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ORIGINAL ARTICLE

Variations of Endocrine Hormones Concentrations in *Tupaia belangeri* under Simulated Seasonal Acclimatized: Role of Leptin Sensitivity

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Seasonal variations in endocrine hormones concentrations are important for the survival of small mammals during acclimatization. In order to understand the role of leptin sensitivity on other endocrine hormones concentrations, we examined body mass, serum leptin level, serum insulin, tri-iodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations in *Tupaia belangeri* under seasonal acclimatized (The simulated temperature and photoperiod in winter: 5°C and SD, 8h:16h Light:Dark; the simulated temperature and photoperiod in summer: 30°C and SD, 16h:8h Light:Dark) for 4 weeks. The results showed that body mass, serum leptin level, serum T_3, T_4 concentrations and T_3/ T_4 showed significant variation, but serum insulin and TSH concentrations showed no variations between treatment group. There were positive correlation between serum leptin level and insulin, T_4 concentrations, and were negative correlation between serum leptin level and body mass, T_3 concentrations. However, no correlation was found between serum TSH concentrations and serum leptin level. The present results suggested *T. belangeri* overcome winter thermogenesis challenges by adjusting body mass and endocrine hormones concentrations. Furthermore, leptin may play an potential role in their body mass regulation in *T. belangeri*.

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Changes of body fat mass is one of main causes for seasonal changes of body mass in small mammals (Klingenspor *et al*., 2000; Bartness *et al*., 2002). Adipose tissue has an important role in small mammal including energy storage and hormone's secretion (Trayhurn and Beattie, 2001), such as *Phodopus sungorus* (Rafael *et al*., 1985) and *Sorex araneus* (Nieminen and Hyvarinen, 2000). Leptin, primarily secreted by fat tissue, can regulate energy intake and energy expenditure (Zhang *et al*., 1994). Insulin is produced by beta cells of the pancreas, and is central to regulate carbohydrate and fat metabolism in the body (Dunn, 2005). Triiodothyronine, also known as T_3, affects almost every physiological process in the body, including growth, development and metabolism (Kelly and Lieberman, 2009). Thyroxine (T_4), is tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism (Kirkegaard and Faber, 1998). Thyroid-stimulating hormone (TSH) is a hormone that stimulates the thyroid gland to produce T_4, and then T_3 which stimulates the metabolism of almost every tissue in the body (Parmentier *et al*., 1989).

Previously studies showed positive correlation between serum leptin levels and body mass in many small mammals including *P. sungorus* (Klingenspor *et al*., 2000), and cold acclimated *Eothenomys miletus* (Zhu *et al*., 2010), *Apodemus chevrieri* (Zhu *et al*., 2011). In mammals, insulin is synthesized in the pancreas within the β-cells, and ob-Rh receptor of β-cells can binding with leptin recombinant, thus leptin and insulin may exist certain relationships (Emilsson, 1997). Energy metabolism was also regulated by the interactions between leptin and thyroid stimulating hormone (Escobar-Morreale *et al*., 1997).

Tupaia belangeri (Mammalia: Scandentia: Tupaiidae) live at the highest latitude, with the Yunnan-Kweichow Plateau being its northern limit (Wang *et al*., 1991). Previous studies demonstrated that environmental factors, such as short photoperiods and cold, are effective cues that influence body mass and thermogenesis in *T. belangeri*, separately (Zhang *et al*., 2011; 2012a; 2012b; 2012c). *T. belangeri* showed a seasonal increased in body mass and thermogenic capacity to adapt to the increase of energy requirements for thermoregulation (Zhu *et al*., 2012). However, we know nothing about the action of simulated seasonal acclimatized with changes in endocrine hormones concentrations in *T. belangeri*. In the present study, we examined the effect on endocrine hormones concentrations in *T. belangeri* under seasonal acclimatized by simulated temperature and photoperiod in winter (5 °C and SD, 8h:16h Light:Dark) and simulated temperature and photoperiod in summer (30 °C and SD, 16h :8h Light: Dark) for 4 weeks.. We predicted that *T. belangeri* change their body mass and endocrine hormones concentrations, and leptin would be involved in the regulation of body mass.

