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RESEARCH ARTICLE

Effect of temperature and food restriction on immune function in striped hamsters (*Cricetulus barabensis*)

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ABSTRACT

Small mammals in temperate areas face seasonal fluctuations of temperature and food availability, both of which may influence their immune responses, which are critical to survival. In the present study, we tested the hypothesis that low temperature and food restriction suppress immune function in striped hamsters (*Cricetulus barabensis*). Thirty-seven adult male hamsters were randomly assigned to warm (23±1°C) and cold (5±1°C) treatment groups, which were further divided into fed and food-restricted groups. Body mass was not affected by cold stress, food restriction or the interaction cold stress×food restriction. Cold stress decreased total body fat mass, haematological parameters including white blood cells, lymphocytes and neutrophilic granulocytes, and immunoglobin (Ig) M titres 5 days after injecting keyhole limpet haemocyanin (KLH). However, cold temperature increased bacterial killing capacity, indicative of innate immunity, and did not affect the mass of the thymus and spleen, intermediate granulocytes, the phytohaemagglutinin (PHA) response and the levels of blood glucose and serum leptin. Corticosterone concentration was affected significantly by the interaction cold stress×food restriction but not by cold stress or food restriction alone. Food restriction reduced thymus mass, but other immunological parameters including body fat mass, spleen mass, haematological parameters, innate immunity, PHA response, the titres of IgM and IgG, and the levels of blood glucose and serum leptin were all not affected by food restriction or the interaction cold stress×food restriction. Innate immunity was positively correlated with leptin levels, whereas no significant correlations were observed in the levels of blood glucose, serum leptin, corticosterone and all the detected immune parameters. Our results show that cold stress suppressed humoral immunity but enhanced innate immunity and did not affect cellular immunity in striped hamsters. Most immunological indices were not influenced by food restriction. Blood glucose, leptin and corticosterone could not explain the changes of innate, cellular and humoral immunity upon cold stress or food restriction in striped hamsters.

KEY WORDS: Corticosterone, Humoral immunity, Leptin, Phytohaemagglutinin response, Innate immunity

INTRODUCTION

The immune system is crucial for the survival of animals by protecting them from infection and the attack of pathogens (Sheldon and Verhulst, 1996; Owens and Wilson, 1999). Immune responses often demonstrate seasonal changes in small mammals in the temperate area (Nelson and Demas, 1996; Nelson, 2004). In winter compared with spring and summer, immune function decreased in some species (Newson, 1962; Lochmiller et al., 1994; Mann et al., 2000), but increased in others (Dobrowolska and Adamczewska-Andrezjewska, 1991; Moshkin et al., 1998; Sinclair and Lochmiller, 2000; Zhang and Wang, 2006). It is clear that winter conditions are associated with stressful environments such as low ambient temperature and reduced food availability, and hence seasonal changes in immune responses probably reflect the composite influences of multiple factors (Demas and Nelson, 1996; Nelson, 2004). Many researchers have investigated the effect of single factors such as temperature or food availability on immunity. For example, cold stress impaired humoral immunity in mice (Cichoń et al., 2002), deer mice (*Peromyscus maniculatus*) (Demas and Nelson, 1996) and grey-sided voles (*Clethrionomys rufocanus*) (Kusumoto and Saitoh, 2008). It also increased the intensity of genital infection in mice (Belay and Woart, 2013). Nevertheless, cold stress did not affect cellular immunity in deer mice (Demas and Nelson, 1998), or the proliferation of lymphocytes and the ability of macrophages to bind bacterial lipopolysaccharide in golden-mantled ground squirrels (*Spermophilus lateralis*) (Maniero, 2002, 2005). Food restriction suppressed cellular immunity in deer mice (*P. maniculatus*) (Demas and Nelson, 1998) and Siberian hamsters (*Phodopus sungorus*) (Bilbo and Nelson, 2004), and humoral immunity in rat-like hamsters (*Cricetulus triton*) (Liang et al., 2004). The immunological memory and spleen-derived antibody-producing B cells were also reduced in food-restricted deer mice (Martin et al., 2007, 2008). In addition, food restriction decreased mitogen-induced T-cell proliferation and humoral immunity in mice (Pocino et al., 1987; Rogers et al., 2008; Ishikawa et al., 2009). However, other researchers obtained different findings in which immune responses were enhanced by food or caloric restriction (Pahlavani, 2000; Jolly, 2004; Ritz and Gardner, 2006; Ahmed et al., 2009; Zysling et al., 2009; Berger, 2013). Our previous study showed that cellular and humoral immunity did not respond to food restriction in Mongolian gerbils (*Meriones unguiculatus*) (Xu et al., 2011). Therefore, it is important that the composite impact of different environmental factors such as low temperature and reduced food availability is studied in more wild mammals (Demas and Nelson, 1996, 1998; Kusumoto, 2009; Zysling et al., 2009).

