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Partial removal of brown adipose tissue enhances humoral immunity in warm-acclimated Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Temperate rodent species experience marked seasonal fluctuations in environmental temperatures. High thermoregulatory demands during winter usually weaken immune function. Brown adipose tissue (BAT) plays a crucial role in adaptive thermoregulatory process. Thus, we proposed the hypothesis that BAT might participate in the regulation of seasonal changes in immune function. The present study examined the trade-off between thermoregulation and immune function and the potential role of BAT in regulating seasonal changes in immune function in Mongolian gerbils. Specifically, surgical removal of interscapular BAT (34% of total BAT) was performed in male gerbils, and subsequently acclimated to either warm (23 ± 1 °C) or cold (4 ± 1 °C) conditions. Gerbils were then challenged with innocuous antigens and the immune responses were measured. Resting metabolic rate (RMR) and nonshivering thermogenesis (NST) were increased under cold conditions. However, the cost of thermoregulation during cold acclimation did not suppress T-cell mediated immunity and humoral immunity or decrease spleen mass, thymus mass and white blood cells. Partial removal of BAT significantly enhanced humoral immunity in warm-acclimated, but not in cold-acclimated gerbils. T-cell mediated immunity, white blood cells and immune organs were not affected by BAT removal under both warm and cold conditions. Collectively, our results imply that BAT has a suppressive effect on humoral immunity in warm-acclimated gerbils and differential effects of BAT on humoral immunity under different temperatures (e.g., summer and winter) might be benefit to their survival.

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1. Introduction

Temperate animals experience marked seasonal fluctuations in environmental conditions, such as reduced food availability and low ambient temperatures during winter. To survive the harsh environmental conditions, animals must maintain a balanced energy budget despite competing physiological functions (e.g., growth, thermoregulation and reproduction) [10,82].

Most animals enhance thermogenic activity, cellular maintenance and other processes that promote survival in winter [68]. During winter, thermoregulation is an important energy-demanding physiological function [67]. Food intake increases during cold exposure in some laboratory and wild rodent species [17,50,54,62]. Cold exposure also elicits a remarkable increase in RMR and in the mass of metabolically active internal organs (including liver, heart, kidney and small intestines) in some animals [50,53,80]. In addition, other body compositions could be affected by cold exposure (including spleen, thymus, lung, gonad and body fat), which may reflect the resource reallocation among different physiological functions [50].

Life history theory assumes that trade-offs exist among various physiological functions in many animals [78]. Many studies suggest that immune function is energetically costly and can be influenced by physiological trade-offs [22,24,29,57,61,73]. A growing body of literature supports that physiological trade-offs mediate seasonal changes in immune function [61]. Immune activity tends to be decreased during winter in the wild but is enhanced in the laboratory during short-day conditions when all other factors are held constant [61,66,67]. Low temperatures and reduced food availability usually suppress immune function during winter; however, this suppression can be ameliorated by short photoperiod [67]. In addition, changes in immune activity are driven by fluctuations in hormones [61], such as leptin and glucocorticoids. Leptin, secreted predominantly by white adipose tissue modulates immune responses in several rodent species [3,23,28,58]. Seasonal changes in leptin concentrations [49,51,56,90] appear to mediate, at least in part, seasonal changes in immunity [33]. Glucocorticoids are secreted in all vertebrates as a result of the activation of the hypothalamic–pituitary–adrenal axis [71]. The immune modulatory effect

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of glucocorticoids, in response to some stressors such as low temperatures and reduced food availability, could also produce seasonal patterns of immune function [61,66].

Brown adipose tissue (BAT) is an important site for nonshivering thermogenesis (NST) in mammals [13]. Partial removal or interruption of blood flow through BAT could reduce animals' capacity for NST, including in new born rabbits (*Oryctolagus cuniculus*) [45], rats (*Rattus norvegicus*) [44], mice (*Mus musculus*) [38] and Djungarian hamsters (*Phodopus sungorus*) [39]. Small mammals improve their ability for NST for their thermoregulation during cold exposure [41,42]. The thyroid hormones, tri-iodothyronine (T_3) and thyroxine (T_4), are tyrosine-based hormones produced by the thyroid gland primarily responsible for the heat produced by NST in BAT and the increase in metabolic rate [32,47]. It has been shown that BAT might have a suppressive effect on T cell-mediated immunity in rats [46].

Mongolian gerbils (*Meriones unguiculatus*) are small seasonally breeding, non-hibernating, and granivorous rodents which are distributed in the desert and semi-arid regions of Mongolia and Northern China [83]. In these regions the average temperature in the winter is −22.3 °C and lasts about 6–7 months, with extreme minimum temperatures below −40 °C [15]. Previous studies have shown that increased adaptive thermogenesis is critical for gerbils in overcoming such harsh winters [55,56,90]. Our previous study has shown that humoral immunity is higher in Mongolian gerbils captured in winter than those in summer [89]. BAT plays an important role in regulating seasonal thermogenesis in Mongolian gerbils [90].

To our knowledge, no data is available regarding the role of BAT in regulating seasonal changes in immune function. The aim of this study was to test the hypothesis that BAT plays a role in regulating seasonal changes in immune function. Specifically, we asked: (i) whether cold exposure compromises immune function (ii) whether surgical partial removal of BAT alters humoral immunity and T cell-mediated immunity and (iii) whether these effects are temperature-dependent. To test this hypothesis, we reduced BAT mass of Mongolian gerbils via surgery and acclimated them to either a warm or cold environment, and examined the effects of these manipulations on both humoral and T cell-mediated immunity. We predicted that partial removal of BAT will enhance immune function, which may be temperature-dependent, and the immune responses will be suppressed in cold-acclimated gerbils.

