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Aquaporins, evaporative water loss and thermoregulation in heat-acclimated Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Evaporative water loss is an essential strategy to maintain stable body temperature in heat-exposed rodents. However, the thermoregulatory role and adjustment of evaporative heat loss capacity is unclear during prolonged heat exposure. Here, we studied the role of evaporative water loss in thermoregulation in Mongolian gerbils during heat acclimation. After 3 weeks of heat acclimation, gerbils exhibited a lower body temperature than the controls, and no difference in evaporative losses of water from the lung or saliva spreading compared with the controls. Heat acclimation did not alter the expression of aquaporin-1 and aquaporin-5 in the lungs and the expression of aquaporin-5 in the salivary glands. The expression of aquaporin-2 in the kidneys was kept stable, while the expression of aquaporin-1 in the kidneys was down-regulated. In addition, resting metabolic rate and non-shivering thermogenesis of heat-acclimated gerbils were reduced to 51% and 55% of the control group, respectively. Taken together, heat-acclimated Mongolian gerbils can reduce the metabolic thermogenesis without enhancing the evaporative water loss capacity for thermoregulation.

1. Introduction

The risk of thermal challenges to wild rodents is increased due to the frequent heat stress under the changing climate (Khaliq et al., 2014; Stillman, 2019). Rodents have a relatively constant body temperature, which provides a stable temperature condition for most biochemical processes in the body (Crompton et al., 1978; Hayes and Garland, 1995; Avaria-Llautureo et al., 2019). This stable body temperature is achieved through a dynamic body heat balance, in which total heat gain in the body equals its heat loss to the environment (Florant and Heller, 1977; Gordon, 1993). While continuous heat stress may lead to adjustments in physiology and alter the strategy for maintaining body heat balance (Ahmed, 2005; Sugimoto et al., 2013).

Heat loss occurs via evaporation, conduction, convection, and radiation (IUPS Thermal Commission, 2001). High ambient temperature decreases the temperature gradient between the animal skin and the air (Gordon, 1993). Hence, non-evaporative heat loss is restricted and evaporative heat loss should be increased (Gordon, 1983; Sugimoto et al., 1999). Evaporative heat loss comprises two mechanisms. The passive mechanism is to vaporize water from the skin surfaces and respiratory surfaces at normal respiration (Gordon, 2012). The active mechanism arises by autonomic thermoeffectors, such as thermal panting and saliva spreading (Furuyama et al., 1998; Gordon, 2012). Rats and mice dissipate body heat through respiratory evaporative water loss and behavioral application of saliva to the skin when exposed to heat (Gordon, 1983; Furuyama et al., 1998; Sugimoto et al., 1999). Continuous heat exposure may lead to physiological adjustments that increases the evaporative heat loss capacity (Gordon, 1993; Sugimoto et al., 2013). However, the adjustment of evaporative heat loss capacity is also affected by metabolic adaptation. Cellular metabolism is the primary mechanism of heat production in rodents, such as basal metabolism and adaptive thermogenesis (Lowell and Spiegelman, 2000; Oelkrug et al., 2015). Metabolic adaptation can reduce the pressure of continuous heat exposure on evaporative heat loss (Rousset et al., 1984; Arieli and Chinet, 1986).

Aquaporins (AQPs) are a family of transmembrane protein channels that function to increase plasma membrane water permeability (Agre, 2004; Verkman, 2005). Some of them may have a role in the adjustment

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of evaporative water loss capacity during heat exposure. AQP1 and AQP5 are expressed in the lung: AQP1 in microvascular endothelia, AQP5 in type I alveolar epithelial cells (Verkman, 2007). Both are involved in the fluid management of peripheral lung (Verkman, 2007). AQP5 is expressed in the apical membranes of acini and acts a pivotal part in saliva secretion (Krane et al., 2001; Akamatsu et al., 2003; Lee et al., 2012; Aure et al., 2014). The up-regulation of AQP5 expression contributes to the secretion of saliva in heat-acclimated rats (Sugimoto et al., 2013). In addition, AQP1 and AQP2 play critical roles in body water homeostasis by regulating kidney function (Gallardo et al., 2005; Urity et al., 2012; Jung and Kwon, 2016).

Mongolian gerbils (*Meriones unguiculatus*) live in desert grasslands, semi-arid steppes and agricultural fields of Northern China, Mongolia and the Baikal region of Russia (Wilson and Reeder, 2005; Liu et al., 2009; Mallon, 2010). This species exhibits a high degree of tolerance for heat (Robinson, 1959). And they can maintain body heat balance during prolonged heat exposure (Guo et al., 2019). Mongolian gerbils show high flexibility of renal AQPs expression to maintain body water homeostasis when water availability is limited (Xu and Wang, 2016). But it is unclear whether this species can cope with the continuous heat stress by increasing its evaporative water loss capacity. Besides, the metabolic rate is a highly flexible phenotypic characteristic of Mongolian gerbils (Wang et al., 2000; Li et al., 2010; Pan et al., 2014; Shi et al., 2015; Guo et al., 2019). We measured the evaporative water loss, AQPs expression, body temperature, and metabolic thermogenesis to determine whether heat-acclimated Mongolian gerbils enhanced evaporative heat loss capacity by regulating the expression of AQPs in the kidney and salivary glands.

2. Material and methods

2.1. Animal

Mongolian gerbils used for this experiment were from a laboratory colony that was the offspring of gerbils captured in the grasslands of Inner Mongolia. They were housed under conditions of ambient temperature of 23 ± 1 °C and a 16L:8D photoperiod (Light on at 0400 h). Gerbils had free access to water and commercial standard rat pellet chow (Beijing Keao Xieli Feed Co.). All animal experiments were approved by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2. Experimental design

Allexperiments were performed with male Mongolian gerbils aged 6–8 months. Gerbils were single-housed in plastic cages (30 × 15 × 20 cm^3) with sawdust as bedding for 2 weeks. Animals were assigned into two groups and exposed to 37 ± 1 °C for heat acclimation ($n = 9$) or 23 ± 1 °C as a control ($n = 9$) over 3 weeks. The duration of temperature acclimation was 3 weeks. Body mass, rectal temperature, and body surface temperature were measured every 3 days at 9–11 a.m. Food intake was determined on days 10–12 and 19–21. On day 21, water intake was measured. After the acclimation, the motor activity of gerbils was quantified according to the distance moved per unit time. Subsequently, the effects of heat acclimation on saliva spreading were assessed by behavioral tests at 37 ± 0.5 °C. On a separate day, all gerbils were sacrificed by CO_2 asphyxiation and then decapitated. Plasma was collected and stored at -80 °C until further analysis. Moreover, kidneys, lungs and salivary glands (parotid, submandibular, and sublingual) were dissected out and weighed (±1 mg). These tissues were then frozen in liquid nitrogen and stored at -80 °C for further analysis of AQPs content.

