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ORIGINAL ARTICLE

Body mass, Thermogenesis and energy metabolism in *Tupaia belangeri* during cold acclimation

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In order to study the relationship between energy strategies and environmental temperature, basal metabolic rate (BMR), nonshivering thermogenesis (NST), the total protein contents, mitochondrial protein contents, state Ⅲ and state Ⅳ respiratory ability, cytochrome C oxidase activity of liver, heart, diaphragm, gastrocnemius and brown adipose tissue (BAT), serum leptin level and serum thyroid hormone levels were measured in tree shrews (*Tupaia belangeri*) during cold exposure (5±1°C) for 1 day, 7 days,14days,21 days. The results showed that body mass increased, BMR and NST increased, the change of liver mitochondrial protein content was more acutely than total protein. The mitochondrial protein content of heart and BAT were significantly increased during cold-exposed, however the skeletal muscle more moderate reaction. The state Ⅲ and state Ⅳ mitochondrial respiration of these tissues were enhanced significantly than the control. The cytochrome C oxidase activity with cold acclimation also significantly increased except the gastrocnemius. Liver, muscle, BAT, heart and other organs were concerned with thermoregulation during the thermal regulation process above cold-exposed. There is a negative correlation between leptin level and body mass. These results suggested that *T. belangeri* enhanced thermogenic capacity during cold acclimation, and leptin participated in the regulation of energy balance and body weight in *T. belangeri*.

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Key words: Tupaia belangeri; adaptive thermogenesis; cold-exposure

Energy metabolism of small rodents always influences their distribution, abundance, reproduction and adaptation (Bozinovic, 1992). The balance of energy obtain and consume is the key to effect their reproduction and adaptation, and the balance of energy metabolism is the balance of the energy budget, thermogenesis, growth, reproduction and other functions (Karasov, 1986). The model of energy distribution decided animals energy budget and physiological adaptation (McNab, 1995), is the key factor to understand animals adaptation model and evolution (Grodzinski *et al*, 1975). Energy metabolism of wildness animals are influenced by some environmental and physiological factors, temperature is the important factor. It influences

animals body mass, energy budget, thermogenesis (Abelenda *et al.*, 2003).

Tree shrew (*Tupaia belangeri*) belongs to Scandebtia, Tupaiidae. They are small mammals of Palaearctic realm and widely distributed at Southern China, India, and Southeast Asia. *T. belangeri* is the widest of distribution and the highest of latitude in their family; Yunnan, Sichuan and the southwest of Guizhou mostly was its north limit and their habitat always was terrestrial, arboreal, mountainous forest and shrub areas (Wang, 1991). The physiological characters of *T. belangeri* showed some transition and some different things. Early studies in our lab showed that RMR, NST (Wang *et al*, 1994; Zhang *et al*, 2001) and the metabolism of energy (Zhang, 2002) of *T. belangeri* presented robust seasonal cycles. We have shown that administration of exogenous melatonin at physiological doses induces seasonal cycle of the thermogenesis in *T. belangeri* (Wang *et al*, 2000) and the thermogenesis in tree shrew was already increased during cold exposured (Wang *et al*, 1995; 1999).

However, the regulation of the adaptive thermogenesis in cell level in *T. belangeri* was never studied now. Some early studies discussed experimental rodents for the adaptive thermogenesis from the cell and molecule level (Abelenda *et al*, 2003), such as *Phodopus sungorus* (Praun *et al*, 2001), and mainly attention to the effects of the cold acclimation (Wang *et al*, 2000) and photoperiod (Zhao and Wang, 2005, 2006a, b). The study of *Merione sunguiculatus* indicated that the weight of BAT, the mitochondrial protein content of BAT and liver, cytochrome C oxidase activity and the content of UCP-1 in winter more than that in summer (Zhang *et al*, 2006). Klaus found that the mitochondrial protein content of BAT and UCP-1 in winter was increased 70% and 30% respectively than in summer. The recently discovered protein, leptin, is a protein which is synthesized primarily by adipose tissue and is secreted into the bloodstream. Discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis (Silva, 2006). Leptin impacts feed intake, the neuroendocrine-axis, metabolism and immunological processes (Barb and Kraeling, 2004). Leptin is hypothesized to contribute to the maintenance of body mass by altering feed intake and energy expenditure (Friedman and halls, 1998). Exogenous injection of leptin can decrease body mass by restraining energy intake and increasing energy utilization (Abelenda *et al*, 2003). The role of leptin in energy expenditure and heat production is an area of current contention in physiology (Hukshorn and Saris, 2004).

By way of further understand the adaptability thermogenesis pattern of *T. belangeri* at Hengduan mountain region, we discussed the changes of BMR, NST, the total protein, mitochondrial protein, the cytochrome C oxidase activity, leptin level and the UCP-1 content, T4-5'DII of BAT of *T. belangeri*, so we wanted to know what changes of these characters during cold exposure and discussed these characters adaptive signification.

MATERIALS AND METHODS

Animals

Tree shrew (*T. belangeri*) were captured (25°25'~26°22' N, 102°13'~102°57'E, 1679 m in altitude) around boskage at Luquan County, Yunnan Province. After being captured, tree shrews were transported to the Sclool of life Science of Yunnan Normal University, Kunming, China (1910m in altitude). Animals were kept individually in wire cages (35x25x20 cm) with no bedding. The animals maintained under 12L:12D (light:dark, lights on 08:00) photoperiod and room temperature that were control under 30±1 ºC. The cold-exposed

animals were maintained under 5±1 ºC and 12L:12D (light: dark, lights on 08:00) photoperiod which contained 1d, 7d, 14d, 21d groups. All pregnant, lactating or young individuals were excluded.

Measurement of metabolic rates

Metabolic rates were measured using AD ML870 open respirometer, gas analysis were using ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5 ℃), breath chamber volume is 500ml, flow is 200ml/min, experiments process more than 1.5 hours. Calculate method of metabolic rate detail in Hill (1972). Before and after the experiment, animal's body mass were measured.

Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) and measured at 25ºC. Two consecutive highest recordings of oxygen consumption in 60 min at each measurement were taken to calculate the NST. The doses of NE were approximately 0.8-1.0 mg/kg body mass according to dose-dependent response curves that were carried out before the experiment.