2. Material and methods

2.1. Experimental animals

Forty adult male Mongolian gerbils (age 6–7 months) were obtained from our laboratory colony, and they were the offspring of Mongolian gerbils trapped in Inner Mongolian grasslands in 1999 and raised in the Institute of Zoology, Chinese Academy of Sciences. Animals were housed individually in plastic cages (30 × 15 × 20 cm) with sawdust as bedding, and maintained at the room temperature of 23 ± 1 °C, under a photoperiod of 16L:8D (16:8 h light–dark cycle, lights on at 0400 h). Commercial standard rat pellet chow (5.1% crude fat, 24.3% crude protein, 25.0% neutral-detergent fiber (NDF), and 13.6% acid-detergent fiber (ADF) (Beijing KeAo Feed Co.) and water were provided *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences.

2.2. Experimental procedures

Half of the animals (n = 20) received interscapular BAT removal (IBATR) and removed IBAT was weighed (±mg) whereas the remaining animals (n = 20) received sham surgeries. Surgeries were performed under anesthesia following administration of sodium Pentobarbital (*ca.* 30 mg kg^{-1}). In IBATR gerbils, their skin was opened by a small (1–2 cm) incision along the mid-dorsal line between the scapulae, the overlying white fat was dissected and retracted, and then the IBAT was freed from surrounding muscles and removed. In sham-operated gerbils we separated the IBAT from surrounding musculature, connective and white adipose tissue, but blood vessels and innervations were left intact. The skin was closed by sewing with absorbable surgical suture. All gerbils were returned to the colony room and allowed to recover. After 10 days recovery, RMR and NST of 11 gerbils in IBATR group and 12 in sham-operated group were measured, respectively. Two weeks recovery later, nine IBATR animals (n = 9) and nine sham-operated animals (n = 9) were randomly transferred to cold conditions (4 ± 1 °C) and maintained for 24 days. The other animals remained at warm conditions (23 ± 1 °C) throughout the test. Ten days after cold exposure, RMR and NST of 5–7 animals in each group were measured. Then thirteen days after cold exposure, all gerbils received a single subcutaneous injection of 100 μg of antigen keyhole limpet hemocyanin (KLH, Sigma 7017), to which all animals were previously naïve, suspended in 0.1 ml sterile saline and were then returned their environment. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*). KLH generates a robust, non-replicating antigenic response in rodents, but does not make the animals sick (e.g., inflammation or fever). On days 5 and 10 post-KLH injection a blood sample was drawn from all animals via the retro-orbital sinus for later measurement of KLH-specific antibodies. Days 5 and 10 incorporate the peak rises in immunoglobulins IgM and IgG, respectively. IgM is the first immunoglobulin class produced following an immune challenge and IgG is the predominant immunoglobulin class present in the blood during the course of the immune response [24,34]. On the day of sampling, animals were lightly anesthetized with isoflurane (Shandong LiNuo Pharmaceutical Co.) and blood samples were drawn from the retro-orbital sinus between 0900 and 1100 h. Samples were allowed to clot for 30 min at 4 °C, and then the samples were centrifuged at 4 °C for 30 min at 3000 r.p.m. Serum aliquots were aspirated and stored in sealable polypropylene microcentrifuge tubes at −80 °C until assayed for IgM and IgG. Each gerbil's body mass and food intake were monitored every three days during the cold acclimation. During the course of the experiment, two gerbils in sham warm (SW) group died by accident, and these two gerbils were not included in the subsequent statistical analyses. So the sample size of each group is as follow: sham warm (SW), n = 9; IBATR warm (RW), n = 11; sham cold (SC), n = 9; IBAT R cold (RC), n = 9.

2.3. Metabolic trials

RMR and NST were quantified as the rate of oxygen consumption, using an open-flow respirometry system (Sable, FoxBox, USA) according to the procedures of Chi and Wang [16]. All the animals will stay at the room temperature (about 25 °C) around 2 h before each metabolic measurement to reduce the effect of great environment change between the housing conditions and testing conditions. In brief, animal was placed in a transparent plastic chamber (volume 1.4 L, 20.5 × 13.4 × 8.4 cm) with small pieces of tissue paper just enough to absorb animal wastes. An incubator (Yiheng Model LRH-250, Shanghai, China) was used to maintain the chamber at a constant ambient temperature of 30 ± 0.5 °C (within the thermal neutral zone of Mongolian gerbils [85]). Air from outside the building was dried using a column filled with DRIERITE desiccants (W.A. Hammond Drierite) then pumped through the chamber at a mass flow rate of 500–600 ml min^{-1}. After passing through the chamber, the gas was subsampled at a

flow rate of approximately 100 ml min^{-1} and dried again using a no chemical gas drier (Sable, ND-2). Then the gas was submitted to oxygen analyzer for analysis (Sable Foxbox). The air baseline was measured twice at the beginning and the end of each metabolic measurement, respectively. The measurement of RMR for an animal lasted for 2 h, recorded at 10 s interval. 5-min continuous stable minimum recordings were taken to calculate RMR [16]. Body mass was weighed before and after each metabolic measurement. Each RMR measurement was followed by another 45 min for measurement of NST at a constant ambient temperature of 25 ± 0.5 °C which is close to the lower critical temperature [84]. The maximum capacity for NST was induced by a subcutaneous injection of noradrenaline (NA) solution (0.2 mg ml^{-1}) with a dosage (NE (mg kg^{-1}) = 2.53 $M^{-0.4}$) suggested by [86]. Maximum NST was considered the highest 3-min average after NE injection [16,40,81].

