Integrative Zoology 2023; 18: 353–371 doi: 10.1111/1749-4877.12678

ORIGINAL ARTICLE

INTEGRATIVE ZOOLOGY

Temperature determines the shift of thermal neutral zone and influences thermogenic capacity in striped hamsters

Shasha LIAO,[1] Song TAN,[1,2] Meizhi JIANG,[1] Jing WEN,[1] Jinsong LIU,[1] Jing CAO,[1] Ming LI[1] and Zhijun ZHAO[1,3]

[1]College of Life and Environmental Science, Wenzhou University, Wenzhou, China, [2]CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China and [3]Zhejiang Provincial Key Laboratory for Water Environment and Marine Biological Resources Protection, Wenzhou University, Wenzhou, China

Abstract

The thermoneutral zone (TNZ) reflects the adaptation of mammals to their natural habitat. However, it remains unclear how TNZ shifts in response to variations in ambient temperature. To test the hypothesis that ambient temperature plays a key role in determining TNZ variations between seasons, we measured metabolic rate, body temperature, and cytochrome c oxidase (COX) activity of several visceral organs in striped hamsters (*Cricetulus barabensis*) either acclimated to semi-natural conditions over a year, or subjected to a gradual decrease in mean temperature from 30 ± 1°C to −15 ± 1°C. The TNZ range in striped hamsters differed seasonally, with a wider TNZ and a lower lower-critical temperature in winter compared to summer. The hamsters showed a considerable leftward shift of lower-critical temperature from 30°C to 20°C after the ambient temperature of acclimation from 30°C down to −15°C, whereas the upper-critical temperature of TNZ remained fixed at 32.5°C. The resting metabolic rate in thermoneutral zone (RMRt), nonshivering thermogenesis (NST), and COX activity of brown adipose tissue, liver, skeletal muscle, brain, and kidneys, increased significantly in hamsters acclimated at lower ambient temperatures. Following acute exposure to 5°C and −15°C, hamsters acclimated to 32.5°C had significantly lower maximal NST and lower serum thyroid tri-iodothyronine (T_3) levels compared to those kept at 23°C. These findings suggest that acclimation to the upper-critical temperature of TNZ impairs the hamsters' thermogenic capacity to cope with extreme cold temperature. Reduced ambient temperature was mainly responsible for the leftward shift of TNZ in striped hamsters, which reflects the adaptation to cold environments.

Key words: metabolic rate, striped hamster, temperature, thermoneutral zone (TNZ), thyroid hormone

Correspondence: Ming Li and Zhijun Zhao, College of Life and Environmental Science, Wenzhou University, Wenzhou, Zhejiang 325035, China.
Email: 20190035@wzu.edu.cn, zhaozj@wzu.edu.cn, zhao73@foxmail.com

INTRODUCTION

The thermoneutral zone (TNZ) is one of the most well recognized concepts of thermal physiology of homeothermic organisms, and is defined as the range of ambient temperatures where metabolic rate is at basal levels (IUPS Thermal Commission 2001; Gordon 2012). Environmental temperatures below the lower-critical

temperature of the TNZ require an organism to increase metabolic heat production by shivering and/or nonshivering thermogenesis in order to maintain a high stable body temperature (T_b) (IUPS Thermal Commission 2001; Gordon 2012). Alternatively, when the temperature rises above the upper-critical temperature of the TNZ, metabolic rate increases due to energy spent on evaporative cooling (IUPS Thermal Commission 2001; Gordon 2012; Speakman & Keijer 2013). The TNZ describes the relationship between metabolic heat production and ambient temperature, providing information on an animal's energetic requirements and ability to live and survive in different climates (Gordon 2012; Zhao *et al.* 2022).

Although there is a tendency to consider the TNZ as a fixed entity, it is a highly variable parameter that varies between species and habitats (Gordon 2012). Early studies by Scholander *et al.* demonstrated that the TNZ of arctic and tropical mammals reflects the adaptations of the animal to its natural habitat (Scholander *et al.* 1950a,b; Ganeshan & Chawla 2017). Arctic mammals such as polar bear cubs (*Ursus maritimus*), white fox (*Alopex lagopus*), and husky (*Canis lupus familiaris*) are heavily insulated in winter due to increased fur density. Additionally, they have an extremely wide TNZ with a very low lower-critical temperature of around −30°C to −40°C and an upper-critical temperature of 30°C (Scholander *et al.* 1950a,b; Gordon 2012). Mammalian species living in cold climates generally have much thicker fur than those living in warm climates (Scholander *et al.* 1950a,b).

There are many factors that influence seasonal environmental conditions, including, temperature, photoperiod, food quality and availability, as well as wind and rainfall. At present, it remains unclear how animals can adapt their TNZ to different natural environments. Many external factors are thought to be involved in interspecies differences in TNZ (Gordon 2012; Ganeshan & Chawla 2017). Although little is known on how animals adapt their TNZ to different natural environments, ambient temperature which differs significantly between arctic and tropical mammals did induce a shift of the TNZ (Scholander *et al.* 1950a,b; Gordon 2012). Cold acclimation and/or cold adaptation in relatively large mammals leads to a leftward shift in the TNZ due to the added insulation of a thicker coat and decreased conductance (Gordon 1993, 2012). In contrast, equatorial mammals, which are better adapted for heat dissipation, exhibit a rightward shift in their TNZ (Scholander *et al.* 1950a,b; Ganeshan & Chawla 2017).

In addition to interspecies differences in the TNZ, changes in TNZ range and thermogenic capacity are also observed within a given species (Gordon 1993; Cannon & Nedergaard 2011; Nedergaard & Cannon 2014). For example, a field study on wild striped hamsters (*Cricetulus barabensis*) found that the range of TNZ differed significantly between individuals captured in summer and winter (Zhao *et al.* 2010a). The lower-critical temperature of striped hamsters is approximately 27.5°C in summer and 20.8°C in winter (Zhao *et al.* 2010a). In addition, Mongolian gerbils (*Meriones unguiculatus*) exhibit a wider thermoneutral zone (26.5–38.9°C), and have considerable fluctuations in the thermogenic capacity when acclimated to different temperatures or seasons (Ding *et al.* 2018; Guo *et al.* 2019; Wen *et al.* 2022). The leftward shift from summer to winter demonstrates that mammals can dynamically modulate their TNZ and thus their susceptibility to cold environmental conditions (Hammond *et al.* 1999; Soriano *et al.* 2002; Ganeshan & Chawla 2017). Nonshivering thermogenesis (NST), a facultative and adaptive form of thermogenesis that can be acutely induced by norepinephrine injection, and/or an increase in resting metabolic rate in thermoneutral zone (RMRt), are typical responses to cold acclimation (Jansky *et al.* 1973; Li *et al.* 2010; Cannon & Nedergaard 2011; Luna *et al.* 2012; Zhou *et al.* 2016; Plasman *et al.* 2020). When exposed to ambient temperatures below the TNZ, many small mammals are shown to depend on NST suggesting a possible link between NST and the TNZ range (Jansky 1973; Haim 1984). For example, the diurnal Mongolian gerbil had a greater RMR and NST, and a wider TNZ compared to the nocturnal midday gerbil (*Meriones meridianus*) (Ding *et al.* 2018). The increased RMR and NST observed in cold-acclimated rodents is usually associated with a significant increase in the rate of mitochondrial state 4 respiration and/or activity of cytochrome c oxidase in metabolically active organs such as brown adipose tissue (BAT), liver, and skeletal muscle (Bourhim *et al.* 1990; Zhao *et al.* 2010a,b; Zheng *et al.* 2014).

The striped hamster (*Cricetulus barabensis*) is a common rodent in northern China and, also occurs in Russia, Mongolia, and Korea (Zhang & Wang 1998). The present study aimed to examine how ambient temperature influences the shift of TNZ and thermogenic capacity in striped hamsters. To examine roles of ambient temperature and photoperiod played in the TNZ shift in the seasonal adaptation of striped hamsters, we (1) acclimated hamsters in outdoor enclosures under the natural ambient temperature and photoperiod regime, (2) acclimated hamsters in the laboratory to short and long photoperiod conditions (8L:16D and 16L:8D) while temperature was kept constant, (3) acclimated hamsters to different

ambient temperatures for 2 weeks. After acclimations of experiments 1, 2, and 3, TNZ in these hamsters were determined by metabolic rate measurement at different ambient temperatures. To examine the changes of thermogenic capacity in hamster from experiment 3, NST and COX activity in several visceral organs were measured with RMRt being calculated. To further examine the effect of the upper critical temperature on physiological response to acute cold exposure, (4) we acclimated hamsters to ambient temperature of 32.5°C for 4 weeks followed by acute cold exposure to ambient temperature 5°C or −15°C, respectively. RMRt, NST, COX activity, and serum thyroid hormone levels were determined in hamsters after acclimation to 32.5°C for 4 weeks and, also acute cold exposure to 5°C or −15°C, respectively. Body temperature and energy intake/digestibility were measured during the acute cold exposures. We predicted that the hamsters in the outdoor enclosures during winter would have a lower lower-critical temperature and a wider TNZ compared to hamsters in outdoor enclosures during summer. Additionally, we predicted that the hamsters acclimated to a lower ambient temperature would decrease their lower-critical temperature, independent of photoperiod, whereas the hamsters acclimated to the upper critical temperature (32.5°C) would have an impaired thermogenic capacity to cope with acute cold exposure.

