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HSP70 plays a role in the defense of acute and chronic heat stress in Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Mongolian gerbils (*Meriones unguiculatus*) show a wide thermal neutral zone (TNZ, 26.5–38.9 °C). Whether heat shock proteins (HSPs) are involved in thermal tolerance for gerbils has still been unknown. We investigated the effects of acute and chronic high temperature within and above TNZ on the expressions of HSP70 and HSP90 and oxidative status in Mongolian gerbils, to test the hypothesis that the gerbils need increase the expression in HSPs to defense the acute and chronic heat stress. In experiment I, 50 Mongolian gerbils were exposed to 23 °C, 27 °C, 37 °C, 40 °C and 43.5 °C for 80 min respectively, and then sacrificed 12 h after treatment. HSP70 expression in the liver increased at 40 °C compared to that at 23 °C, but did not change after 27 °C, 37 °C or 43.5 °C exposure. There were no differences in HSP90 expression, oxidative stress parameters such as malonaldehyde (MDA) and hydrogen peroxide (H_2O_2), or antioxidant parameters such as superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in the liver. HSP70 and HSP90 expression both in the heart and brain showed no differences among groups. In experiment II, another set of 30 gerbils were acclimated to 23 °C, 27 °C and 37 °C for 21 days, respectively. During chronic acclimation, HSP70 expression increased and H_2O_2 level decreased in the liver in 37 °C group compared to other two groups. Both H_2O_2 and SOD in the brain decreased in 37 °C group, but there were no differences in HSP70, MDA or T-AOC in the brain. These data indicate that Mongolian gerbils can maintain basal levels of HSPs after acute exposure to temperatures within the wide TNZ, but rely on increased HSP70 in the liver to protect from heat damage at temperatures above TNZ and during chronic heat acclimation. The increased HSP70 expression in the liver may contribute to keeping from heat damage in desert rodents.

1. Introduction

Small mammals living in temperate zone are faced with seasonal variations in ambient temperature and food resources in their natural habitats (Wang et al., 2000; Xu and Wang, 2016). Ambient temperature is one of the most important environmental cues that influence the behavior and physiology of small mammals (McNab, 2002). Extreme weather events occur more frequently with the climate changes. The small mammals due to their small body size are prone to be affected directly by the extreme weather (Coumou and Rahmstorf, 2012), and the species with narrower thermal tolerance ranges have more risks to be threatened than those with wider thermal tolerance ranges (Gilman et al., 2006; Khaliq et al., 2014). Heat stress may lead to excessive hyperthermia, thermoregulatory deficits, systemic inflammation, DNA damage, oxidative damage, impaired hypothalamo-pituitary-adrenocortical axis and decreased survival (Li et al., 2013; Mizrahi et al., 2016).

Heat shock proteins (HSPs) are induced by high temperature and other environmental and biological stressors, such as radiation, heavy metals, hypoxia, oxidative stress, inbreeding and high density (Parsell and Lindquist, 1993; King and MacRae, 2015). HSPs function as chaperons to assist protein folding and repairing in stressful and normal physiological conditions (Feder and Hofmann, 1999). HSPs are divided into different families based on their molecular weights, of which HSP70 and HSP90 were mostly studied and involved in the process of chaperone activity in high temperature (King et al., 2002; Sorensen et al., 2003). Early studies illustrated that the expression of HSPs could increase stress tolerance in cell lines and ectotherms (Tomanek and Somero, 2000). Induction of HSP expression is also associated with improvement of thermal tolerance in small mammals (Flanagan et al., 1995; King et al., 2002). After 5-day heat acclimation, HSP70 expression

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in the liver and lung increased in mice (Sareh et al., 2011). The increase in HSPs expression in internal organs after heat exposure was considered to be the first defense against heat stress (Feder and Hofmann, 1999; Horowitz, 2002).

In mammals, acute and chronic exposures to high temperature may also cause oxidative stress to the metabolic organs (Yu et al., 2010; Pearce et al., 2012). In normal physiological condition, the production of reactive oxygen species (ROS) in the body is maintained in homeostasis, which play vital roles in maintenance of signal transduction, gene expression, and activation of receptors (Ashkenazi and Haim, 2013). In hot or other stress conditions, ROS are excessively accumulated, which are generally decomposed by the antioxidant system, including non-enzymatic components and a number of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). The imbalance between ROS and antioxidant capacity will lead to damage of DNA, protein and lipid (Durackova, 2010), and the increased HSPs may help to repair the wrongly folded proteins by chaperone activity in stress conditions (Feder and Hofmann, 1999; King and MacRae, 2015). Single high temperature exposure induced the increase in antioxidant system to defense oxidative damage in fibroblast cell and mice (Paul et al., 2009), but a few papers reported that acute and chronic high temperature tolerance was independent on antioxidant capacity (King et al., 2002; Li et al., 2013).

Mongolian gerbils (*Meriones unguiculatus*) mainly distributed in typical steppe, desert steppe and desert habitats in Northern China, Mongolia and Trans-Baikal region of Russia (Zhang et al., 2016; Deng et al., 2017). The annual range of ambient temperature is from -47.5 °C to 35.3 °C. In summer ambient temperature fluctuates from 3 °C during the night to 52 °C in the day (Wang et al., 2000). In the wild, Mongolian gerbils from the desert population have a lower basal metabolic rate (BMR) and total evaporate water loss (TEWL) due to down regulation of tricarboxylic acid cycle (TCA) metabolites than gerbils from typical steppe (Shi et al., 2015). Mongolian gerbils have wide thermal neutral zone (TNZ, 26.5–38.9 °C). After exposure at 38 °C for 3 h, Mongolian gerbils increased small molecular antioxidants to deal with the oxidative stress (Shi and Wang, 2016), and could also remodel mitochondrial membrane to help to reduce respiration and thus keep BMR stable (Pan et al., 2014). In addition, brown adipose tissue played a thermoregulatory role at 37 °C during chronic heat acclimation (Guo et al., 2019). However, whether the gerbils rely on HSPs and antioxidant ability to keep from heat stress has still been unknown. Therefore, the present study was to investigate the effect of acute and chronic high temperature within and above TNZ on the expressions of HSP70 and HSP90 and the levels of oxidative stress in Mongolian gerbils. We hypothesized that the gerbils need increase the expression in HSPs to defense the acute and chronic heat stress.

2. Material and method

2.1. Animals and experimental designs

All the animal procedures were carried out in agreement of Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Mongolian gerbils were the offspring of laboratory colony trapped in Inner Mongolian grassland. The gerbils were housed in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding at 23 ± 1 °C under a 16 h light: 8 h dark photoperiod (with lights on at 4:00 a.m.). Gerbils were housed individually with food and water *ad libitum*. Commercial rat chow pellets (Beijing KeAo Feed Co.) were used as the standard food.