2.4. Humoral immunity

To assess humoral immunity, serum anti-KLH IgG and IgM concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) according to the method of [28,87]. Microtitre plates were coated with antigen by incubating overnight at 4 °C with 0.5 mg ml^{-1} KLH in sodium bicarbonate buffer (pH 9.6), phosphate buffered saline (PBS; pH = 7.4) containing 0.05% Tween 20 (PBS-T; pH = 7.4), then blocked with 5% nonfat dry milk in PBS-T overnight at 4 °C to reduce nonspecific binding, and washed again with PBS-T. Thawed serum samples were diluted 1:20 with PBS-T, and 150 μl of each serum dilution was added in duplicate to the wells of the antigen-coated plates. Positive control samples (pooled sera from gerbils previously determined to have high levels of anti-KLH antibodies, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naïve gerbils, similarly diluted with PBS-T) were also added in duplicate to each plate; plates were sealed, incubated at 37 °C for 3 h, then washed with PBS-T. A secondary antibody (alkaline phosphatase-conjugated anti-mouse IgG (Sigma chemical, St Louis, MO, USA) diluted 1:2000 with PBS-T; or anti-mouse IgM (Sigma Chemical, St. Louis, MO) diluted 1:500 with PBS-T) was added to the wells, and the plates were sealed and incubated for 1 h at 37 °C. Plates were washed again with PBS-T and 150 μl of the enzyme substrate *p*-nitrophenyl phosphate (Sigma Chemical, St. Louis, MO; 1 mg ml^{-1} in diethanolamine substrate buffer) was added to each well. Plates were protected from light during the enzyme–substrate reaction, which was terminated after 20 min by adding 50 μl of 1.5 M NaOH to each well. The optical density (OD) of each well was determined using a plate reader (VersaMax™) equipped with a 405 nm wavelength filter and the mean OD for each set of duplicate wells was calculated. To minimize intra-assay variability, the mean OD for each sample was expressed as a percent of its plate positive control OD for statistical analyses.

2.5. T cell-mediated immunity

To determine the T cell-mediated immunity of all the gerbils at the last day of cold acclimation, we measured the footpad thickness of their left hind feet with a micrometer (Tesa Shopcal, Swiss) to ±0.01 mm at 0900 h. Immediately thereafter, we injected subcutaneously 0.1 mg of PHA (PHA-P, Sigma L-8754) dissolved in 0.03 ml of sterile PBS (pH 7.4) in the middle of the footpad around 0900 h. After 6 h of injection, we measured the footpad thickness. The PHA response was calculated as the difference between pre- and post-injection measurements divided by initial footpad thickness (PHA response = (post-PHA − pre-PHA)/pre-PHA). Each measurement of PHA response was replicated six times [6,77]. Previous result showed that the maximum PHA response occurs after 6 h of PHA injection [88].

2.6. White blood cells (WBCs) and blood glucose concentrations measurement

White blood cells (WBCs; or leukocytes), which are fundamental to immune responses against pathogens, are useful to estimate overall health [11]. Blood glucose is an immediate energy provision. It has been reported that decreased glucose availability leads to immunosuppression in deer mice (*Peromyscus maniculatus*) and Siberian hamsters (*P. sungorus*) [25,60]. Following the measurement of PHA response, each gerbil was euthanized by CO_2 asphyxiation and trunk blood was collected around 15:00 h for the measurements of WBC and blood glucose concentrations. 20 μl whole blood was diluted immediately in 0.38 ml solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. The total number of WBC was determined by counting all leukocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9 cells/l) [88]. At the same time, another 20 μl whole blood was obtained immediately for measuring blood glucose concentrations with FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc. Alameda, USA) according to the manufacturer's instructions. The range tested of blood glucose was 1.1–27.8 mmol/l. The within-lot and -vial variabilities were <5.6% and <4.1%, respectively. The rest of the blood samples were allowed to clot for 30 min at 4 °C, and then the samples were centrifuged at 4 °C for 30 min at 3000 r.p.m. The serum was collected and then stored at −80 °C until assayed for serum leptin, corticosterone and thyroid hormones.

2.7. Circulating hormones

Letpin may be involved in the regulation of brown adipose tissue thermogenesis under different temperatures [2,12,18]. Serum leptin concentrations were quantified by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. No. XL-85 K, Millipore Corporation, MO, USA). The lower and upper limits of the assay kit were 1 and 50 ng ml^{-1}, and 100 μl serum (no dilution) was used. The inter- and intra-assay coefficients of variation were <8.7% and <3.6%, respectively [54,91].

Several studies showed that corticosterone acts to inhibit nonshivering thermogenesis of brown adipose tissue [64,79]. Serum corticosterone concentrations were quantified by rat corticosterone ELISA (enzyme-linked immunosorbent assay) kit (Cat. No. HR083, RapidBio Lab. Calabasas, CA, USA). The lowest level of corticosterone that could be detected by this assay was 0.2 ng ml^{-1}, and 25 μl serum (no dilution) was used. Inter- and intra-assay variations for corticosterone ELISA were <7% and <5%, respectively [88].

Thyroid hormones are usually responsible for the regulation of metabolism, and they act to increase the metabolic rate [32]. Serum thyroid hormones (T_3 and T_4, ng ml^{-1}) were determined by radio-immunoassay using RIA kits from Beijing Institute of Northern Biotech, and 100 μl serum (no dilution) was used. Inter- and intra-assay variations were 15% and 10% for both T_3 and T_4, respectively [91].

2.8. Body compositions

At termination of the experiment all gerbils were sacrificed, and the remaining sites of BAT (interscapular, dorsal cervical, subscapular, intrathoracic, perirenal and inguinal) were removed and weighed (±1 mg). Then the visceral organs, including heart, thymus, lung, liver, spleen, kidneys, gonad (testis and seminal vesicle)

and the digestive organs with contents (i.e., stomach, small intestine, cecum and colon) were dissected and weighed (±1 mg). The remaining carcass were dried in an oven at 60 °C to constant mass, and then weighed again to obtain the dry carcass mass. Total body fat was extracted from the dried carcass by petroleum ether extraction in a Soxhlet apparatus [54,91].