Serum bacterial killing capacity is usually used to evaluate innate immunity, which is one arm of the immune system (Tieleman et al., 2005; Demas et al., 2011; Yang et al., 2013). Adaptive immunity, comprising cellular and humoral immunity, is another arm of the immune system. Cellular immunity, which is generally responsible for controlling intracellular pathogens, is often assessed by the phytohaemagglutinin (PHA) response (Smits et al., 1999; Goüy de Bellocq et al., 2006; Xu and Wang, 2010). Humoral immunity, which

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primarily controls extracellular pathogens, is usually evaluated by antibody production following immunochallenge with a specific antigen such as keyhole limpet haemocyanin (KLH) (Demas et al., 2003; Zysling and Demas, 2007; Zysling et al., 2009). Immune organs such as the thymus and spleen are also indirect immunological parameters, as the thymus is the site of primary T-cell development and a larger spleen implies stronger immune responses (Savino and Dardenne, 2000; Calder and Kew, 2002; Smith and Hunt, 2004). Similarly, haematological parameters including white blood cells, lymphocytes, intermediate granulocytes and neutrophilic granulocytes play important roles in mounting immune responses against pathogens (Calder and Kew, 2002).

Leptin, which has regulatory role in energy balance and immune responses, is a cytokine-like hormone and is mainly produced by white adipose tissues (Zhang et al., 1994; Fantuzzi and Faggioni, 2000; Faggioni et al., 2001; Matarase et al., 2005; Lam and Lu, 2007; Lago et al., 2007). Adipose tissues are not only energy reserves but also considered as important endocrine and immune organs (Pond, 1996; Ahima and Flier, 2000; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al., 2007). Stressful conditions such as cold stress and food restriction usually activate the hypothalamic-pituitary–adrenal axis and hence stress hormones such as corticosterone increase, which often has a suppressive effect on immunity (Bligh-Tynan et al., 1993; Sapolsky et al., 2000; Demas and Nelson, 1998; Webster Marketon and Glaser, 2008).

The striped hamster (*Cricetulus barabensis*), which is mainly distributed in northern China, Russia, Mongolia and Korea, is granivorous, nocturnal and feeds mainly on stems and leaves of plants during summer and on forage crop seeds in winter (Lu et al., 1987; Zhang and Wang, 1998; Song and Wang, 2003). The climate is arid and characterised by warm and dry summers (extreme maximum temperature 42.6°C) and cold winters (extreme minimum temperature below −20°C). Thus, this species is confronted with great seasonal fluctuations in temperature and food availability (Zhang and Wang, 1998; Zhao et al., 2010). Understanding how immune function varies in the face of fluctuations in temperature and food resources can help us to clarify the adaptive strategies of striped hamsters to environmental changes. It can also help us to understand their distribution and population dynamics in the field from the immunological perspective. In the present study, we tested the hypothesis that cold stress and food restriction would suppress innate, cellular and humoral immunity in striped hamsters.