Experiment I was designed to test effects of acute temperature exposure within and over TNZ on HSPs in the liver, heart and brain, and the oxidative responses in serum and liver. The gerbils were exposed to 23 °C (room temperature), 27 °C (lower critical temperature, T_{lc}), 38 °C (upper critical temperature, T_{uc}), 40 °C, or 43.5 °C for 80 min, respectively ($n = 10$ per group, 5 males and 5 females). The animals were put back to room temperature and had free access to food and water to recover. Animals were kept in room temperature for 12 h to allow synthesis of HSPs in tissues based on our pilot experiment. Then they were sacrificed with overdose of CO_2. Serum was collected for MDA and SOD measurement. The liver was collected for SOD, T-AOC, MDA, H_2O_2, HSP70, and HSP90 measurements. The heart and brain were collected for HSP70 and HSP90 measurements.

Experiment II was designed to investigate the effect of chronic high temperature acclimation on the expression of HSP70 and HSP90 and the oxidative responses in the gerbils. Another set of Mongolian gerbils were divided into three groups and acclimated to 23 °C, 27 °C or 37 °C for 21 days, respectively. The liver and brain were collected for SOD, T-AOC, MDA, H_2O_2, HSP70, and HSP90 measurements ($n = 10$ per group, male 5 and female 5).

2.2. Western blot analysis of HSPs

HSP70 and HSP90 expression in the liver, heart and brain was measured as mentioned in the previous study with some modifications (King et al., 2002). The liver and heart were washed with 0.9% saline thoroughly to avoid blood contamination. Tissue was lysed in homogenization buffer (29.18 mM sucrose, 0.5 mM Tris-HCl, 1 mM PMSF, 1 mM DTT, 100 PIC, pH = 6.8). The homogenates were then centrifuged at 10,000 g for 15 min at 4 °C. Protein concentration was measured by Bradford method. Protein sample was added to 10% separated SDS-GAGE gels and blotted into polyvinylidene difluoride (PVDF) membranes. Then PVDF membranes were incubated with mouse anti-HSP antibodies (HSP70, 1:8000, H5147; HSP90, 1:8000, H1775, Sigma-Aldrich USA; mouse anti-GAPDH, 1:8000, Yeasen, China) and horseradish peroxidase conjugated secondary antibody (1:3000, Yeasen China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was taken as the internal reference. The immunoblot was revealed by ECL (Yeasen China) and densitometric analysis was done using Image lab software (Bio-Rad Laboratories, Inc., USA). The density of each HSP band was normalized to GAPDH density.

2.3. Measurement of oxidative parameters

For the preparation of tissues (liver, heart and brain), we followed the instructions from kit manufactures (Nanjing Jiancheng, Nanjing, China). Liver, heart and brain were weighted using a digital balance (Sartorious BP121s, Germany). Then tissues were lysed in 0.9% saline in ice bath, with the ratio of weight of tissue to volume of saline (1:9). The homogenates were then centrifuged at 2500 rpm/min for 10 min at 4 °C. Protein concentration was measured by Bradford method.

Malonaldehyde (MDA) indicates the level of lipid peroxidation (Bhusari et al., 2008). MDA level was measured by a thiobarbituric acid reactive substances (TBARS) assay kit (Nanjing Jiancheng, Nanjing, China) following instructions from manufacturer (Yang et al., 2013; Khakisahneh et al., 2019). The absorbance of the eluent was monitored spectrophotometrically at 532 nm (Molecular Devices Spectra Max i3). Variations within- and among-sample for this assay were below 1.5%. Lipid peroxidation level was expressed as nmol MDA mg^{-1} protein or nmol MDA ml^{-1} serum.

The ROS level in tissue was indicated by the level of hydrogen peroxide (H_2O_2). H_2O_2 level was measured by a commercial kit (Nanjing Jiancheng, Nanjing, China), following instructions from manufacturer. H_2O_2 can react with molybdic acid and then peroxo-polymolybdic acid was produced, which has peak absorption at 405 nm.

2.4. Measurement of antioxidant parameters

Superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) were also measured by commercial kits (Nanjing Jiancheng, Nanjing, China). The amount of enzyme, which results in 50% inhibition of superoxide radicals produced by the reaction between xanthine and

xanthine oxidase at 37 °C, was defined as one unit of SOD (Yang et al., 2013; Khakisahneh et al., 2019).

One unit of T-AOC was defined as the extent to which optical density is increased by 0.01 per milligram protein per min. Antioxidants in cells can react with Fe^{3+} to produce Fe^{2+}. Then Fe^{2+} combined with Fehling reagent and produced a stable complex compound, which has peak absorption at 520 nm.

2.5. Statistics

Data are analyzed by SPSS 20.0 (IBM, Armonk, NY, USA). Before statistical analysis, data were checked for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests, respectively. HSP70 and HSP90 expression and markers of oxidative stress were analyzed by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. $P < 0.05$ was considered to be significantly different in all tests. Data were presented as means ± SE.

3. Results

3.1. HSP70 and HSP90 expression in the liver, heart and brain after acute temperature exposure

There were no significant differences in the parameters between females and males, so the data from females and males were pooled together for analysis. HSP70 expression in the liver kept stable after exposure to 27 °C and 38 °C, and increased by 131.3% after 40 °C exposure when compared with 23 °C group ($F_{4,45} = 7.214$, $P < 0.05$, Fig. 1A). But the value of HPS70 at 43.5 °C was similar to that observed at 23 °C. There was no difference in HSP90 expression in the liver among all the groups ($F_{4,45} = 0.552$, $P = 0.698$, Fig. 1B). The expressions of HSP70 ($F_{4,45} = 0.790$, $P = 0.540$) and HSP90 ($F_{4,45} = 2.140$, $P = 0.091$) in the heart did not show significant differences among different temperature groups (Table 1). Neither HSP70 ($F_{4,45} = 0.609$, $P = 0.659$, Table 1) nor HSP90 ($F_{4,45} = 1.252$, $P = 0.303$, Table 1) in the brain differed among groups.

3.2. Oxidative and antioxidant parameters in the liver after acute temperature exposure

There were no group differences either in serum MDA ($F_{4,45} = 0.952$, $P = 0.443$, Fig. 2A) or SOD ($F_{4,45} = 1.383$, $P = 0.255$, Fig. 2B). There were also no differences in MDA ($F_{4,45} = 1.194$, $P = 0.326$, Fig. 2C), H_2O_2 ($F_{4,45} = 2.352$, $P = 0.084$, Fig. 2D), SOD ($F_{4,45} = 1.342$, $P = 0.269$, Fig. 2E) or T-AOC ($F_{4,45} = 1.056$, $P = 0.390$, Fig. 2F) in the liver after different temperature exposures.