Isolation of mitochondria and measurement of biochemical characters

Isolation of mitochondria of tissues

Between control group and cold acclimation group, after collecting trunk blood, the liver, heart and diaphragm muscle was removed rapidly, weighed, and placed in ice-cold isolation medium (250 mM sucrose, 10 mM Tris·HCl, 1 mM EGTA, and 0.1% BSA, pH 7.4). The liver was then cut into small cubes with a razor blade and rinsed with cold isolation medium to remove blood. Tissue (10% wt/vol in isolation medium) was transferred to an ice-cold glass-Teflon homogenizer and homogenized with five to six strokes of the pestle. The homogenate was transferred to chilled centrifuge tubes and centrifuged at 2,000 *g* for 7 min at 4°C. The supernatant was decanted into clean centrifuge tubes and centrifuged at 10,000 *g* for 10 min (4°C). The supernatant was discarded, and the pellet was gently suspended in isolation medium using a cooled glass rod and centrifuged at 10,000 *g* for 10 min (4°C), then discarded the supernatant and stored on ice (Cannon and Lindberg, 1979). The distill method of mitochondria of heart and diaphragm muscle details in Oufara (1987) and Goglia (1993).

Measurement of mitochondrial respiration activity

Using the CHLOTOLAB2 oxygen electrode (Hansatech Instruments Lab) mensurate mitochondrial state Ⅳ respiratory, increased of 5 μmol ADP measuring state Ⅲ (Wang, 1996) ; succinate for respiratory substrate. The total volume of reaction cup was 2 ml and the reaction temperature was 30 ºC.

The cytochrome C oxidase activity was measured by oxygen electrode also, measurement of activities of COX details in Sundin *et al* (1987). Proteins were measured by Folin-Phenol method.

Measurement of uncoupling protein 1

BAT were weighed and placed in ice-cold isolation medium A (250 mM sucrose, 10 mM Trisbase, 2 mM EDTA). The BAT was then cut into small cubes with a razor blade on ice. Tissue (20% wt/vol in isolation medium) was transferred to an ice-cold glass-Teflon homogenizer and homogenized with five to six strokes of the pestle. The homogenate was transferred to chilled centrifuge tubes and centrifuged at 1,500 *g* for 10 min at 4°C. The supernatant was discarded, and centrifuged at 500 *g* for 5 min (4°C). The supernatant was transferred to chilled centrifuge tubes and centrifuged at 10,000 *g* for 10 min (4°C), then discarded the supernatant and suspend the

deposition with isolation medium B (20mM MOPS,20mM Na_2S0_4,2mM EDTA).

Measurement of uncoupling protein 1 (UCP1) were details in Thompson *et al* (1989).

Measurement of the T_4-5' deiodinase activity of BAT

The Thyromicrosomal Antibody RIA Kit from the isotope academe of china Institute of Atomic Energy was used to measure the concentration of T_3, T_4 in the serum (Brzezinska and Slebodzinski, 1993).

Measurement of serum leptin level

Serum leptin level were using radicalized immune reagent box, the equipment was SN-682 radicalized immune γ- arithmometer, details in Li *et al* (2004). The lowest level of leptin that could be detected by this assay was 1.0·ng·ml–1 when using a 100μl sample.

Statistical analysis

Data were analyzed with the SPSS16.0 software package (Windows version 10.0) by one-way analysis. All values were expressed as mean ± SE and statistical significance was determined at $P < 0.05$.

RESULTS

Body weight, organs weight, serum leptin level, BMR and NST

During the cold exposure, body weight of *T. belangeri* increased gradually and reached a notable level ($P<0.05$) at 21d (Figure 1). Compared to the control, the weight of the liver of 1d was increased insignificantly ($P>0.05$), but increased significantly after 7d ($P<0.05$), and increased significantly by 65.3% ($P<0.01$) at 21d. The weight of heart increased significantly by 16.0% and 29.6% respectively at 14d and 21d ($P<0.01$), the diaphragm and sural muscle stand during the cold exposure, however, the weight of BAT increased by 11.6%,37.2% and 60.5% respectively at 7d,14d and 21d ($P<0.05$) (Figure 2). There has a negative correlation between leptin level and body mass during cold acclimation ($r= -0.62$, $P<0.01$) (Figure 3). BMR increased during the cold exposure. Compared to the control, BMR increased 41.18% in 21d (Figure 4). NST increased during the cold exposure. Compared to the control, NST increased 19.23% in 21d (Figure 5).

The metabolized activity of mitochondria

The change of total protein and mitochondrial protein content

The total protein content of liver increased insignificantly at 1d ($P>0.05$) and significantly at 7d and 21d ($P<0.001$). The mitochondrial protein content of liver had no variance comparing to the control in the beginning of cold exposure ($P>0.05$), however, it increased by 14.9% and 33% respectively at 14d and 21d ($P<0.01$) ; the total protein of heart increased significantly by 18.2% at 21d ($P<0.01$), while the mitochondrial protein by 59.7%; the total protein of diaphragm muscle increased significantly during the cold exposure, and increased by 28.5% at 21d ($P<0.01$) and the mitochondrial protein increased by 19.7% ($P<0.05$); The total protein and mitochondrial protein content of sural muscle changed little during the cold exposure. The total protein of BAT increased by 26.9%, 31.2% and 49.6% at 7d,14d and 21d, respectively ($P<0.001$), the mitochondrial protein content of BAT increased by 16.5%, 47.3% and 67% at 7d,14d and 21d, respectively ($P<0.001$). The uncoupling protein one (UCP1) increased by 6.3%, 19.% and 36% respectively ($P<0.01$) (Figure 6, 7 and 8).

The change of the state* Ⅲ *and state* Ⅳ *respiration of mitochondrial on some organs

The results showed that the state Ⅲ mitochondrial respiration of liver in the1d, 7d, 14d

and 21d groups were more enhanced than that control (P<0.01, 62.76%, 102.89%, 106.84% and 132.86%) but had no significant difference in diaphragm muscle and sural muscle (P>0.05). (Figure 9). The mitochondrial state Ⅳ respiration of some organs was significantly affected by cold exposure. The mitochondrial state Ⅳ respiration of liver begun to increased from 7d and reached a significant level at 14d and 21d, with a 88.5% and 99.0% increase respectively (P<0.01); the mitochondrial state Ⅳ respiration of heart increased by 26.4% (P<0.05), 70.0%, 70.7% and 71.4% (P<0.01) at 1d, 7d, 14d and 21d respectively; the mitochondrial state Ⅳ respiration of diaphragm muscle increased by 49.5%, 41.1% (P<0.05), 68.2% and 121.5% (P<0.01) at 1d, 1d, 7d, 14d and 21d; the mitochondrial state Ⅳ respiration of sural muscle increased by 33.0% and 41.8% (P<0.05) at 7d and 14d respectively; The mitochondrial state Ⅳ respiration of BAT had a significant increase with the extension of cold exposure, and increased by 47.1%, 44.7% and 64.3% at 7d, 14d and 21d respectively (Figure 10).