MATERIALS AND METHODS

Animals and experimental design

All animal procedures were carried out according to the guidelines of the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Adult male striped hamsters used in this study were captured from Jiuxian Mountain (35°46.275′N, 116°59.976′E) in Qufu, Shangdong province. The hamsters were housed individually in plastic cages (30 cm×15 cm×20 cm) with sawdust as bedding under a constant photoperiod of 12 h light:12 h dark and temperature of 23±1°C. Standard rat pellets (Beijing KeAo Feed Co., Beijing, China) and water were provided throughout the experiment. The diet macronutrients were 6.2% crude fat, 18% crude protein, 23.1% neutral fibre, 5% crude fibre, 12.5% acid detergent fibre and 10.0% ash, and the caloric value was 17.5 kJ g^{-1}. Generally, body mass of wild-captured gerbils tends to increase after laboratory feeding (Stuermer et al., 2003). We also found that body mass of wild-caught striped hamsters tended to increase after laboratory feeding. After body mass stabilised, 37 male hamsters were selected and randomly assigned to one of four experimental groups: (1) hamsters were fed *ad libitum* and kept at a constant temperature of 23±1°C [the warm–fed (WF) group, n=9]; (2) hamsters were subjected to food restriction at 23±1°C [the warm–food-restricted (WR) group, n=10]; (3) hamsters were fed *ad libitum*, but the ambient temperature was maintained at 5±1°C [the cold−fed (CF) group, n=9]; (4) hamsters were food restricted at 5±1°C [the cold−food-restricted (CR) group, n=9]. Striped hamsters kept at room temperature began to die after 20 days of 10% food restriction (i.e. fed 90% of baseline food intake when hamsters ate *ad libitum*), 17 days of 20% food restriction, 7 days of 30% food restriction and 6 days of 40% food restriction (Professor Zhao Zhijun, Wenzhou University, personal communication). Therefore, we decided that hamsters would be subjected to 10% food restriction for 19 days in the present study, given their sensitivity to food shortage (Zhao, 2012; Xu and Xu, 2015). Baseline food intake (g day^{-1}) of hamsters subjected to food restriction was measured for 6 days (once every other day). Average food consumption per day was calculated for each individual, and the restricted food amount was 90% of the baseline food intake. In the experimental timeline, day 0 and day n represented the initial day and the number of days post-cold exposure/post-restriction exposure, respectively.

Body composition

Body composition was measured according to Xu and Wang (2010). In brief, immune system organs including the thymus and spleen were dissected and weighed (±1 mg). All the visceral organs were removed to obtain carcass wet mass. Perigonadal fat, mesenteric fat, retroperitoneal fat and subcutaneous fat were also dissected carefully and weighed. Subcutaneous fat was isolated as follows: the skin was cut open with ophthalmic scissors along the dorsal skin centre line, the skin was removed and the subcutaneous fat pads of the shoulder, neck and buttocks were dissected carefully with tweezers. At the same time, connective tissue was removed. For retroperitoneal fat, all organs in the peritoneal cavity were removed and the fat remaining at the back of the cavity was carefully isolated with tweezers.

Haematological parameter assays

At the end of the experiment, trunk blood was collected and 20 µl whole blood was diluted immediately in 4 ml diluent; red blood cells, white blood cells, lymphocytes, intermediate granulocytes (including eosinophil and basophil granulocytes) and neutrophilic granulocytes were counted in a Hematology Analyzer (Auto Counter 910EO^{+}).

Innate immunity assay

Serum bacterial killing capacity indicative of innate immunity was assessed in a sterile laminar flow cabinet to gauge the functional response of the animal's innate immune system against a relevant pathogen, *Escherichia coli* (Tieleman et al., 2005; Demas et al., 2011; Yang et al., 2013). Briefly, serum samples were diluted 1:20 in CO_2-independent medium (Gibco no. 18045, Carlsbad, CA, USA). A standard number of colony-forming units (CFUs) of *E. coli* (ATCC no. 8739, Microbial Culture Collection Centre of Guangdong Institute of Microbiology, China) was added to each sample in a 1:10 ratio, and the mixture of *E. coli* dissolved in CO_2-independent medium, serum samples and CO_2-independent medium was allowed to incubate at 37°C for 30 min to induce bacterial killing. After incubation, 50 µl of each sample was added to tryptic soy agar plates in duplicate. All plates were covered and left to incubate upside down at 37°C for 24 h, and then total CFUs

