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Brown adipose tissue plays thermoregulatory role within the thermoneutral zone in Mongolian gerbils *(Meriones unguiculatus)*

Yang-Yang Guo[a,b], Qing-Sheng Chi[a], Xue-Ying Zhang[a,b], Wei Liu[a,b], Shao-Yan Hao[c], De-Hua Wang[a,b,*]

[a] State Key laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Hebei University; Shijiazhuang 050024, China

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ABSTRACT

Brown adipose tissue (BAT) plays an important role in thermoregulation and many metabolic processes in small mammals, especially in cold adaptation. However, in warm adaptation, ambient temperature cannot directly activate BAT by sympathetic nervous system. Mongolian gerbils exhibit a wider thermoneutral zone (26.5–38.9 °C). We hypothesized that BAT atrophied near the lower critical temperature and further atrophied near the upper critical temperature. Male gerbils were acclimated to 23 °C, 27 °C or 37 °C, respectively, for 3 weeks. Results showed that regulatory non-shivering thermogenesis did not change in gerbils acclimated to 27 °C compared with 23 °C group, whereas it was reduced by 43.5% in gerbils acclimated to 37 °C. Bigger lipid droplet in BAT was observed in gerbils acclimated to 27 °C and 37 °C compared with 23 °C group, while the expression of uncoupling protein 1 and tyrosine hydroxylase was only reduced in gerbils acclimated to 37 °C. Further, thermoneutral acclimation did not change BAT thermogenesis by down-regulation of peroxisome proliferator-activated receptor gamma coactivator-1α, PR domain containing 16, peroxisome proliferator-activated receptor-α or peroxisome proliferator activated receptor-γ gene expression in BAT. In addition, body temperature was reduced in gerbils acclimated to 37 °C compared with 23 °C group, which was associated with a decreased resting metabolic rate and regulatory non-shivering thermogenesis. In conclusion, BAT does not atrophy near the lower critical temperature, whereas it atrophies near the upper critical temperature, suggesting that BAT may play thermoregulatory role within the TNZ in Mongolian gerbils.

1. Introduction

Brown adipose tissue (BAT) plays an important role in thermoregulation of small mammals in cold environment (Cannon and Nedergaard, 2004; Oelkrug et al., 2015). It is specialized in thermogenesis by uncoupling oxidative phosphorylation of uncoupling protein 1 (UCP1) in mitochondria (Nicholls and Locke, 1984; Fedorenko et al., 2012). BAT generates heat with fatty acids and glucose as substrate in response to cold or excess feeding, thus also playing critical roles in energy balance, glucose homeostasis and triglyceride clearance (Stock, 1989; Bartelt et al., 2011; Stanford et al., 2012; Albert et al., 2016).

BAT evolves as a thermogenic organ whose activation and functional are associated with ambient temperature (T_a) (Cannon and Nedergaard, 2004; Oelkrug et al., 2015). Its thermogenesis is controlled by central nervous networks (Morrison et al., 2012, 2014). Cold exposure activates BAT by stimulating the sympathetic nervous system (SNS) to release noradrenaline (Thomas and Palmiter, 1997; Contreras et al., 2015). Moreover, prolonged cold exposure leads to enhanced BAT thermogenic capacity by increasing UCP1 expression, mitochondrial biogenesis, angiogenesis, sympathetic nerve fiber density and proliferation (Bukowiecki et al., 1982; Murano et al., 2009; Xue et al., 2009; Nedergaard and Cannon, 2013). Mechanistically, the transcriptional factors, such as PR domain containing 16 and peroxisome proliferator activated receptor-γ, contribute to these adaptive changes in BAT (Lowell and Spiegelman, 2000; Seale, 2015). However, less is known about the effects of warm exposure on BAT.

Thermoneutral zone (TNZ) defines a range of T_a at which thermoregulation is achieved without regulatory changes in metabolic heat production or evaporative heat loss (IUPS Thermal Commission, 2001). Within this range, temperature cannot directly induce BAT thermogenesis by activating the SNS (Nakamura and Morrison, 2010). Recent studies also demonstrate that chow-fed mice housed around 30 °C

* Correspondence to: Institute of Zoology, Chinese Academy of Sciences, No.1 Beichen Xilu, Chaoyang, Beijing 100101, China.
E-mail address: wangdh@ioz.ac.cn (D.-H. Wang).

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shows decreased expression of UCP1 and thermogenic program in BAT (Cui et al., 2016; Small et al., 2018). However, these studies cannot reflect the complete responses of BAT within the TNZ. Mice exhibit a weight-dependent lower critical temperature (LCT, 26–28 °C), and the upper critical temperature (UCT) is around 32 °C (Gordon, 2012; Speakman and Keijer, 2013). Moreover, overall metabolic status other than temperature may also mediate remodeling of BAT function (Tupone et al., 2014). UCP1-ablated induces obesity in mice housed at 29 °C, suggesting that BAT plays a role in energy balance within the TNZ (Feldmann et al., 2009). In addition, studies have showed that rats exposed to T_a within the TNZ exhibit similar signs of thermal stress, such as elevated body temperature and dilated tail vessel (Swift and Forbes, 1939; Hainsworth, 1967; Romanovsky et al., 2002). Further, chronic thermal stress above TNZ results in reduction of BAT thermogenic capacity in rats (Rousset et al., 1984). Therefore, T_a within the TNZ may have different effects on BAT.