The change of the mitochondrial cytochrome C oxidase activity of some organs

During the cold exposure, the mitochondrial cytochrome C oxidase activity of liver and muscle (except sural muscle) gradually increased with the extension of cold exposure. The cytochrome C oxidase activity of liver increased significantly by 40.8%,77.8% and 99.2% at 7d,14d and 21d respectively (P<0.01). In the heart, the cytochrome C oxidase activity of 7d,14d and 21d increased by 38.9%,58.5% and 73.0% (P<0.01) respectively at 7d,14d and 21d. However, it changed little in the sural muscle. The cytochrome C oxidase activity of the BAT increased significantly with the extension of cold exposure as well, by 11.6%,31.3%and 60.8% at 7d,14d and 21d respectively (P<0.01) (Figure 11).

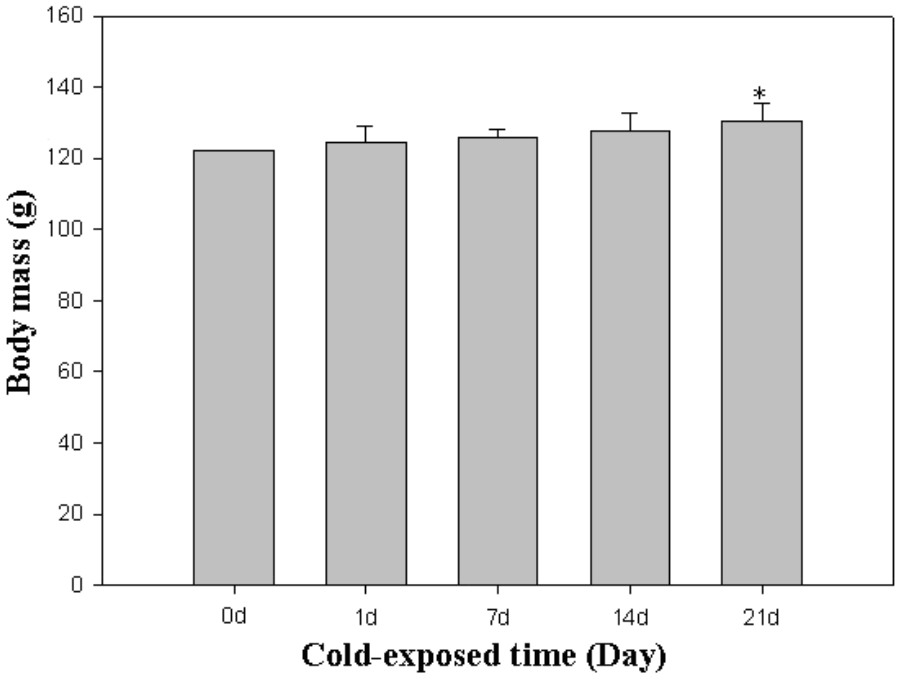

Figure 1 Effects of cold exposure on body mass in tree shrews.
*P<0.05, compared with control

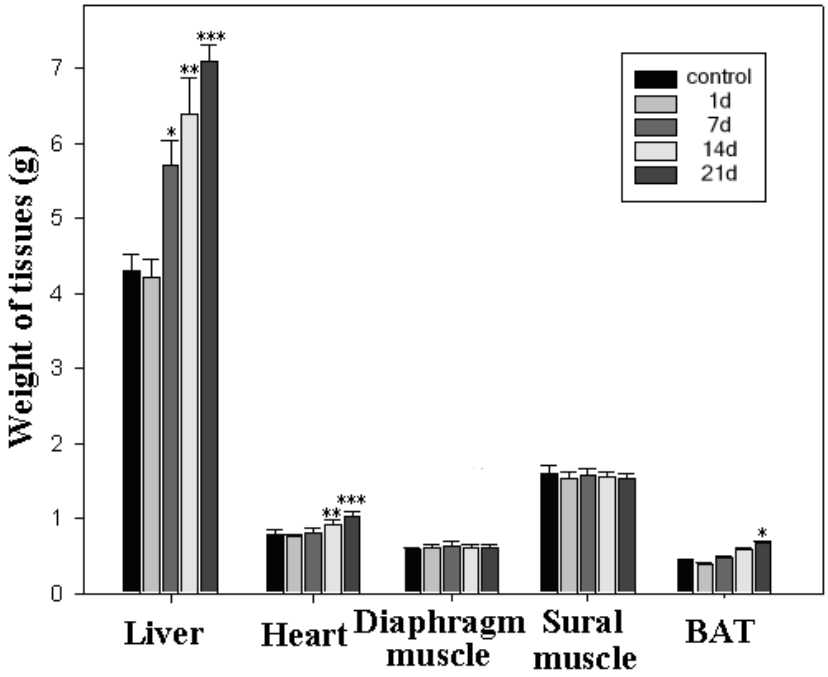

Figure 2 Effects of cold exposure on organ weight in tree shrews.
*P<0.05, **P<0.01, ***P<0.001, compared with control

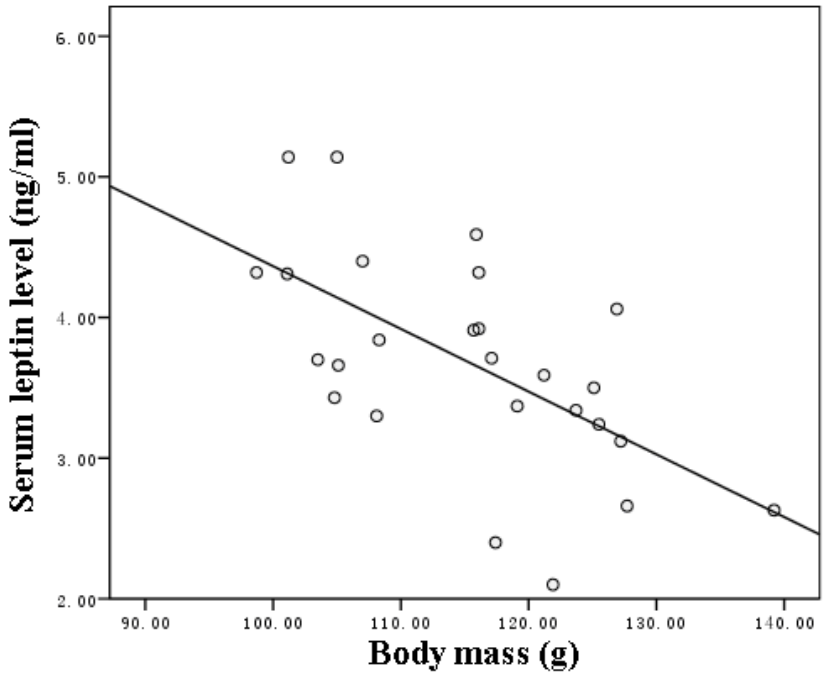

Figure 3 Correlation of serum level concentration with body mass in tree shrews during cold exposure