MATERIALS AND METHODS

All experimental procedures involving animals were reviewed and approved by the Animal Care and Use Committee of the Wenzhou University (approval no. WZU-086). We followed the Reporting in Vivo Experiments (ARRIVE) guidelines developed by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs).

Animals

We obtained striped hamsters from the colony maintained at Liaocheng University and Wenzhou University, which began in 2008 with animals trapped from farmland in the center of Hebei Province (115°13′E, 38°12′S) in the North China Plain. Hamsters were housed in plastic cages ($29 \times 15 \times 18$ cm^3) with sawdust bedding, on a 12/12 h light: dark cycle (12L:12D; lights on at 0800 hours) at an ambient temperature of 23 ± 1°C. Food (standard rodent chow; produced by Beijing KeAo Feed, Beijing) and water were provided *ad libitum*.

Striped hamster feeds mainly on plant stems and leaves during the summer and forages on crop seeds in the winter (Zhang & Wang 1998; Song & Wang 2002, 2003). Previous studies have shown that the lower-critical temperature of the TNZ is lower in hamsters in winter and in those acclimated to cold conditions (5°C) compared to hamsters in summer and those acclimated to warm conditions (30°C) (Zhao *et al.* 2010a,b, 2014a). Thus, Experiment 1 was designed to examine seasonal changes in TNZ by excluding the food effects. Thirty-two male hamsters (3.5- to 4.5-month-old) were transferred from the laboratory to outdoor enclosures during mid-April where they were maintained individually for 12 months in plastic cages ($29 \times 18 \times 16$ cm) with fresh sawdust bedding. The hamsters kept in outdoor enclosures had free access to food (standard rodent chow), but were subject to natural photoperiod and ambient temperature across 4 seasons (Spring: March–May, mean daily maximum and minimum temperature is 20.2°C and 8.5°C; Summer: June–August, 31.3°C and 21.6°C; Autumn: September–November, 19.2°C and 9.8°C; Winter: December–February, 4.1°C and −4.6°C). TNZ estimation through metabolic rate measurements in hamsters (details see below) were performed during summer (the end of July, daily maximal and minimal temperatures were 33°C and 24°C, respectively; 14.5L:9.5D; $n = 8$ male hamsters, 36.3 ± 0.7 g), autumn (early November, 7°C and −4°C; 10.5L:13.5D; $n = 8$ male hamsters, 36.9 ± 0.9 g), winter (mid-January, −2°C and −11°C; 9.5L:14.5D; $n = 8$ male hamsters, 37.0 ± 0.6 g), and spring (mid-April, 17°C and 9°C; 13L:11D; $n = 8$ male hamsters, 38.1 ± 0.6 g).

Metabolic rate measurement and TNZ estimation

The metabolic rate of the hamsters was measured using an open-flow respirometry system (Sable Systems International, Inc.; Las Vegas, NV, USA) as described previously (Zhao 2011a). Fresh air was pumped at a rate of 800–900 mL·min^{-1} through a cylindrical Perspex chamber (diameter: 10 cm and length: 35 cm) placed in an incubator (5–50°C, ±0.1°C). Air leaving the chamber is dried (silica gel) and subsampled through the oxygen analyzer (FC-10a, Sable Systems) at a flow rate of 300 mL·min^{-1} (SS4 gas analyzer sub-sampler/pump). PVC transparent tubes (diameter: 6 mm and pipe wall thickness: 1 mm) were used to connect the pump, chamber, subsampling unit, and oxygen analyzer. Eight animals were measured in parallel (8-channel multiplexer, Sable RM-8 Respirometry Flow Multiplexer) with 1 minute per animal. The O_2 consumption rate was determined by calculating the difference in O_2 concentration between each channel

and the reference air using the equation: $VO_2 = FR (FiO_2 - FeO_2)/(1 - FiO_2 \times (1 - RQ))$, where FR is the flow rate, FiO_2 is O_2 concentration of the air reference, FeO_2 is excurrent fractional concentration of O_2 from the animal chamber, and RQ is respiratory quotient (Arch *et al.* 2006). Here, RQ was assumed to be 0.85 (Withers 1977; Chi & Wang 2011). CO_2 in the outflow stream was not absorbed prior to gas analysis to minimize error when converting oxygen consumption to energy expenditure when the respiratory quotient is unknown (Koteja 1996; Speakman 2000; Johnson *et al.* 2001; Król *et al.* 2003). The data were averaged and collected every 10 s using a computer connected to an analogue-to-digital converter (UI2, Sable system), and analyzed using standard software (Expedata, Sable system). Metabolic rate was measured at 37.5°C, 35°C, 32.5°C, 30°C, 27.5°C, 25°C, 22.5°C, 20°C, 15°C, 10°C, and 5°C, for 1.5 h at each temperature. The baseline measurements were performed prior to each measurement. The lowest reading of oxygen consumption of 2 stable and consecutive bouts was taken to estimate the metabolic rate at each temperature, and expressed as $mLO_2 \cdot g^{-1} \cdot h^{-1}$.

Experiment 2 was designed to examine the effect of photoperiod on TNZ range. Twenty male hamsters (3.5- to 4.5-month-old) were randomly selected and assigned to either a long-day (LD, 16L:8D, $n = 10$) or a short-day (SD, 8L:16D, $n = 10$) group for 6 weeks. Both groups were kept at a constant ambient temperature of 23°C and fed *ad libitum*. At the end of the experiment, the metabolic rate of hamsters was measured across the range of temperatures from 37.5°C to 5°C, using the same methods described in experiment 1.

Experiment 3 was designed to examine the changes of TNZ in striped hamsters acclimated to a gradual decrease in temperature. To acclimate the hamsters at different ambient temperatures, a total of 96 male hamsters were randomly assigned to 1 of 6 temperature groups: 30°C ($n = 16$), 23°C ($n = 16$), 10°C ($n = 16$), 0°C ($n = 16$), −8°C ($n = 16$), and −15°C ($n = 16$). The hamsters used in this experiment were from the same colony as those in experiment 1 and 2 but were maintained at Wenzhou University.

Metabolic trails

At the end of the experiment 8 hamsters were randomly selected from each group to measure metabolic rate across the range of temperatures from 37.5°C to 5°C, using an open flow respirometry system (LabMaster, TSE system, Germany). As described previously (Wen *et al.* 2018a,b), air was pumped at a rate of 1000 $mL \cdot min^{-1}$ (Calosys Pump, 994620-AirP-YP-VC, TSE, Germany) through a cylindrical sealed Perspex chamber (diameter: 10 cm and length: 35 cm). Air leaving the chamber were dried using a special drier (Air drying Process Control Unit, TSE system, Germany) and directed through an oxygen and carbon dioxide analyzer (Ultramat/Oxymat 6, Siemens, Germany) at a flow rate of 380 $mL \cdot min^{-1}$. Metabolic rate measured as oxygen consumption was given automatically at intervals of 9 min by the respiratory system. The resting metabolic rate at each ambient temperature was calculated as the average of the lowest, stable, and consecutive 2 data points. An open flow respirometry system (TSE, Germany) was also used to quantify NST as the rate of oxygen consumption. Maximum NST (NST_{max}) was defined as the maximum rate of oxygen consumption in response to noradrenaline (NA) and was induced by a subcutaneous injection of NA at 25 ± 0.5°C (Nespolo *et al.* 2001). The mass-dependent dosage of NA (Shanghai Harvest Pharmaceutical Co, Ltd) was calculated according to the equation: NA ($mg \cdot kg^{-1}$) = 6.6 $Mb^{-0.458}$ (g) (Mb, body mass) (Heldmaier 1971; Zhao 2011b). The NST_{max} was measured for 1.5 h and calculated from continuous stable maximal recordings over 10 min. Additionally, a 3-h baseline measurement of metabolic rate was obtained before the NE injection. Regulative NST (NSTr) was calculated as NST_{max} minus RMR. All measurements were performed between 1000 and 1700 to correct for a possible effect of the circadian rhythm (Zhao *et al.* 2022).

COX activity

The animals were sacrificed by decapitation at the end of each temperature (the day after NST measurements). Interscapular BAT, liver, kidneys, brain, heart, and skeletal muscle, taken from hind leg, were quickly removed, weighed and immediately frozen using liquid nitrogen. These tissues were stored at −80°C for COX measurements. Mitochondrial protein of these tissues was prepared as described previously (Wiesinger *et al.* 1989; Zhao & Wang 2005). In brief, tissues were weighed and homogenized (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 μM BSA, pH 7.2). The homogenate was centrifuged at 12 096 *g* for 10 min at 4°C, the supernatant was discarded, and the precipitate was resuspended with ice cold medium B (containing 250 mM sucrose, 10 mM TES, 1 mM EGTA, 64 μM BSA, pH 7.2) and centrifuged at 500 g for 10 min at 4°C. The supernatant was then centrifuged at 8740 g for 10 min at 4°C, and the resulting pellet was resuspended

(1:1, w/v) with ice-cold medium C (containing 100 mM KCl, 20 mM TES, 1 mM EGTA, pH 7.2). Mitochondrial protein concentrations were determined by the Folin phenol method with bovine serum albumin as the standards (Lowry *et al.* 1951). COX activity was measured with a polarographic method using an oxygen electrode (Hansatech Instruments Ltd., England) (Sundin *et al.* 1978; Zhao & Wang 2005).