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were counted and bactericidal capacity was calculated as 100% minus the mean number of CFUs for each sample divided by the mean number of CFUs for the positive controls (containing only medium and standard bacterial solution), i.e. the percentage of bacteria killed relative to the positive control.

Cellular immunity assay

We measured PHA response as in previous studies (Goüy de Bellocq et al., 2006; Xu and Wang, 2010). Specifically, hamsters in the WF, WR, CF and CR groups on day 16 were caught (Fig. 1), and we measured the footpad thickness of the left hind foot with a micrometre (Digimatic Indicator ID-C 547-301, Mitutoyo, Kawasaki, Japan) to ±0.01 mm. Immediately after, hamsters in the four groups were injected subcutaneously with 0.1 mg of PHA (PHA-P, Sigma L-8754, St Louis, MO, USA) dissolved in 0.03 ml of sterile saline (pH 7.4) in the middle of the footpad. Footpad thickness was measured 6, 12, 24 and 48 h after injection. The PHA response (i.e. cellular immunity) was calculated as the difference between pre- and post-injection measurements divided by the initial footpad thickness [PHA response=(post-PHA−pre-PHA)/pre-PHA]. Six measures of footpad thickness were taken at each of the four time points to obtain a value for each hamster (Xu and Wang, 2010; Xu et al., 2011). Only PHA responses after 6 h of PHA injection were included in the results because they were representative of the maximal response.

Humoral immunity assay

Hamsters in the four groups on day 9 received a single subcutaneous injection of 100 µg of KLH (Sigma LH7017) suspended in 0.1 ml sterile saline in order to assess humoral immunity. Five days after KLH injection (i.e. on day 14), hamsters in all groups were lightly anaesthetised with isoflurane (Shandong LiNuo Pharmaceutical Co., Jinan, Shandong, China) and blood samples (~200 µl) were drawn from the retro-orbital sinus. Blood samples were allowed to clot for 1 h and were then centrifuged at 4°C for 30 min at 4000 rpm. Sera were collected and stored in polypropylene microcentrifuge tubes at −20°C for later measurement of anti-KLH IgM and IgG concentrations. After another 5 days (i.e. on day 19), each hamster was killed and trunk blood was collected for measurement of anti-KLH IgM and IgG, haematological parameters, blood glucose, leptin and corticosterone. IgM is the first immunoglobulin class and IgG is the predominant immunoglobulin class present in the blood produced following an immune challenge (Demas et al., 2003; Zysling and Demas, 2007). Blood samples were allowed to clot for 1 h and were then centrifuged at 4°C for 30 min at 4000 rpm. Sera were collected and stored in polypropylene microcentrifuge tubes at −20°C until assayed.

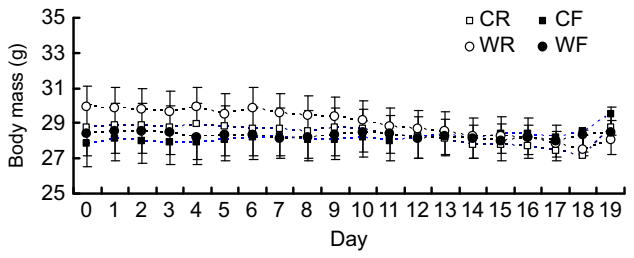

Fig. 1. Changes of body mass during cold and food-restriction treatment in striped hamsters. CR, cold and food-restricted group; CF, cold and fed group; WR, warm and food-restricted group; WF, warm and fed group.