3.3. HSP70 and HSP90 expression in the liver and brain after chronic temperature acclimation

HSP70 expression in the liver showed no change after 27 °C acclimation, but increased by 106.9% after 37 °C acclimation compared with that in 23 °C group ($F_{2,27} = 5.759$, $P < 0.05$, Fig. 3A). HSP90 expression in the liver kept in basal level among all the groups ($F_{2,27} = 0.699$, $P = 0.506$, Fig. 3B). HSP70 expression in the brain showed differences between 23 °C and 27 °C, with a 54.6% decrease in 27 °C group ($F_{2,27} = 3.343$, $P = 0.05$, Fig. 3C). The value of HSP70 at 37 °C was between 23 °C and 27 °C, but didn't differ significantly from 27 °C or 23 °C groups. HSP90 expression in the brain showed no change after heat acclimation ($F_{2,27} = 0.268$, $P = 0.767$, Fig. 3D).

3.4. Oxidative and antioxidant parameters in the liver and brain after chronic temperature acclimation

There was no difference in MDA level in the liver among different groups ($F_{2,27} = 0.471$, $P = 0.630$, Fig. 4A). The level of H_2O_2 in 37 °C group decreased by 24.6% compared with that in 23 °C group ($F_{2,27} = 3.851$, $P = 0.036$, Fig. 4B). There were no differences in SOD ($F_{2,27} = 0.931$, $P = 0.410$, Fig. 4C) or T-AOC ($F_{2,27} = 0.041$, $P = 0.960$, Fig. 4D) in the liver among different groups.

MDA level in the brain showed no group difference ($F_{2,27} = 0.693$, $P = 0.509$, Fig. 5A). However, the levels of H_2O_2 decreased by 58.5% in 27 °C group and by 66.5% in 37 °C group ($F_{2,27} = 28.878$, $P < 0.001$, Fig. 5B). SOD decreased by 45.9% in the brain in 37 °C group compared with 23 °C group ($F_{2,27} = 3.654$, $P = 0.039$, Fig. 5C). There was no significant difference in T-AOC in the brain among different groups ($F_{2,27} = 1.614$, $P = 0.218$, Fig. 5D).

4. Discussion

In the present study, we found that HSP70 expression in the liver increased after exposure to 40 °C, and the expression of HSP90 in the liver was unchanged after acute exposure to different temperatures. Neither HSP70 nor HSP90 expression in the heart and brain was affected by acute exposure to different temperatures. The oxidative stress and antioxidant parameters in the serum and liver didn't change, too. After chronic heat acclimation at 37 °C (around T_{uc}), HSP70 expression in the liver increased, and H_2O_2 levels both in the liver and brain decreased

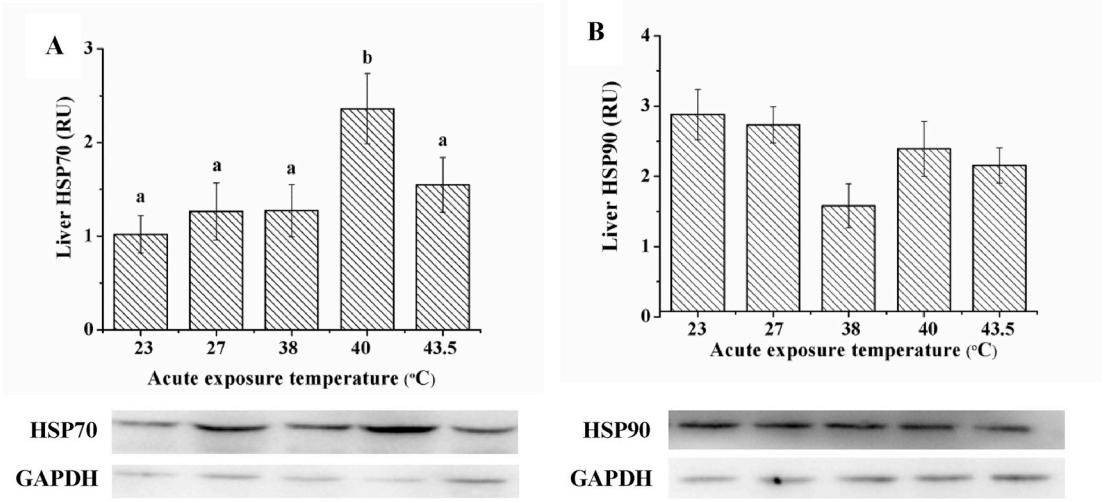

Fig. 1. Heat shock protein 70 (HSP70) and HSP90 expressions in the liver after acute exposure to different temperatures in Mongolian gerbils. Significant differences are indicated by different letters if $P < 0.05$. Data are mean ± SE ($n = 10$ for each group).

Table 1
The expressions of heat shock protein 70 (HSP70) and 90 (HSP90) in the heart and brain after exposure to different temperatures in Mongolian gerbils.

	23 °C	27 °C	38 °C	40 °C	43.5 °C	F	P
Heart							
HSP70	0.922 ± 0.071	2.522 ± 1.302	2.134 ± 0.469	1.438 ± 0.413	1.737 ± 0.451	0.790	0.540
HSP90	1.062 ± 0.204	1.918 ± 0.472	1.811 ± 0.357	2.431 ± 0.454	1.456 ± 0.133	2.140	0.091
Brain							
HSP70	1.428 ± 0.418	0.998 ± 0.272	1.360 ± 0.466	1.122 ± 0.323	1.857 ± 0.575	0.609	0.659
HSP90	4.033 ± 1.773	1.470 ± 0.285	2.225 ± 0.763	1.888 ± 0.570	4.036 ± 1.346	1.252	0.303

Data are mean ± SE ($n = 10$ for each group).