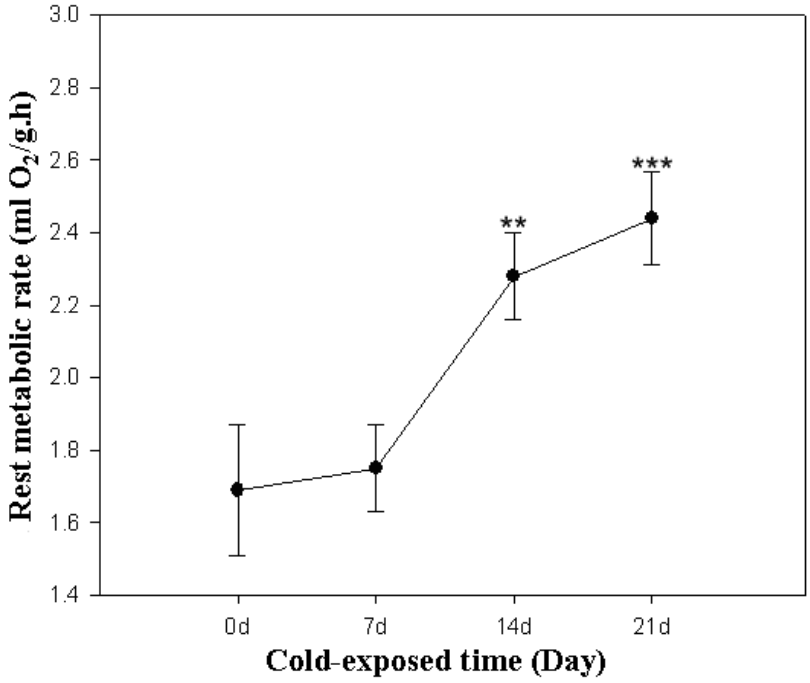

Figure 4 Effects of low temperature and short-lighted on BMR of tree shrew
*P<0.05, **P<0.01, ***P<0.001, compared with control

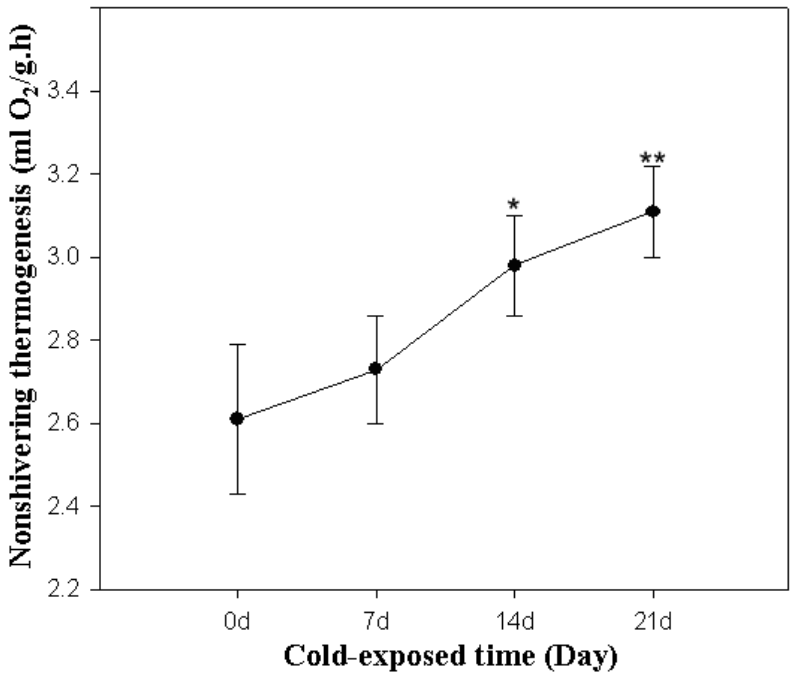

Figure 5 Effects of low temperature and short-lighted on NST of tree shrew
*P<0.05, **P<0.01, ***P<0.001, compared with control

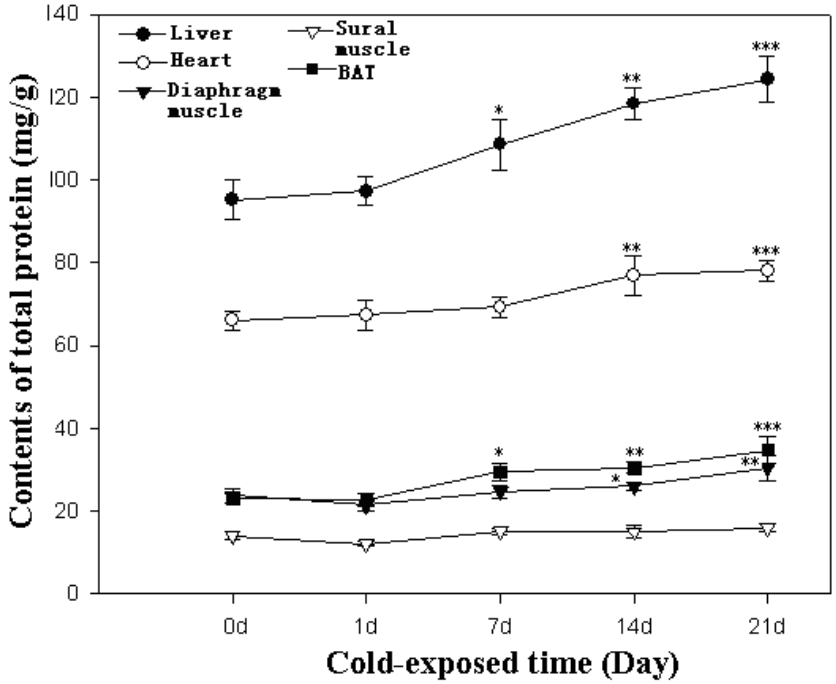

Figure 6 Effects of cold exposure on the content of total protein of organ in tree shrews
*P<0.05, **P<0.01, ***P<0.001, compared with control