Mongolian gerbils (*Meriones unguiculatus*) are widely distributed in semi-arid steppes, desert grasslands, and agricultural fields of Northern China, Mongolia and Russia's Baikal area (Wilson and Reeder, 2005; Mallon, 2010). This species exhibits intraspecific variations in the TNZ characteristics with LCT values of 28–32 °C and UCT values of 32–39 °C (Gordon, 1993). We previously showed that Mongolian gerbils had a wide TNZ, from 26.5° to 38.9°C (Wang et al., 2000; Pan et al., 2014). Within the TNZ, the metabolic rate is minimal and stable, while the body temperature is elevated significantly when the T_a is above 34 °C (Wang et al., 2000). Furthermore, high concentrations of oxidative stress-related metabolites are observed in the liver and serum of gerbils exposed to 38 °C (Shi and Wang, 2016). We hypothesized that BAT atrophied near the LCT and further atrophied near the UCT.

2. Material and Methods

2.1. Animals

All animal experiments were licensed by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Mongolian gerbils in this study were from our laboratory colony, which were the offspring of Mongolian gerbils captured in the grasslands of Inner Mongolia and raised in the animal room at 23 ± 1 °C under a photoperiod of 16 L:8D (Light on at 0400 h) with ad libitum access to commercial standard rat pellet chow (Beijing KeAoXieLi Feed Co.) and water.

Male gerbils, 6 or 7 months old, were single-housed in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding for 2 weeks before temperature acclimation. They had an ad libitum access to food and water throughout the experiments. Three independent sets of gerbils (n = 9 per group) were used in each experiment. The control group was acclimated to 23 ± 1 °C, while the other two groups were acclimated to 27 ± 1 °C or 37 ± 1 °C for three weeks, respectively.

2.2. Body mass, food Intake and body temperature

During the acclimation, gerbils were weighed using a digital balance (PL2001-L, to 0.1 g, Mettler Toledo, Switzerland) every 3 days. Daily food intake was measured at the middle and the end of the acclimation (10th to 12th day and 9th to 21st day). To measure the food intake, 50 g food was provided to each gerbil. After 3 days, the food residues were collected and weighed. Food intake was calculated from the difference between food provided and food residues. After the acclimation, body temperature (rectal temperature) was measured using a digital thermometer (TES-1310, TES Electrical Electronic Corp, China). The probe of the thermometer was inserted 3 cm into the rectum and the highest stable temperature reading was taken within 30 s. To determine whether the body temperature pattern among groups was stable, we measured T_b at the end of each metabolic trial.

2.3. Metabolic trials

To investigate the effect of thermoneutral acclimation on thermogenic capacity of BAT, resting metabolic rate (RMR) and non-shivering thermogenesis (NST) were determined. The regulatory NST was calculated from the difference between max NST and RMR. RMR was determined using an open-flow respiratory system (TSE LabMaster Calorimetry System, Germany) after the acclimation. The gerbils were placed in respiratory chambers individually (TSE, type I for mice, volume 2.7 L). The temperature of the chamber was maintained at 30 ± 0.5 °C using an incubator (Sanyo, MIR-554). The incurrent flow rate was 0.8 L/min and the sample rate was 0.39 L/min. Each RMR measurement was lasting for 3 h and no food or water was provided during the measurement. RMR of each individual was calculated by averaging 12-min least variable and lowest readings of oxygen consumption (Pan et al., 2014).

NST was determined using the same open-flow respiratory system the next day. The temperature of the metabolic chamber was maintained at 25 ± 0.5 °C. The maximum capacity for NST was induced by a subcutaneous injection of noradrenaline (NA) (Shanghai Harvest Pharmaceutical Co. Ltd). The dosage of NA was calculated according to the equation: NA dosage (mg/kg) = $6.6\,W^{-0.458}$, where W is body mass (g) (Heldmaier, 1971; Li and Wang, 2005; Wang and Wang, 2006). Each NST measurement was lasting for 1 h. Maximum NST (max NST) of each individual was calculated by averaging 3-min readings of the highest oxygen consumption (Heldmaier et al., 1982). Body mass was measured after each metabolic trial. All measurements were performed between 08:00 and 20:00 h.

2.4. Body composition analysis

After NST measurement, all gerbils were sacrificed by CO_2 asphyxiation. Interscapular brown adipose tissue (BAT), heart, liver, lung and kidneys were rapidly removed and weighed (± 1 mg). Then brain and digestive tracts, including stomach, small intestine and large intestine were also removed. The carcass was put into the oven at 60 °C, and dried to constant weight. Total body fat was extracted from the dried carcass using a Soxhlet apparatus (Soxtex Avanti 2055, Sweden).

2.5. Histological analysis

To further investigate potential mechanisms underlying changes in thermogenic capacity of BAT, BAT morphology was analyzed. A part of BAT was fixed in Bouin's solution for 24 h. The remaining BAT was immediately frozen in liquid nitrogen and stored at − 80 °C for other analyses. Then, BAT samples were dehydrated in ethanol and embedded in paraffin. Paraffin-embedded BAT was cut into 6-μm sections. Sections were stained with hematoxylin and eosin. The sections were examined by Nikon Eclipse 80i microscope. The size of lipid droplet and cell density in BAT were quantified by analyzing pictures taken at 200 × magnification.