Experiment 4 was designed to examine the effect of acclimation at upper critical temperature on thermogenic capacity

A total of 96 hamsters (aged 3.5–4 months) were randomly assigned into 2 groups, each acclimated to either ambient temperature of 23°C ($n = 48$) or 32.5°C (the upper critical temperature of the TNZ of this species, Zhao *et al.* 2010a, $n = 48$) for 4 weeks, respectively. After the temperature acclimation, 16 hamsters from each group continued to be maintained at the same ambient temperature as before. Another 16 hamsters from each group were exposed to mild cold conditions (5°C) for 24 h (23–5°C, $n = 16$, or 32.5–5°C, $n = 16$). The 16 hamsters left in each group were exposed to extreme cold conditions (−15°C) for 24 h (23°C to −15°C, $n = 16$, or 32.5°C to −15°C, $n = 16$).

RMRt and NST measurements

RMRt and NST were measured in hamsters that continued to be maintained at 23°C ($n = 8$) or 32.5°C ($n = 8$) and those after acutely exposed to 5°C ($n = 8$) or −15°C for 24 h ($n = 8$). Metabolic rates were quantified as the rate of oxygen consumption using the same open-flow respirometry system (TSE Labmaster, Germany) as described in experiment 3. To exclude the effects of heat exposure and injection of NA on the physiology of hamsters, 8 animals used in the metabolic trails were not further going through the food trails or used to determine the T_b, COX activity and serum thyroid hormone levels.

T_b measurement, food trials, and COX activity and state 4 respiratory determinations

T_b was measured at 3-h intervals over 24 h in hamsters acutely exposed to ambient temperature of 5°C ($n = 8$) or −15°C ($n = 8$), also in hamsters that continued to be maintained at the original ambient temperature of 23°C ($n = 8$) or 32.5°C ($n = 8$) as control. In detail, under general anesthesia (induction 3–5%, maintenance 1–2% isoflorane), an encapsulated thermo-sensitive passive transponder (LifeChip with Bio-Thermo^{TM}, diameter 2 mm and length 14 mm; Destron Fearing, South St Paul, USA) was implanted via intraperitoneal injection with a syringe into each animal under general anesthesia (induction 3–5%, maintenance 1–2% isoflorane). After surgery, each animal was returned to its cage and given a week to recover before T_b recording began. The LifeChip with Bio-Thermo^{TM} is an implantable radio frequency microchip preloaded into an individualized sterile syringe and is used to measure temperature. The microchip communicates its information to a Destron Fearing^{TM} Pocket Reader EX, which has been programmed with software to read temperature. The LifeChip with Bio-Thermo^{TM} is calibrated to the normal animal temperature range of 29°C to 41°C (resolution is 0.1°C). It uses the energy in the radio signal from the Destron Fearing Pocket Reader EX to create the power it requires to operate. Upon receiving this radio signal, the microchip's sensor determines the temperature and transmits the information to the reader.

Energy intake and digestibility

The hamsters subjected to T_b measurement were simultaneously used to determine gross energy intake (GEI), digestive energy intake (DEI), and digestibility over the same 24 h period, during which changes of body mass in all these hamsters were also monitored. Four out of 8 hamsters showed inactivity and reduced food intake after being transferred from 32.5°C to −15°C, and the T_b decreased below 34°C; hence, we terminated the −15°C exposure experiment. GEI and digestibility measurements were performed in the remaining 5 groups (23°C, 23–5°C, 23°C to −15°C, 32.5°C, and 32.5–5°C). As previously described (Grodzinski & Wunder 1975; Liu & Wang 2007), a known quantity of food was provided and after 24 h any uneaten food and orts mixed with the bedding material were collected, along with feces from each animal. Food and feces were separated manually after drying at 60°C to constant mass. The gross energy content of food and feces were determined using an IKA C2000 oxygen bomb calorimeter (IKA, Germany). GEI, DEI, gross energy of feces (GEF), and digestibility were calculated according to the following equations (Grodzinski & Wunder 1975; Zhao *et al.* 2020):

$$\text{GEI}\left(\text{kJ}\cdot\text{d}^{-1}\right) = \text{food take}\left(\text{g}\cdot\text{d}^{-1}\right) \times \text{dry matter content of food (\%)} - \text{dry spillage of food} \times \text{gross energy content of food}\left(\text{kJ}\cdot\text{g}^{-1}\right);$$

$$\text{GEF}\left(\text{kJ}\cdot\text{d}^{-1}\right) = \text{dry feces mass}\left(\text{g}\cdot\text{d}^{-1}\right) \times \text{energy content of feces}\left(\text{kJ}\cdot\text{g}^{-1}\right);$$

$$\text{DEI}\left(\text{kJ}\cdot\text{d}^{-1}\right) = \text{GEI} - \text{GEF};$$

$$\text{Digestibility (\%)} = (\text{DEI}/\text{GEI}) \times 100\%.$$

COX activity and state 4 respiratory

After the T_b measurements and food trails being completed in 24 h, hamsters were euthanized by decapitation. As described above, scapular BAT, liver, and muscle were removed, weighed, and immediately frozen in liquid nitrogen and stored at −80°C until required for analysis. Tissues were homogenized, and the COX activities were measured using the methods outlined in experiment 3. The rate of state 4 respiration was measured in the absence of 1.0 mmol·L^{-1} ADP, with the substrates of 5.0 mmol·L^{-1} succinate and 3.75 mol·L^{-1} rotenone (Demin *et al.* 1998).

Serum T_3 and T_4

Trunk blood was collected from each animal and left to clot for 2 h. The samples were then centrifuged at 4°C (3500 rpm·min^{-1} for 15 min) and serum aliquots extracted and stored at −20°C until required for T_3 and T_4 measurements. Serum T_3 and T_4 levels were determined using radioimmunoassay I^{125} RIA kits (Beijing North Institute of Biological Technology, Beijing, China). The volumes used for the measurements were 50 μL for T_3 and 50 μL for T_4. The intra- and inter-assay coefficients of variation were 2.4% and 8.8% for T_3, and 4.3% and 7.6% for T_4.

Statistical analysis

The data were means ± SE.

Experiment 1 and Experiment 2: The lower- and upper-critical temperature of TNZ was estimated using a linear regression approach as described previously (Corp *et al.* 1997). At temperatures within the TNZ, metabolic rate is at a minimum and is independent of temperature. However, at below lower-critical temperature, an animal increases its metabolic rate to maintain a constant body temperature. To calculate the TNZ of striped hamsters for each season, the metabolic rate corresponding to the highest temperature was removed and the r^2 was recalculated using the remaining data. Repeating this procedure resulted in a sequential removal of data. A greater r^2 would result from the exclusion of temperatures above the lower-critical temperature. Similarly, a significantly greater r^2 would result from the exclusion of temperatures below upper- critical temperature.

Experiment 3: The effect of temperature on RMRt, NST_{max}, NSTr, as well as COX activity of several tissues was analyzed using one-way ANOVA, followed by Student–Newman–Keuls (SNK) *post hoc* test if required. Pearson's correlation was performed to examine relationships between COX activity and RMRt, NST_{max} and NSTr.

Experiment 4: The effect of high temperature acclimation and extreme cold exposure on body mass, energy intake, RMRt, NST_{max}, NSTr, and T_b, as well as COX activity and serum T_3 and T_4 levels was examined using 2-way ANOVA, followed by SNK *post hoc* test where required. The level of significance was set at $P < 0.05$.