2.9. Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL). Prior to all statistical analyses, data were examined for normality of variance using the Kolmogorov–Smirnov test. During the course of cold exposure, differences in body mass were analyzed by two-way (IBATR × cold) repeated-measures ANOVA, while differences in food intake were analyzed by two-way repeated-measures ANCOVA with body mass as covariate followed by Tukey's honestly significant difference post hoc comparisons. Differences in RMR and NST measured during recovery period were analyzed by independent-samples *t* test. Differences in RMR, NST, immune responses (serum anti-KLH IgM, serum anti-KLH IgG, PHA response, WBCs), blood glucose concentrations and circulating hormones (leptin, corticosterone and T_3/T_4) were analyzed by a two-way ANOVA followed by Tukey's honestly significant difference post hoc comparisons. Group differences in body compositions were analyzed by a two-way ANCOVA with body mass as covariate followed by the Tukey's honestly significant difference post hoc tests. Finally, Pearson correlation analysis was performed to determine the correlations between BAT mass and humoral immunity, and the correlations between CORT and RMR or food intake, and the correlation between T_3/T_4 and RMR. Differences between group means were considered statistically significant at $P < 0.05$.

3. Results

3.1. Body mass and food intake

Body mass on day 0 (the day in cold exposure) among SW, RW, SC and RC groups did not differ significantly (all $F < 1.40$, $P > 0.25$; Fig. 1a). At the end of the experiment (day 24), IBATR, cold exposure or the interaction had no significant effect on body mass (all $F < 1.57$, $P > 0.22$). However, gerbils showed an increase in body mass over the time, and the interaction with cold exposure had a suppressive effect on the increase of body mass (all $F > 3.48$, $P < 0.05$).

Food intake on day 0 among SW, RW, SC and RC groups did not differ significantly ($P > 0.05$; Fig. 1b). At the end of experiment, food intake of cold-acclimated gerbils was increased by 75% ($F_{1,31} = 131.15$, $P < 0.001$), compared with those acclimated to warm conditions, while IBATR and the interaction with cold exposure had no significant effect (all $F < 0.17$, $P > 0.68$).

3.2. BAT mass

Cold-acclimated gerbils had a significantly more other BAT mass (BAT mass out of IBAT mass) compared with those acclimated to warm conditions ($F_{1,33} = 9.51$, $P = 0.004$; Fig. 2). There was no effect of IBATR or interaction with cold exposure on other BAT mass (all $F < 0.95$, $P > 0.33$).

Both IBATR ($F_{1,33} = 17.12$, $P < 0.001$; Fig. 2) and cold exposure ($F_{1,33} = 8.85$, $P = 0.005$) significantly affected total left BAT mass. There was no significant interaction between these two treatments ($F_{1,33} = 0.174$, $P > 0.05$). IBATR gerbils had significantly less total left BAT mass compared with sham-operated gerbils independent of cold exposure, while cold-acclimated gerbils had significantly more total left BAT mass than those acclimated to warm conditions. Specifically, surgical removal of IBAT reduced total BAT mass by about 34% in warm-acclimated gerbils, and by about 25% in cold-acclimated gerbils.

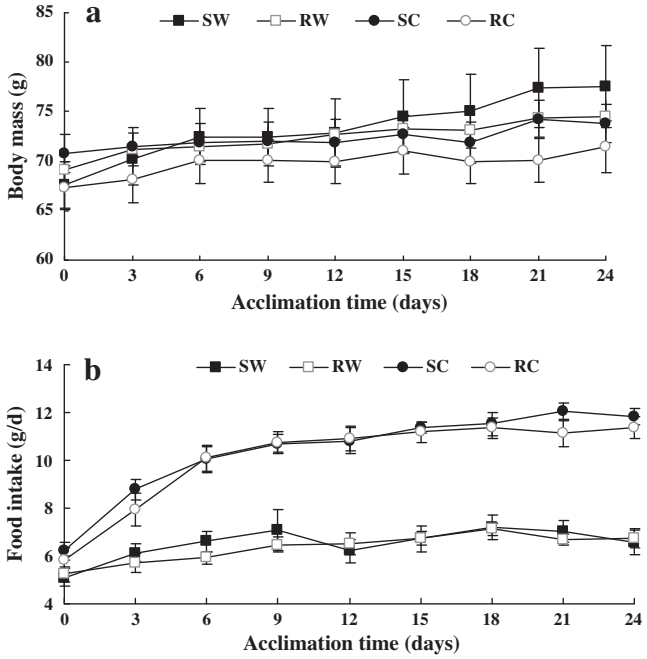

Fig. 1. Body mass (a) and food intake (b) over time in Mongolian gerbils received the surgical removal of IBAT and acclimated to either a warm (23 ± 1 °C) or cold (4 ± 1 °C) environment. Values are means ± SE. SW, sham and warm; RW, surgical removal and warm; SC, sham and cold; RC, surgical removal and cold. Values are means ± SE.

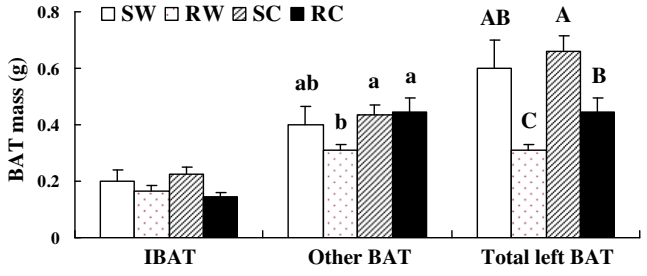

Fig. 2. Interscapular brown adipose tissue (IBAT) mass, other BAT mass (BAT mass out of IBAT) and total left BAT mass in Mongolian gerbils received the surgical removal of IBAT or sham surgery and acclimated to either a warm (23 ± 1 °C) or cold (4 ± 1 °C) environment. The surgical removal of IBAT of RW and RC were not counted in the total left BAT mass. Significant differences in each column are indicated by different letters (a,b or A,B) if $P < 0.05$. Values are means ± SE.