2.6. Protein isolation and Western blot

Protein contents of UCP1 and tyrosine hydroxylase (TH) in BAT were quantified by western blotting (Zhao and Wang, 2005; Liu et al., 2012). The BAT samples were lysed in a RIPA buffer (Bordone et al., 2006). Then the BAT lysate was centrifuged at 12,000 rpm for 10 min. Total protein concentrations of BAT were measured by the Folin phenol method (Lowry et al., 1951). Proteins (60 mg) were separated in a 10% polyacrylamide gel (4% stacking gel and 10% running gel) and then transferred onto PVDF membranes (Millipore, USA, IPVH00010). The PVDF membranes were blocked using non-fat dried milk for 1.5 h and incubated overnight at 4 °C with the primary antibody glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (30201ES60, Yeasen, Shanghai, China), TH (AB152, Millipore, Darmstadt, Germany) or UCP1

Table 1
Primers used for real-time PCR.

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
UCP1	AGCCATCTGCATGGGATCAAA	GGGTCGTCCCTTTCCAAAGTG
PGC-1α	TGCTAGCGGTTCTCACAGAG	AGTGCTAAGACCGCTGCATT
PRDM16	CCACCAGCGAGGACTTCAC	GGAGGACTCTCGTAGCTCGAA
PPARα	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
PPARγ	CTCCAAGAATACCAAAGTGCGA	GCCTGATGCTTTATCCCCACA
GAPDH	AGTATGACTCTACCCACGGC	ACTCCACAACATACTCGGCA

(ab10983, Abcam, Cambridge, UK). Secondary antibody with the appropriate horseradish peroxidase-linked was added. Finally, the PVDF membranes were visualized with ECL (Beyotime, China). Blots were analyzed with *Image-Pro ® Plus* version 6.0 and normalized to GAPDH.

2.7. RNA isolation and qPCR

To avoid the effects of other experiments on thermogenesis-related gene expression in BAT, another 27 gerbils ($n = 9$) were acclimated to 23 ± 1 °C, 27 ± 1 °C or 37 ± 1 °C for 3 weeks, respectively. After the acclimation, all subjects were sacrificed by CO2 asphyxiation. The BAT was rapidly removed and frozen in liquid nitrogen. Total RNA was isolated using TRIzol® reagent (15596026, Invitrogen, USA) according to the manufacturer's instructions. Then 1000 ng total RNA was purified and reverse-transcribed to cDNA using the PrimeScript RT reagent Kit with gDNA Eraser (RR047A, TaKaRa, Japan). Real-time PCR was carried out on Mx3000P quantitative PCR system (Stratagene, La Jolla, CA, USA) using TB Green *Premix Ex Taq* II (RR820A, TaKaRa, Japan). Real-time PCR primer sequences showed in Table 1 were designed according to sequences of homologous genes in mice. Homology analysis showed that PCR products using these primers came from the target genes. All reactions were carried out using the following cycling parameters: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 61 °C for 1 min; 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 1 min. Relative gene expression was determined by the comparative C_T method (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008).

2.8. Statistical analyses

All data were examined for normality using the Shapiro-Wilk test. Body mass during the acclimation were analyzed using repeated measures ANOVA followed LSD comparison. Daily food intake, RMR, max NST, regulatory NST, organ mass and body composition were analyzed using one-way ANCOVA with body mass as the covariate followed Bonferroni comparisons. Correlations between body temperature and residuals of RMR or regulatory NST were made using Pearson's correlation. Body temperature, morphology of BAT, protein content and genes mRNA expression were analyzed using one-way ANOVA. When equal variance was determined, analysis of variance was performed by Tukey's *post hoc* test. In case of unequal variances, Dunnett's *post hoc* test was followed. Difference at P values < 0.05 was considered to be statistically significant in all tests. Statistical analyses were performed using IBM SPSS.v.20.0 software. Data were presented as means ± SE.

3. Results

3.1. Body mass and food intake

Gerbils acclimated to 23 °C or 27 °C showed stable body mass, while gerbils acclimated to 37 °C showed reduction in body mass from the 9th day of acclimation (group × time effect, $F_{14, 168} = 3.624$, $P < 0.001$; time effect, 23 °C, $F_{7, 56} = 0.778$, $P = 0.608$; time effect, 27 °C, $F_{7, 56} = 0.624$, $P = 0.734$; time effect, 37 °C, $F_{7, 56} = 5.869$, $P < 0.001$; repeated measures ANOVA; Fig. 1A). In addition, a significant difference in daily food intake among groups was observed (Fig. 1B). In the middle of acclimation, average daily food intake was reduced significantly by 18.5% and 51.3% in gerbils acclimated to 27 °C and 37 °C compared with 23 °C group, respectively ($F_{2, 23} = 45.375$, $P < 0.001$; ANCOVA using body mass as the covariate). Similarly, At the end of acclimation, average daily food intake was reduced by 23.2% and 51.2% in gerbils acclimated to 27 °C and 37 °C compared with 23 °C group, respectively ($F_{2, 23} = 26.825$, $P < 0.001$).

3.2. Body temperature

After the acclimation, there was no difference in body temperature (T_b) of gerbils acclimated to 27 °C compared with 23 °C group, while average T_b decreased by 1.0 °C in gerbils acclimated to 37 °C ($F_{2, 24} = 9.154$, $P = 0.001$; One-way ANOVA; Fig. 2A). A similar T_b pattern was seen after RMR measurement, there was no difference in T_b of gerbils acclimated to 27 °C compared with 23 °C group, while an average reduction of 1.7 °C was observed in gerbils acclimated to 37 °C ($F_{2, 24} = 7.874$, $P < 0.05$; One-way ANOVA; Fig. 2B). However, after NST measurement, there was no difference in T_b among groups ($F_{2, 24} = 2.697$, $P = 0.088$; Fig. 2B).