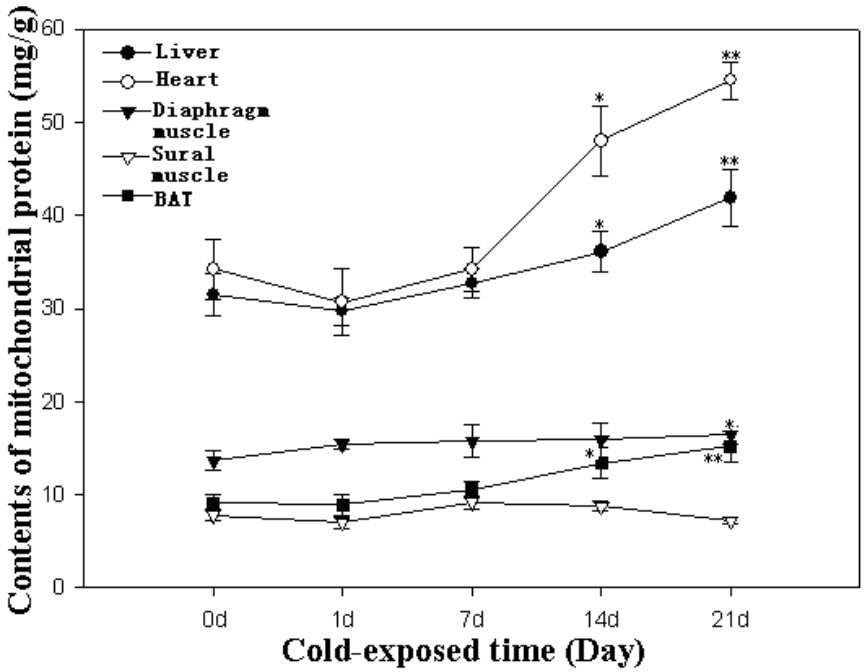

Figure 7 Effects of cold exposure on the content of mitochondrial protein of organ in tree shrews.
*P<0.05, **P<0.01,***P<0.001, compared with control

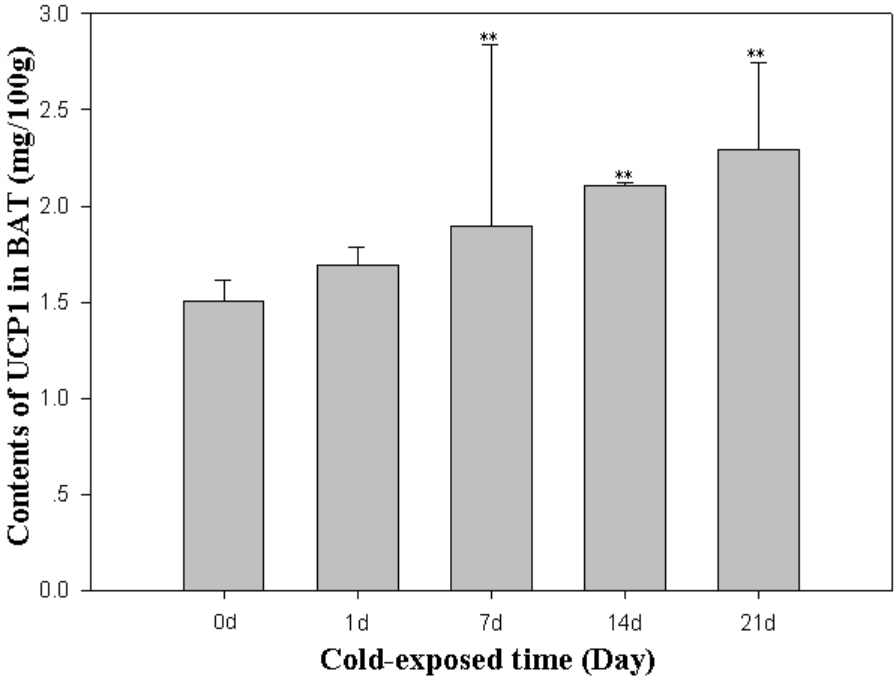

Figure 8 Effects of cold exposure on the content of the uncoupling protein one of BAT in tree shrews.
*P<0.05, **P<0.01,***P<0.001, compared with control

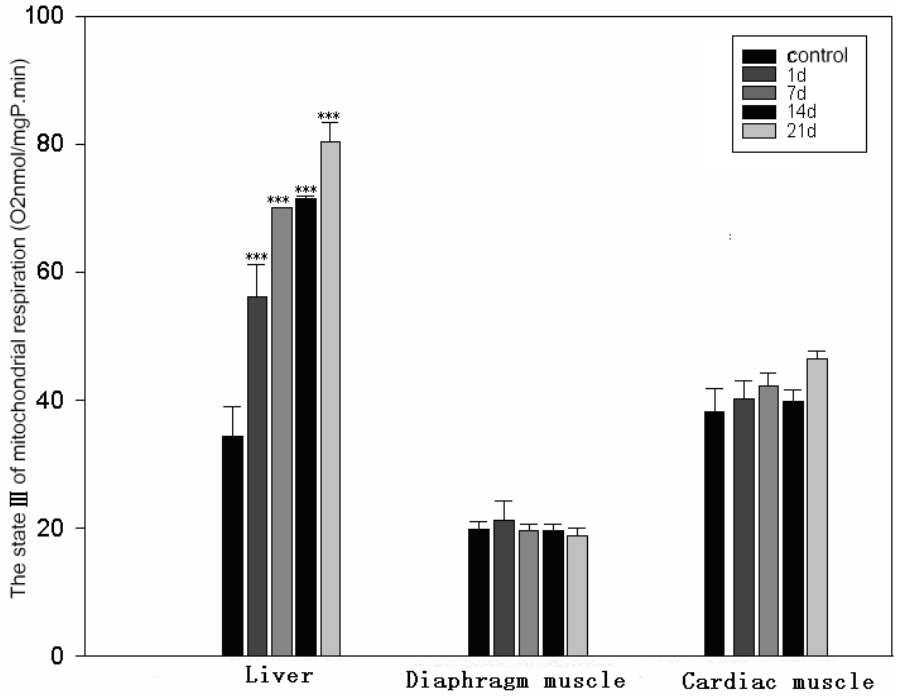

Figure 9 Effects of cold exposure on state Ⅲ of mitochondrial respiration on some tissue in tree shrews.
*P<0.05, **P<0.01,***P<0.001, compared with control

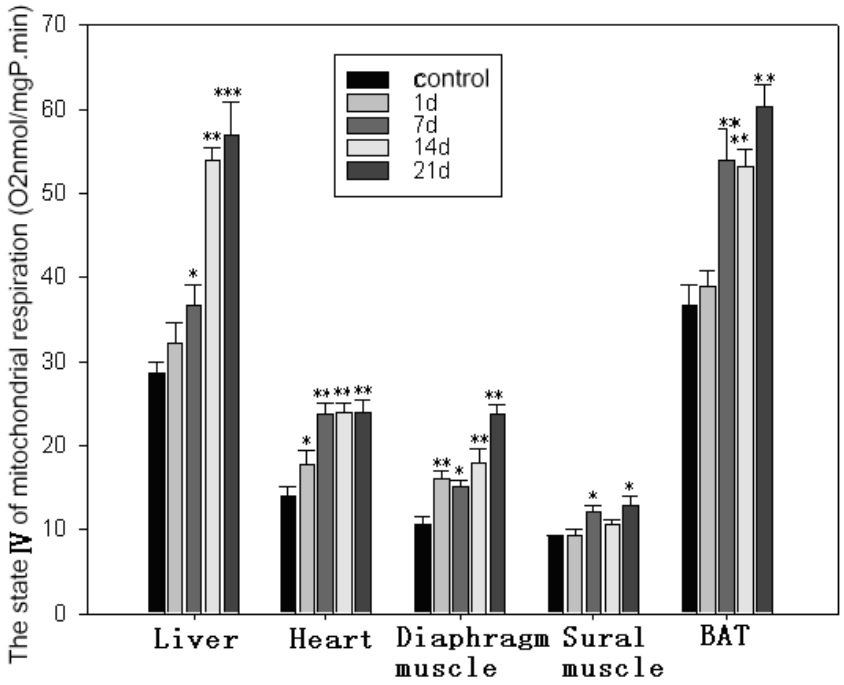

Figure 10 Effects of cold exposure on state Ⅳ of mitochondrial respiration on some tissue in tree shrews.