MATERIALS AND METHODS

Samples

T. belangeri were captured (25°25´-26°22´ N, 102°13´-102°57´ E, 1679 m in altitude) around boscage at Luquan County in 2011. The average yearly temperature was 15.6 °C, mean monthly temperature ranges from 7.8 °C in winter to 19.6 °C in summer. After being captured, *T. belangeri* were brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1910 m in altitude). Each weight-matched tree shrew was housed individually in a wire cage (40 cm×40 cm×40 cm) with no bedding; all animals (60 males) were healthy adults. The photoperiod, ambient temperature and humidity were maintained at 12 L: 12D (light on at 08.00 h), 25 (±1)°C , and 85%–92% relative humidity, respectively. Animals were kept for at least two weeks, and 60b adults with similar body mass were divided into two treatment groups. One group was transferred to winter simulated group (5 °C and SD, 8h:16h, 30 males), the other group was at summer simulated group (30 °C and SD, 16h:8h, 30 males) for 4 weeks (0, 7, 14 21 28 days, each group=6). *T. belangeri* were fed mixed food containing 25.0% crude protein, 6.3% crude fat, 4.6% crude fibred, 7.4% ash, and 0.96 kJ/g gross energy (Zou *et al*., 1991); every two-day interval apples, pears, other fruits, and water were provided ad libitum. *T. belangeri* were fed once daily at 10:00 h. On day 0, 7, 14, 21 and 28, body mass were weighed, then animals were killed and blood was centrifuged at 800 g for 30 min, and serum was sampled and stored at −20 °C for later measurement. All shrews were dissected to evaluate organ morphology. Pregnant, lactating or young individuals were excluded.

Measurement of hormone concentration

Serum leptin levels were determined by radioimmunoassay (RIA) with the ^{125}I Multi-species Kit (Cat. 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.

Serum insulin concentrations were measured by radioimmunoassay (RIA) with a ^{125}I human kit (Atom HighTech Co., Ltd., Beijing, CHN). The lower and upper limits of the assay kit were 5 and 160 ng ml^{-1} and the intra- and inter-assay variations were <10 and 15%, 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. Thyroid stimulating hormone (TSH) concentrations were determined by radioimmunoassay kit (Linco Co. USA) (Du and You, 1992).

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 and endocrine hormones concentrations were analyzed by a two-way analysis of covariance (ANCOVA) with body mass as a covariate. Pearson's correlation was performed to detect possible correlations among serum leptin and body mass, and endocrine hormones concentrations. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass and serum leptin level

Prior to acclimation, no group differences were found between acclimation *T. belangeri* (t=-0.096, P>0.05). During the acclimation, body mass in winter simulated group exhibited greater increases and decreased in summer simulated group (group effect, F=8.053, P<0.01; day effect, F=0.781, P>0.05; interaction group×day, F=4.539, P<0.01; fig. 1). Body mass in winter simulated group was 16.55% higher than that in summer simulated group. Serum leptin level showed a significant differences between two treatment group (group effect, F=7.126, P<0.01; day effect, F=2.801, P<0.05; interaction group×day, F=5.606, P<0.01; fig. 2). Correlation analysis indicated there had negative correlation between serum leptin level and body mass during the acclimation (r = -0.676, P<0.01, fig. 3).

Serum insulin concentrations

During the acclimation, serum insulin concentrations observed no differences between winter simulated group and summer simulated group (group effect, F=2.737, P>0.05; day effect, F=0.313, P>0.05; interaction group×day, F=1.118, P>0.05). Correlation analysis indicated there had no correlation between serum insulin concentrations and body mass during the acclimation (r = -0.270, P>0.05), however, it indicated there had positive correlation between serum insulin concentrations and serum leptin level during the acclimation (r = 0.336, P < 0.05, fig. 4).