3.3. RMR and NST

There was no difference in RMR and NST of gerbils acclimated to 27 °C compared with 23 °C group, while RMR and max NST were decreased by 49.4% and 45.1% in gerbils acclimated to 37 °C, respectively (RMR, $F_{2, 23} = 10.274$, $P = 0.001$; Max NST, $F_{2, 23} = 65.698$, $P < 0.001$; ANCOVA using body mass as the covariate; Fig. 3A). Furthermore, there was no difference in regulatory NST of gerbils acclimated to 27 °C compared with 23 °C group, while regulatory NST was decreased by 43.5% in gerbils acclimated to 37 °C ($F_{2, 23} = 27.729$, $P < 0.001$; ANCOVA using body mass as the covariate; Fig. 3B).

3.4. Body temperature in relation to RMR and regulatory NST

Pearson correlation analysis showed that the body temperature of gerbils after acclimation was positive correlated with residuals of RMR ($r = 0.518$, $P = 0.006$, Fig. 4A) and regulatory NST ($r = 0.394$, $P = 0.042$, Fig. 4B).

3.5. Organ mass and body composition

There was no significant difference in BAT mass, liver mass and lung mass between groups (BAT mass, $F_{2, 23} = 1.401$, $P = 0.$ 0.267; liver mass, $F_{2, 23} = 0.634$, $P = 0.539$; lung mass, $F_{2, 23} = 2.077$, $P = 0.148$; ANCOVA using body mass as the covariate; Table 2). Acclimation at 37 °C decreased heart mass and kidney mass compared with acclimation at 23 °C (heart mass, $F_{2, 23} = 7.106$, $P < 0.$ 05; kidney mass, $F_{2, 23} = 0.634$, $P < 0.05$; ANCOVA using body mass as the covariate; Table 2). In addition, there was no significant difference in dry carcass mass, body water mass, body fat mass and lean body mass (dry carcass mass, $F_{2, 23} = 3.057$, $P = 0.$ 066; body water mass, $F_{2, 23} = 0.125$, $P = 0.883$; body fat mass, $F_{2, 23} = 1.173$, $P = 0.327$; lean body mass, $F_{2, 23} = 1.173$, $P = 0.327$; ANCOVA using body mass as the covariate; Table 2). Acclimation at 37 °C decreased carcass mass compared with acclimation at 23 °C ($F_{2, 23} = 3.471$, $P < 0.05$; ANCOVA using body mass as the covariate; Table 2).

3.6. Histological of BAT

Morphology of BAT was analyzed using hematoxylin-eosin staining (Fig. 5A-C). There was no significant difference in the cell nucleu number per area (200 μm × 200 μm) of the BAT sections among groups ($F_{2, 24} = 1.096$, $P = 0.350$; One-way ANOVA; Fig. 5D). However, larger lipid droplet was observed in gerbils acclimated to 37 °C compared with 23 °C group (lipid droplet number per area, $F_{2, 24} = 99.835$,

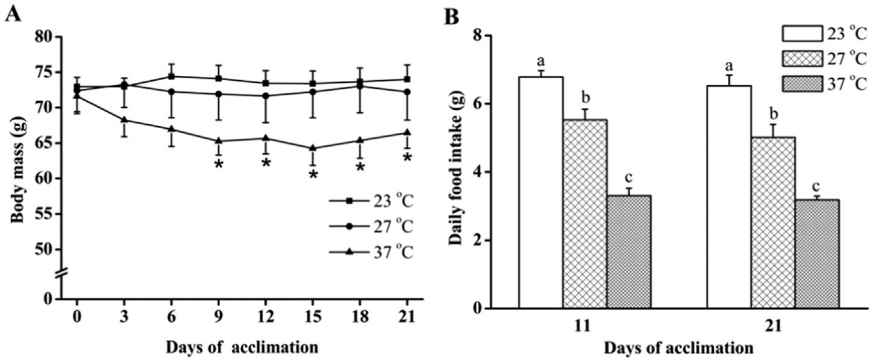

Fig. 1. Body mass (A) and daily food intake (B) of Mongolian gerbils acclimated to 23 °C, 27 °C or 37 °C. All data were mean ± SE. $n = 9$ per group. * $P < 0.05$ vs 23 °C, and data were analyzed using repeated measures ANOVA with LSD comparisons. Different letters indicated significant differences between groups ($P < 0.05$), and data were analyzed using one-way ANCOVA with Bonferroni comparisons.

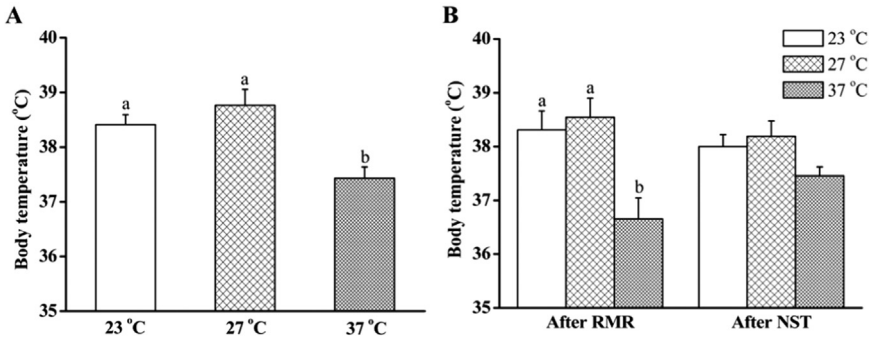

Fig. 2. Body temperature of Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. (A) Body temperature was measured after the acclimation. (B) Body temperature was measured after resting metabolic rate (RMR) measurement and maximum non-shivering thermogenesis (Max NST) measurement, respectively. Values were means ± SE ($n = 9$ per group). Data were analyzed using one-way ANOVA with Tukey HSD comparisons. Different letters indicated significant differences ($P < 0.05$) between groups.