In a second study, gerbils were randomly assigned to two groups and acclimated at 23 ± 1 °C ($n = 9$) or 37 ± 1 °C ($n = 9$) for 3 weeks. To estimate evaporative water loss capacity through respiration in heat-acclimated gerbils, the total evaporative water loss (TEWL) was quantified at 30 ± 0.5 °C (Shi et al., 2015). Subsequently, resting metabolic rate (RMR) and non-shivering thermogenesis (NST) were performed.

2.3. Body mass, body temperature, food intake, and water intake

Body mass (±0.1 g) was measured using a digital balance (PL2001-L, Mettler Toledo, Switzerland). To determine food intake, 50 g of food was provided to each gerbil. Food residues were collected and weighed after 3 days. Daily food intake was calculated from the difference between the food provided and the residues. Rectal temperature was measured at 23 °C with 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 value was taken within 30 s. To measure body surface temperature, gerbil was transferred to a plastic cage (30 × 15 × 20 cm3). Its infrared image was taken at 23 °C using a thermal camera (FLIR C2, FLIR Systems Inc, USA) and analyzed with FLIR Tools. Besides, water intake was calculated from the difference in the weight of the water bottle within one day.

2.4. Examination of saliva spreading and motor activity

Gerbils were placed individually in a clear respiratory chamber (TSE, type I for mice, volume 2.7 L) covered with wire mesh. The temperature in the chamber was maintained at 37 ± 0.5 °C using an incubator (Sanyo, MIR-554). At this temperature, the gerbil would spread saliva on the fur or stretch its body. The air in the incubator was continuously exchanged with outside air. Behaviors of body stretching and saliva spreading were recorded with a video camera for 1 h. The duration of these behaviors and the time taken for them to first appear were analyzed according to the captured video. At the end of the trial, gerbil was immediately transferred to a plastic cage and its body surface temperature was measured. The chamber was cleaned with ethanol following each trial.

The motor activity of gerbils was recorded directly by a video camera mounted above the cage. The distance moved by gerbils per unit time was analyzed using EthoVision software.

2.5. TEWL measurement

TEWL was measured using an open-flow respiratory system (TurboFOX Complete Field System, Sable Systems International Inc., Las Vegas, USA) as described previously (Shi et al., 2015; Xu and Wang, 2016). Gerbil was placed in the respiratory chamber (TSE, type I for mice, volume 2.7 L) contained within an incubator (Sanyo, MIR-554). The temperature in the respiratory chamber was maintained at 30 ± 0.5 °C (within the thermoneutral zone of Mongolian gerbils) (Pan et al., 2014). The incurrent air was dried with a Drierite desiccant (W.A. Hammond Drierite Co Ltd., USA) and warmed using a copper and pumped through the respiratory chamber at a flow rate of 600–700 mL/min. The excurrent air was sampled at a flow rate of 100 mL/min. The water vapor density of the sampled air was measured using a humidity module. Water vapor density values were recorded every 15 s. Each individual was in the chamber for 3 h. TEWL was calculated as:

$$TEWL = (Ve \times \rho out – Vi \times \rho in) \times 60$$

Where, Ve = flow rate of air exiting the chamber (mL/min), Vi = flow rate of air entering the chamber (mL/min), ρin = water vapor density (mgH_2O/mL) of the air entering chamber, ρout = water vapor density (mgH_2O/mL) of the air exiting the chamber (Shi et al., 2015; Williams and Tieleman, 2000).

TEWL was estimated from the average of the 20 lowest consecutive data points of water vapor density for each gerbil (Shi et al., 2015; Xu and Wang, 2016). Body mass was weighed for further analysis before each measurement.

2.6. Metabolic trials

RMR was quantified as the rate of oxygen consumption using an open-flow respiratory system (TSE LabMaster Calorimetry System, Germany). Gerbils were placed individually in a respiratory chamber (TSE, type I for mice, volume 2.7 L) contained within an incubator (Sanyo, MIR-554) set at 30 °C (Wang et al., 2000). Air was pumped through the chamber at a rate of 0.8 L min^{-1}. Subsequently, excurrent air was sampled at a rate of 0.39 L min^{-1}. Each individual was in the chamber for a period of 3 h and no food or water was provided. RMR was estimated as the oxygen consumption over 12-min least variable and lowest values (Pan et al., 2014). Body mass was weighed for further analysis before each run.

The capacity of gerbils for NST was determined using the open-flow respiratory system. The temperature in the respiratory chamber was maintained at 25 ± 0.5 °C. Maximum NST (max NST) was estimated from the oxygen consumption response to noradrenaline (NA) (Shanghai Harvest Pharmaceutical Co. Ltd). The NA dosage injected subcutaneously was calculated according to the equation: NA dosage (mg/kg) = 6.6 $W^{-0.458}$, where W is body mass (g) (Heldmaier, 1971). Each individual was in the chamber for a period of 1 h and no food or water was provided. Max NST was estimated by averaging 3-min highest oxygen consumption (Heldmaier et al., 1982). Body mass was weighed before each run.

Besides, thermal conductance was estimated by the following equation:

$$C = MR / (T_b - T_a)$$

Where *C* is thermal conductance, *MR* is RMR (mL O_2/h), T_b is the body temperature (°C) of gerbils, and T_a is the ambient temperature (°C) (Bradley and Deavers, 1980).

2.7. Measurement of serum antidiuretic hormone (ADH) concentrations and serum osmolality

Serum ADH concentration was determined by enzyme-linked immunosorbent assay (Gerbil ADH ELISA kit) from Enzyme-linked Biotechnology (Shanghai, China), according to the instructions of the supplier. Serum osmolality was determined using a freezing-point osmometer (SMC-30B, Tian-he medical instruments, Tianjin, China).

