen. Comp. Endocrinol.*, **136**: 30-36.

Kadenbach, B., Huttemann, M., Arnold, S., Lee, I., Bender, E. (2000) Mitochondrial energy metabolism is regulated via nuclear-coded subunits of cytochrome c oxidase. *Free Radical. Biol. Med.*, **29**: 211-221.

Klein, J.R. (2006) The immune system as a regulator of thyroid hormone activity. *Exp. Biol. Med.*, **231**: 229-236.

Klingenspor, M., Niggemann, H., Heldmaier, G. (2000) Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. *J. Comp. Physiol. B*, **170**: 37-43.

Korhonen, T., Mustonen, A.M., Nieminen, P., Saarela, S. (2008) Effects of cold exposure, exogenous melatonin and short-day treatment on the weight-regulation and body temperature of the Siberian hamster (*Phodopus sungorus*). *Regul. Peptides.*, **149**: 60-66.

Krotkiewski, M. (2002) Thyroid hormones in the pathogenesis and treatment of obesity. *Eur. J. Pharmacol.*, **440**: 85-98.

Lanni, A., Moreno, M., Lombardi, A., Goglia, F. (2003) Thyroid hormone and uncoupling proteins. *FEBS Lett.*, **543**: 5-10.

Leonard, J.L., Mellen, S.A., Larsen, R.P. (1983) Thyroxine 5'-deiodinase activity in brown adipose tissue. *Endocr*., **112**: 1153-1155.

Li, X.S., Wang, D.H., Yang, M. (2004) Effects of cold acclimation on body weight, serum leptin level, energy metabolism and thermogenesis in the Mongolian gerbil *Meriones unguiculatus*. *Acta Zool. Sin.*, **50**: 334-340.

Li, X.S., Wang, D.H. (2005) Regulation of body weight and thermogenesis in seasonally

acclimatized Brandt's voles (*Microtus brandti*). *Horm. Behav.*, **48**: 321-328.

Liu, J.S., Sun, R.Y., Wang, D.H. (2006) Thermogenic properties in three rodent species from Northeastern China in summer. *J. Therm. Biol.,* **31**: 172-176.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.

Molik, E., Misztal, T., Romanowicz, K., Zieba, D.A. (2010) The effects of melatonin on prolactin and growth hormone secretion in ewes under different photoperiods, during the early post partum period. *Small Ruminant Res.*, **94**: 137-141.

Moore, A.F., Menaker, M. (2011) The effect of light on melatonin secretion in the cultured pineal glands of *Anolis* lizards. *Comp. Biochem. Physiol.*, **160**: 301-308.

Mustonen, A.M., Nieminen, P., Hyvarinen, H., Asikainen, J. (2000) Exogenous Melatonin Elevates the Plasma Leptin and Thyroxine Concentrations of the Mink (*Mustela vison*). *Z. Naturforsch.*, **55**: 806-813.

Paradies, G., Petrosillo, G., Paradies, V., Reiter, R.J., Ruggiero, F.M. (2010) Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *J. Pineal Res.*, **48**: 297-310.

Puig-Domingo, M., Guerrero, J.M., Reiter, R.J., Tannenbaum, H.J., Hurlbut, E.C., Gonzalez-Brito, A. (1988) Thyroxine 5'-deiodination in brown adipose tissue and pineal gland: implications for thermogenic regulation and role of melatonin. *Endocri*., **123**: 677-680.

Rios-Lugo, M.J., Cano, P., Jimenez-Ortega, V., Fernandez-Mateos, M.P., Scacchi, P.A., Cardinali, D.P., Esquifino, A.I. (2010) Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat–fed rats. *J. Pineal Res.*, **49**: 342-348.

Tan, D.X., Manchester, L.C., Fuentes-Broto, L., Paredes, S.D., Reiter, R.J. (2011) Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. *Obes. Rev.*, **12(3)**: 167-188.

Song, Y.M., Chen, M.D. (2009) Effects of melatonin administration on plasma leptin concentration and adipose tissue leptin secretion in mice. *Acta Biol. Hung*., **60**: 399-407.

Steffen, J.M., Roberts, J.C. (1977) Temperature acclimation in the Mongolian g*erbil (Meriones unguiculatus*): biochemical and organ weight changes. *Comp. Biochem. Physiol.*, **58**: 237-243.

Steinlechner, S., Heldmaier, G. (1982) Role of photoperiod and melatonin in seasonal acclimatization of the Djungarian hamster, *Phodopus sungorus*. *Int. J. Biometeor.*, **26**: 329-337.

Sundin, U., Moore, G., Nedergaard, J., Cannon, B. (1987) Thermogenin amount and activity in hamster brown fat mitochondria: effect of cold acclimation. *Am. J. Physiol.*, **252**: 822-832.

Wolden-Hanson, T., Mitton, D.R., McCants, R.L., Yellon, S.M., Wilkinson, C.W., Matsumoto, A.M., Rasmussen, D.D. (2000) Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology*, **141(2)**: 487-497.

Zhang, Y., Proenca, R., Maffei, M., Barone, M.,

Leopold, L., Friedman, J.M. (1994) Positional cloning of the mouse obese gene and its human homologue. *Nat*., **372**: 425-432.

Zhu, J.Q., Zhang, H.R., Li, F.D., Zhang, Y. (2005) Effect of implanting melatonin on the weight and oestrus of sheep. *J. Gansu Agricul. Uni.*, **4**: 448-451.

Zhu, W.L., Jia, T., Lian, X., Wang, Z.K. (2008) Evaporative water loss and energy metabolism in two small mammals, voles (*Eothenomys miletus*) and mice (*Apodemus chevrieri*) in Hengduan mountains region. *J. Therm. Biol.*, **33**: 324-331.

Zhu, W.L., Jia, T., Lian, X., Wang, Z.K. (2010a) Effects of cold acclimation on body mass, serum leptin level, energy metabolism and thermognesis in *Eothenomys miletus* in Hengduan Mountains region. *J. Therm. Biol.*, **35**: 41-46.

Zhu, W.L., Cai, J.H., Lian, X., Wang, Z.K. (2010b) Adaptive character of metabolism in *Eothenomys miletus* in Hengduan Mountains region during cold acclimation. *J. Therm. Biol.*, **35**: 417-421.

Zhu, W.L., Wang, B., Cai, J.H., Lian, X., Wang, Z.K. (2011a.) Thermogenesis, energy intake and serum leptin in *Apodemus chevrieri* in Hengduan Mountains region during cold acclimation. *J. Therm. Biol.*, **36**: 181-186.

Zhu, W.L., Cai, J.H., Lian, X., Wang, Z.K. (2011b) Effects of photoperiod on energy intake, thermogenesis and body mass in *Eothenomys miletus* in Hengduan Mountain region. *J. Therm. Biol.*, **36**: 380-385.

Zhu, W.L., Yang, S.C., Wang, Z.K. (2012) Adaptive characters of energy metabolism, thermogenesis and body mass in *Eothenomys miletus* during cold exposure and rewarming. *Anim. Biol.*, **62**: 263-276.