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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.

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