2.8. AQPs measurements

The protein expression of AQPs in the lungs, salivary glands, and kidneys were quantified by Western blot. The samples were homogenated in dissecting buffer containing 250 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 10 mM triethanolamine, and protease inhibitor cocktail (P8340, Sigma, USA). Homogenates were centrifuged at 3, 000 g for 10 min at 4 °C after standing for 30 min. The supernatants were pooled and centrifuged at 100, 000 g for 1 h. The resultant pellets were resuspended in dissecting buffer and stood for 2 h. The supernatants were pooled for future analysis after centrifugation for 30 min at 4 °C. The total protein concentration in each sample was measured by the Folin phenol method (Lowry et al., 1951). Equal amounts of protein were separated by 12% SDS-PAGE gels and then transferred onto polyvinylidene fluoride membranes (PVDF) (Millipore, USA, IPVH00010). Then the membranes were blocked for 1.5 h at room temperature in non-fat dried milk and incubated overnight at 4 °C with the primary antibody. The primary antibody was mouse anti-β-tubulin (diluted 1:4000, 30301ES60, Yeasen, Shanghai, China), rabbit anti-AQP1 (diluted 1 : 4000, AB32722-50UL, Millipore, USA), goat anti AQP2 (diluted 1: 4000, SC-98882, Santa Cruz, USA) or rabbit anti-AQP5 (diluted 1: 3000, AB78486-50UL, Millipore, USA). A secondary antibody with the horseradish peroxidase-linked was added. Finally, the immunoblot was visualized with ECL (Beyotime, China) and analyzed with Image-Pro® Plus version 6.0 and normalized to β-tubulin.

2.9. Statistical analyses

Statistical analyses were performed using IBM SPSS.v.20.0 software. All data were tested for normality using the Shapiro-Wilk test. Data on body mass and body temperature during temperature acclimation were analyzed using repeated measures ANOVA followed by Bonferroni's *post hoc* testing. The differences in metabolic rate, TEWL, organ mass, food intake and water intake between two groups were analyzed using one-way ANCOVA with body mass as the covariate followed Bonferroni's *post hoc* testing. Differences in other indicators between two groups were analyzed using independent-sample *t*-test. Data were expressed as means ± standard error (SE). Statistical significance was defined as $P < 0.05$, as indicated by asterisks in the figure panels.

3. Results

3.1. Body mass, food intake, and rectal temperature during heat acclimation

During the heat acclimation period, the body mass decreased (time effect, $F_{7, 56} = 5.172$, $P < 0.001$; group × time effect, $F_{7, 56} = 4.786$, $P < 0.001$; repeated measures ANOVA) and was significantly lower than that of the control group on days 9, 12, 18, and 21 ($P < 0.05$) (Fig. 1 A). The rectal temperature decreased in response to acclimation at 37 °C (time effect, $F_{7, 56} = 7.752$, $P < 0.001$; group × time effect, $F_{7, 56} = 10.277$, $P < 0.001$; repeated measures ANOVA) and was lower than that of the control group from the 9th day (group effect, $F_{1, 8} = 8.410$, $P = 0.020$) (Fig. 1 B). The average daily food intake on days 11 and 21 was 48.8% of the control group (days 11, $F_{1, 15} = 98.901$, $P < 0.001$; day 21, $F_{1, 15} = 77.181$, $P < 0.001$; ANCOVA using body mass as the covariate; Fig. 1 C). At the end of heat acclimation, the temperature gradient between the body core and the surrounding environment was only 4.4% of the control group ($t = 77.179$, $df = 16$, $p < 0.001$; independent-samples *t* tests; Fig. 1 D).

3.2. Body surface temperature during heat acclimation

In most laboratory situations, rodents dissipate their body heat primarily through radiation (Gordon, 1993). As expected, interscapular surface temperature (time effect, $F_{7, 56} = 36.975$, $P < 0.001$; group × time effect, $F_{7, 56} = 10.695$, $P < 0.001$; repeated measures ANOVA) and lumbar surface temperature (time effect, $F_{7, 56} = 49.233$, $P < 0.001$; group × time effect, $F_{7, 56} = 17.946$, $P < 0.001$) were elevated during acclimation at 37 °C (Fig. 2A,B and C). The temperature gradient between the body surface and the surrounding environment was small. No body surface temperature above 37 °C was observed during this period. Besides, the interscapular surface temperature ($t = 4.758$, $df = 16$, $p < 0.001$; independent-samples *t* tests) and lumbar surface temperature ($t = 7.592$, $df = 16$, $p < 0.001$) of the heat-acclimated gerbils decreased when exposed to 23 °C for 30 min (Fig. 2D and E).

3.3. Metabolic thermogenesis

After heat acclimation, the RMR was reduced to 50.6% of the control group ($F_{1, 15} = 22.439$, $P < 0.001$; ANCOVA using body mass as the covariate; Fig. 3 A). Max NST was reduced to 54.9% of the control group ($F_{1, 15} = 54.500$, $P < 0.001$; Fig. 3 A). Moreover, there was no difference in motor activity between the heat-acclimated gerbils and control gerbils ($t = -1.638$, $df = 16$, $p = 0.121$; independent-samples *t* tests; Fig. 3 B). Besides, heat acclimation reduced the thermal conductance to 59.4% of that in controls ($t = 5.220$, $df = 16$, $p < 0.001$; independent-samples *t* tests; Fig. 3 C).