$P < 0.001$; lipid droplet area, $F_{2,\ 24} = 40.425$, $P < 0.001$; one-way ANOVA; Fig. 5E–F).

3.7. UCP1and TH expression in BAT

There was no difference in expression of UCP1 and TH of gerbils acclimated to 27 °C compared with 23 °C group, while they were decreased in gerbils acclimated to 37 °C (UCP1, $F_{2,\ 24} = 13.831$, $P < 0.001$; TH, $F_{2,\ 24} = 5.623$, $P = 0.010$; one-way ANOVA; Fig. 6).

3.8. UCP1 and BAT-specific transcriptional regulators gene expression

We also explored potential molecular mechanisms which regulated the BAT thermogenesis. Relative mRNA levels of UCP1 were not different between gerbils acclimated to 23 °C and 27 °C, while it was decreased in gerbils acclimated to 37 °C compared with 23 °C group ($F_{2,\ 24} = 4.521$, $P = 0.022$; one-way ANOVA, Tukey comparison; Fig. 7A). In addition, both acclimation at 27 °C and 37 °C did not alter relative mRNA levels of PR domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1-α) in BAT (PRDM16, $F_{2,\ 24} = 0.932$, $P = 0.407$; PGC1-α, $F_{2,\ 24} = 13.831$, $P = 0.233$; one-way ANOVA, Tukey comparison; Fig. 7B-C). Moreover, acclimation at 27 °C did not alter peroxisome proliferator-activated receptor-α (PPARα) and peroxisome proliferator activated receptor-γ (PPARγ) gene expression in BAT, while acclimation at 37 °C increased PPARα and PPARγ gene expression in BAT compared with acclimation at 23 °C (PPARα, $F_{2,\ 21} = 12.675$, $P < 0.001$, 8 subjects per group due to the missing samples; PPARγ, $F_{2,\ 24} = 3.844$, $P = 0.036$, $n = 9$ subjects per group; one-way ANOVA, Tukey comparison; Fig. 7D–E).

4. Discussion

In this study, we addressed the thermoregulatory role of BAT within the TNZ of Mongolian gerbils. Our study showed that BAT thermogenic capacity did not change in gerbils acclimated to 27 °C, while it was significantly reduced in gerbils acclimated to 37 °C by decreasing UCP1 expression and sympathetic stimulation. Changes in BAT may contribute to the thermoregulation during thermoneutral acclimation. Moreover, down-regulation of BAT thermogenesis-regulated genes was not observed. Instead, peroxisome proliferator-activated receptor-α (PPARα) and peroxisome proliferator activated receptor-γ (PPARγ) gene were up-regulated.

4.1. Metabolic TNZ of Mongolian gerbils

TNZ describes the relationship between ambient temperature and

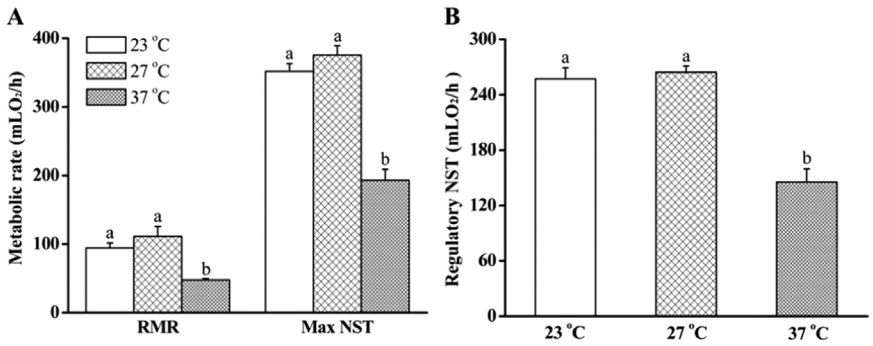

Fig. 3. RMR and max NST (A) and regulatory NST (B) of Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. RMR was determined at 30 ± 0.5 °C. NST was determined at 25 ± 0.5. Values were means ± SE ($n = 9$ per group). Different letters indicated significant differences ($P < 0.05$) between groups. Data were analyzed using one-way ANCOVA.

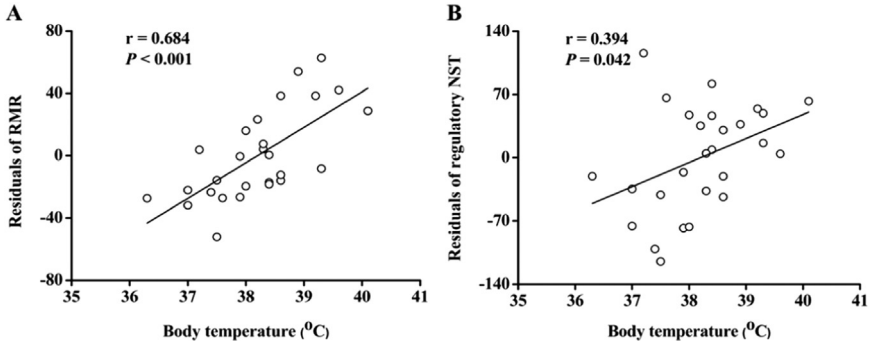

Fig. 4. Body temperature in relation to RMR (A) and regulatory NST (B) in Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. Data were analyzed using Pearson's correlation.