ELISA was used to assess serum IgM and IgG concentration as described previously (Demas et al., 2003; Zysling and Demas, 2007; Xu et al., 2011). Specifically, microtitre plates were coated with 100 µl 0.5 mg ml^{-1} KLH in sodium bicarbonate buffer (pH 9.6) overnight at 4°C. Plates were washed with 200 µl phosphate-buffered saline containing 0.05% Tween 20 (PBS-T, pH 7.4) three times, then blocked with 5% non-fat dry milk in PBS-T overnight at 4°C to reduce non-specific binding, and washed again with PBS-T three times. 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 hamsters repeatedly challenged with KLH, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naive hamsters, similarly diluted with PBS-T) were added in duplicate. Plates were sealed, incubated at 37°C for 3 h, and then washed with PBS-T three times. Secondary antibody (alkaline phosphatase-conjugated anti-mouse IgG diluted 1:2000 with PBS-T, Sigma; alkaline phosphatase-conjugated anti-mouse IgM diluted 1:500 with PBS-T, Sigma) was added to the wells, and the plates were sealed and incubated for 1 h at 37°C. Plates were then washed again with PBS-T and 150 µl enzyme substrate *p*-nitrophenyl phosphate (Sigma; 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 30 min by adding 50 µl of 1.5 mol l^{-1} NaOH solution to each well. The optical density (OD) of each well was determined using a plate reader (Bio-Rad, Benchmark, Richmond, CA, USA) equipped with a 405 nm wavelength filter, and the mean OD for each set of duplicate wells was calculated. To minimise inter- and intra-assay variability, the mean OD for each sample is expressed relative to the positive control OD for statistical analysis (Demas et al., 2003; Zysling and Demas, 2007).

Blood glucose assays

Blood glucose levels were measured with a FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc., Alameda, CA, USA) according to the manufacturer's instructions. The range of blood glucose tested was 1.11–30.56 mmol l^{-1}. The within-lot and within-vial precision are <5.6% and <4.1%, respectively.

Serum leptin assays

Serum leptin titres were determined by a hamster leptin ELISA kit (cat. no. XL-85K, Linco Research Inc., St Charles, MO, USA) following the manufacturer's instructions. The range detected by this assay was 0.3–8 ng ml^{-1} when using a 10 µl sample (see manufacturer's instructions).

Serum corticosterone assays

Serum corticosterone concentration was determined by a hamster corticosterone ELISA kit (cat. no. HR083, RapidBio Lab, Calabasas, CA, USA) following the manufacturer's instructions. The range detected by this assay was 8–150 ng ml^{-1} when using a 10 µl sample (see manufacturer's instructions for the hamster corticosterone ELISA kit).

Statistical analysis

Data were analysed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. The ratio values of the PHA response, IgM and IgG levels were subjected to arcsine transformation. The difference of body mass on day 0 was analysed by a one-way analysis

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of variance (ANOVA), while body mass at other times of the experimental course was analysed by two-way ANOVA (temperature×food restriction) followed by Bonferroni *post hoc* tests. Group differences in thymus and spleen mass, fat mass from different areas with body mass as the covariate were analysed by general linear model (GLM) multivariate analysis followed by Bonferroni *post hoc* tests. Group differences in other parameters (body composition, haematological parameters, PHA response, concentrations of IgM, IgG, blood glucose, leptin and corticosterone) were analysed by a two-way ANOVA followed by Bonferroni *post hoc* tests. Significant group differences were further evaluated by one-way ANOVA followed by Turkey's *post hoc* tests or GLM multivariate analysis followed by Bonferroni *post hoc* tests. As IgG and IgM concentrations were measured at two different times, a repeated measure of ANOVA was carried out to analyse changes in these two parameters with time. Pearson correlation analysis was performed to determine the correlations of PHA response, IgM and IgG titres with body fat mass, blood glucose, leptin and corticosterone levels for all hamsters. The results are presented as means±s.e.m, and $P<0.05$ was considered to be statistically significant.