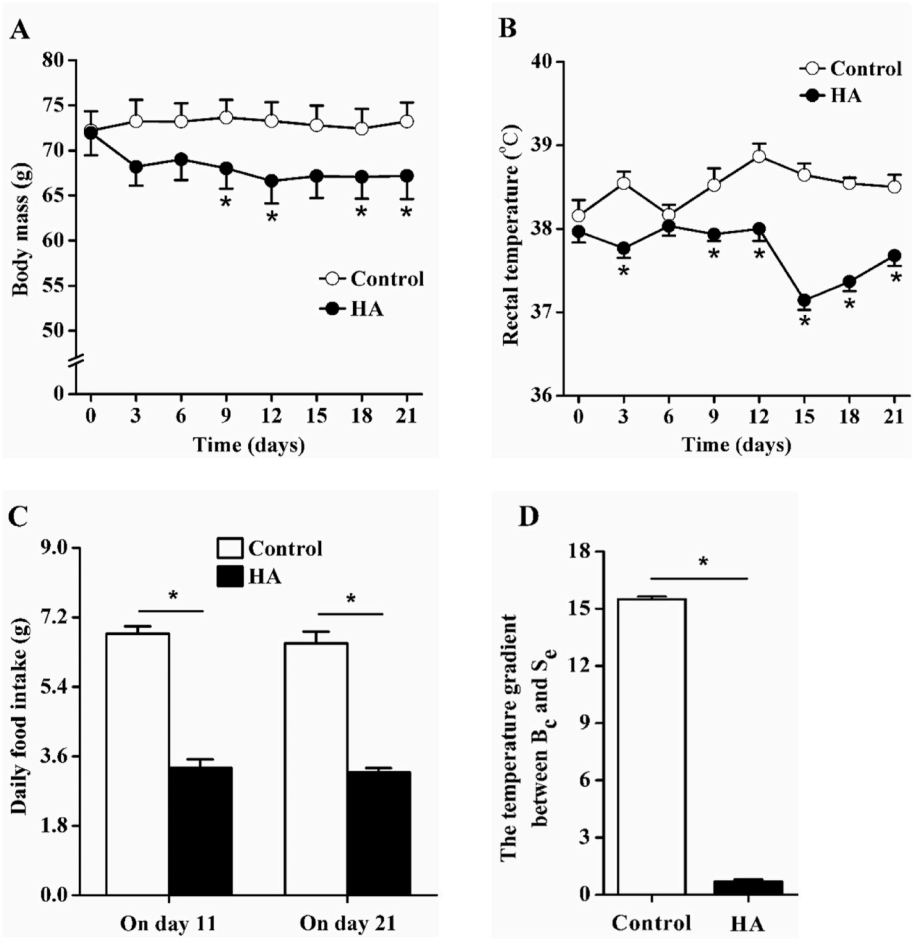

Fig. 1. Heat acclimation (at 37 ± 1 °C) reduces body mass, rectal temperature and food intake in Mongolian gerbils. (A) Body mass, (B) rectal temperature and (C) daily food intake were determined during temperature acclimation (at 37 ± 1 °C or 23 ± 1 °C). (D) The temperature gradient between the body core and the surrounding environment was calculated based on the rectal temperature at the end of temperature acclimation. HA, Heat acclimation; B_c, the body core; S_e, the surrounding environment. Values were mean ± SE. n = 9 per group. *P < 0.05 vs control (A and B, repeated measures ANOVA; C, one-way ANCOVA with body mass as the covariate; D, independent-sample t-test).

3.4. Behaviors in response to heat exposure

Gerbils exhibited behaviors of body stretching and saliva spreading when exposed to heat. Heat acclimation reduced the duration of the body stretching (t = 5.387, df = 16, p < 0.001; independent-samples t tests; Fig. 4 A) in response to heat exposure without changing the duration of saliva spreading (t = 1.403, df = 16, p = 0.180). Moreover, the time required for body stretching to first appear of heat-acclimated gerbils increased when subjects were exposed to heat (t = -3.892, df = 16, p = 0.001; independent-samples t tests; Fig. 4 B). No difference was observed in the time required for saliva spreading to first appear between heat-acclimated gerbils and control gerbils (t = -0.994, df = 16, p = 0.335; Fig. 4 B). In addition, interscapular surface temperature and lumbar surface temperature of heat-acclimated gerbils were lower than those of control gerbils after the behavior test (interscapular surface temperature, t = 3.301, df = 16, p = 0.005; lumbar surface temperature, t = 2.636, df = 16, p = 0.018; independent-samples t tests; Fig. 4C and D).

3.5. Body water homeostasis in gerbils after heat acclimation

At the end of heat acclimation, gerbils had a higher water intake than the controls ($F_{1,\ 15}$ = 10.477, P = 0.006; ANCOVA using body mass as the covariate; Fig. 5 E). But the TEWL did not differ between the two groups ($F_{1,\ 13}$ = 0.277, P = 0.607; ANCOVA using body mass as the covariate; Fig. 5 A). After dissection, reduced kidney mass ($F_{1,\ 15}$ = 13.904, P = 0.002; ANCOVA using body mass as the covariate; Fig. 5 B) and increased lung mass ($F_{1,\ 15}$ = 5.953, P = 0.028) in heat-acclimated gerbils were observed. There was no difference in parotid gland mass ($F_{1,\ 15}$ = 0.033, P = 0.857; ANCOVA using body mass as the covariate), submandibular gland mass ($F_{1,\ 15}$ = 1.453, P = 0.247) and sublingual gland mass ($F_{1,\ 15}$ = 2.332, P = 0.148) between the two groups (Fig. 5 B). In addition, no difference was observed in serum ADH concentration (t = -0.428, df = 14, p = 0.675; independent-samples t tests; Fig. 5 C) and serum osmolality (t = -1.645, df = 14, p = 0.125; Fig. 5 D) between heat-acclimated gerbils and control gerbils.

3.6. The expression of AQPs in the lung, salivary glands and kidney

AQPs are involved in maintaining body water homeostasis by regulating the expression in the lungs, salivary glands and kidneys (Jung and Kwon, 2016; Sugimoto et al., 2013; Verkman, 2007). After heat acclimation, the expression of AQP1 (t = 1.054, df = 16, p = 0.307; independent-samples t tests; Fig. 6 A) and AQP5 (t = -1.836, df = 16, p = 0.098; Fig. 6 B) in the lungs was kept stable compared with the control group. There was no difference in the expression of AQP5 in the parotid gland (t = 0.886, df = 16, p = 0.389; independent-samples t tests; Fig. 6 C) and sublingual gland (t = 2.045, df = 16, p = 0.058; Fig. 6 D) between the two groups. Moreover, down-regulation of AQP1 expression in the kidneys was observed after heat acclimation (t = 2.490, df = 16, p = 0.024; independent-samples t tests; Fig. 6 E). There was no difference in the expression of AQP2 in the kidneys between the two groups (t = 0.417, df = 16, p = 0.682; Fig. 6 F).