Serum thyrotropin concentrations

During the acclimation, serum T_3, T_4 and T_3/T_4 concentrations showed significantly differences between winter simulated group and summer simulated group (T_3: group effect, F=2.173, P<0.05; day effect, F=1.353, P>0.05; interaction group×day, F=4.191, P<0.01, fig. 5), (T_4: group effect, F=2.831, P<0.05; day effect, F=1.470, P>0.05; interaction group×day, F=3.790, P<0.05, fig 6; T_3/T_4: group effect, F=0.103, P>0.05; day effect, F=0.348, P>0.05; interaction group×day, F=7.945, P<0.01). But serum TSH concentrations showed no differences between winter simulated group and summer simulated group (T_3: group effect, F=0.438, P>0.05; day effect, F=0.493, P>0.05; interaction group×day, F=1.885, P>0.05). Serum leptin level was negatively correlated with serum T_3 concentration (r=-0.280, P<0.05; fig. 7A) and positive correlation with serum T_4 concentration (r=0.293, P<0.05; fig 7B), but showed no correlation with TSH concentrations (r=0.080, P>0.05).

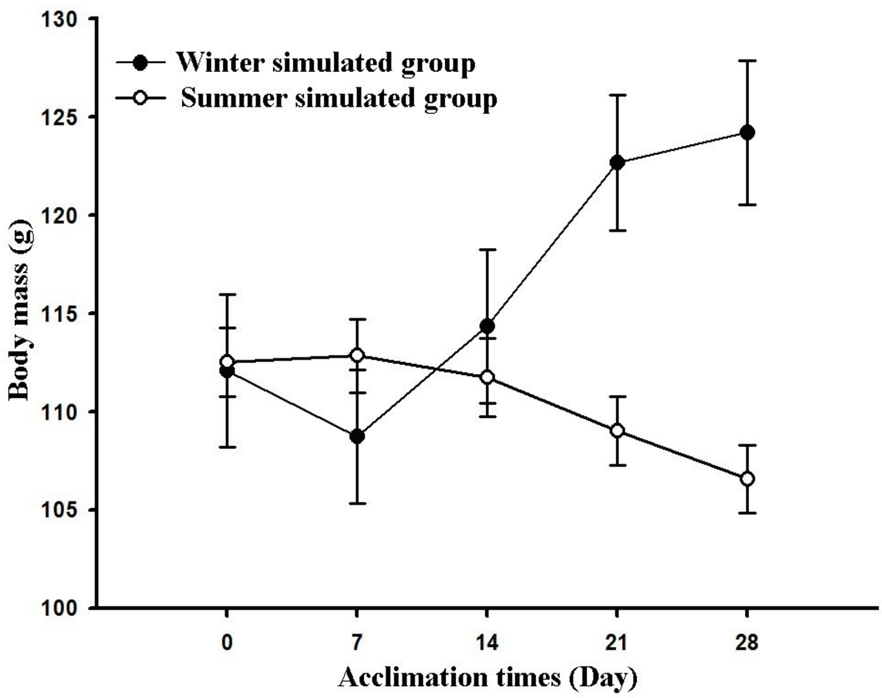

Figure 1 Changes of body mass under winter simulated group and summer simulated group in *Tupaia belangeri*