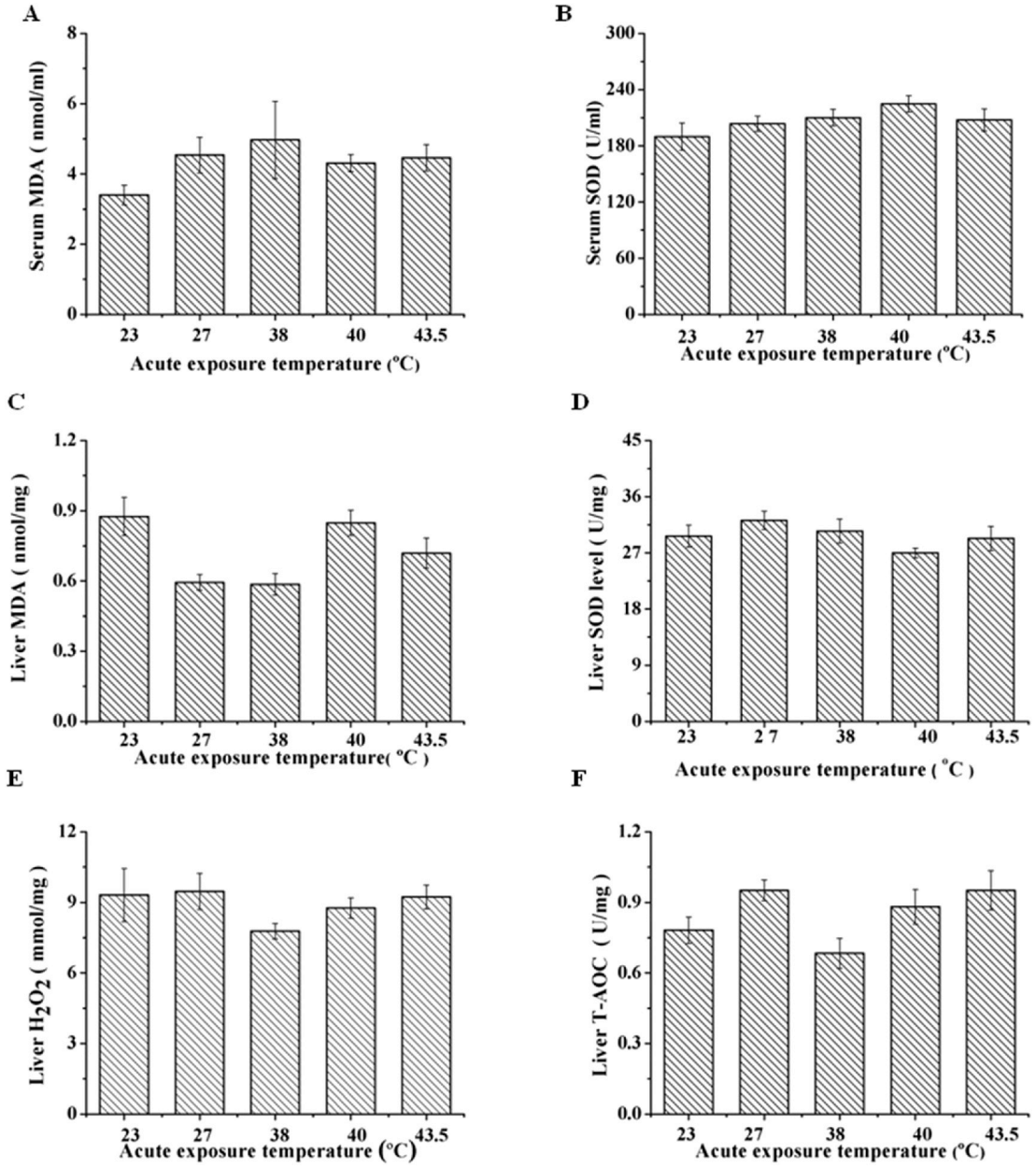

Fig. 2. Serum malonaldehyde (MDA) and superoxide dismutase (SOD) after exposure to different temperatures in Mongolian gerbils. The levels of malonaldehyde (MDA), hydrogen peroxide (H_2O_2), total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) in the liver after acute exposure to different temperatures in Mongolian gerbils. Data are mean ± SE ($n = 10$ for each group).

compared to those in 23 °C. Our data indicate that the gerbils could keep stable HSPs and oxidative status at acute temperatures within TNZ, and mainly increased HSP70 in liver to keep from heat damage at mild heat exposure above TNZ and during chronic heat acclimation at T_{uc}.

4.1. HSP70 plays a role in thermal tolerance in Mongolian gerbils

HSPs, as highly conserved proteins, were expressed under all types of stress conditions to protect the cells and organisms from injuries (Feder

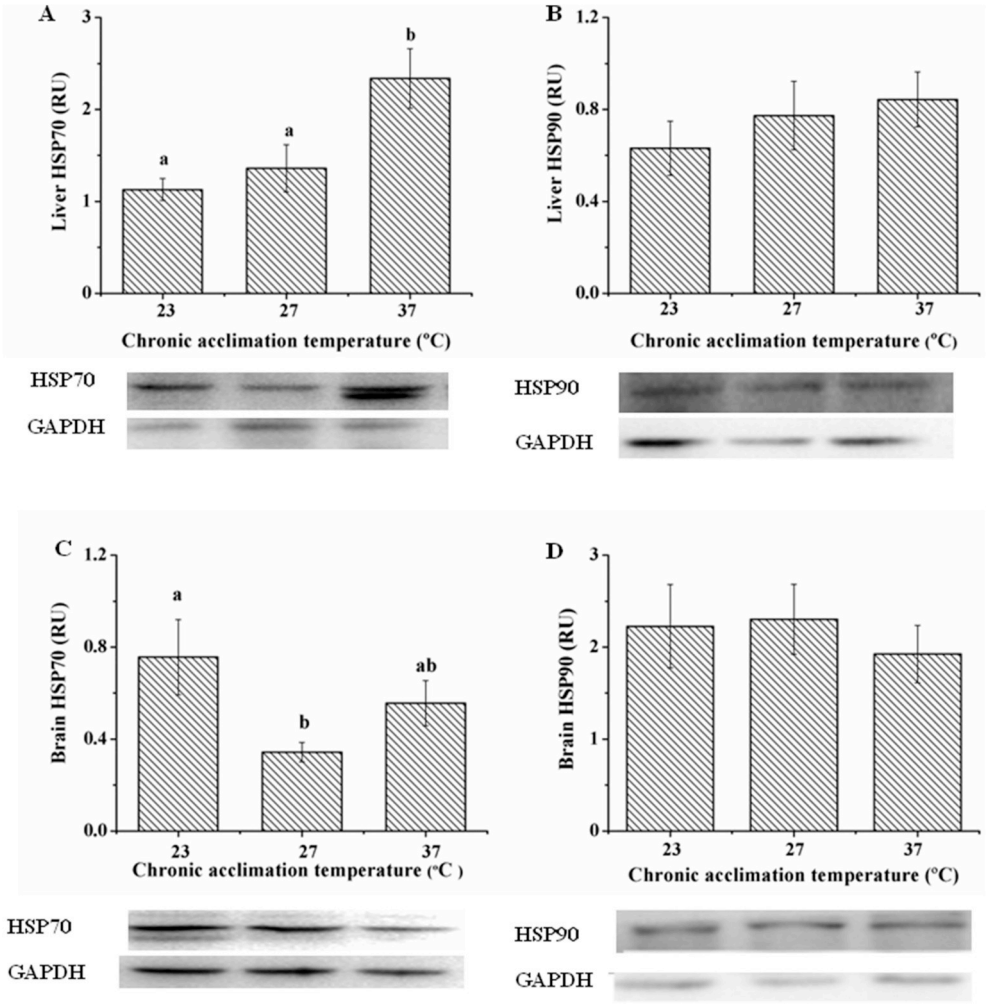

Fig. 3. Heat shock protein 70 (HSP70) and HSP90 expressions in the liver after chronic acclimation to different temperatures in Mongolian gerbils. Significant differences are indicated by different letters if $P < 0.05$. Data are mean ± SE ($n = 10$ for each group).