RESULTS

The TNZ shift across 4 seasons and the effects of photoperiod

Experiment 1: Removing metabolic rate data responding to ambient temperature above 30°C from the summer group significantly increased the r^2 (from $r^2 = 0.532$, to $r^2 = 0.648$, $F_{1,76} = 4.31$, $P < 0.05$), and therefore 30°C represents the lower–critical temperature for hamsters in the summer group (Fig. 1). Removing data below 32.5°C significantly increased the r^2 (from $r^2 = 0.176$, to $r^2 = 0.649$, $F_{1,44} = 11.77$, $P < 0.001$), and therefore 32.5°C represents the upper-critical temperature for the summer group. For the autumn group, the r^2 was significantly increased when excluding data above 25°C (from $r^2 = 0.462$ to $r^2 = 0.736$, $F_{1,76} = 8.69$, $P < 0.01$), and when excluding data below 32.5°C (from $r^2 = 0.187$ to $r^2 = 0.611$, $F_{1,44} = 8.54$, $P < 0.01$), and therefore 25°C and 32.5°C represent the lower- and upper-critical temperatures, respectively, for hamsters in the autumn group. Similarly, the r^2 was significantly increased in the winter group following exclusion of data above 20°C (from $r^2 = 0.711$ to $r^2 = 0.774$, $F_{1,60} = 5.67$, $P < 0.05$) and below 32.5°C (from $r^2 = 0.135$ to $r^2 = 0.315$, $F_{1,44} = 2.91$, $P < 0.05$), and therefore the lower- and upper-critical temperatures for hamsters in the winter group was 20°C and 32.5°C, respectively. Additionally, excluding data above 25°C (from $r^2 = 0.595$ to $r^2 = 0.728$,

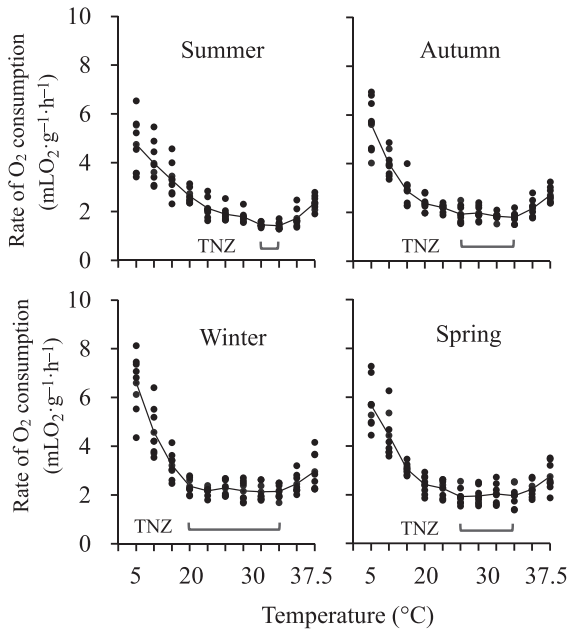

Figure 1 The rate of O_2 consumption of striped hamsters across 4 seasons. Striped hamsters were acclimated to natural photoperiod and temperature in an enclosure. TNZ, thermoneutral zone.

$F_{1,76} = 10.01$, $P < 0.001$) and below 32.5°C (from $r^2 = 0.042$ to $r^2 = 0.347$, $F_{1,76} = 3.93$, $P < 0.05$) resulted in a significant increase in the r^2 in the spring group, and therefore 25°C and 32.5°C represent the lower- and upper-critical temperatures, respectively. The TNZ varied considerably across seasons becoming wider during winter and narrower during summer (Fig. 1). The lower-critical temperature of the TNZ decreased from summer to winter (summer, 30°C; autumn, 25°C; winter, 20°C; spring, 25°C), whereas the upper-critical temperature was fixed at 32.5°C for all 4 seasons. The regression equation below the lower-critical temperature of TNZ was $y = -0.136x + 5.363$ ($F_{1,54} = 151.46$, $P < 0.0001$) for summer, $y = -0.191x + 6.179$ ($F_{1,38} = 127.59$, $P < 0.0001$) for autumn, $y = -0.338x + 8.195$ ($F_{1,22} = 49.92$, $P < 0.0001$) for winter, and $y = -0.1999x + 6.4560$ ($F_{1,38} = 136.36$, $P < 0.0001$) for spring. There was a significant difference in slopes between seasons with the slope in winter being significantly lower than that in summer ($F_{1,76} = 26.31$, $P < 0.001$), autumn ($F_{1,60} = 11.18$, $P < 0.001$) and spring ($F_{1,60} = 9.67$, $P < 0.001$). For experiment 2 the TNZ was 27.5–32.5°C, and it was the same between the LD and SD acclimated hamster groups (Fig. 2).

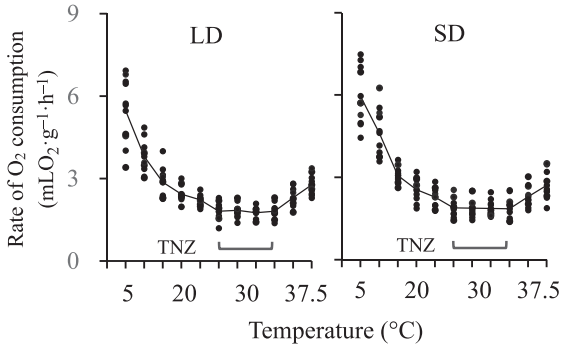

Figure 2 The rate of O_2 consumption of striped hamsters acclimated to long day (LD) or short day (SD) photoperiod. TNZ, thermoneutral zone.

TNZ, RMRt and NST in hamsters acclimated to different ambient temperatures

Experiment 3: The metabolic rate of striped hamsters varied considerably across the temperature range of 5°C to 37.5°C (Fig. 3a–f). The metabolic rate of all 6 groups of hamsters increased as temperatures decreased below 23°C, or increased above 32.5°C. The TNZ range changed considerably between the 6 temperature groups, with the −15°C group having the widest range (20°C to 32.5°C), and the 30°C group having the narrowest range (30°C to 32.5°C, Fig. 4). The TNZ was characterized by a fixed upper point of 32.5°C, but the lower-critical temperature decreased from 30°C to 20°C. RMRt was significantly different between the 6 temperature groups with the maximum value in hamsters acclimated to −15°C and the minimum value in hamsters acclimated to 30°C ($F_{5,42} = 15.69$, $P < 0.01$, Fig. 3g). NSTmax and NSTr were significantly higher in the hamsters acclimated to 0°C, −8°C and −15°C compared to those acclimated to 30°C and 23°C (NSTmax, 30°C, 4.04 ± 0.37 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 23°C, 5.12 ± 0.41 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 10°C, 5.66 ± 0.52 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 0°C, 6.26 ± 0.37 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; −8°C, 7.01 ± 0.30 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; and −15°C, 7.35 ± 0.22 $mLO_2 \cdot g^{-1} \cdot h^{-1}$, $F_{5,42} = 10.83$, $P < 0.01$, Fig. 3h; NSTr, 30°C, 2.56 ± 0.38 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 23°C, 4.12 ± 0.51 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 10°C, 3.44 ± 0.42 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; 0°C, 4.46 ± 0.37 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; −8°C, 5.11 ± 0.27 $mLO_2 \cdot g^{-1} \cdot h^{-1}$; and −15°C, 5.28 ± 0.22 $mLO_2 \cdot g^{-1} \cdot h^{-1}$, $F_{5,42} = 7.01$, $P < 0.01$, Fig. 3i).

The COX activity in hamsters acclimated to different ambient temperatures

There was a significant effect of temperature on COX activity of BAT, liver, skeletal muscle, brain and

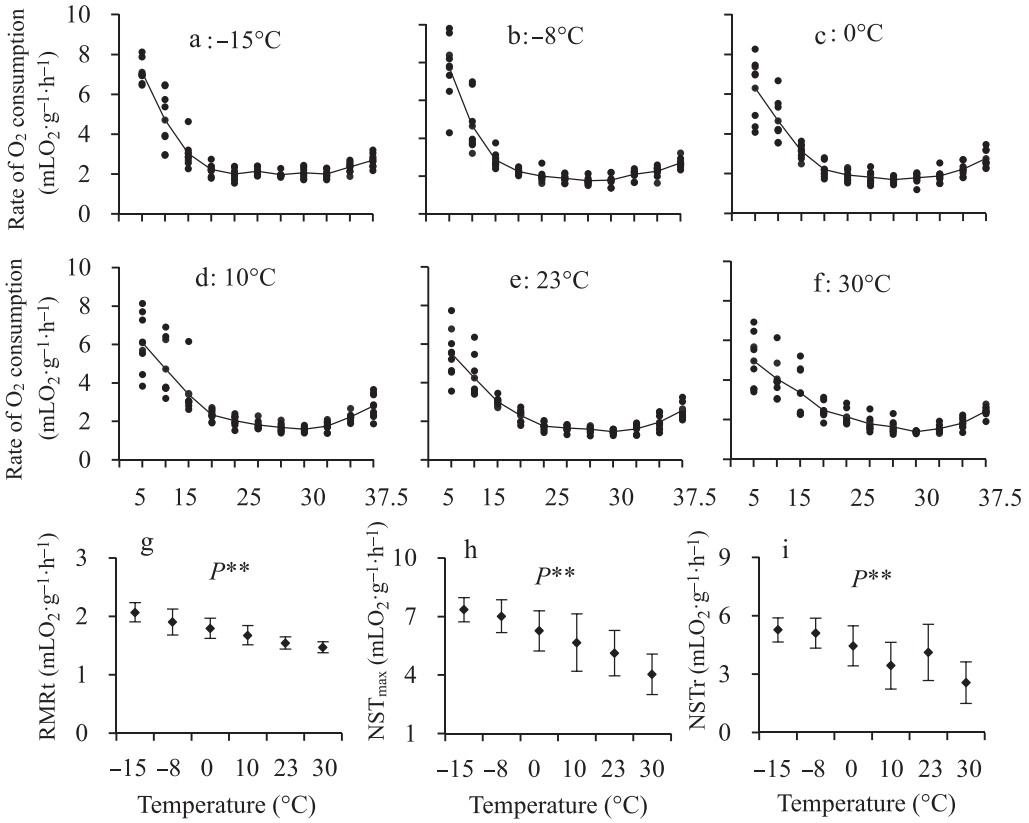

Figure 3 The rate of O_2 consumption, RMRt, NSTmax, and NSTr of striped hamsters. The striped hamsters were acclimated to a gradual decrease in temperatures from 30°C to −15°C. Data are plotted or are means ± SE. ** indicates a significant effect of temperature ($P < 0.01$).