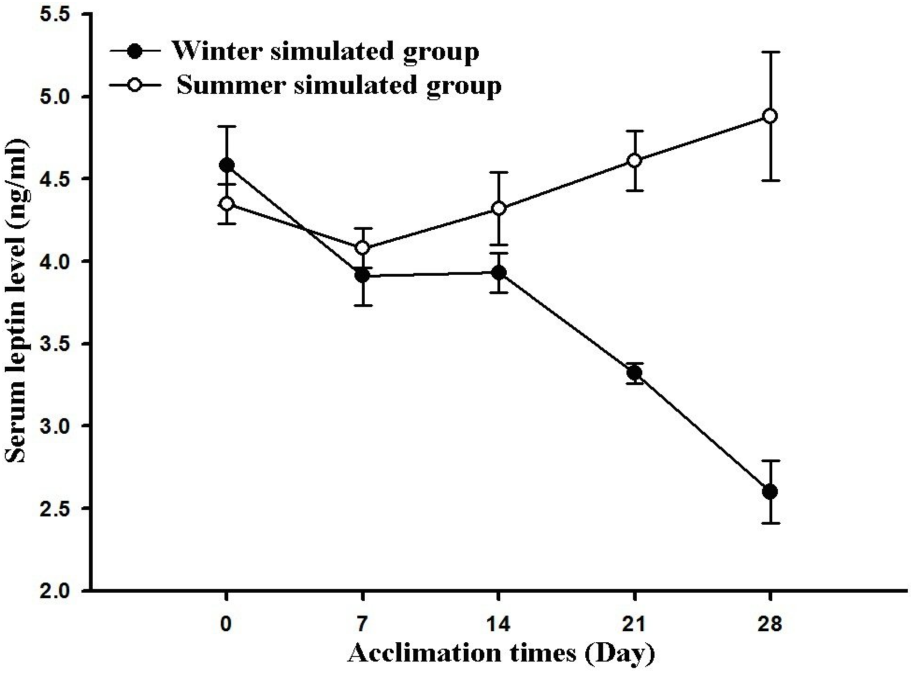

Figure 2 Changes of serum leptin level under winter simulated group and summer simulated group in *Tupaia belangeri*

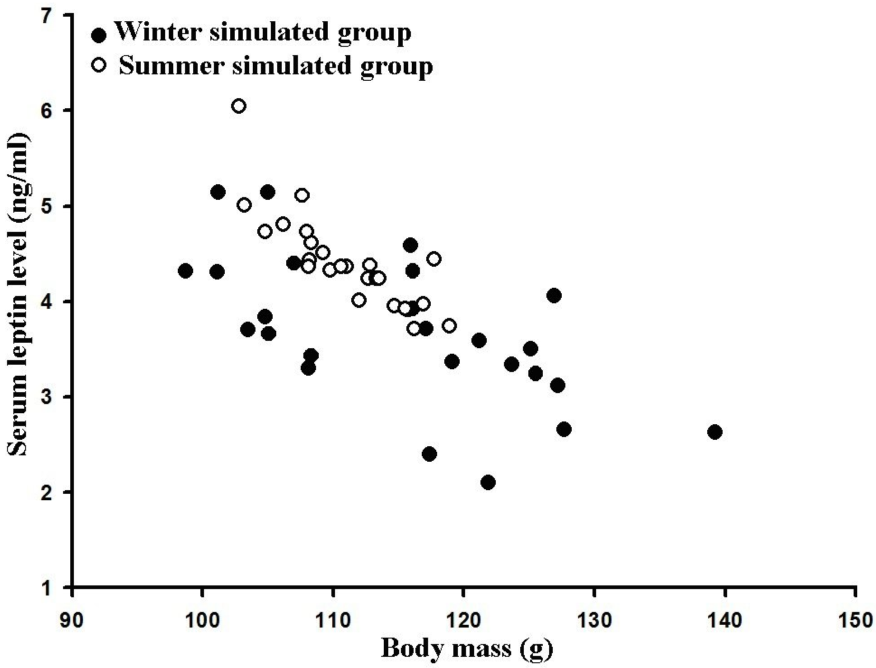

Figure 3 Correlation between serum leptin level and body mass under winter simulated group and summer simulated group in *Tupaia belangeri*