4. Discussion

Heat-acclimated Mongolian gerbils maintained body heat balance by reducing the metabolic rate rather than enhancing the evaporative water loss capacity. Heat acclimation greatly reduced the RMR and NST of Mongolian gerbils. And no changes were observed in evaporative

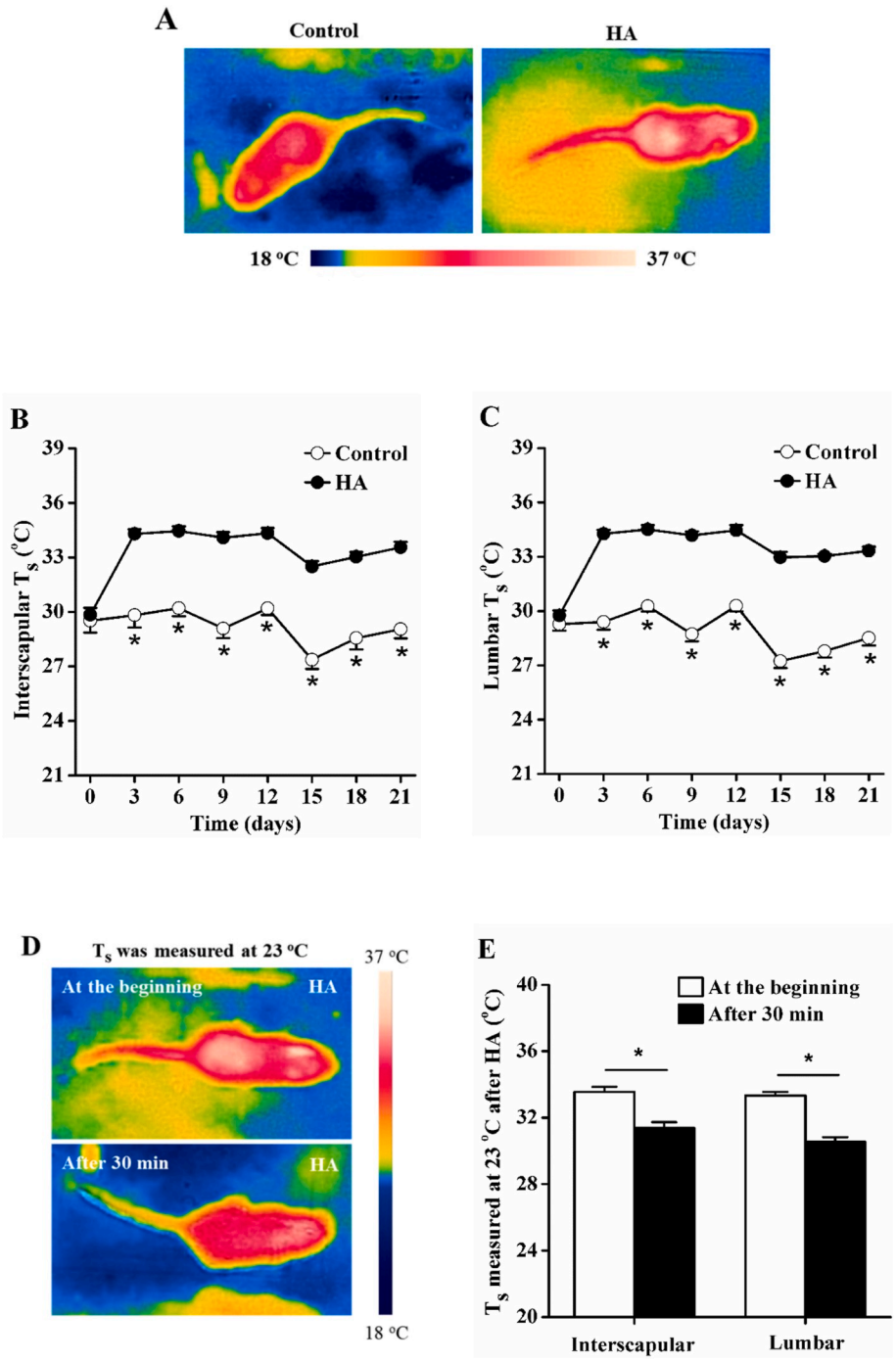

Fig. 2. The body surface temperature of Mongolian gerbils is elevated during heat acclimation. (A) Infrared images were taken at 23 °C after temperature acclimation (at 37 ± 1 °C or 23 ± 1 °C). (B) Interscapular surface temperature and (C) lumbar surface temperature were measured at 23 °C during temperature acclimation. (D) The infrared images of heat-acclimated subjects were taken at the beginning and after 30 min of exposure to 23 °C. (E) Interscapular surface temperature and the lumbar surface temperature decreased after 30 min exposure to 23 °C. HA, Heat acclimation; T_s, surface temperature. Values were mean ± SE. $n = 9$ per group. *$P < 0.05$ vs control (B and C, repeated measures ANOVA; E, independent-sample *t*-test).

water loss through respiratory surfaces or saliva spreading. The expression of AQP1 and AQP5 in the lung and AQP5 in salivary glands were not altered after heat acclimation. Moreover, heat acclimation decreased the body temperature in Mongolian gerbils.

4.1. Heat acclimation reduced the metabolic thermogenesis

In this study, the RMR and max NST were reduced after heat acclimation. The reduced metabolic rates may decrease the demand for evaporative heat loss during heat acclimation. Rats also show a decreased oxygen consumption after 3 weeks of exposure to 34 °C (Rousset et al., 1984). Brown adipose tissue is the main heat source of max NST (Cannon and Nedergaard, 2004). Thus, decreased function of brown adipose tissue may play a role in the reduction of metabolic thermogenesis (Guo et al., 2019). Moreover, we speculate that the reduction in metabolic rates is related to the decline function of metabolically active organs (Konarzewski and Diamond, 1995). Studies in rats show that heat acclimation reduces the oxidative capacities of kidneys, liver, and heart (Ray et al., 1968; Kerr et al., 1975). In addition, metabolic rate following heat acclimation is associated with thyroid hormone levels (Arieli and Chinet, 1986; Chaffee and Roberts, 1971). Serum triiodothyronine level in Mongolian gerbils decreases after heat acclimation (Khakisahneh et al., 2019). Above all, metabolic adaptation is an efficient way for Mongolian gerbils to overcome the challenge of continuous heat stress.