3.3. Resting metabolic rate (RMR) and nonshivering thermogenesis (NST)

Compared with sham-operated gerbils, no significant effects of IBATR on RMR ($T = -1.59$, $df = 14.24$, $P > 0.05$; Fig. 3a) and NST ($T = -0.447$, $df = 21$, $P > 0.05$; Fig. 3a) were found during surgical recovery period. The RMR and NST measured during the course of cold exposure varied significantly with cold exposure ($F_{1,19} = 34.526$, $P < 0.001$; $F_{1,19} = 4.617$, $P = 0.045$; Fig. 3b), such that cold-acclimated gerbils had significantly higher RMR and NST regardless of IBATR. There was still no effect of IBATR or interaction with cold exposure on RMR and NST (all $F < 0.05$, $P > 0.73$; Fig. 3b).

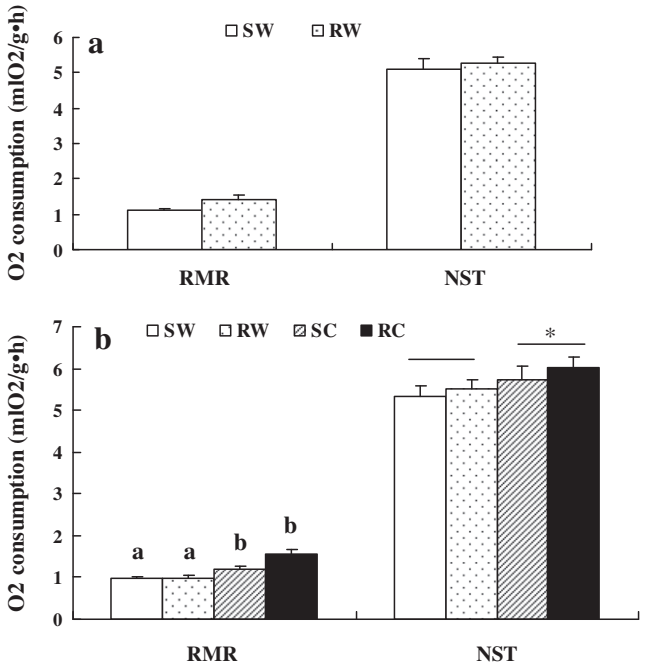

Fig. 3. RMR and NST in Mongolian gerbils measured during surgical recovery period (a) and during cold exposure (b) in Mongolian gerbils received the surgical removal of IBAT or sham surgery and acclimated to either a warm (23 ± 1 °C) or cold (4 ± 1 °C) environment. Significant differences are indicated by different letters if $P < 0.05$. Asterisk indicates the significant main effect of cold acclimation on NST. Values are means ± SE.

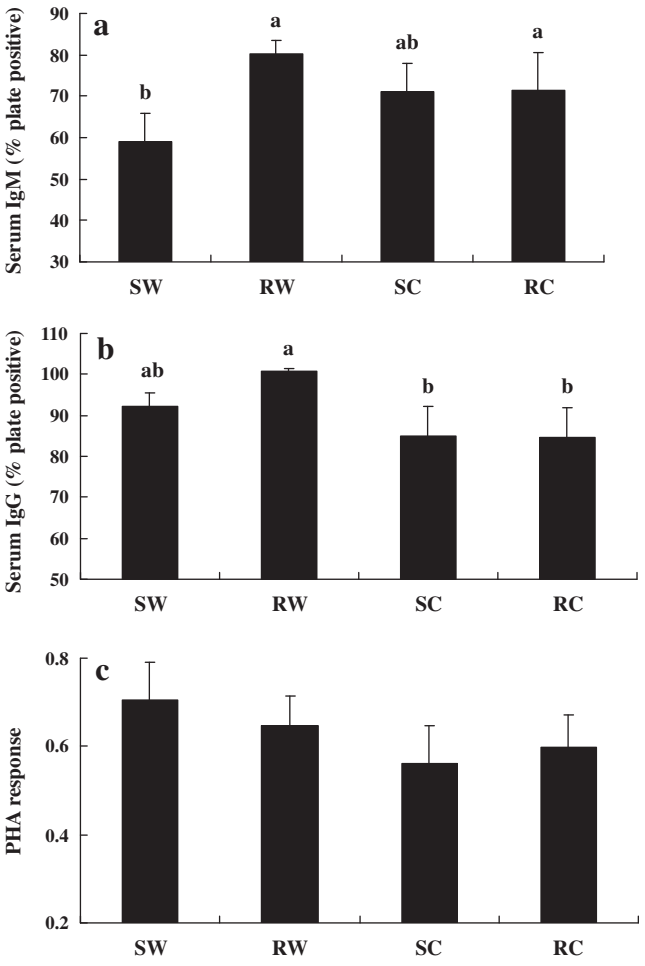

Fig. 4. Serum anti-KLH IgM (a) and IgG (b) levels and PHA response (c) in Mongolian gerbils received the surgical removal of IBAT and acclimated to either a warm (23 ± 1 °C) or cold (4 ± 1 °C) environment. Significant differences are indicated by different letters if $P < 0.05$. Values are means ± SE.

3.4. Humoral immunity

IBATR significantly increased circulating IgM levels ($F_{1,33} = 7.09$, $P = 0.012$; Fig. 4a), while there were no effects of cold exposure or interaction on IgM levels (all $F < 0.85$, $P > 0.36$). Specifically, IgM levels were significantly higher in IBATR gerbils compared with sham-operated gerbils under warm conditions ($P = 0.013$), but not under cold conditions ($P > 0.05$).

Cold-acclimated gerbils displayed significantly lower IgG levels when compared with those acclimated to warm conditions ($F_{1,33} = 4.83$, $P = 0.035$; Fig. 4b), while there were no effects of IBATR or interaction on IgG levels (all $F < 0.67$, $P > 0.42$). Specifically, IBATR gerbils acclimated to cold conditions had significantly lower IgG levels compared with IBATR gerbils acclimated to warm conditions ($P = 0.033$), but not in sham-operated gerbils ($P > 0.05$). In addition, IBATR gerbils acclimated to warm conditions had a significant higher IgG levels compared with sham-operated ones acclimated to warm conditions (independent samples t-test, $T = -2.50$, $P = 0.038$; Fig. 4b), but not in those acclimated to cold conditions ($P > 0.05$). Total BAT mass was negatively correlated with anti-KLH IgM but not with anti-KLH IgG percent plate positive values ($r = -0.428$, $P = 0.008$; $r = -0.218$, $P > 0.05$; Fig. 5a and b).