RESULTS

Body mass

There was no significant difference of body mass among the WF, WR, CF and CR groups at the beginning of the experiment (day 0: $F_{3,33}=0.472$, $P=0.704$; Fig. 1). At any time point, body mass was not affected by cold stress (day 1: $F_{1,33}=0.309$, $P=0.582$; day 19: $F_{1,33}=0.545$, $P=0.466$), food restriction (day 1: $F_{1,33}=0.713$, $P=0.405$; day 19: $F_{1,33}=0.243$, $P=0.625$) or the interaction cold stress×food restriction (day 1: $F_{1,33}=0.062$, $P=0.805$; day 19: $F_{1,33}=0.028$, $P=0.868$; Fig. 1).

Body composition

Cold stress reduced perigonadal, subcutaneus and total body fat mass in striped hamsters, but had no effect on wet carcass mass, and retroperitoneal and mesenteric fat mass (Table 1). In particular, perigonadal fat was less for CR than for WF hamsters, but all other pairwise comparisons were similar among the treatment groups (Table 1). All the above parameters were not influenced by food restriction or the interaction cold stress×food restriction (Table 1).

Immune organs

Wet thymus mass was decreased by food restriction ($F_{1,33}=9.791$, $P=0.004$), but was not affected by cold stress ($F_{1,33}=0.004$, $P=0.948$) or the interaction cold stress×food restriction ($F_{1,33}=0.401$, $P=0.531$; Fig. 2A). In addition, wet spleen mass was not influenced by food restriction ($F_{1,33}=3.492$, $P=0.071$), cold stress ($F_{1,33}=3.182$, $P=0.084$) or the interaction cold stress×food restriction ($F_{1,33}=0.309$, $P=0.582$; Fig. 2B).

Haematological parameters

White blood cells, lymphocytes and neutrophilic granulocytes were decreased by cold stress, whereas they were not influenced by food restriction or the interaction cold stress×food restriction (Table 2). Red blood cells and intermediate granulocytes were also not affected by cold stress, food restriction or the interaction cold stress×food restriction (Table 2).

Innate immunity

Bacterial killing capacity was increased significantly by cold stress ($F_{1,33}=5.115$, $P=0.030$), but was not affected by food restriction ($F_{1,33}=0.734$, $P=0.389$) or the interaction cold stress×food restriction ($F_{1,33}=0.030$, $P=0.863$; Fig. 3). No significant correlation was detected between bacterial killing capacity and total body fat mass ($r=-0.173$, $P=0.305$).

Cellular immune response

No significant effect on PHA response was observed for cold stress ($F_{1,33}=0.019$, $P=0.891$), food restriction ($F_{1,33}=0.307$, $P=0.583$) or

Table 1. Effect of low temperature and food restriction on body composition in striped hamsters

					Temperature		Food		Temperature×food	
	WF (9)	WR (10)	CF (9)	CR (9)	$F_{1,33}$	P	$F_{1,33}$	P	$F_{1,33}$	P
Carcass (g)	17.5±0.7	17.0±0.7	16.5±0.7	16.2±0.8	0.279	0.601	1.479	0.233	0.014	0.908
Perigonadal fat (mg)	1293±77^{a}	1201±72^{a,b}	1022±52^{a,b}	961±76^{b}	70.622	<0.001	1.719	0.199	0.526	0.473
Mesenteric fat (mg)	205±13	197±15	230±41	199±28	0.021	0.885	0.323	0.574	0.176	0.678
Retroperitoneal fat (mg)	65±11	86±19	54±13	52±23	2.759	0.106	0.651	0.426	0.416	0.524
Subcutaneus fat (mg)	314±36	402±55	280±48	263±68	5.426	0.026	1.161	0.289	1.071	0.308
Total fat (mg)	1877±117^{a}	1886±144^{a}	1585±105^{b}	1474±144^{b}	35.991	<0.001	0.002	0.966	0.341	0.563