and Hofmann, 1999; Sorensen et al., 2003). The frequent heat waves in summer is an extreme threat not only to ectotherms but also to endotherms (Stillman and Tagmount, 2009; Mizrahi et al., 2016). Although the ectotherms rely more on HSPs as the first defense against high ambient temperature, the endotherms also need to regulate HSP expression to protect from damage caused by high temperature (Shabtay and Arad, 2005). For example the laboratory mice increased HSP70 expression in the liver and muscle when they were exposed to 43.5 °C for 30 min (Leon et al., 2006), and the rats increased HSP70 in the liver after exposed to 41 °C for 17.2 min (Flanagan et al., 1995). In Mongolian gerbils, we found that HSP70 expression in the liver showed no change at 27 °C and 38 °C (within TNZ), and increased after exposure to 40 °C. But the level of HSP70 in the liver did not increase after 43.5 °C exposure in comparison with 23 °C. We also found that neither HSP70 nor HSP90 expression both in the heart and brain showed differences after exposure to different temperatures. Within TNZ, the animals can keep stable metabolism and need not regulate metabolic heat production or evaporative heat loss to maintain thermoregulation. Therefore, HSP70 showed the same level when the gerbils were exposed to temperatures within TNZ for a short period. HSPs are induced in a specific temperature range for organisms (Evgen'ev et al., 2014). During this temperature range, the animals can regulate HSPs to protect from the heat damage. If the ambient temperature is too high, however, the animals will abandon to defend by HSPs and the apoptotic response will be induced (Evgen'ev et al., 2014). That may be the reason why HSP levels did not increase at 43.5 °C in Mongolian gerbils. The stress responses in HSP expression both in mice and rats are organ-specific (Flanagan et al., 1995; Leoni et al., 2000), suggesting that the sensitivity or tolerance to high temperature is diverse for different tissues. In addition, the accumulation rate of HSP70 was determined by intrinsic characteristics of cell types (Beck et al., 1995). It was reported that accumulation rate of HSP70 was lower in the heart and brain than that in the liver and colon after heat exposure (Beck et al., 1995). The HSP expression is also related with the habitat environments. The human skin fibroblast cells originating from desert people synthesized much more HSPs than those from the moderate environment after acute heat exposure (Lyashko et al., 1994). The study in land snails (*Theba pisana*) showed that the HSP70 expression in feet from different geographic populations was positively correlated with habitat temperature (Mizrahi et al., 2016).

The desert species Mongolian gerbils have a wide TNZ from 26.5 °C to 38.9 °C (Pan et al., 2014), whereas both mice and rats have relatively narrow TNZs from 28 °C to 34 °C and from 30 °C to 34 °C, respectively (Gordon, 2012). The wide TNZ indicates that the animals can maintain a stable BMR over a large temperature range and may be more thermal tolerant (Khaliq et al., 2014). Moreover, the thermo-tolerant species usually maintain a high level of basal HSP70 and are not much disturbed by environmental fluctuation (Dong et al., 2008). We observed that all the gerbils could survive after exposure to 43.5 °C for 80 min in the present study. However, the survival rates of mice were 72.2 ± 14.3% and 36.1 ± 14.0% after exposure to 41 °C for 45 or 60 min (King et al., 2002). These data suggest that the desert species can maintain basal levels of HSPs after acute exposure to temperatures within the wide TNZ,

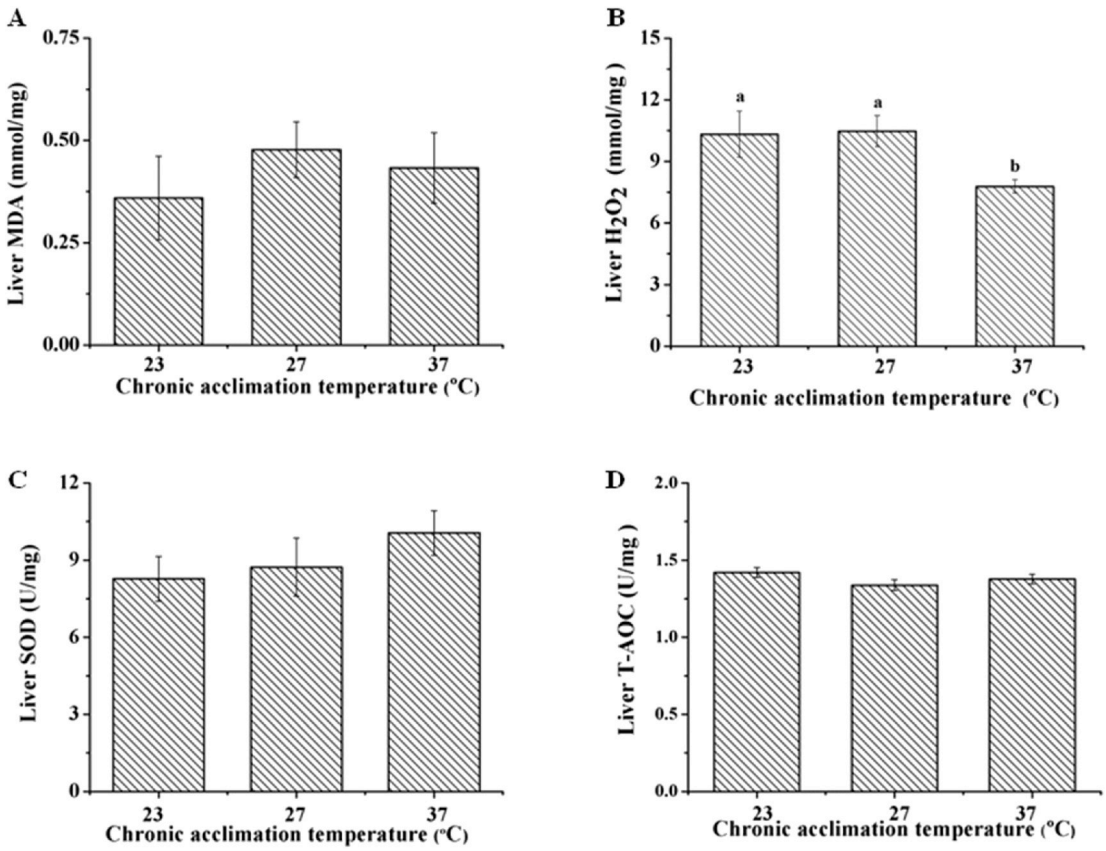

Fig. 4. The levels of malonaldehyde (MDA), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in the liver after chronic acclimation to different temperatures in Mongolian gerbils. Significant differences are indicated by different letters if $P < 0.05$. Data are mean ± SE ($n = 10$ for each group).

but need increase HSP70 expression to defend heat stress at moderate temperatures above TNZ.