3.5. T cell-mediated immunity and white blood cells

There were no effects of IBATR, cold exposure or interaction on T cell-mediated immunity (all $F < 1.56$, all $P > 0.22$; Fig. 4c). There were also no effects of IBATR, cold exposure or interaction on white blood cells (all $F < 1.5$, $P > 0.23$; Table 1).

3.6. Body compositions

There were no significant effects of IBATR, cold exposure or an interaction between these two treatments on immune organs mass (spleen and thymus) (all $F < 2.07$, $P > 0.16$; Table 2). The reproductive organs (testis and seminal vesicle) of cold-acclimated gerbils were significantly smaller compared with warm-acclimated gerbils (all $F > 4.35$, $P < 0.05$). Cold-acclimated gerbils had significantly larger heart, liver, lung, kidneys and alimentary tract mass (stomach, small intestine, colon, and cecum; mass with content) than those of gerbils acclimated to warm conditions (all $F > 6.52$, $P < 0.015$). Wet carcass mass and body fat mass were significantly decreased by cold exposure (all $F > 4.53$, $P < 0.05$). Overall, body fat mass of cold-acclimated gerbils was decreased by 41.2% compared with those acclimated to warm conditions. There were no effects of IBATR or interaction with cold exposure for these parameters (all $P > 0.05$).

3.7. Blood glucose concentrations and circulating hormones

There were no effects of IBATR, cold exposure or interaction on blood glucose concentrations (all $F < 0.99$, $P > 0.33$; Table 1).

Serum leptin concentrations were lower in cold-acclimated gerbils when compared with those acclimated to warm conditions ($F_{1,34} = 7.64$, $P = 0.009$; Table 1), however, there were no significant effects of IBATR ($F_{1,34} = 1.45$, $P > 0.05$), or an interaction between IBATR and cold exposure treatments ($F_{1,34} = 3.343$, $P > 0.05$). No significant correlations were found between leptin and humoral immunity (data not shown).

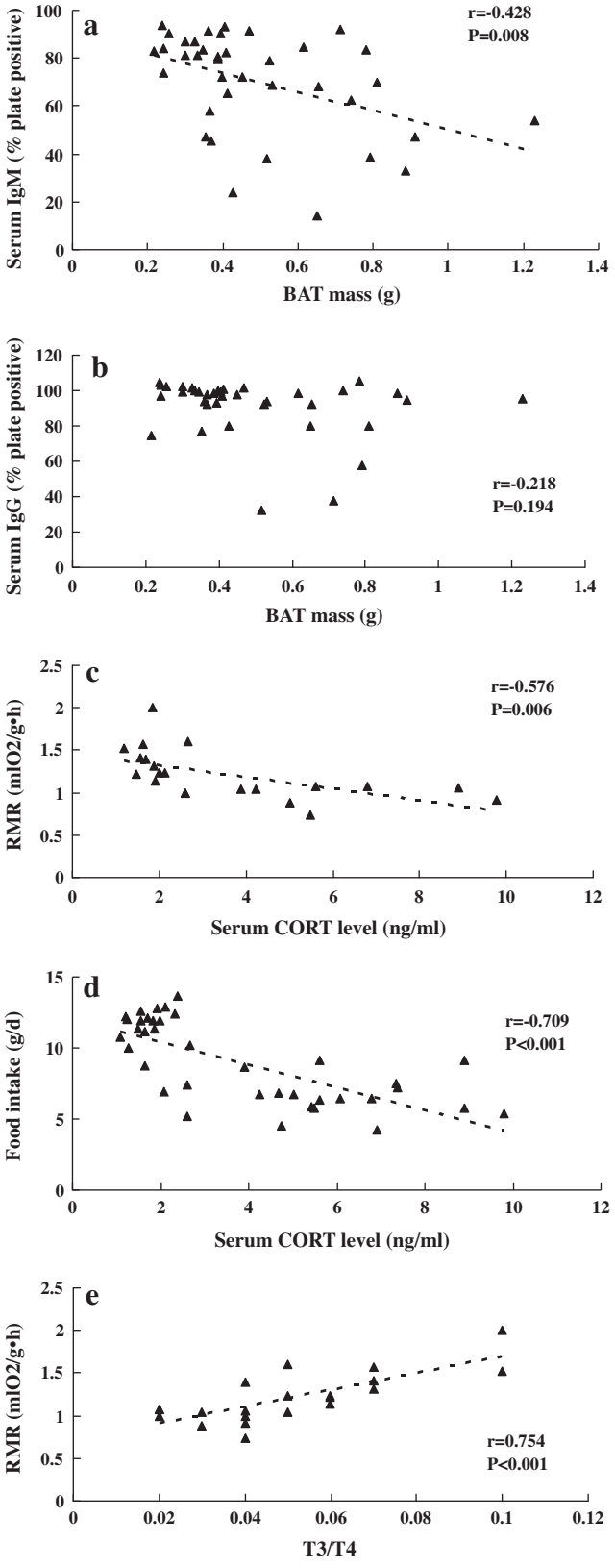

Fig. 5. Pearson correlations between BAT mass and IgM (a) or IgG (b); the correlations between serum CORT levels and RMR (c) or food intake (d); and the correlation between T3/T4 and RMR (e).

Cold-acclimated gerbils had significantly lower serum corticosterone concentrations than those of gerbils acclimated to warm conditions ($F_{1,34} = 56.70$, $P < 0.001$; Table 1) regardless of IBATR manipulation. There were no effects of IBATR or interaction with cold exposure on corticosterone concentrations (all $F < 0.04$, $P > 0.85$). Serum corticosterone levels were significantly and negatively correlated with resting metabolic rate and food intake ($r = -0.576$, $P = 0.006$; $r = -0.709$, $P < 0.001$; Fig. 5c and d). However, no significant correlations were detected between corticosterone and humoral immunity (data not shown).