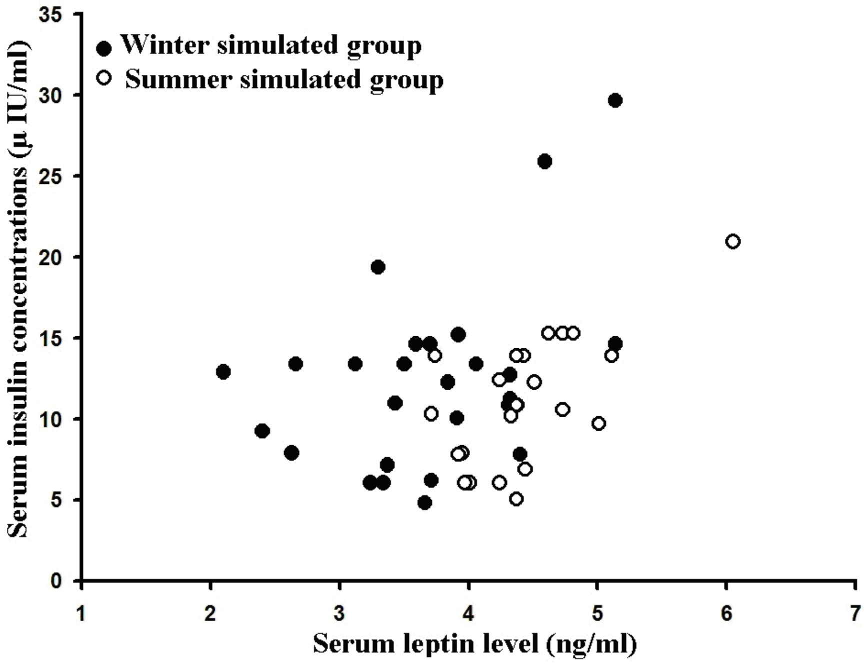

Figure 4 Correlation between serum insulin concentrations and serum leptin level under winter simulated group and summer simulated group in *Tupaia belangeri*

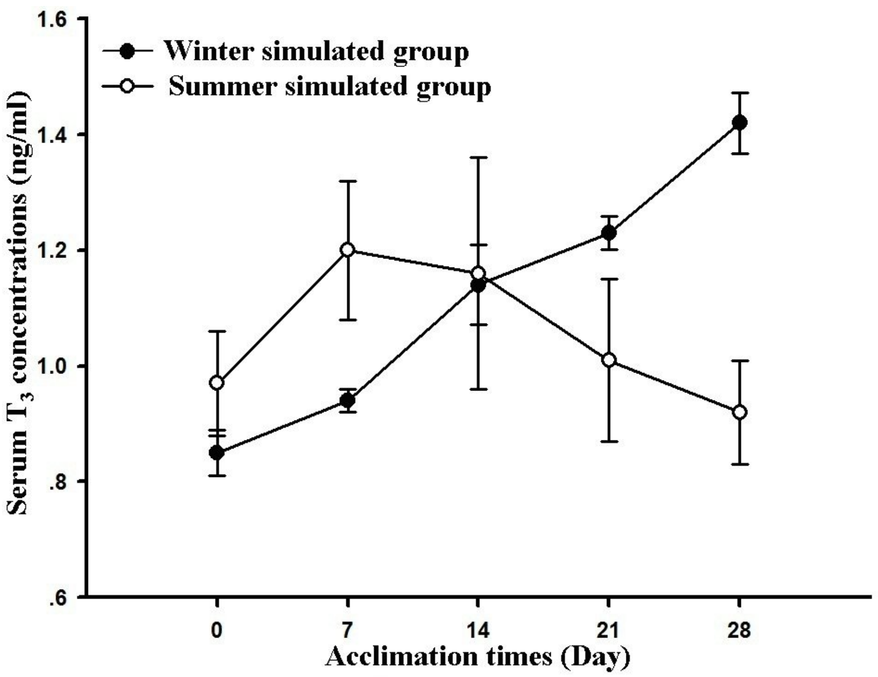

Figure 5 Changes of serum T_3 concentrations under winter simulated group and summer simulated group in *Tupaia belangeri*

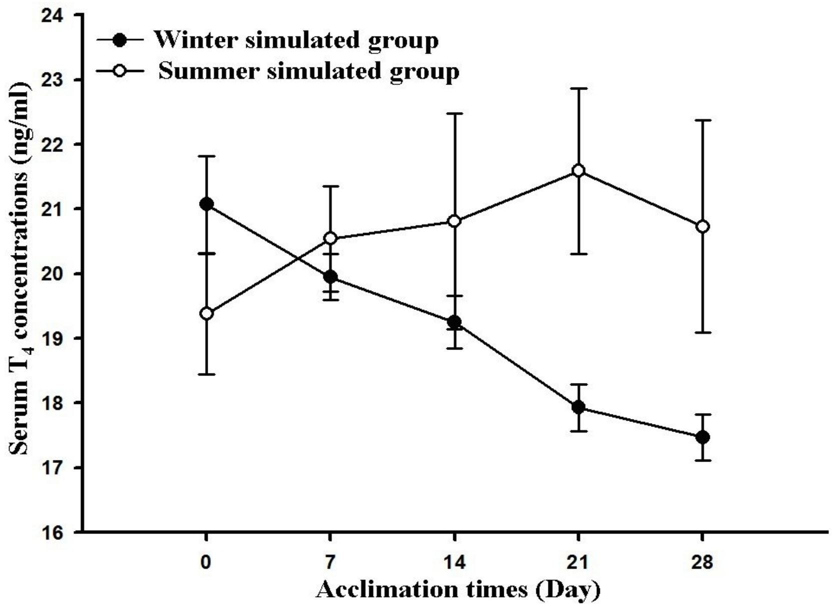

Figure 6 Changes of serum T_4 concentrations under winter simulated group and summer simulated group in *Tupaia belangeri*