thermoregulation in mammals, which provides lots of information on the ability of a species to live and survive in different environments (Gordon, 2012; Bozinovic et al., 2014; Khaliq et al., 2014). In practice, a classic and widely used method for determining TNZ is based on metabolic rate (Scholander et al., 1950; IUPS Thermal Commission, 2001; Bozinovic et al., 2014). However, using this method to define UCT is not satisfactory (Gordon, 2012). The metabolic rate does not reflect the regulatory changes in evaporation heat loss near the UCT. Rats exhibit significant variation in rapid eye movement within the TNZ (Szymusiak and Satinoff, 1981). In gerbils, body temperature tends to increase at T_a close to UCT (Robinson, 1959; Wang et al., 2000; Pan et al., 2014).

In this study, RMR and NST did not change in gerbils acclimated to 27 °C, but they were reduced in gerbils acclimated to 37 °C. Studies have demonstrated that chronic thermal stress above TNZ induces an adaptive physiological process that improves the ability to cope with heat challenge (Horowitz, 2002; Sareh et al., 2011). The reduction in RMR may decrease the hyperthermia and evaporative water loss, and the UCT is presumably shifted upward (Hart, 1971; Luebbert et al., 1979). Moreover, heart mass and kidney mass were reduced after acclimation at 37 °C. Heart and kidney are metabolically active organs. The sizes of them are crucial determinant of RMR (Gordon, 1993; Konarzewski and Diamond, 1995). In addition, the reduction in NST is associated with adaptive changes in BAT (Cannon and Nedergaard, 2004). Our data are in line with observations in mice acclimated to 30 °C (Xiao et al., 2015; Small et al., 2018). Similar results are also observed in rats exposed to heat (Rousset et al., 1984; Arieli and Chinet, 1985). Together, within the TNZ, thermal stress near the UCT may lead to metabolic adaptations in gerbils.

4.2. The thermoregulatory role of BAT within the TNZ

The present study suggested that the acclimation temperature near LCT only reduced BAT activation, while the acclimation temperature near UCT reduced BAT activation and thermogenic capacity. In this study, regulatory NST did not change in gerbils acclimated to 27 °C, while it was significantly reduced in gerbils acclimated to 37 °C. The major source of regulatory NST is BAT (Cannon and Nedergaard, 2004). At the cellular level, acclimations to 27 °C or 37 °C increased the size of lipid droplet in brown adipocytes. Lipid droplets are the main source of fatty acids used for oxidative phosphorylation and UCP1 activation (Fedorenko et al., 2012). The size of lipid droplets is negatively correlated with UCP1 activation (Bartelt et al., 2011; Geerling et al., 2014). Moreover, lipid droplets associate with mitochondria function (Blanchette-Mackie and Scow, 1983). They can directly interact with mitochondria and remodel metabolism of BAT (Yu et al., 2015). Therefore, the large size of lipid droplets may also impede the interaction with mitochondria and decrease metabolism of BAT. At the molecular level, acclimation at 37 °C decreased the expression of UCP1 and TH in BAT. UCP1 supports the specialized function of thermogenesis, and its expression level reflects the thermogenic capacity of BAT (Golozoubova et al., 2006). TH, the rate-limiting enzyme in noradrenalin synthesis, correlates with sympathetic activity in BAT (Stanford et al., 2012; Shi et al., 2013). The reduction in expression of TH indicates attenuation of sympathetic activity.

BAT is a metabolically active organ, and changes in BAT may contribute to the thermoregulation in warm adaptation. In mammals, a stable body temperature is maintained by the balance of heat production and heat loss (Nakamura, 2011). In this study, acclimation at 27 °C did not alter the body temperature and BAT thermogenic capacity, suggesting that BAT may have a thermoregulatory role here. But acclimation at 37 °C reduced the body temperature and BAT thermogenic capacity. Furthermore, the reduced body temperature was associated with decreased RMR and regulatory NST. Our data are in line with observations in rats that thermal stress above TNZ results in adaptive changes of thermoeffectors, including decreased oxygen consumption

Table 2
Effects of 3 weeks thermoneutral acclimation on organs and body composition of Mongolian gerbils.

Parameters	23 °C	27 °C	37 °C	*P* value
Organ mass (g)				
BAT	0.165 ± 0.012	0.190 ± 0.027	0.143 ± 0.009	0.267
Heart	0.340 ± 0.015	0.318 ± 0.018	0.242 ± 0.015*	0.004
Liver	2.427 ± 0.066	2.421 ± 0.183	2.109 ± 0.077	0.539
Lung	0.437 ± 0.026	0.410 ± 0.013	0.368 ± 0.015	0.148
Kidney	0.632 ± 0.009	0.590 ± 0.023	0.511 ± 0.019*	0.002
Carcass mass (g)	57.708 ± 2.106	57.536 ± 3.268	54.394 ± 1.859*	0.048
Dry carcass mass (g)	22.700 ± 1.189	23.066 ± 2.456	20.899 ± 1.123	0.066
Body water mass (g)	35.008 ± 1.447	34.470 ± 1.149	33.496 ± 0.946	0.883
Body fat mass (g)	7.822 ± 1.349	7.726 ± 1.779	6.532 ± 0.882	0.327
Lean body mass (g)	66.189 ± 1.496	64.507 ± 2.486	59.935 ± 1.443	0.327

NOTE. Values are means ± SE (n = 9 per group). Data were analyzed using one-way ANOVA with Tukey HSD comparisons.
* $P < 0.05$ vs 23 °C.