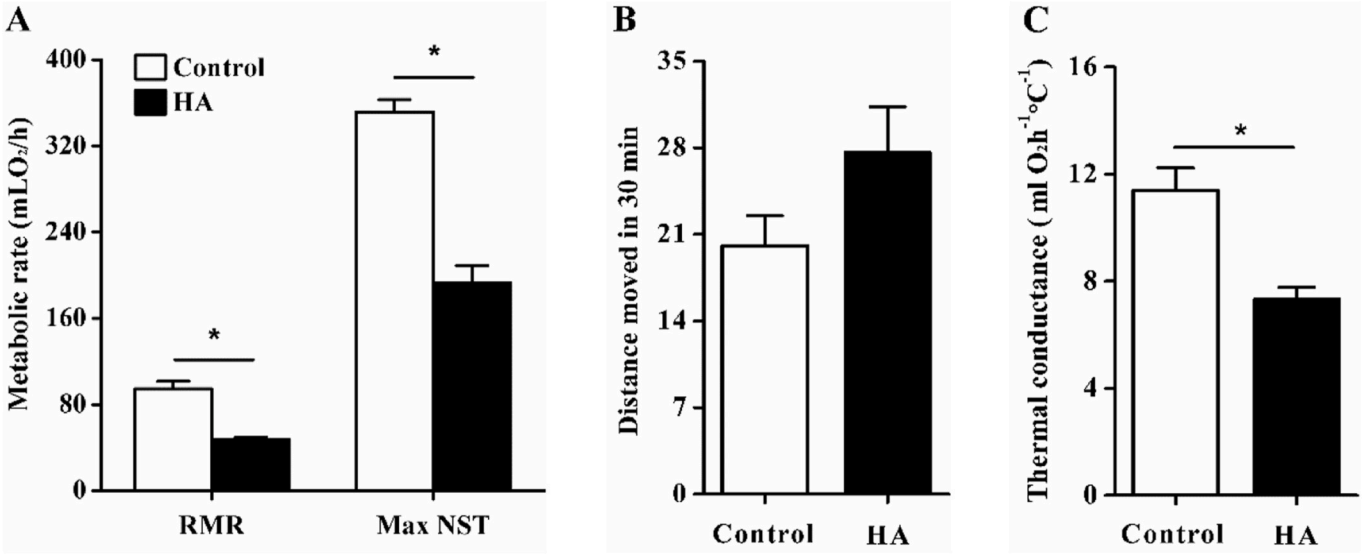

Fig. 3. Heat acclimation (at 37 ± 1 °C) reduces metabolic thermogenesis in Mongolian gerbils. (A) RMR and max NST were determined after temperature acclimation (at 37 ± 1 °C or 23 ± 1 °C). (B) Motor activity was estimated by the distance moved in 30 min. (C) Thermal conductance was estimated from RMR, body temperature, and ambient temperature. HA, Heat acclimation. Values were mean ± SE. $n = 9$ per group. *$P < 0.05$ vs control (A, one-way ANCOVA with body mass as the covariate; B and C, independent-sample *t*-test).

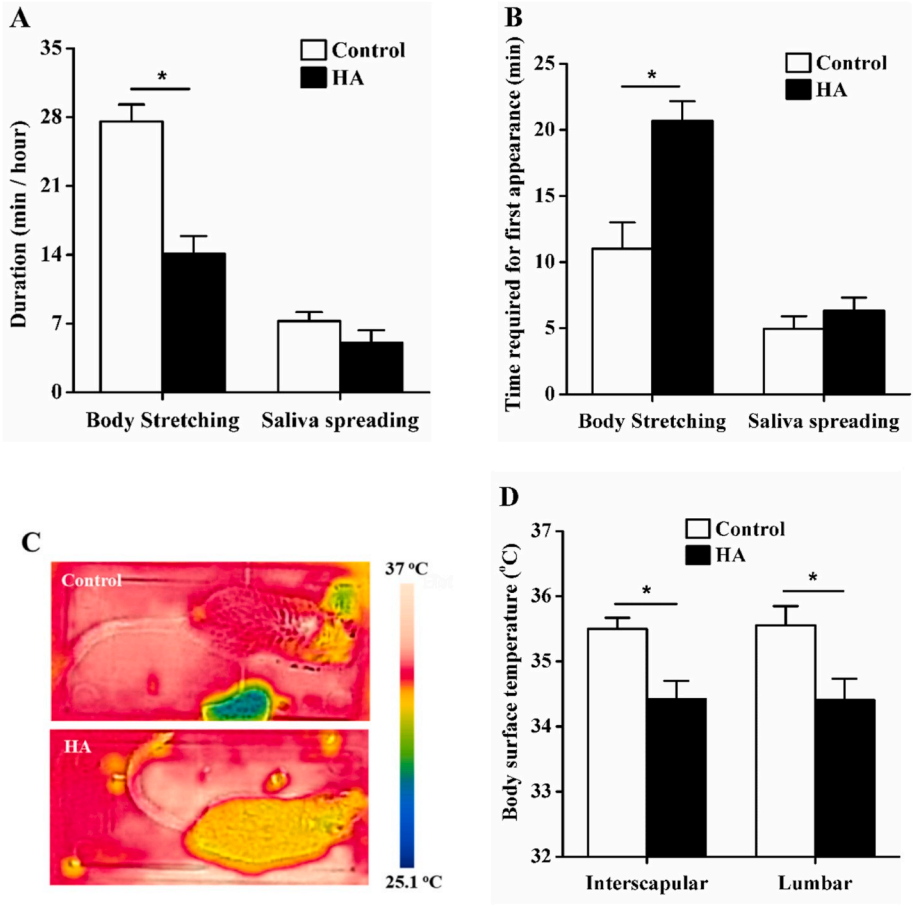

Fig. 4. Heat acclimation (at 37 ± 1 °C) changes the behavior of Mongolian gerbils in response to heat exposure. (A) The duration of body stretching and saliva spreading and (B) the time taken for them to first appear were measured at 37 ± 0.5 °C for 1 h. (C) Infrared images were taken at 23 °C after heat exposure. (D) Interscapular surface temperature and lumbar surface temperature of Mongolian gerbils. HA, Heat acclimation. Values were mean ± SE. $n = 9$ per group. *$P < 0.05$ vs control (independent-sample *t*-test).

4.2. Heat acclimation did not enhance evaporative water loss capacity

Rodents must rely on evaporation to dissipate heat when the ambient temperature is near or above their body temperature (Gordon, 1993). They enhance evaporative water loss mainly through respiration and saliva spreading because their sweat glands are nonfunctional in thermoregulation (Sugimoto et al., 1999; Robertshaw, 2006; Xu and Wang, 2015). In our study, heat acclimation did not alter respiratory