kidneys, with a higher level of activity in cold acclimated hamsters compared to warm acclimated hamsters (BAT, $F_{5,41} = 3.06$, $P < 0.05$, Fig. 5a; liver, $F_{5,41} = 2.75$, $P < 0.05$, Fig. 5b; skeletal muscle, $F_{5,41} = 3.27$, $P < 0.05$, Fig. 5c; brain, $F_{5,41} = 2.86$, $P < 0.05$, Fig. 5d; kidney, $F_{5,41} = 3.58$, $P < 0.01$, Fig. 5f). The COX activity of heart did not differ significantly between the 6 groups ($F_{5,41} = 0.50$, $P > 0.05$, Fig. 5e). BAT COX activity was significantly correlated with RMR, NSTmax and NSTr (Fig. S1A–C, Supporting Information). COX activity of the liver was not statistically correlated with RMR (Fig. S2D, Supporting Information), whereas it was positively correlated with NSTmax and NSTr (Fig. S2E,F, Supporting Information). COX activity of skeletal muscle was positively correlated with RMR (Fig. S2G, Supporting Information), but not NSTmax or NSTr (Fig. S2H,I, Supporting Information). There were significant positive correlations between brain COX activity and RMR, NSTmax and NSTr (Fig. S1J–L, Supporting Information). No significant correlations were observed between heart COX activity and RMR, NSTmax or NSTr (Fig. S1M–O, Supporting Information). COX activity of the kidney was significantly correlated with RMR, NSTmax and NSTr (Fig. S1P–R, Supporting Information).

Effects of acclimation at upper critical temperature on body mass, energy intake and their response to acute cold exposure

Experiment 4: There was no significant difference in body mass between hamsters continuously maintained at 23°C and those at 32.5°C ($F_{1,35} = 0.40$, $P > 0.05$). Body mass also did not change significantly after the acute cold exposure to 5°C or −15°C ($F_{1,35} = 0.03$, $P > 0.05$, Fig. 6a). GEI of hamsters at 23°C was significantly higher than that of hamsters at 32.5°C (Fig. 6b). In

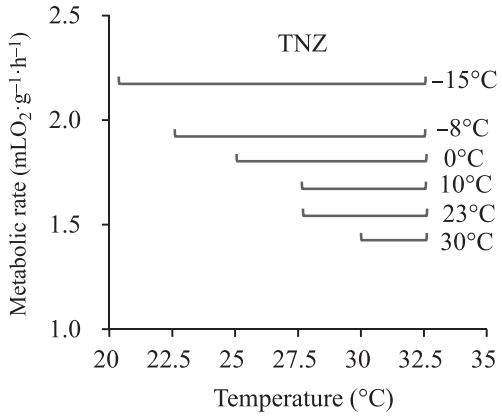

Figure 4 The thermoneutral zone (TNZ) of striped hamsters acclimated to a gradual decrease in temperatures from 30°C to −15°C.

hamsters acclimated at 23°C, acute cold exposure to 5°C or −15°C induced considerable increase of GEI by 39.5% and 72.4%, respectively. GEI of hamsters acclimated at 32.5°C also increased significantly following exposure to 5°C (Fig. 6b). There was no such data for 32.5°C to −15°C group since the cold exposure to −15°C terminated in the middle of the experiment. Additionally, hamsters acclimated at 23°C had a significantly higher DEI and produced more feces than those at 32.5°C. Acute cold exposure resulted in significant increases in DEI and GEF for both the 23°C and 32.5°C groups of hamsters (Fig. 6c,d), but the increases were greater for the 23°C compared to the 32.5°C group. Digestibility did not differ between hamsters acclimated at 23°C and those at 32.5°C and there was also no significant change in digestibility following acute cold exposure ($F_{1,35} = 0.40$, $P > 0.05$, Fig. 6e).

Effects of acclimation at upper critical temperature on RMRt, NST and their response to acute cold exposure

Hamsters acclimated to 32.5°C had a lower RMR than hamsters maintained at 23°C ($F_{1,34} = 5.72$, $P < 0.05$, Fig. 7a). Acute exposure to 5°C resulted in a significant increase in RMRt by 21.1% and 27.9% for hamsters in the 23°C and 32.5°C groups, respectively ($F_{1,34} = 20.21$, $P < 0.01$, Fig. 7a). Both NSTmax and NSTr were significantly lower in hamsters acclimated to 32.5°C compared to those kept at 23°C (NSTmax, $F_{1,34} = 24.74$, $P < 0.01$, Fig. 7b; NSTr, $F_{1,34} = 20.67$, $P < 0.01$, Fig. 7c). For hamsters kept at 23°C, acute exposure to 5°C and −15°C increased NSTmax by 24.2% and 26.7%, respectively, and increased NSTr by 25.2% and 29.7%, respectively. In contrast, there was no effect of acute cold exposure on the NSTmax or NSTr of hamsters acclimated to 32.5°C.

Effect of acclimation at upper critical temperature on T_b and its response to acute cold exposure

There was no difference in T_b between hamsters kept at 23°C and 32.5°C prior to cold exposure (h0, $F_{1,32} = 1.75$, $P > 0.05$, Fig. 8). Following cold exposure to 5°C, the T_b of the hamsters acclimated at 23°C did not change significantly however there was a brief significant reduction in T_b following exposure to −15°C (h9, h12, and h15, SNK *post hoc*, $P < 0.05$). In contrast, acute exposure to 5°C and −15°C resulted in a significant reduction in T_b for the hamsters acclimated to 32.5°C (to 5°C: h15 and thereafter, h24, $t_{14} = 8.69$, $P < 0.05$; to −15°C: h3, $F_{1,32} = 9.35$, $P < 0.01$). Four out of 8 individuals developed hypothermia with $T_b < 34$°C, lethargic behavior and ceasing of feeding behavior, when exposed to −15°C. Hence, the animals were removed and the −15°C exposure experiment was terminated (Fig. 8).

Effect of acclimation at upper critical temperature on COX activity and state 4 respiratory and their response to acute cold exposure

Acclimation at the upper critical temperature (32.5°C) significantly reduced COX activity on average by 50.6% compared to that in hamsters kept at 23°C ($F_{1,38} = 42.02$, $P < 0.01$, Fig. 9a). There were significant increases in BAT COX activity in hamsters kept at 23°C in response to acute exposure to 5°C (70.1%) and −15°C (133.8%) ($F_{2,38} = 4.71$, $P < 0.05$, SNK *post hoc*, $P < 0.05$). However, COX activity in hamsters acclimated to 32.5°C did not change significantly following acute cold exposure to either 5°C or −15°C (SNK *post hoc*, $P > 0.05$). The rate of mitochondria state 4 respiration was significantly lower in hamsters acclimated to 32.5°C compared to those kept at 23°C ($F_{2,38} = 35.10$, $P < 0.01$, Fig. 9b). Following acute cold exposure, state 4 respiration was significantly higher in hamsters acclimated to 23°C (SNK *post hoc*, $P < 0.05$), but not those acclimated to 32.5°C (SNK *post hoc*, $P > 0.05$). Liver COX activity and state 4 respiration were significantly lower in the groups acclimated to