*P<0.05, **P<0.01,***P<0.001, compared with control

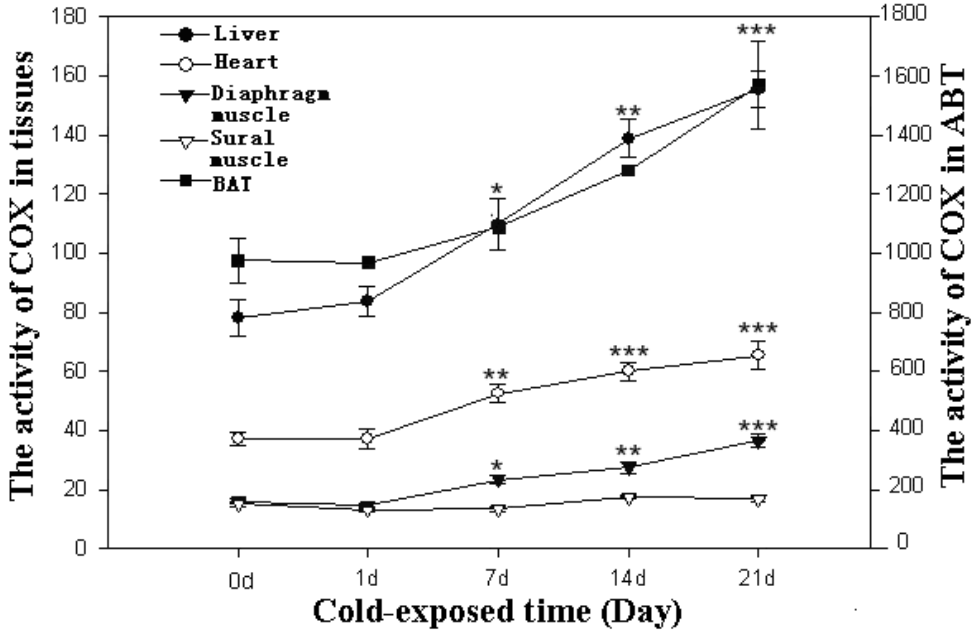

Figure 11 Effects of cold exposure on the activities of cytochrome C in tree shrews.

*P<0.05, **P<0.01,***P<0.001, compared with control

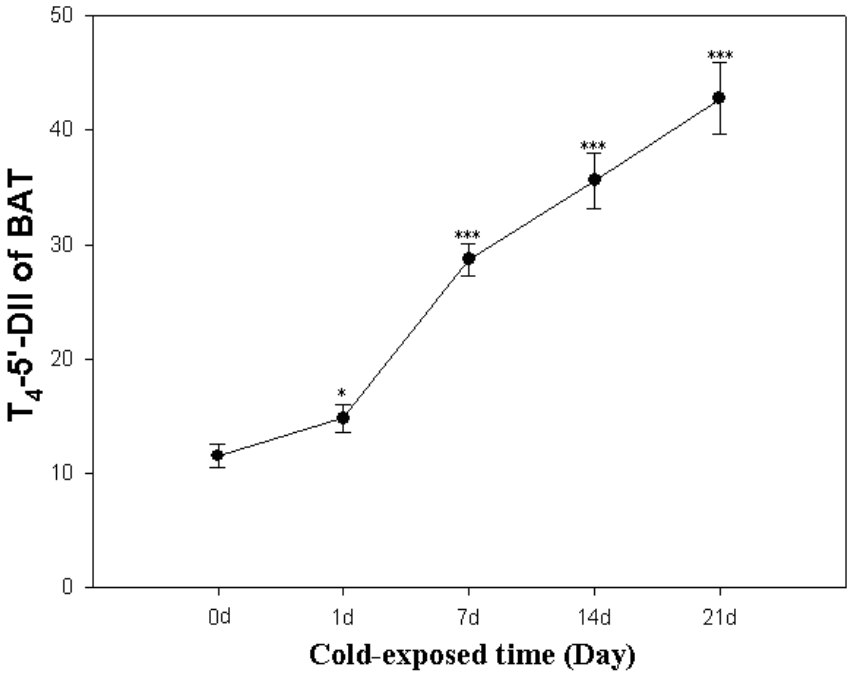

Figure 12 Effects of cold exposure on the activities of T_4-5'-DII of BAT in tree shrews.
*P<0.05, **P<0.01,***P<0.001, compared with control

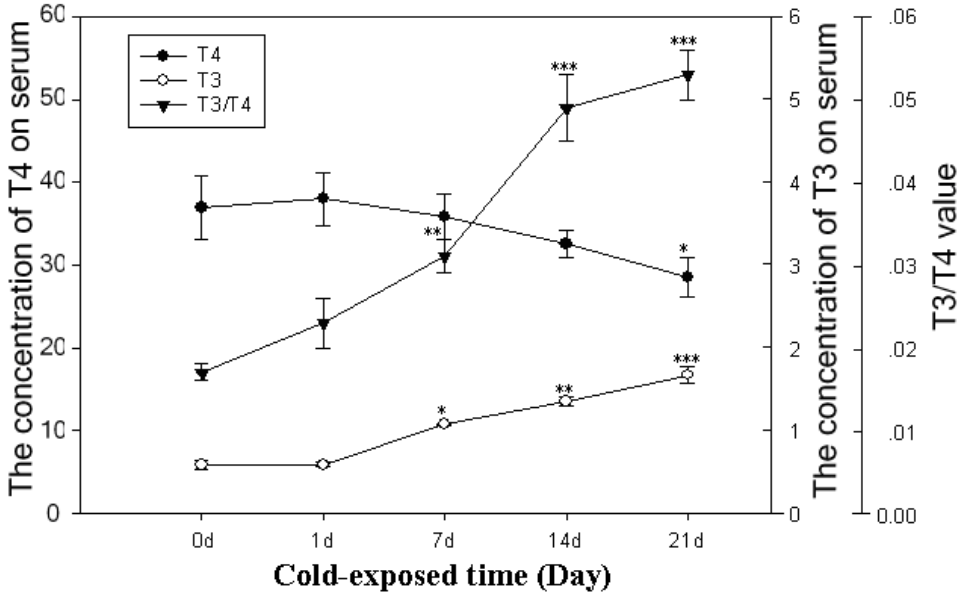

Figure 13 Effects of cold exposure on the concentration of serum thyroid hormones in tree shrews.
*P<0.05, **P<0.01,***P<0.001, compared with control