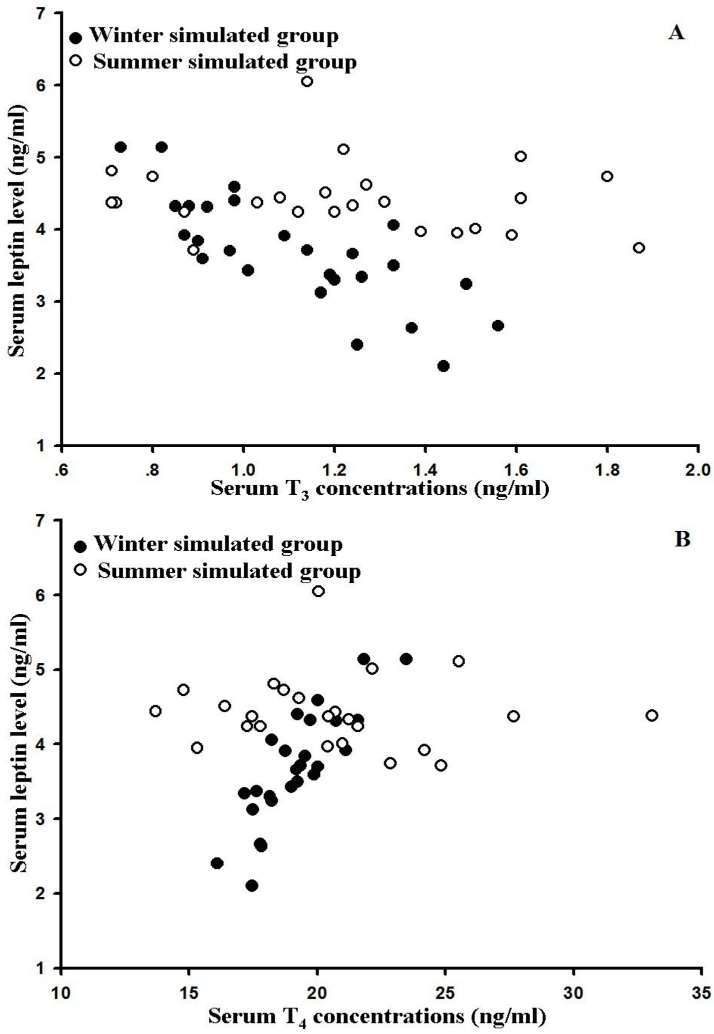

Figure 7 Correlation between serum leptin level and serum T_3 concentrations (A) and serum T_4 concentrations (B) under winter simulated group and summer simulated group in *Tupaia belangeri*

DISCUSSION

Body mass and serum leptin level

Many small mammals reduced body mass in winter or under cold acclimation (Bartness *et al*., 2002), such as *Microtus ochrogaster* (Voltura and Wunder, 1998), *M. pennsylvanic* (Iverson and Turner, 1974). In winter or under cold condition, reducing body mass is a way of reducing energy consumption (Klingenspor *et al*., 1996). In contrast, some small mammals increased body mass under cold acclimation, such as *Dicrostonga groenlandicus* (Nagy and Negus, 1993). In addition, body mass in some small mammals did not shown seasonal variation, such as *Meriones unguiculatus* (Li *et al*., 2004). Body mass in *T. belangeri* gradually increased under winter simulated group, similar to the results that under seasonal acclimatized (Zhu *et al*., 2012). Increasing in body mass was advantageous in decreasing heat loss of individuals and increasing the capacity during cold tolerance (Li *et al*., 2001). Leptin, a hormone secreted from adipose tissue, which can regulate body mass and energy intake (Zhang *et al*., 1994). Klingenspor found that serum leptin level decreased by 54.8% and 77.4% under short photoperiod for 66 days and 116 days in *Phodopus sungorus* (Klingenspor *et al*., 2000). Further, serum leptin level declined in rats under cold acclimation (Bing *et al*., 1998), the common shrew also decreased leptin secretion in winter (Nieminen and Hyvarinen, 2000). In addition, serum leptin level was positive with body mass, it indicated that leptin can act as a signal perception in seasonal changes in body fat storage condition (Rousseau *et al*., 2003). In the present study, serum leptin levels had remarkable decreased under winter simulated group in *T. belangeri*, and increase in body mass, so it showed a negative correlation between serum leptin levels and body mass in *T. belangeri*.