	Warm vs cold				Fed vs food restricted			
	WF+WR (19)	CF+CR (18)	$F_{1,34}$	P	WF+CF (18)	WR+CR (19)	$F_{1,34}$	P
Carcass (g)	17.0±0.5	16.6±0.5	0.247	0.622	17.3±0.5	16.4±0.5	1.530	0.224
Perigonadal fat (mg)	1244±52^{a}	991±45^{b}	69.389	<0.001	1157±56	1087±58	0.479	0.493
Mesenteric fat (mg)	201±10	215±24	0.021	0.885	218±21	198±15	0.330	0.569
Retroperitoneal fat (mg)	76±11	53±13	2.946	0.095	59±8	70±15	0.699	0.409
Subcutaneus fat (mg)	361±34^{a}	271±40^{b}	5.605	0.024	297±29	336±45	1.150	0.291
Total fat (mg)	1881±91^{a}	1530±88^{b}	38.101	<0.001	1731±84	1691±110	0.011	0.916

Data are means±s.e.m. (sample size is given in parentheses). CR, cold and food-restricted group; CF, cold and fed group; WR, warm and food-restricted group; WF, warm and fed group.

Values for WF, WR, CF and CR treatments groups were first analysed by a two-way ANCOVA with body mass as the covariate and Bonferroni *post hoc* tests. Group differences were further analysed by general linear model (GLM) multivariate analysis followed by Bonferroni *post hoc* tests; different superscript letters indicate a significant difference ($P<0.05$).

Group differences in the warm versus cold or fed versus food-restricted groups (warm group, WF+WR; cold group, CF+CR; fed group, WF+CF; food-restricted group, WR+CR) in fat mass (perigonadal, mesenteric, retroperitoneal and subcutaneous) with body mass as the covariate were analysed by GLM multivariate analysis followed by Bonferroni *post hoc* tests; different superscript letters indicate a significant difference ($P<0.05$).

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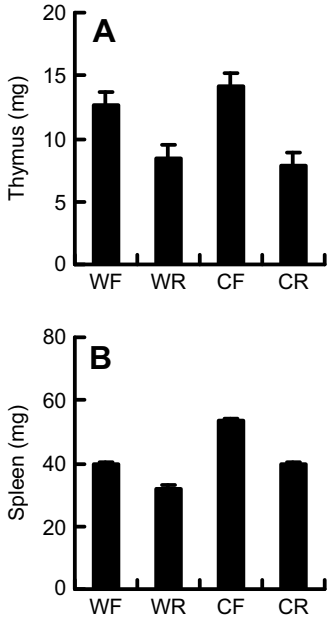

Fig. 2. Effect of cold and food restriction on immune system organs in striped hamsters. (A) Thymus mass. (B) Spleen mass. Groups are as described in Fig. 1.

the interaction cold stress×food restriction ($F_{1,33}$=4.021, P=0.053; Fig. 4). It was not correlated with total body fat mass (r=−0.059, P=0.730).

Humoral immunity

IgG concentration increased significantly with time ($F_{1,33}$=42.896, P<0.001), but there was no interaction of time×group (WF, WR, CF and CR groups; $F_{3,33}$=0.799, P=0.503). IgG levels 5 days ($F_{1,33}$=0.784, P=0.382) and 10 days ($F_{1,33}$=0.285, P=0.597) after KLH challenge were not influenced by cold stress. Likewise, food restriction had no effect on IgG titres 5 days ($F_{1,33}$=0.175, P=0.678) and 10 days ($F_{1,33}$=1.320, P=0.259) after KLH challenge. IgG concentration 5 days ($F_{1,33}$=1.935, P=0.173) and 10 days ($F_{1,33}$=2.850, P=0.101) after of KLH challenge was also not influenced by the interaction cold stress×food restriction (Fig. 5A). Additionally, total body fat mass was not correlated with IgG levels 5 days (r=−0.033, P=0.845) and 10 days (r=0.136, P=0.421) after KLH challenge.