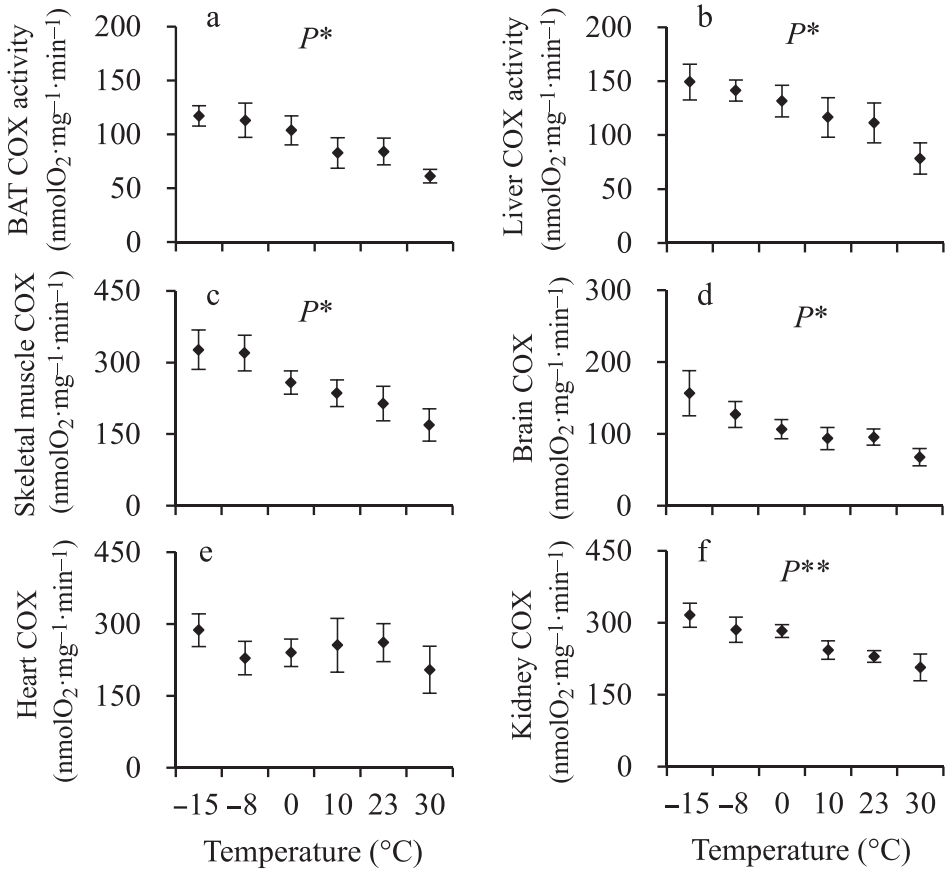

Figure 5 The cytochrome c oxidase (COX) activity of BAT (a), liver (b), skeletal muscle (c), brain (d), heart (e), and kidney (e) of striped hamsters. Striped hamsters were acclimated to a gradual decrease in temperatures from 30°C to −15°C. Data are means ± SE. * indicates a significant effect of temperature ($P < 0.05$), **, $P < 0.01$.

32.5°C compared to those acclimated to 23°C (COX, $F_{1,38} = 10.76$, $P < 0.01$, Fig. 9c; state 4, $F_{1,38} = 9.69$, $P < 0.01$, Fig. 9d). There was no significant change in liver COX activity or state 4 respiration following acute cold exposure (COX, $F_{2,38} = 1.28$, $P > 0.05$, Fig. 9c; state 4, $F_{2,38} = 1.26$, $P > 0.05$, Fig. 9e).

COX activity and state 4 respiration of skeletal muscle did not significantly differ between the groups acclimated to 32.5°C and those kept at 23°C (COX, $F_{1,38} = 3.22$, $P > 0.05$, Fig. 9e; state 4, $F_{1,38} = 2.17$, $P > 0.05$, Fig. 9f). Acute cold exposure resulted in a significant increase in COX activity and state 4 respiration in hamsters kept at 23°C (COX, $F_{2,38} = 4.42$, $P < 0.05$, SNK *post hoc*, $P < 0.05$; state 4, $F_{2,38} = 4.74$, $P < 0.05$, SNK *post hoc*, $P < 0.05$). However, there was no significant effect on COX activity or state 4 respiration in hamsters acclimated to 32.5°C (COX, SNK *post hoc*, $P > 0.05$; state 4, SNK *post hoc*, $P > 0.05$).

Effect of acclimation at upper critical temperature on serum T3 and T4 levels and their response to acute cold exposure

Serum T_3 was on average 32.1% lower in hamsters acclimated to 32.5°C compared to those kept at 23°C ($F_{1,42} = 11.54$, $P < 0.01$, Fig. 10a). Acute cold exposure also had a significant effect on serum T_3 levels ($F_{2,42} = 28.78$, $P < 0.01$, Fig. 10a), with a significantly higher level of T_3 in hamsters acclimated to 32.5°C (SNK *post hoc*, $P < 0.05$) and those kept at 23°C (SNK *post hoc*, $P < 0.05$) following exposure to −15°C. The exposure to 5°C significantly increased serum T_3 for the hamsters acclimated to 23°C (SNK *post hoc*, $P < 0.05$), but have no significant effect on T_3 for hamster acclimated to 32.5°C (SNK *post hoc*, $P > 0.05$). Acute exposure to −15°C resulted in a significant reduction in serum T_4 in hamsters kept at 23°C ($F_{1,42} = 14.16$, $P < 0.01$, SNK

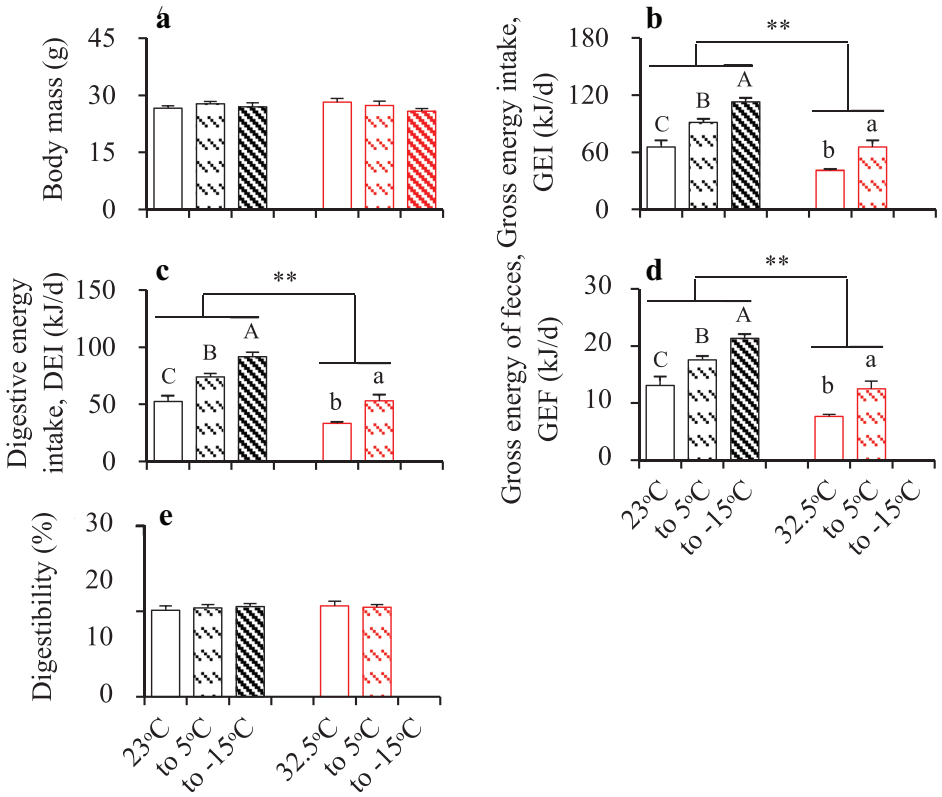

Figure 6 Body mass (a), gross energy intake, GEI (b), digestive energy intake, DEI (c), gross energy of feces, GEF (d), and digestibility (e) in striped hamsters transferred from 23°C and 32.5°C to 5°C and −15°C, respectively. Data are means ± SE. **indicates the significant effect of high temperature ($P < 0.01$). Different letters above the columns indicate a significant effect of transfer to 5°C and −15°C (capital letters were used between the 23°C groups, and lower cases were used between the 32.5°C groups).

post hoc, $P < 0.05$), but not for hamsters acclimated to 32.5°C (SNK *post hoc*, $P > 0.05$, Fig. 10b). The ratio of T_3 to T_4 was significantly lower in the hamsters acclimated to 32.5°C than those kept at 23°C ($F_{1,42} = 16.99$, $P < 0.01$, Fig. 10c). The ratio of T_3 to T_4 was significantly higher following acute exposure to −15°C for hamsters acclimated to 32.5°C and those kept at 23°C ($F_{2,42} = 15.58$, $P < 0.01$, SNK *post hoc*, $P < 0.05$).

DISCUSSION

In the present study, we observed a significant seasonal change in the range of TNZ in striped hamsters acclimated to natural photoperiod and temperature, whereas we observed no difference in the range of TNZ between hamsters acclimated to short photoperiod and those to long photoperiod. When acclimated to a gradient of ambient temperatures from 30°C to −15°C, hamsters at lower ambient temperatures showed a wider TNZ range due to a decreased lower-critical temperature. These findings are consistent with a previous field study in which we found a seasonal difference in the TNZ of wild-caught striped hamsters, with a wider TNZ range in winter compared to summer (Zhao *et al.* 2014a). In the present study, hamsters acclimated at semi-natural conditions were fed *ad libitum* with a constant supply of food, so we were able to eliminate the possible effects of food availability on the shift in TNZ. Thus, the shift in TNZ of striped hamsters should mainly due to the changes in ambient temperature. Arctic mammals have a very low lower-critical temperature and an extremely wide TNZ compared to equatorial mammals (Scholander *et al.* 1950a,b; Ganeshan & Chawla 2017). Thus, changes of TNZ in mammals might reflect a general adaptation in thermal physiology to the variations of ambient temperature in their habitats.