Serum insulin concentrations and serum leptin level

Insulin is the most important factor to effect on leptin synthesis and secretion (Saad *et al*., 1998). In rat cultured fat cells, insulin can enhance the expression of leptin mRNA and secretion (Saladin *et al*., 1995), leptin mRNA expression in adipose tissue decreased under fasting while increased after refeeding, which was consistent with changes of insulin concentration (Cusin *et al*., 1995). Many studies indicated that leptin can be both directly and indirectly inhibits the secretion of insulin, leptin and insulin may exist in a feedback control loop, long time exposed to physiological concentrations of leptin in islet cell can inhibit glucose-stimulated insulin transcription levels, also reduce the secretion of insulin, and high concentration of leptin is rapid inhibitory effects on insulin secretion (Ceddia *et al*., 1999). But the regulating mechanism of leptin on insulin secretion is still not completely clear, insulin may play a signaling role on adipose tissue, promote its synthesis and secretion of leptin, leptin became negative feedback medium in pancreas, which inhibited insulin secretion, thereby reducing fat assimilation to reduce fat storage (McMinn *et al*., 1998). In our results, leptin was positively correlated with insulin, it indicated that insulin can indeed positive regulation of leptin synthesis and secretion in *T. belangeri*.

Serum thyrotropin concentrations and serum leptin level

Thyroid hormones play an important role in the regulation of mammalian adaptive thermogenesis (McNabb, 1992), including regulated by complex physiological and biochemical mechanism (Wu *et al*., 1991), and also by environmental temperature (Tomasi *et al*. 1987) and photoperiod (Nagy *et al*., 1995). In the present study, serum T_3 concentration increased, serum T_4 concentration decreased, T_3/T_4 increased gradually in *T. belangeri* under winter simulated group. In previous studies, cold exposure can cause rats rapid increase of serum TSH concentrations (Ducommun *et al*., 1966). The serum level of TSH in 30 minutes can improve 1.5 times in rats under cold acclimation (Hershman *et al*., 1970). Serum TSH concentrations after 2 hours can promote serum thyroxine concentrations increase, which can last 48 hours (Hefco *et al*., 1975). In our study, low temperature short photoperiod conditions, TSH levels increased first and then dropped under winter simulated group in *T. belangeri*, probably because of low temperature and short photoperiod stimulates the thyroid stimulating hormone secretion, and increase in serum thyroid hormone levels, when thyroid level reaches a certain concentration, a high level of thyroid hormone through the hypothalamus-pituitary-thyroid axis (HPT) feedback effects to adjust the thyroid stimulating hormone secretion, thereby to maintain the body's endocrine hormone balance. Leptin secretion was negatively regulated by HPT axis function, which reduced the level of Thyrxine releasing hormone (TRH) (Kakucaka *et al*., 1995). Thyroid hormone had the interaction with serum leptin concentration in rodents (Escobar-Morreale *et al*., 1997), and thyroid hormone can influence body fat content and TSH in the regulation of leptin (Pinkney *et al*., 1998). In the present study, serum leptin level was negatively correlated with serum T_3 concentration, and was positively correlated with serum T_4 concentration, but showed no correlation with TSH concentrations in *T. belangeri*, suggested that leptin might be involved in the regulation of hormones concentrations.

In conclusion, the present results suggested *T. belangeri* overcome winter thermogenesis challenges by adjusting body mass and endocrine hormones concentrations. Furthermore, leptin may play an potential role in their body mass regulation in *T. belangeri*.

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