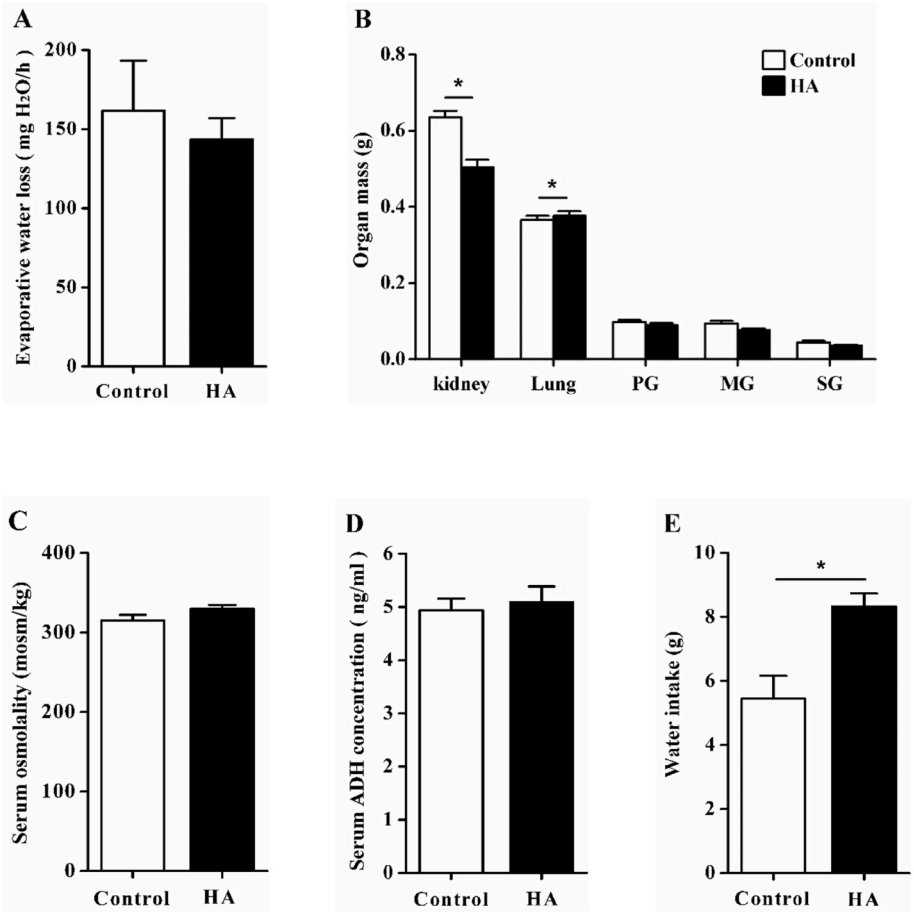

Fig. 5. Heat acclimation (at 37 ± 1 °C) changs the body water homeostasis of Mongolian gerbils. (A) The total evaporative water loss was determined at 30 ± 0.5 °C for 3 h. (B) The organ mass of kidney, lung, and salivary glands was measured after temperature acclimation (at 37 ± 1 °C or 23 ± 1 °C). (C) Serum osmolality and (D) serum ADH concentrations were determined. (E) Water intake was measured on the last day of temperature acclimation. HA, Heat acclimation; PG, parotid gland; MG, submandibular gland; SG, sublingual gland; ADH, antidiuretic hormone. Values were mean ± SE. $n = 9$ per group. *$P < 0.05$ vs control (independent-sample t-test). (A, B, and E, one-way ANCOVA with body mass as the covariate; C and D, independent-sample t-test).

evaporative water loss capacity. And the duration of saliva spreading was not changed. Although acute heat exposure increases the respiratory frequency in mice, information on respiratory frequency response to heat acclimation is lacking (Gordon, 2012). Our data suggest that the respiratory frequency of Mongolian gerbils may return to basal values after heat acclimation. In addition, studies in rats show that heat acclimation decreases saliva production (Horowitz et al., 1983; Horowitz and Meiri, 1985). Overall, heat-acclimated Mongolian gerbils may not increase their evaporative water loss capacity by enhancing saliva spreading.

AQP1 and AQP5 play a major role in water transport between airspace and capillary compartments (King et al., 1996; Carter et al., 1997; Ma et al., 2000). In this study, no changes in AQP1 and AQP5 expression were observed in the lungs after heat acclimation. These data confirm that the heat-acclimated Mongolian gerbils do not show enhanced evaporative water loss capacity through respiration. Moreover, heat acclimation did not change the expression of AQP5 in the parotid and submandibular glands. Hence, the salivary secretion of heat-acclimated Mongolian gerbils may be at a basic level. However, studies in rats show increased expression of AQP5 in the submandibular glands after 5 days of heat exposure (Sugimoto et al., 2013). The difference in AQP5 expression may be related to the time of acclimation. But further study is needed regarding the patterns of AQP5 expression in salivary glands during long-term heat acclimation.

Body water homeostasis is critically dependent on kidney function. AQP1 is expressed in the proximal tubule and descending thin limb of the nephron, where it mediates water permeability in these segments (Nielsen et al., 1993b, 1995b). Studies in mice have shown that the deletion of AQP1 leads to reduced renal water reabsorption and marked polyuria (Ma et al., 1998; Chou et al., 1999). In this study, heat acclimation resulted in decreased kidney mass and down-regulation of AQP1 expression in the kidneys. Therefore, the water permeability in proximal tubule and descending thin limb may decrease. Our data suggest that Mongolian gerbils may maintain body water homeostasis by regulating kidney function during heat acclimation. Additionally, AQP2 is abundant in the principal cells of the renal collecting duct and is the fundamental target for ADH regulation of collecting duct water reabsorption (Fushimi et al., 1993; Nielsen et al., 1993a, 1995a; Loonen et al., 2008; Kwon et al., 2009). Renal collecting duct water reabsorption is the key event for the maintenance of body water homeostasis (Kwon et al., 2009). The long-term regulation of this process is achieved through the regulation of AQP2 expression (Lankford et al., 1991; DiGiovanni et al., 1994). In this study, heat acclimation did not alter AQP2 expression in the kidneys. No change was observed in serum ADH concentration and serum osmolality in heat-acclimated Mongolian gerbils. These results suggest that AQP2 may not contribute to the adjustment of kidney function during heat acclimation.