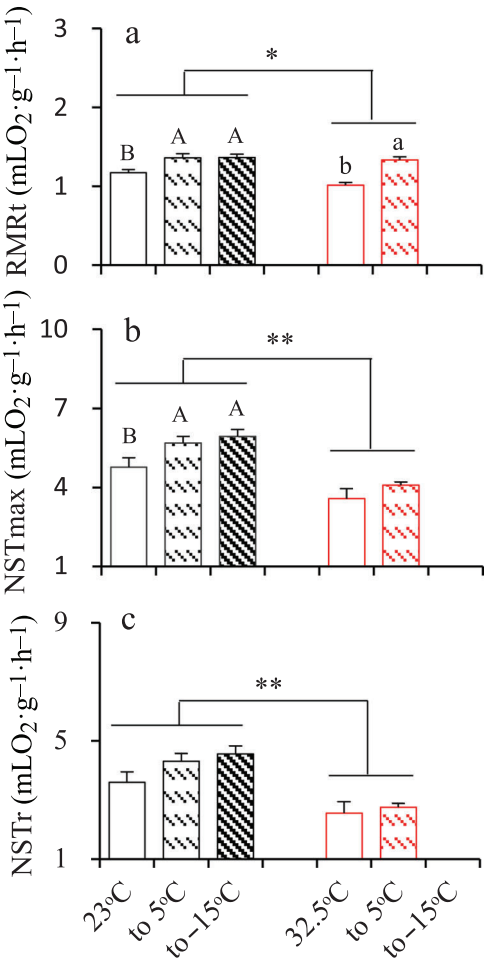

Figure 7 Resting metabolic rate, RMRt (a), maximal nonshivering thermogenesis (NSTmax) (b), and regulative NST (NSTr) (c) in striped hamsters transferred from 23°C and 32.5°C to 5°C and −15°C, respectively. Data are means ± SE. * indicates the significant effect of high temperature ($P < 0.05$), **, $P < 0.01$. Different letters above the columns indicate the significant effect of transfer to 5°C and −15°C (capital letters were used between the 23°C groups, and lower cases were used between the 32.5°C groups).

Photoperiod might be not involved in the shift of TNZ in striped hamsters

Changes in photoperiod have been shown to be an early cue for seasonal adjustments in energy metabolism and thermoregulation in many rodent species (Heldmaier *et al.* 1982a,b; Haim *et al.* 1999; Haim & Zisapel 1999; Wang *et al.* 1999, 2006; Jefimow *et al.* 2004; Peacock *et al.* 2004; Król *et al.* 2005). Acclimation to long photoperiod regimes (16L:8D) was found to decrease RMRt and elevate the upper-critical temperature in Levant voles (*Microtus guentheri*), migratory hamsters (*Cricetulus migratorius*), and Macedonian mice (*Mus macedonicus*), resulting in a wider TNZ compared to individuals acclimated to short (8L:16D) photoperiod regimes (Haim *et al.* 1998). In this study, the range of TNZ in striped hamsters acclimated to a short day (SD) photoperiod did not differ from that in the individuals acclimated to a long day (LD) photoperiod, indicating that photoperiod might not have a significant effect on TNZ shift in striped hamsters. This suggests that the effect of photoperiod on RMRt and TNZ may be species-specific. Natural photoperiod changes gradually, and seasonally, rather than abruptly as experienced by the animals in this study, which were either transferred to a SD or LD photoperiod regime. Therefore, seasonal photoperiod cannot be completely excluded as a potential external factor influencing the shift in TNZ in striped hamsters although we did not observe a significant difference in the TNZ between the SD and LD groups.

Decreases in ambient temperature cause the leftward shift of TNZ in striped hamsters

Temperature is likely the most effective and direct factor in inducing significant changes in behavior, energy budget, and metabolic thermogenesis, as well as many other aspects of physiology in animals, particularly in those exposed to extreme cold or hot temperatures (Scholander *et al.* 1950a,b; Consolazio *et al.* 1973; Gordon 1993, 2012; Han *et al.* 2020; Zhao *et al.* 2020; Giroux *et al.* 2022; Ren *et al.* 2022). In this study, striped hamsters acclimated to a gradient of ambient temperatures from 30°C to −15°C showed significant differences in TNZ ranges, with a wider TNZ range at a lower acclimation ambient temperature. Importantly, our finding that the lower-critical temperature gradually decreased in hamsters acclimated to a lower ambient temperature, may be a general pattern of intraspecies TNZ shift in response to cold acclimation. Interestingly, we found that the upper-critical temperature remained at 32.5°C in striped hamsters acclimated to a gradient of ambient temperatures. This suggests that striped hamsters may be vulnerable or susceptible to extreme high temperature.

Previous studies in larger mammals found that cold acclimation leads to a leftward shift in TNZ due to the added insulation of a fur coat (Gordon 2012). For example, Arctic mammals such as polar bear cubs, white foxes, and husky are heavily insulated and have a very low lower-critical temperature and, an extremely

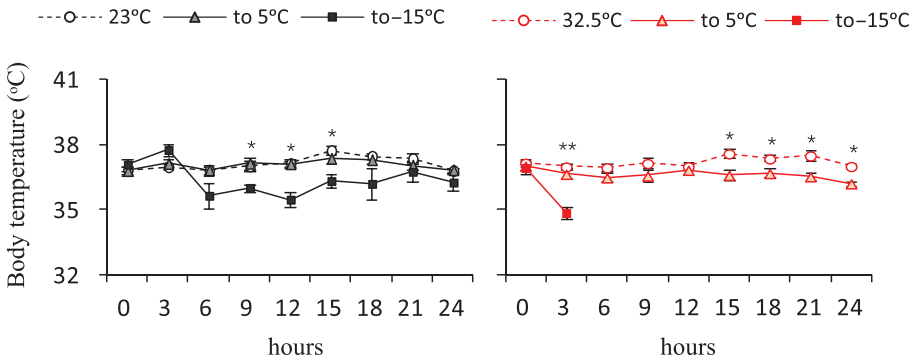

Figure 8 Body temperature in striped hamsters transferred from 23°C and 32.5°C to 5°C and −15°C, respectively. Data are means ± SE. * indicates the significant effect of transfer to 5°C and −15°C ($P < 0.05$), **, $P < 0.01$.

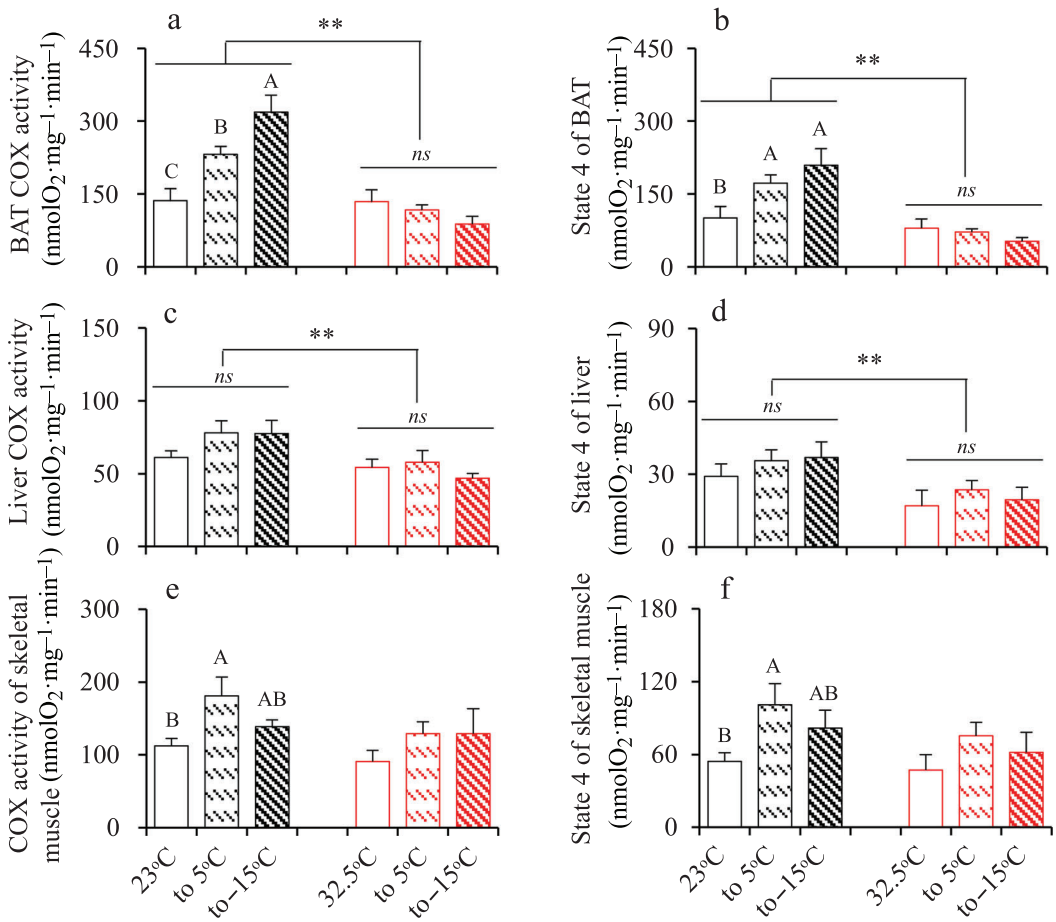

Figure 9 Cytochrome c oxidase (COX) activity and rate of mitochondria state 4 respiration of brown adipose tissue (BAT, a,b), liver (c,d), and skeletal muscle (e,f) in striped hamsters transferred from 23°C and 32.5°C to 5°C and −15°C, respectively. Data are means ± SE. **indicates the significant effect of high temperature ($P < 0.01$). Different letters (A, B, or C) above the columns indicate significant effect of acute cold exposure.