During chronic heat acclimation, HSP70 expression increased in the liver in 37 °C (T_{uc}) group compared with 23 °C and 27 °C group. HSP70 expression in the brain at 27 °C was lower than that at 23 °C, but at 37 °C HSP70 showed no difference from that at 23 °C and 27 °C. HSP90 expression either in the liver or brain showed no any changes among groups. The similar responses to chronic heat acclimation were also observed in mice, with an increase in HSP70 expression but no changes in HSP90 in the liver (Sareh et al., 2011). Other studies showed that HSP70 expression increased in rabbit testis and rat skeletal muscles after chronic heat acclimation (Pei et al., 2012). Liver was a target organ for a series of internal and environmental stress, such as hyperthermia, ischemia, heavy metal, drugs and viral hepatitis (Li et al., 2013). HSP70 could effectively bind to the misfolded cellular proteins and thus prevent their aggregation based on its chaperone activity, whereas HSP90 didn't participate in the process of denatured protein refolding but helped to transport denatured protein to HSP70 for 3D structure restoration (Nollen and Morimoto, 2002). The increase in HSP70 expression contributed to not only defending the current heat damage, but also being prepared for another future high temperature period. Moreover, this kind of physiological plasticity enabled the animals to be tolerant with a broader thermal window in the face of climate change (Bozinovic et al., 2011). Combined with our previous study which showed that body temperature and BMR of Mongolian gerbils decreased after chronic 37 °C acclimation (Guo et al., 2019), we can conclude that the gerbils could adapt to chronic high temperature acclimation by combined strategies such as increased HSP70 expression in the liver and decreased metabolic rate.

4.2. Antioxidant ability was not involved in thermal tolerance in Mongolian gerbils

ROS were generated as by-products of metabolic process (Balaban et al., 2005). MDA was a stable lipid peroxidation product and was often used as a biomarker of lipid peroxidation (Bhusari et al., 2008). The MDA levels indicated the degree of oxidative degradation of polyunsaturated fatty acids and cellular deterioration in the liver. Superoxide radical anion (O_2-) is the primary oxygen reactant, whereas H_2O_2 released from mitochondria was the main ROS (Azad et al., 2010). SOD decomposed superoxide anion into H_2O_2 and singlet oxygen, which were later neutralized by catalase and vitamin E (Monaghan et al., 2009). In normal physiological condition, antioxidants and ROS could be fully neutralized, and thus animals were in a balance state between oxidative stress and antioxidant protection. But the balance between ROS and antioxidants could also be broken by environmental stressors and internal stressors.

In the present study, we found that acute high temperature exposure within and above TNZ did not affect the oxidative stress parameters (such as MDA and H_2O_2) and antioxidant ability (such as SOD and T-AOC) in the liver. In rats, however, MDA level increased and SOD activity decreased in the liver after water bath at 45 °C for 25 min (Khafagaa et al., 2019). Our previous data showed that after exposure at 38 °C for 3 h small molecular antioxidants (such as uric acid and inosine) were increased to protect from oxidative damage in the gerbils (Shi and Wang, 2016). Together with keeping stable BMR between 26.5 and 38.9 °C (Pan et al., 2014), the data suggest that Mongolian gerbils were not subjected to oxidative damage during acute heat exposure.

During chronic 37 °C acclimation, H_2O_2 levels in the liver and brain, and SOD activity in the brain decreased in 37 °C group compared to

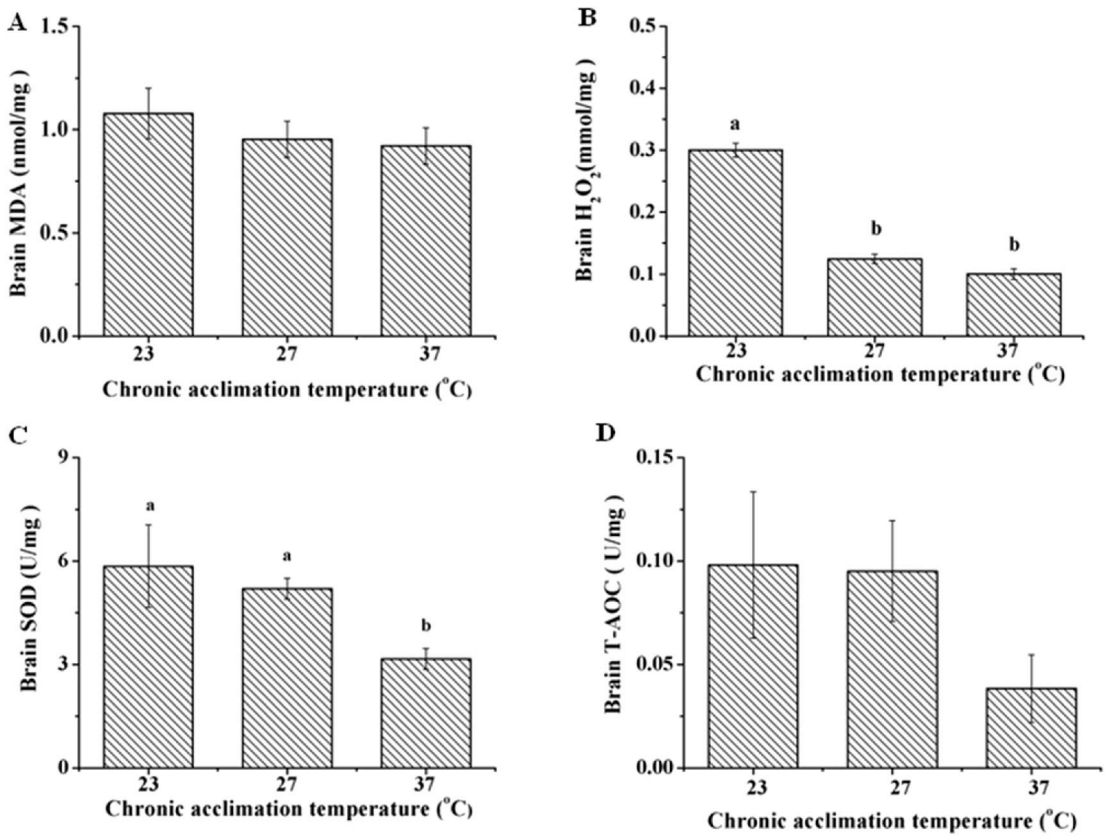

Fig. 5. The levels of malonaldehyde (MDA), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in the brain after chronic acclimation to different temperatures in Mongolian gerbils. Significant differences are indicated by different letters if $P < 0.05$. Data are mean ± SE ($n = 10$ for each group).