Table 1 Comparision of the Thermogenesis in 5 species of small mammals

	Cold exposure	BMR (ml O_2/g·h)	NSTmax (ml O_2/g·h)	$\frac{NST\max - BMR}{BMR} \times 100\%$	Reference
Microtus brandti	5℃，30 d	3.57±0.06	9.81±0.13	174.6±2.0(+)	Li *et al*, 1994
Microtus oeconomus	5℃，20 d	2.97±0.29	8.80±0.61	196.3±11.0(+)	Wang *et al*, 1996
Ochotona curzoniae	5℃，28 d	2.19±0.10	3.95±0.21	80.36±9.1(+)	Liu and Li, 1996
Apodemus chevrieri	5℃，28 d	3.81±0.15	5.88±0.24	55.46±7.9(-)	Jiang *et al*, 2009
Eothenomys miletus	5℃，28 d	3.42±0.27	5.93±0.3	73.39±5.5(-)	Zhu *et al*, 2008
Tupaia belangeri	5℃，21 d	2.42±0.09	3.13±0.10	29.33±3.6(-)	This study

"+"express raised, "-" express dropped.

T_4-5' deiodinase activity of BAT

The T_4-5' deiodinase activity of BAT increased with the extension of cold exposure, significantly ($P<0.05$) in the cold exposure of 1d, and by 272% at 21d (Figure 12).

The hypothyroid hormone in serum

During the cold exposure, the concentration of T_4 in serum decreased slowly and had a negative relation with the time of cold exposure (T_4=37.064-0.3161t, r=-0.33684,P=0.0415), while the T3 increased, and the regression equation of T3 level and the time of cold exposure can be described as T3=0.7540+0.0436t (r=0.8889,P<0.001). T_3/T_4 (T') had a persistent increase as well (T_3/T_4=0.0210+0.00167t, r=0.8443, P<0.001) (Figure 13).

DISCUSSION

Body weight, serum leptin level, BMR and NST

The seasonal change of body mass was the adaptation of energy intake and energy demand in many small mammals (Speakman *et al*, 2003). Many physiological parameters were influenced during cold stress, such as body mass. The change of body mass related with increase the ability of adopt cold environment for mammals (Swanson, 2001).There had lots of studies about body mass, especially in rodents (Heldmaier *et al*, 1982). Body mass in *T. belangeri* gradually increased during the cold acclimation, the maximal difference appeared in 21days, in other studies it showed similar results, such as *Dicrostonga groenlandicus* and *Mesocricetus auratu* (Nagy and Negus, 1993).

Energy metabolism of small rodents always influences species distribution, abundance, reproduction and adaptation (Speakman, 2007). Body mass were influenced by energy intake, energy consume and digestion (Ernest, 2005), so body mass were lie on the balance of energy intake and consume. BMR and NST increased during cold acclimation, it indicated that increase energy intake and thermogenesis were a strategy to reply in a low temperature (Johnstone *et al*, 2005).When serum leptin level decreased, NST increased during cold acclimation, they have a significant negative correlation. Leptin may participate in the regulation of energy balance; maintain a relative balance of thermogenesis. On the other hand, when the energy intake is not satisfy to the require of thermogenesis, mammals need to employ fat to apply the energy expenditure of thermogenesis (Nieminen and Hyvarinen, 2000). So leptin is hypothesized to contribute to the maintenance of body mass by

altering energy intake and energy expenditure (Friedman and Halls, 1998). It is an important strategy for *T. belangeri* to survive in Hengduan mountains region.

Furthermore, there were two hypothesis of origin in tree shrew. One is mainland origin; the other is Bornean origin of tree shrew. A mainland origin may be better supported by fossil evidence (Jacobs, 1980). There were also some evidences from the distribution of living scandentians is equally supportive of a Bornean origin of tree shrews (Olson *et al*, 2005). If the tree shrew were originated in mainland, the contribution of thermogenesis of NST may similar to the mammals in north. But in our studies, the contribution of thermogenesis of NST were different to the mammals in north (Table 1), and the ratio of (NST-BMR) /BMR were also lower than *Eothenomys miletus* and *Apodemus chevrieri* in Hengduan maintains region, it may indicate that tree shrew were diffused from south to north, and it is supportive of a Bornean origin of tree shrews indirectly.

Thermogenesis of Liver

During the cold exposure, the weight of liver in tree shrews were significant increased (Fig 2).The trend was similar with the change of the seasonal BMR (Wang *et al*, 1994) and cold acclimation (Wang, 1995) in tree shrews. BMR and the weight of the liver of Rodents in cold exposure also increased, such as *Gerbillus campestris* (Oufara *et al*, 1988), *Eliomys querinus* (Lanni *et al*, 1990), *Microtus agrestis* (McDevitt and Speakman, 1994a). So the increase of BMR was related with the increase of liver (McDevitt and Speakman, 1994a, 1994b).

During the cold-induce, the content of total protein of liver in *Gerbillus campestris* and the content of mitochondrial protein in *Eliomys querinus* were significant increased, the increase of the proportion of mitochondrial protein were higher than that of total protein, it may related with the increase of BMR in cold temperature (Lanni *et al*, 1990); during the cold exposure, the content of total protein and mitochondrial protein of liver in tree shrews were increased. The trend was accord with the change of its BMR. Therefore, the increases of the content of total protein of liver in tree shrews, especially of mitochondrial protein were importance factors of increase thermogenesis.

State Ⅲ of mitochondrial respiration of liver in *T. belangeri* gradually increased during the cold acclimation, it may related with the increased of synthesize of ATP. The state Ⅳ of mitochondrial respiration were influenced by the cold temperature, the state Ⅳ of mitochondrial respiration were increase during the cold exposure. To 21 day, it was the 198.95% of the control. Mitochondrial respirations of liver were positive correlation with the cold exposure. The change was accordant to its BMR. Commonly, about 70% of BMR were from bowel organizes (Claussen *et al*, 1991), especially for liver, its thermogenesis occupy total BMR 20~25％ (Brand *et al*, 1991). So the increase of the thermogenesis in tree shrews were sometimes relative with the increase of its mitochondrial oxidation ability, in rodents has similar phenomenon, such as *Eliomys querinus* (85.9%) (Lanni *et al*, 1990); *Gerbillus campestris* (95.3%) (Oufara *et al*, 1988). The activities of cytochrome C in tree shrews were influence by cold exposure, to 21day, it was 198.7% of the control, and it was positive correlation with the cold exposure. The trend was similar with the mitochondrial oxidation ability.