Triiodothyronine to thyroxine (T_3/T_4) ratios were significantly affected by cold exposure ($F_{1,34} = 50.23$, $P < 0.001$; Table 1); T_3/T_4 were significantly higher in cold-acclimated gerbils compared with those acclimated to warm conditions independent of IBATR. There were no effects of IBATR or interaction (all $F < 0.37$, $P > 0.55$). T_3/T_4 was positively correlated with RMR ($r = 0.754$, $P < 0.001$; Fig. 5e).

4. Discussion

In the present study, we found that partial removal of BAT enhanced humoral immunity in warm-acclimated, but not in cold-acclimated gerbils. Partial removal of BAT increased KLH-specific IgM production, and a similar trend in KLH-specific IgG production in warm-acclimated gerbils. In addition, partial removal of BAT had no effect on T cell-meditated immunity (PHA response), immune organ mass (spleen and thymus) and white blood cells. These results indicate that BAT has a suppressive effect on humoral immunity in warm-acclimated gerbils. Our data support the idea that BAT can influence specific immune component in gerbils and the effects of BAT on immunity can vary according to environmental temperatures.

NST did not decrease in IBAT removed gerbils compared with sham-operated ones. We found that surgical removal of IBAT significantly reduced the NST by about 12% in the same gerbils before and after surgery (unpublished data).Thus, IBAT removal might still decrease the NST in the present study, at least in those acclimated to warm conditions. However, whether the reduction of NST in IBATR gerbils is the direct reason of enhancement of humoral immunity in warm-acclimated gerbils needs to be clarified. The negative correlation between BAT mass and KLH-specific IgM presented in our experiment showed BAT may influence humoral immunity. One possibility of immunosuppressive activity of BAT is that the expression of a biologically active substance produced by the tissue [43]. A few studies in this area have indicated that extracts obtained from brown adipose tissue of hibernators are capable of suppressing the production of antibody [75,76], which might be caused by AMP [5]. Another possibility is that the immune effect of BAT is due to a release of fatty acids from the tissue [31]. The suppressive effect of BAT on anti-KLH IgM disappeared under cold conditions, which might be due to the reduced effect of surgical removal in BAT mass compensated by cold acclimation or the changed active substance produced by BAT under cold conditions [14,74]. In addition, the discrepancy between humoral immunity and T cell-mediated immunity implies that humoral immunity might be more sensitive to BAT manipulation and cold acclimation. However, we still do not know about the precise mechanism.

Cold acclimation decreased KLH-specific IgG production in IBAT removed gerbils. Anti-KLH IgM followed a similar trend. Optimal BAT mass or thermogenic function is important for protecting animals against cold [21,35]. Considerable evidence indicates that mild core hypothermia directly impairs immune function, including antibody production [30,74]. It can be speculated that intact BAT might be critical for maintaining normal humoral immunity of cold-acclimated gerbils. Natural antibody production in response to select antigen depends upon innate immune cells and already present census of T lymphocytes [1]. The difference between KLH-specific IgM and IgG production raises the possibility that gerbils can mediate appropriate immune responses to seasonal-spe-

Table 1
Effect of surgical removal of IBAT and cold acclimation on serum hormone levels, WBCs and blood glucose levels.

Parameters	Group				Statistical summary		
	SW	RW	SC	RC	Cold	IBATR	Cold × IBATR
Leptin (ng/ml)	31.7 ± 3.3[a]	22.6 ± 3.0[a,b]	17.8 ± 2.7[b]	19.7 ± 2.9[b]	0.009	ns	ns
Corticosterone (ng/ml)	5.8 ± 0.8[a]	5.6 ± 0.6[a]	1.8 ± 0.1[b]	1.7 ± 0.2[b]	<0.001	ns	ns
T_3/T_4	0.029 ± 0.004[b]	0.031 ± 0.002[b]	0.072 ± 0.007[a]	0.072 ± 0.008[a]	<0.001	ns	ns
WBCs ($\times 10^9$ cells/L)	6.6 ± 0.9	6.0 ± 0.9	7.8 ± 0.9	6.9 ± 0.7	ns	ns	ns
Blood glucose levels (mg/dL)	105.2 ± 4.2	102.6 ± 6.5	104.6 ± 9.8	93.8 ± 5.0	ns	ns	ns

Groups with different letters indicate statistically significant differences among group means ($P < 0.05$). Values are means ± SE.
Cold, cold acclimation; IBATR, surgical removal of IBAT; cold × IBATR, interaction of cold and IBATR; ns, no significant difference.

Table 2
Effects of surgical removal of IBAT and cold acclimation on body compositions.

Parameters	Group				Statistical summary		
	SW	RW	SC	RC	Cold	IBATR	Cold × IBATR
Sample size	9	11	9	9			
Wet organ mass (mg)							
Heart	358 ± 23[a]	352 ± 17[a]	436 ± 14[b]	415 ± 15[b]	<0.001	ns	ns
Liver	2668 ± 281[a]	2558 ± 97[a]	3118 ± 93[b]	3002 ± 169[b]	<0.001	ns	ns
Spleen	74 ± 15	73 ± 8	68 ± 5	48 ± 6	ns	ns	ns
Lung	457 ± 25[a]	463 ± 24[a]	555 ± 39[b]	489 ± 33[a,b]	<0.05	ns	ns
Kidneys	741 ± 34[a]	738 ± 24[a]	864 ± 32[b]	826 ± 34[b]	<0.001	ns	ns
Thymus	28 ± 6	33 ± 2	38 ± 5	38 ± 5	ns	ns	ns
Testis	872 ± 53[a,b]	926 ± 27[a]	714 ± 92[a,b]	629 ± 91[b]	<0.05	ns	ns
Seminal vesicle	305 ± 70[a]	294 ± 62[a]	111 ± 39[a,b]	56 ± 12[b]	<0.01	ns	ns
Alimentary tract mass (mass with content, mg)							
Stomach	1142 ± 134[a]	1082 ± 114[a]	1616 ± 148[b]	1758 ± 168[b]	<0.001	ns	ns
Small intestine	2010 ± 133[a]	2068 ± 83[a]	3713 ± 95[b]	3456 ± 223[b]	<0.001	ns	ns
Colon	1451 ± 81[a]	1441 ± 81[a]	2041 ± 73[b]	2143 ± 115[b]	<0.001	ns	ns
Cecum	794 ± 90[a]	750 ± 37[a]	1180 ± 54[b]	1006 ± 85[b]	<0.001	ns	ns
Wet carcass mass (g)	58.8 ± 3.1[a]	56.1 ± 1.4[a]	51.7 ± 1.6[b]	51.1 ± 2.0[b]	<0.01	ns	ns
Body fat mass (g)	11.6 ± 1.9[a]	8.1 ± 0.8[a,b]	5.8 ± 0.5[b]	5.8 ± 0.8[b]	<0.001	ns	0.078