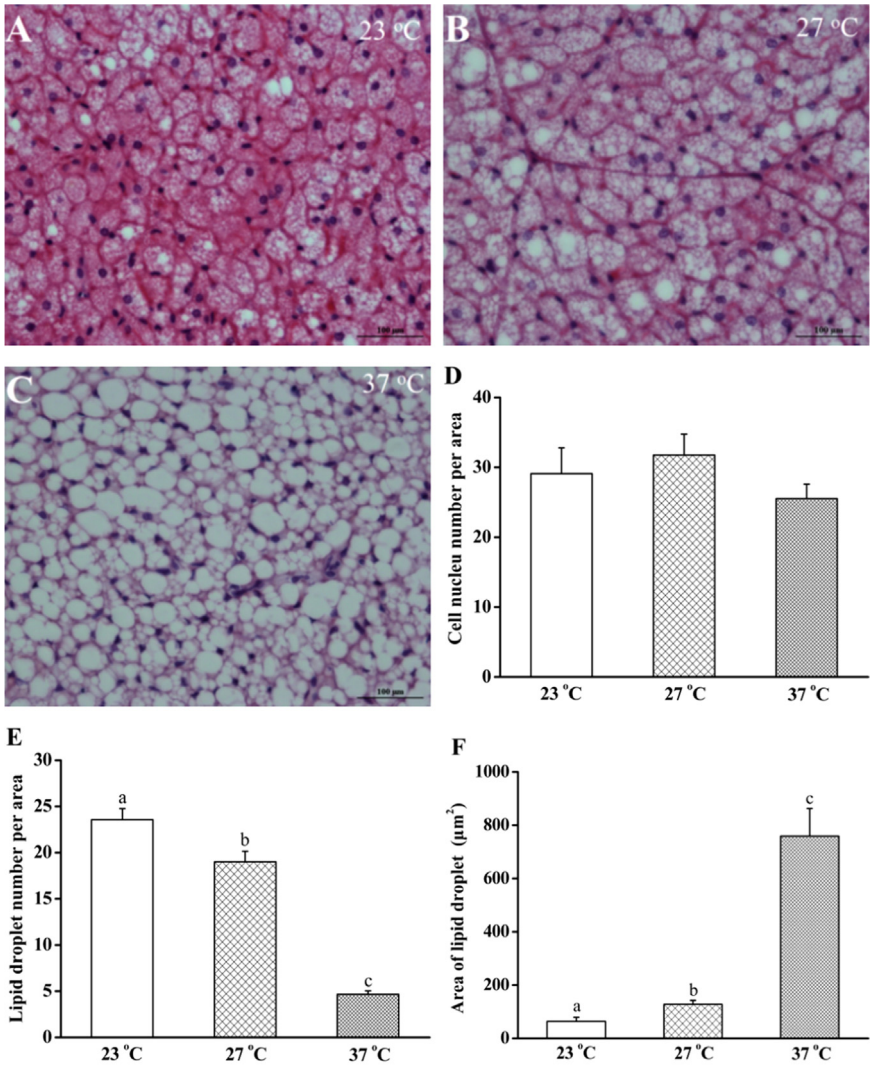

Fig. 5. Morphology of BAT in Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. (A-C) Sections of BAT stained by hematoxylin-eosin. (D) Cell nucleu number per area (200 μm × 200 μm) in sections. (E) Lipid droplet number per area (100 μm × 100 μm) in sections. (F) Lipid droplet area in sections. Values were means ± SE (n = 9 per group). Data were analyzed using one-way ANOVA. Different letters indicated significant differences (P < 0.05) between groups. Scale bars represent 100 μm in panels (A, B and C).

and BAT function (Rousset et al., 1984; Arieli and Chinet, 1985; Horowitz et al., 1988). Therefore, gerbils maintain a lower body temperature by reduction of BAT metabolism and RMR during adaptation near UCT.

Network of transcription factors regulates the differentiation and thermogenesis of brown adipocytes. PPARγ coordinates the general program of brown adipocyte differentiation with CCAAT/enhancer-binding proteins and regulates the transcription of UCP1 gene (Barbera et al., 2001; Seale, 2015). PPARα can directly activate UCP1, PRDM16 and PGC1-α (Hondares et al., 2011). PRDM16 can bind and enhance the

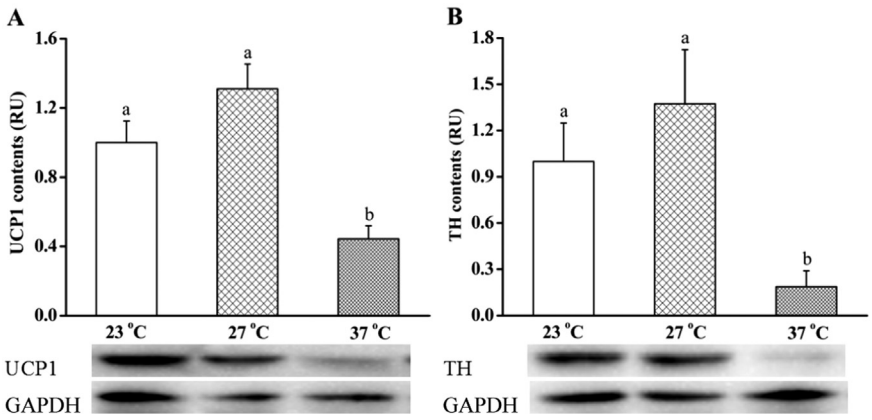

Fig. 6. Uncoupling protein 1 (UCP1, A) and tyrosine hydroxylase (TH, B) contents in BAT of Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. Protein level was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level. Values were means ± SE (n = 9 per group). Different letters indicated significant differences (P < 0.05) between groups. Data were analyzed using one-way ANOVA.