other groups, whereas other parameters did not differ among groups. In the 3-week aged mice, MDA, SOD and caspase-3 levels in the liver didn't change after acclimation to 39 °C for 1.5 h every day and continuously for 6 weeks (Li et al., 2013). Another study in mice showed the similar results with no changes in SOD or catalase activity in a hyperthermia state (King et al., 2002). The study in stripped hamsters (*Cricetulus barabensis*) also showed that acclimation to 32.5 °C for 6 weeks had no effect on either the oxidative stress or antioxidant ability in the liver, heart and other internal organs (Zhou et al., 2015). The mass of several metabolically active organs and BMR decreased after chronic heat acclimation in Mongolian gerbils (Guo et al., 2019), which may contribute to decreased accumulation of H_2O_2 in the liver and brain. Our previous study showed that both MDA level and SOD activity in the liver decreased, whereas all oxidative and antioxidant parameters in the heart, testis and small intestine did not change after 37-day acclimation at 32 °C compared with 23 °C group in Mongolian gerbils (Xu et al., 2019). However, acclimation to 37 °C for 3 weeks led to increased protein carbonyl levels in the liver in Mongolian gerbils (Khakisahneh et al., 2019). Increased SOD and catalase activity were observed in the liver in mice after acclimation to 34 °C for 2 weeks (Bhusari et al., 2008). Acclimation to 35 °C for 7 days also led to the increase in MDA but not in SOD or CAT in skeletal muscle in *Sus scrofa* (Ganesan et al., 2018). The animals exhibited diverse responses to different high temperatures and the responses were species-specific due to different capacities of heat tolerance. These data indicate that chronic exposure to the high temperature around T_{uc} is a huge threat to induce oxidative stress to some metabolic tissues, and the mammals will balance the oxidative stress and antioxidant protection to survive during chronic heat acclimation.

5. Conclusion

The present study investigated the responses in HSPs and oxidative stress to acute temperature exposure and chronic heat acclimation in a desert mammal species. Collectively, our data showed that HSP70 expression in the liver increased in response to acute heat exposure above T_{uc} and chronic T_{uc} acclimation in Mongolian gerbils. HSP90 expression in different tissues was not affected by acute heat exposure or chronic acclimation. The gerbils could maintain low levels of H_2O_2 and MDA and keep from the oxidative damage after acute heat exposure and chronic heat acclimation. Our data suggest that the gerbils could maintain stable HSPs and oxidative status at acute temperatures within TNZ, and mainly rely on the increased HSP70 in the liver to keep from heat damage at mild heat exposure above TNZ and during chronic T_{uc} acclimation. The desert rodents have evolved wide TNZ which may not only reduce energy cost but also confer heat tolerance without oxidative damage at acute exposure to hot in the desert environments. It may also give some implications that the desert rodents may increase HSP70 expression in the liver for survive in response to climate warming.

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References

Ashkenazi, L., Haim, A., 2013. Effect of Light at Night on oxidative stress markers in Golden spiny mice (*Acomys russatus*) liver. Comp. Biochem. Physiol. A 165, 353–357.

Azad, M.A., Kikusato, M., Sudo, S., Amo, T., Toyomizu, M., 2010. Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken. Comp. Biochem. Physiol. A 157, 266–271.

Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. Cell 120, 483–495.

Beck, S.C., Paidas, C.N., Tan, H.,., Yang, J., Maio, A., De, 1995. Depressed expression of the inducible form of HSP 70 (HSP 72) in brain and heart after in vivo heat shock. Am. J. Physiol. 269, 608–613.

Bhusari, S., Hearne, L.B., Spiers, D.E., Lamberson, W.R., Antoniou, E., 2008. Transcriptional profiling of mouse liver in response to chronic heat stress. J. Therm. Biol. 33, 157–167.

Bozinovic, F., Calosi, P., Spicer, J.I., 2011. Physiological correlates of geographic range in animals. Annu. Rev. Ecol. Evol. Systemat. 42, 155–179.

Coumou, D., Rahmstorf, S., 2012. A decade of weather extremes. Nat. Clim. Chang. 2, 491–496.

Deng, K., Liu, W., Wang, D.H., 2017. Inter-group associations in Mongolian gerbils: quantitative evidence from social network analysis. Integr. Zool. 12, 446–456.

Dong, Y.W., Miller, L.P., Sanders, J.G., Somero, G.N., 2008. Heat-shock protein 70 (Hsp 70) expression in four limpets of the genus Lottia: interspecific variation in constitutive and inducible synthesis correlates with in situ exposure to heat stress. Biol. Bull. 215, 173–181.

Durackova, Z., 2010. Some current insights into oxidative stress. Physiol. Res. 59, 459–469.

Evgen'ev, M.B., Garbuz, D.B., Zatsepina, O.G., 2014. Heat Shock Proteins and Whole Body Adaptation to Extreme Environments. Springer.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.

Flanagan, S.W., Ryan, A.J., Gisolfi, C.V., Moseley, P.L., 1995. Tissue-specific HSP70 response in animals undergoing heat stress. Am. J. Physiol. 268, 28–32.

Gilman, S.E., Wethey, D.S., Helmuth, B., 2006. Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. Proc. Natl. Acad. Sci. 103, 9560–9565.

Ganesan, S., Brownstein, A.J., Pearce, S.C., Hudson, M.B., Gabler, N.K., Baumgard, L.H., Rhoads, R.P., Selsby, J.T., 2018. Prolonged environment-induced hyperthermia alters autophagy in oxidative skeletal muscle in *Sus scrofa*. J. Therm. Biol. 74, 160–169.

Gordon, C.J., 2012. Thermal physiology of laboratory mice: defining thermoneutrality. J. Therm. Biol. 37, 654–685.

Guo, Y.Y., Chi, Q.S., Zhang, X.Y., Liu, W., Hao, S.Y., Wang, D.H., 2019. Brown adipose tissue plays thermoregulatory role within the thermoneutral zone in Mongolian gerbils (*Meriones unguiculatus*). J. Therm. Biol. 81, 137–145.

Horowitz, M., 2002. From molecular and cellular to integrative heat defense during exposure to chronic heat. Comp. Biochem. Physiol. A 131, 475–483.