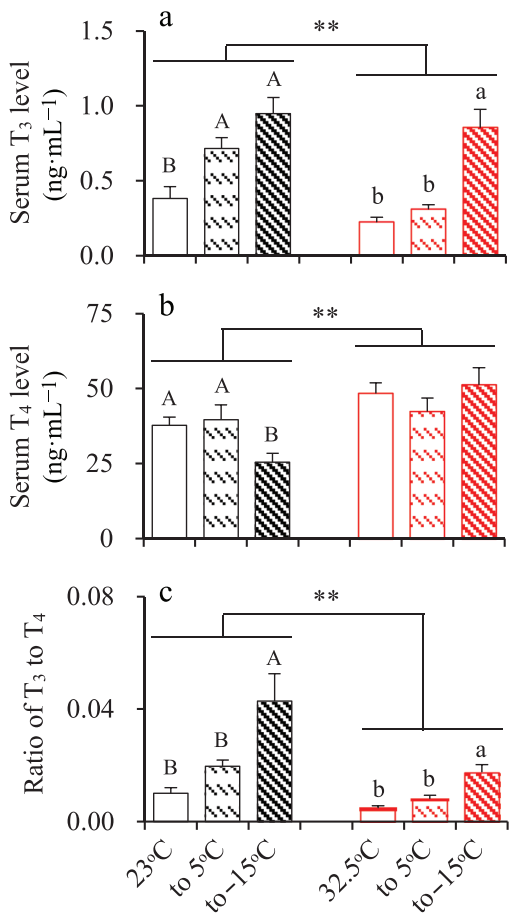

Figure 10 Serum T_3 (a) and T_4 (b) level, and the ratio of T_3 to T_4 (c) in striped hamsters transferred from 23°C and 32.5°C to 5°C and −15°C, respectively. Data are means ± SE. ** indicates the significant effect of high temperature ($P < 0.01$). Different letters (A or B; a or b for the 23°C and 32.5°C groups, respectively) above the columns indicate the significant effect of acute cold exposure.

wide TNZ (Scholander *et al.* 1950a,b). In contrast, for small mammals any shift in TNZ of cold acclimation is likely to be very small due to their small size and thin insulation. For example, the lower-critical temperature of mice acclimated to warm (26°C) or mild cold (4°C) for 4 weeks was found to be nearly the same (Gordon 2012). Additionally, the density of hair was considerably changed in wild hamsters captured in winter compared to those caught in summer, while acclimation to 5°C and short day did not increase insulation (Zhao & Cao 2014). This suggests that the seasonal change in insulation of hair may partly contribute to the leftward shift of TNZ in winter. Even though there was no significant change in the density of hair in response to cold exposure, the thermal conductance of hamsters acclimated to a gradual decrease in temperature may have changed as a result of an increasing difference between body temperature and ambient temperature (Zhao & Cao 2014). Therefore, the cold-induced widening of the TNZ may also be influenced by changes in thermal conductance.

The leftward shift of TNZ is associated with elevated RMRt and NST

In addition to the leftward shift of TNZ, the hamsters in our study showed significant increases in RMRt and NST following a gradual decrease in ambient temperature of acclimation. An increase in thermogenic capacity is recognized as being one of the most effective mechanisms in small mammals enabling them to cope with low temperatures (Heldmaier 1971; Jansky *et al.* 1973; Heldmaier *et al.* 1982a; Haim & Zisapel 1999; Nespolo *et al.* 2001; Cannon & Nedergaard 2011). Studies on small mammals have demonstrated that increases in RMRt and NST are more related to cold acclimation than insulative adaptations (Gordon 1993, 2012; Broekman *et al.* 2006). Elevated RMRt and NST in cold-acclimated small mammals have been linked to the metabolic rate of specific active organs and tissues (Johnson *et al.* 2001; Król *et al.* 2003, 2005). In this study, we found that COX activity of BAT, liver, skeletal muscle, brain, and kidneys significantly increased following a gradual decrease in acclimation temperature from 30°C to −15°C. RMRt and NST were also significantly elevated in a similar pattern. As these organs are highly aerobic active, an increase in COX activity in response to cold acclimation would contribute to an overall increase in heat production and thus be advantageous for mammals living in a cold environment (Gordon 1993, 2012). Taken together, an elevated thermal capacity contributed by metabolic active organs, associated with the leftward shift in the TNZ, appeared to be the main thermal physiological responses in striped hamster during cold acclimation.

In this study, we found that striped hamsters acclimated to 32.5°C (the upper-critical temperature) consumed less food, had a significantly lower RMRt and NST, and consequently performed worse in mild (5°C) and extreme (−15°C) cold exposure compared to those acclimated to 23°C (close to lower-critical temperature). In response to extreme cold exposure, hamsters acclimated to the lower-critical temperature showed a significant increase in COX activity and rate of state 4 respiration in BAT, liver, and skeletal muscle, whereas hamsters at the upper-critical

temperature did not. Actually, hamsters acclimated to upper-critical temperature failed to keep normothermic when acutely exposed to −15°C. The results suggest that acclimation to a high ambient temperature like the upper-critical temperature may impair not only the thermogenic capacity and also the motivation of metabolic active organs when facing a cute cold exposure.

Thyroid hormones in hamsters after acclimation at upper critical temperature and the following cute cold exposure

Thyroid hormones (THs) are well known for their unique ability to stimulate basal thermogenesis, also known as BMR or RMRt (Silva 1995, 2001; Lannia *et al.* 2003). In a variety of small mammals in the field, seasonal enhancement of either RMRt or NST during winter conditions was associated with increased THs secretion and production (Freake & Oppenheimer 1995; Lannia *et al.* 2003; Kim 2008; Silva 2011). Striped hamsters acclimated to the upper-critical temperature had significantly lower serum T_3 concentrations than those kept at the lower-critical temperature. The conversion rate of T_4 to T_3 was also significantly lower in hamsters acclimated to the upper-critical temperature. Following mild or extreme cold exposure, T_3 level and T_3/T_4 ratio increased significantly in both hamsters acclimated at the lower-critical temperature and those at the upper-critical temperature; however, magnitudes of the increase were significantly lower in hamsters acclimated at the upper-critical temperature. In comparison with the hamsters acclimated to 23°C, the lower serum T_3 concentrations and T_3/T_4 ratio in hamsters acclimated to the upper-critical temperature are in parallel with the decreased thermogenic capacity, indicated by lower RMRt and NST after the cute cold exposure. This suggests that the pathway involved in stimulating heat production through thyroid hormones may also be involved in the physiological response to acute cold exposure. The impairment of thermogenic capacity during acute cold exposure in hamsters acclimated to the upper-critical temperature may be partly due to the down regulation of the T_3 pathway.

CONCLUSION

Striped hamsters acclimated to semi-natural conditions showed considerable seasonal differences in the range of TNZ, with a wider TNZ and a lower lower-critical temperature in winter compared to that in summer. Changes of ambient temperature but not photoperiod induced a shift of TNZ. Specifically, hamsters acclimated to lower ambient temperatures showed a considerable leftward shift of TNZ, whereas the upper critical temperature of the TNZ remained fixed. RMRt, NST, as well as COX activity of several metabolically active organs also increased significantly in hamsters acclimated to lower ambient temperatures. The leftward shift of the lower-critical temperature associated with the elevated thermal capacity may reflect a general adaptive response in some small mammals to the seasonal decrease of ambient temperature in their natural habitats. Furthermore, striped hamsters acclimated to the upper-critical temperature showed a significant decreased capacity to cope with acute cold exposure, which was mainly due to the impaired thermogenic capacity and may partly be regulated by T_3 pathway.

ACKNOWLEDGMENTS

This study was supported by grants from National Natural Science Foundation of China (Nos. 31670417, 31870388).

CONFLICT OF INTEREST

All authors declare no conflicts of interest and have approved the final version of the manuscript.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 The relationships between cytochrome c oxidase activity of BAT, liver, skeletal muscle, brain, heart, and kidney with RMRt, NST and NSTr of striped hamsters. Striped hamsters were acclimated to a gradual decrease in temperatures from 30°C to −15°C. * indicates a significant correlation ($P < 0.05$), **, $P < 0.01$.

Cite this article as:

Liao S, Tan S, Jiang M *et al.* (2023). Temperature determines the shift of thermal neutral zone and influences thermogenic capacity in striped hamsters. *Integrative Zoology* **18**, 353–71. https://doi.org/10.1111/1749-4877.12678