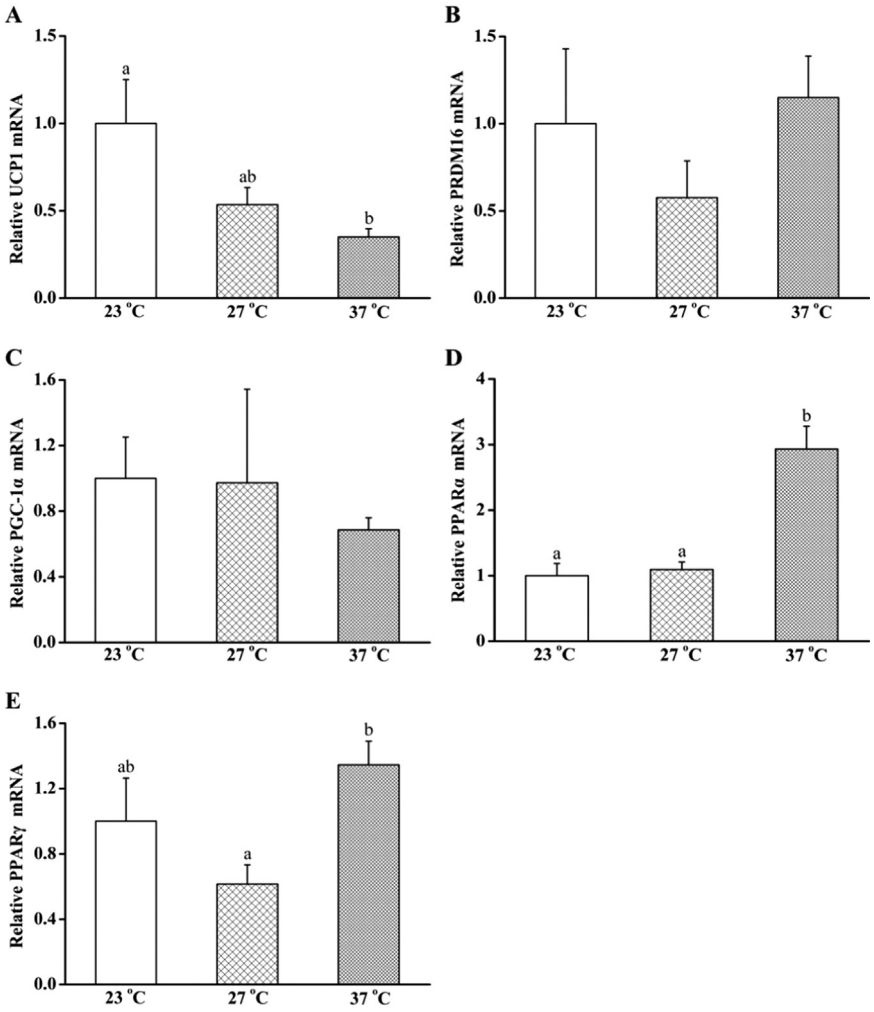

Fig. 7. The mRNA expression of UCP1 (A), PR domain containing 16 (PRDM16, B), peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1-α, C), peroxisome proliferator-activated receptor-α (PPARα, D) and peroxisome proliferator activated receptor-γ (PPARγ, E) in BAT of Mongolian gerbils after 3 weeks acclimation at 23 °C, 27 °C or 37 °C. Values were means ± SE (n = 9 per group). Different letters indicated significant differences ($P < 0.05$) between groups. Data were analyzed using one-way ANOVA.

transcription of PPARγ, PPARα and PGC-1a (Seale et al., 2007, 2008; Hondares et al., 2011). PGC1-α modulates oxidative metabolism, mitochondrial biogenesis and thermogenic gene activation by interaction with various transcription factors (Wu et al., 1999; Bagattin et al., 2010). In contrast, lack of PPARγ disrupts the differentiation of brown adipocytes (Barak et al., 1999). Brown adipocytes lacking PRDM16 show large lipid droplets and reduced UCP1 expression and mitochondria (Harms et al., 2014). Lack of PGC1-α reduces the thermogenic gene expression and BAT thermogenesis (Uldry et al., 2006). However, down-regulation of these genes expression in BAT was not observed in our study. Therefore, our data do not support the idea that thermoneutral acclimation decreases BAT thermogenesis by down-regulation of PRDM16, PGC1-α, PPARα or PPARγ gene. In addition, PPARα and PPARγ gene expression was up-regulated in acclimation near the UCT. A possible explanation is that the up-regulated PPARγ may activate the transcription of genes involved in lipid storage (Rosen and MacDougald, 2006). But further studies are needed to fully explain the function of these transcription factors in warm adaptation.

4.3. Conclusions

In summary, BAT did not atrophy near the LCT, while it atrophied near the UCT. The present findings suggest that BAT may play a role during adaptation near LCT. Furthermore, atrophic BAT contributes to thermoregulation during adaptation near UCT. Therefore, our study rather suggests that BAT plays thermoregulatory role in the warm adaptation of Mongolian gerbils.

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