Khakisahneh, S., Zhang, X.Y., Nouri, Z., Hao, S.Y., Chi, Q.S., Wang, D.H., 2019. Thyroid hormones mediate metabolic rate and oxidative, anti-oxidative balance at different temperatures in Mongolian gerbils (*Meriones unguiculatus*). Comp. Biochem. Physiol. C 216, 101–109.

Khaliq, I., Hof, C., Prinzinger, R., Bohning-Gaese, K., Pfenninger, M., 2014. Global variation in thermal tolerances and vulnerability of endotherms to climate change. Proc. Biol. Sci. 281, 20141097.

Khafagaa, A.F., Noreldinb, A.E., Taha, A.E., 2019. The adaptogenic anti-ageing potential of resveratrol against heat stress mediated liver injury in aged rats: role of HSP70 and NF-kB signalling. J. Therm. Biol. 83, 8–21.

King, A.M., MacRae, T.H., 2015. Insect heat shock proteins during stress and diapause. Annu. Rev. Entomol. 60, 59–75.

King, Y.T., Lin, C.S., Lin, J.H., Lee, W.C., 2002. Whole-body hyperthermia-induced thermotolerance is associated with the induction of Heat Shock Protein 70 in mice. J. Exp. Biol. 205, 273–278.

Leon, L.R., Blaha, M.D., DuBose, D.A., 2006. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. J. Appl. Physiol. 100, 1400–1409.

Leoni, S., Brambilla, D., Risuleo, G., de Feo, G., Scarsella, G., 2000. Effect of different whole body hyperthermic sessions on the heat shock response in mice liver and brain. Mol. Cell. Biochem. 204, 41–47.

Li, C.M., Li, L., Bai, J.Y., Wu, J., Huang, S., Wang, G.L., 2013. Correlation between heat shock protein 32 and chronic heat-induced liver injury in developing mice. J. Therm. Biol. 38, 513–519.

Lyashko, V.N., Vikulova, V.K., Chernicov, V.G., Ivanov, V.I., Ulmasov, K.A., Zatsepina, O. G., Evgenev, M.B., 1994. Comparison of the heat shock response in ethnically and ecologically different human populations. Proc. Natl. Acad. Sci. U. S. A. 91, 12492–12495.

Mizrahi, T., Goldenberg, S., Heller, J., Arad, Z., 2016. Geographic variation in thermal tolerance and strategies of heat shock protein expression in the land snail Theba pisana in relation to genetic structure. Cell Stress Chaperones 21, 219–238.

McNab, B.K., 2002. The Physiological Ecology of Vertebrates. Cornell Univ. Press, Ithaca.

Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. 12, 75–92.

Nollen, E.A.A., Morimoto, R.I., 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. J. Cell Sci. 115, 2809–2816.

Pan, Q., Li, M., Shi, Y.L., Liu, H., Speakman, J.R., Wang, D.H., 2014. Lipidomics reveals mitochondrial membrane remodeling associated with acute thermoregulation in a rodent with a wide thermoneutral zone. Lipids 49, 715–730.

Parsell, D.A., Lindquist, S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27, 437–496.

Paul, C., Teng, S., Saunders, P.T.K., 2009. A single, mild, transient scrotal heat stress causes hypoxia and oxidative stress in mouse testes, which induces germ cell death. Biol. Reprod. 80, 913–919.

Pearce, S.C., Mani, V., Boddicker, R.L., Johnson, J.S., Weber, T.E., Ross, J.W., Baumgard, L.H., Gabler, N.K., 2012. Heat stress reduces barrier function and alters intestinal metabolism in growing pigs. J. Anim. Sci. 4, 257–259.

Pei, Y.L., Wu, Y.J., Qin, Y.H., 2012. Effects of chronic heat stress on the expressions of heat shock proteins 60, 70, 90, A2, and HSC70 in the rabbit testis. Cell Stress Chaperones 17, 81–87.

Sareh, H., Tulapurkar, M.E., Shah, N.G., Singh, I.S., Hasday, J.D., 2011. Response of mice to continuous 5-day passive hyperthermia resembles human heat acclimation. Cell Stress Chaperones 16, 297–307.

Shabtay, A., Arad, Z., 2005. Ectothermy and endothermy: evolutionary perspectives of thermo protection by HSPs. J. Exp. Biol. 208, 2773–2781.

Shi, Y.L., Wang, D.H., 2016. Implication of metabolomic profiles to wide thermoneutral zone in Mongolian gerbils (*Meriones unguiculatus*). Integr. Zool. 11, 282–294.

Shi, Y.L., Chi, Q.S., Liu, W., Fu, H.P., Wang, D.H., 2015. Environmental metabolomics reveal geographic variation in aerobic metabolism and metabolic substrates in Mongolian gerbils (*Meriones unguiculatus*). Comp. Biochem. Physiol. D 14, 42–52.

Sorensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025–1037.

Stillman, J.H., Tagmount, A., 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, Petrolisthes cinctipes. Mol. Ecol. 18, 4206–4226.

Tomanek, L., Somero, G.N., 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (*Genus Tegula*) from different tidal heights. Physiol. Biochem. Zool. 73, 249–256.

Wang, D.H., Wang, Y.S., Wang, Z.W., 2000. Metabolism and thermoregulation in the Mongolian gerbil Meriones unguiculatus. Acta Theriol. 45, 183–192.

Xu, D.L., Xu, M.M., Wang, D.H., 2019. Effects of air temperatures on antioxidant defense and immunity in Mongolian gerbils. J. Therm. Biol. 84, 111–120.

Xu, M.M., Wang, D.H., 2016. Water deprivation up-regulates urine osmolality and renal aquaporin 2 in Mongolian gerbils (*Meriones unguiculatus*). Comp. Biochem. Physiol. A 194, 37–44.

Yang, D.B., Xu, Y.C., Wang, D.H., Speakman, J.R., 2013. Effects of reproduction on immuno-suppression and oxidative damage, and hence support or otherwise for their roles as mechanisms underpinning life history trade-offs, are tissue and assay dependent. J. Exp. Biol. 216, 4242–4250.

Yu, J., Yin, P., Liu, F.H., Cheng, G.L., Guo, K.J., Lu, A., Zhu, X.Y., Luan, W.L., Xu, J.Q., 2010. Effect of heat stress on the porcine small intestine: a morphological and gene expression study. Comp. Biochem. Physiol. A 156, 119–128.

Zhang, X.Y., Chi, Q.S., Liu, W., Wang, D.H., 2016. Studies of behavioral and physiological ecology in Mongolian gerbils (*Meriones unguiculatus*). Sci. Sin. Vitae 46, 120–128.

Zhou, S.S., Cao, L.L., Xu, W.D., Cao, J., Zhao, Z.J., 2015. Effect of temperature on oxidative stress, antioxidant levels and uncoupling protein expression in striped hamsters. Comp. Biochem. Physiol. A 189, 84–90.