Thermogenesis of heart

In the experiments of the rodent animals, durable cold exposition greatly influenced the animal cardiovascular system (Gordon, 1993). For the cold tractable little white rat, the amount of pulse output

and coronary artery increased (Hirata and Nagaska, 1981). The content of the myoglobin (Gordon, 1993) and the oxygen consumption of the cardiac muscle of heart cells obviously increased (Claussen *et al*, 1991). These change may had a close correlation with the increase of thermogenesis and acceleration of blood cycle of the animal in the low temperature (Gordon, 1993). During the durable low temperature treatment cardiac muscle mitochondrial of *tupaia belangeri*, heart weight and the activity of cytochrome C oxidase both obviously enhanced except the enhancement of the state Ⅳ and the increase of the mitochondrial protein content (Wang, 1996).

Thermogenesis of skeleton muscle

The thermogenesis of the muscle played an important role in the animal adaption in the low temperature, which had a correlation with the larger proportion of the muscle in the animal (Claussen *et al*, 1991). During the durable low temperature treatment diaphragm muscle mitochondrial respiration of *T. belangeri*, the activity of cytochrome C oxidase and the mitochondrial content of protein which means the diaphragm muscle contributing to the thermogenesis of *T. belangeri* during the cold-exposed (Wang, 1995). These may indicated the difference of the thermogenesis type between the *T. belangeri* and the typical north small mammal under cold intimidation.

Thermogenesis of BAT

The weight of BAT in tree shrews was positive correlation with the cold exposure, but it was different to other tree shrews live in torrid zone, theirs BAT didn't increase during cold exposure (Chaffee *et al*, 1969). It indicated that during the diffused process, effects of BAT in thermogenesis were bigger than that live in torrid zone, appear adapt characters of some mammals live in high latitude area. Simultaneity, the content of total protein and mitochondrial protein of liver in tree shrews were increased, increase the mitochondrial oxidation ability and the activities of cytochrome C, were positive correlation with the cold exposure, similar with type north some small mammals, such as *Phodopus sungorus* added 10 doubles; *Apodemus* (3 times) ; *Gerbillus campertris* (4 times), the stage Ⅳ added 60% (Oufara *et al*, 1988). Therefore, BAT have important adaptive significance for them to cope with cold exposure.

NST were increased during cold exposure, it was decided by the increase of UCP in mitochondrial inner membrane (Kluas *et al*, 1991) ; cold-stress may stimulate the increase of the UCP synthesize (Jaccobsson *et al*, 1994) ; lead the increase of the uncoupling thermogenesis (Himms-Hagen, 1990). In our study, the content of UCP of BAT in tree shrews was positive correlation with the cold exposure. To 21 day, it is 142.7% of the control. It indicated that during cold exposure (Wang *et al*, 1995) or winter (Wang *et al*, 1994), the increase of NST was related with its increase of UCP protein. The trend model and the change of NST (Wang *et al,* 1995) were similar with that in laboratory or type northern mammals. Thereby, during cold exposure the change model of UCP may reflect a diffused model that tropic species diffuse to northern or high altitude area.

BAT T_4-5'-DⅡ

During the cold exposure, the activities of T_4-5'-DⅡ of BAT in tree shrews were significant increased, and may had important effect on the regulation of T_3 level in blood (Raasmaja, 1990), such as, T_3 level in blood of Hamster added 6-8times (Wu *et al,* 1987); T_4-5'-DⅡ of Rat added 5.9 times (Pazos-Moura *et al,* 1991); New Zealand White Rabbit (3.65 times) (Brzezinska and Slebodinski, 1993). The trend of the activities of T_4-5'-DⅡ was similar with that of T_3 level in blood. To 28 day, the activities of T_4-5'-DⅡ was added 2.42 times, had

positive correlation with the cold exposure. So T_4-5'-DII of BAT may had important effect on the regulation of T_3 level in blood.

During the cold exposure, the activities of T_4-5'-DII of BAT in tree shrews may had important effect on the thermoregulation in BAT. The increase of the activities of T_4-5'-DII lead the increase of T_3 level in blood (Bianco *et al*, 1992), increased the content of UCP (Rabelo *et al*, 1995). However, the increase proportions of the activities of T_4-5'-DII were lower than that of some rodents, such as, after cold exposure (8 hours), the activities of T_4-5'-DII of Rat added 20 times (Jones *et al*, 1986). In tree shrews, to 1 day, the activities of T_4-5'-DII added 28.7%, persisted and maintain higher level. To 21 day, it added 2.72 times. Thus, the change model of the activities of T_4-5'-DII was different to that of laboratory or type northern mammals. The activities of T_4-5'-DII always mushroom in a short time of laboratory or type northern mammals (Jones *et al*, 1986). *T. belangeri* was typical tropic specie, now here rare studies to research the thermoregulation of tropic small mammals, especially for the activities of T_4-5'-DII during cold exposure. Tropic species have low NST and thermogenesis of BAT (Haim and Izhaki, 1993). *T. belangeri* were distributed northernmost, the character of thermogenesis may reserved some characters of tropic mammals, also appeared character of thermogenesis of mammals live in temperate zone.

Concentration of serum thyroid hormones

The manage function of serum thyroid hormones was to stimulate cell to enhance thermogenesis (McNabb, 1992), the level of serum thyroid hormones reflect the characters of thermoregulation in cold condition (Tomasi and Michell, 1994). In our study, the level of T_4 were decreased or held the line, but the level of T_3 were significant increased during cold exposure, it may adapt cold condition (Gordon, 1993), such as, after 4 weeks cold exposure, BMR of *Mesocricetus auratus* added 17%, the level of T_3 added 13 times (Tomasi and Michell, 1994) ; similar phenomenon in *Scalopus aquaticus* (Leach *et al*, 1962). The level of T_4 were decreased or held the line simultaneity. To 21 day, the concentration of serum thyroid hormones in tree shrews added 189%, the level of T_3 was positive correlation with the cold exposure; but the level of T_4 was negative correlation with the cold exposure. It may relate with the increase of T_4 using rate, lead to decrease of the level of T_4 (Tomasi, 1991). So the level of T_3 was an important stimulus regulator during the cold surrounding.

In conclusion, the ability of thermogenesis, liver, muscle, BAT, heart and other organs in *T. belangeri* increased. It was the biochemistry foundation for it to enhance BMR, the ability of thermogenesis of liver and BAT had significant increased, was one of important adapt strategies in *T. belangeri* under cold exposure.

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