4.3. *Changes in body temperature of heat exposed Mongolian gerbils*

Mongolian gerbils marked adaptive changes in body temperature over the time course of heat acclimation. Studies have demonstrated that acute heat exposure increases the body temperature of Mongolian gerbils (Pan et al., 2014; Wang et al., 2000). The body temperature rapidly rises to 39–41 °C when the ambient temperature is 38.9 °C (Pan et al., 2014). Similarly, the body temperature of mice is elevated significantly when exposed to 42 °C (Sareh et al., 2011). In addition, the body temperature of rats is elevated when exposed to 35 °C, and returns to basal levels rather than decreased after 2 days of heat exposure (Miova et al., 2008). In this study, the body temperature decreased from

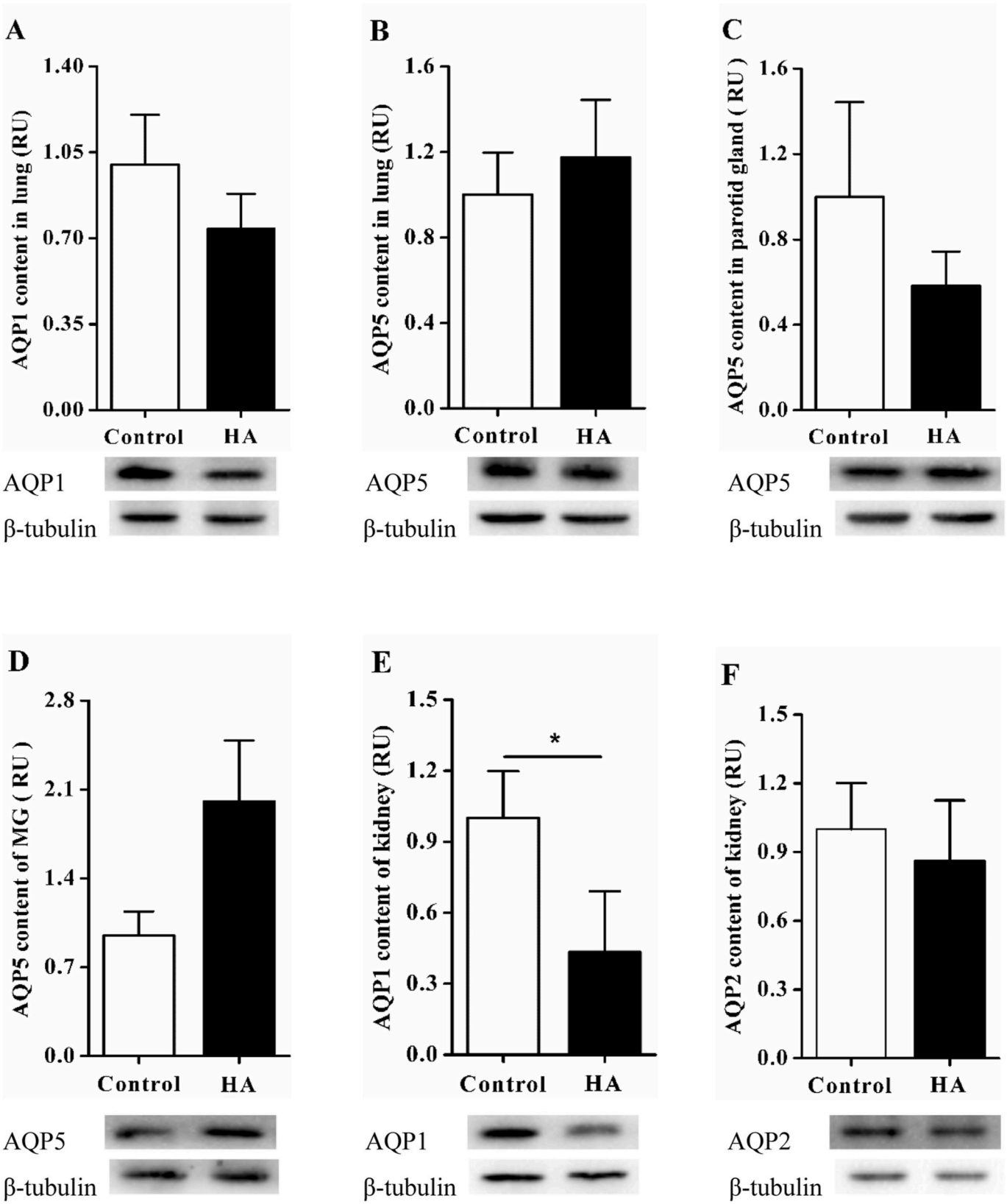

Fig. 6. Effects of heat acclimation (at 37 ± 1 °C) on aquaporins (AQPs) expression in lungs, salivary glands, and kidneys of Mongolian gerbils. (A) AQP1 expression and (B) AQP5 expression in the lung were determined and normalized to β-tubulin expression. (C) AQP5 expression in the parotid gland and (D) submandibular gland was determined and normalized to β-tubulin expression. (E) AQP1 and (F) AQP2 expression in the kidney was determined and normalized to β-tubulin expression. HA, Heat acclimation; MG, submandibular gland. Values were mean ± SE. $n = 9$ per group. *$P < 0.05$ vs control (independent-sample t-test).

the ninth day during acclimation at 37 °C. But the mechanism remains unclear. Studies show that heat acclimation may induce adjustments in peripheral thermoeffectors and thermoregulatory centers (Horowitz, 2002). The cooler body temperature may be related to the reduction in metabolic rates. Moreover, body temperature is governed by a synaptic network (Hammel, 1965). The changes in body temperature may have a neuronal basis (Matsuzaki et al., 2009; Shido and Matsuzaki, 2015). But further studies are needed.

This study also suggests that body temperature adaptation reduces the temperature gradient between the body and the surrounding environment. During heat acclimation, the body surface temperature was raised. The temperature gradient between the body core, body surface, and surrounding environment was almost negligible after heat acclimation. Therefore, we propose that heat loss through conduction, convection, and radiation will become ineffective when Mongolian gerbils are acclimated at 37 °C. In addition, heat-acclimated Mongolian gerbils exhibited lower body surface temperature after acute heat exposure than the controls, suggesting a reduced cooling requirement (Gordon, 2012).

In summary, metabolic adaptation is the primary strategy for heat-acclimated Mongolian gerbils to maintain body heat balance when heat stress cannot be avoided through behavioral means. This strategy may greatly reduce the impact of continuous heat exposure on evaporative water loss. Our report suggests the potential of Mongolian gerbils to tolerate for continuous heat challenges.

Declaration of competing interest

No conflict of interest exits in the submission of the manuscript entitled “Aquaporins, evaporative water loss and thermoregulation in heat-acclimated Mongolian gerbils (Meriones unguiculatus)”, and manuscript is approved by all authors for publication. We declare that the work described was original research, has not been published in whole or in part, and has not been considered for publication elsewhere.

CRediT authorship contribution statement

Yang-Yang Guo: Writing - original draft. **Shaoyan Hao:** Investigation. **Meng Zhang:** Methodology. **Xueying Zhang:** Methodology. **Dehua Wang:** Writing - review & editing.

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