Different letters indicate statistically significant differences among group means ($P < 0.05$). Values are means ± SE. Cold, cold acclimation; IBATR, surgical removal of IBAT; cold × IBATR, interaction of cold and IBATR; ns, no significant difference.

cific challenges. Contrary to our prediction, cold exposure did not compromise all the immune components in sham-operated gerbils, suggesting no trade-off between thermoregulation and immune function. A remarkable increase in food intake, RMR, the mass of metabolically active internal organs and the decrease of reproductive organs assured us that costs of thermoregulation caused by cold exposure were substantial. It has been suggested that increased food intake might have a positive feedback on metabolically active organs (e.g., gut), causing hypertrophy of these organs [37,50]. The increased food intake may compensate the high energy requirement for the cold-induced thermoregulation, but not to reduce the energy resources allocated to immunity. The present result is contrast with those of some studies in some rodent species indicating a trade-off between thermoregulation and immunity [17,67,74,80]. Demas and Nelson [27] found that low ambient temperature alone does not suppress T cell-mediated immunity in deer mice. Humoral immunity and T cell-mediated immunity in gerbils are not compromised by chronic food restriction [87]. Thus, the interaction between cold exposure and other environmental factors (e.g., food availability) on immunity need to be investigated in the gerbils. In addition, humoral immunity of mice exposed to long-lasting cold exposure was significantly lower but not in short-lasting cold-exposed mice [17,52].

Stressful conditions such as low ambient temperatures and reduced food availability usually elevate glucocorticoids concentrations [7,9,65]. Elevated glucocorticoids tend to suppress immune function [59,63,71]. Surprisingly, serum corticosterone concentrations were decreased by cold exposure in Mongolian gerbils in the present study. 'Stress hormone' corticosterone has been used by ecologists as indicator of physiological stress in wild vertebrates [70], and it seems that cold conditions may be more comfortable for gerbils. Reductions in corticosterone concentrations in response to chronic cold exposure might be responsible for maintaining the normal immune responses in cold-acclimated gerbils. Studies in European starlings (*Sturnus vulgaris*) also find that exposure to chronic psychological stress significantly reduce corticosterone [19,69], and this suggests that starlings dampen corticosterone response to avoid pathology such as impaired immune function [19]. Thus, more research is necessary before corticosterone concentrations can be used to assess chronic stress. Physiologically appropriate concentrations of naturally secreted corticosterone have major stimulatory effects on energy expenditure and food intake [4,20], and several studies have showed that corticosterone decreases NST in brown adipose tissue [64,79]. The low corticosterone circulating levels under cold exposure may counteract the excessive catabolic rate of basal metabolism evoked by prolonged cold exposure.

Some studies have shown the direct link between body fat and immunity in Siberian hamsters (*P. sungorus*), which is mediated by leptin [26,28]. Although chronic cold exposure decreased the body fat (i.e., energy stores), and simultaneously leptin, which can mediate energy homeostasis (i.e., reduces food intake and increases energy expenditure), and leptin-induced increase in energy expenditure is thought to be through increased thermogenesis in BAT [4,36,72]. We did not find the same correlation between leptin and immunity in gerbils, at least under chronic cold exposure (in the present study) or chronic food restriction [87]. However, the

role of leptin in mediating immunity in gerbils can not be excluded. Thus, more studies should be conducted to illustrate the mechanisms of leptin in mediating immunity in more species under different environmental conditions.

As we expected, ratios of T_3 to T_4 were increased in cold-acclimated gerbils, and intracellular conversion of T_4 to T_3 is required for the optimal thermogenic function of BAT [8]. The protective effect of thyroid hormones against cold-induced hypothermia is largely the result of the stimulation of thermogenesis in BAT [47]. In addition, thyroid hormones stimulate diverse metabolic activities of most tissues, leading to an increase in resting metabolic rate [32]. One consequence of this activity is to increase body heat production [48]. Actually, there was a significantly positive correlation between T_3/T_4 and RMR in the present study.

Although we do not detect any correlations between the hormones (corticosterone, leptin and T_3/T_4) and immune function in gerbils in the present study, changes in these hormones must be helpful for their survival adaptation (including maintain normal immune responses) during cold exposure. Thus, gerbils might have evolved a set of elaborate mechanisms to maintain normal immune responses for promoting their survival under low temperatures during harsh winter.

In summary, our data support the idea that partial removal of BAT affects humoral immune responses in warm-acclimated gerbils, probably either through the reduction in NST or changes of substances secreted by BAT. However, the effects of partial removal of BAT on humoral immune responses disappeared in gerbils acclimated to cold environment. In addition, cold acclimation decreased humoral immunity in IBAT removed gerbils. Taken together, BAT may play an important role in the regulation of seasonal immune function. Cold acclimation does not elicit a trade-off between thermoregulation and immune function in sham-operated gerbils. The results provide some new information to understand the physiological role of BAT in regulating seasonal changes in immunity.

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