IgM concentration was higher 5 days after than 10 days after KLH injection ($F_{1,33}$=4.787, P=0.036), but there was no interaction of time×group ($F_{3,33}$=2.169, P=0.110). IgM titre 5 days after KLH challenge in the CR hamsters was lowest among the four groups ($F_{1,33}$=4.525, P=0.041), but IgM levels 10 days after KLH challenge were not affected by cold stress ($F_{1,33}$=0.003, P=0.955; Fig. 5B). Moreover, IgM concentration 5 days ($F_{1,33}$=1.597, P=0.215) and 10 days ($F_{1,33}$=0.219, P=0.643) after KLH challenge was not affected by food restriction (Fig. 5B). IgM concentration 5 days ($F_{1,33}$=1.474, P=0.233) and 10 days ($F_{1,33}$=1.402, P=0.245) after KLH challenge was also not influenced by the interaction cold stress×food restriction (Fig. 5B). In addition, total body fat mass was not correlated with IgM levels 5 days (r=0.286, P=0.086) and 10 days (r=0.160, P=0.345) after KLH challenge.

Blood glucose

Blood glucose concentration was not impacted by cold stress ($F_{1,33}$=1.642, P=0.209), food restriction ($F_{1,33}$=1.344, P=0.255) or the interaction cold stress×food restriction ($F_{1,33}$=0.690, P=0.412; Fig. 6). It was not correlated with bacterial killing capacity (r=−0.094, P=0.579), PHA response (r=−0.294, P=0.077), IgG titre 5 days (r=0.052, P=0.759) and 10 days (r=0.101, P=0.552) after KLH challenge or IgM titre 5 days (r=−0.066, P=0.698) and 10 days (r=0.040, P=0.812) after KLH challenge.

Serum leptin concentration

Leptin concentration was not impacted by cold stress ($F_{1,33}$=0.088, P=0.769), food restriction ($F_{1,33}$=3.287, P=0.079) or the interaction cold stress×food restriction ($F_{1,33}$=0.057, P=0.812; Fig. 7). It was positively correlated with bacterial killing capacity (r=0.443, P=0.006), but was not correlated with PHA response (r=−0.046, P=0.788), IgG levels 5 days (r=0.040, P=0.815) and 10 days (r=−0.142, P=0.402) after KLH challenge or IgM levels 5 days (r=−0.046, P=0.788) and 10 days (r=0.137, P=0.417) after KLH challenge.

Serum corticosterone concentration

Corticosterone concentration was affected significantly by the interaction cold stress×food restriction ($F_{1,33}$=5.249, P=0.028). However, it was not influenced by cold stress ($F_{1,33}$=4.106, P=0.051) or food restriction ($F_{1,33}$=0.421, P=0.521; Fig. 8). No significant correlations were observed between corticosterone levels and bacterial killing capacity (r=0.062, P=0.717), PHA response (r=0.164, P=0.333), IgG titre 5 days (r=0.065, P=0.701) and 10 days (r=−0.194, P=0.249) after KLH challenge or IgM titre 5 days (r=0.204, P=0.227) and 10 days (r=−0.184, P=0.275) after KLH challenge.

Table 2. Effect of low temperature and food restriction on haematological parameters in striped hamsters

					Temperature		Food		Temperature×food	
	WF (9)	WR (10)	CF (9)	CR (9)	$F_{1,33}$	P	$F_{1,33}$	P	$F_{1,33}$	P
RBCs (10^{12} l^{-1})	10.1±0.4	9.3±0.3	9.6±0.4	9.1±0.4	1.121	0.297	3.234	0.081	0.160	0.692
WBCs (10^{9} l^{-1})	5.4±0.5^{a}	5.5±0.6^{a}	3.4±0.4^{b}	3.8±0.3^{a,b}	14.615	0.001	0.251	0.620	0.101	0.752
Lymphocytes (10^{9} l^{-1})	3.8±0.2^{a}	4.0±0.5^{a}	2.5±0.3^{b}	2.7±0.3^{a,b}	14.742	0.001	0.318	0.577	0.036	0.850
% Lymphocytes	73.2±3.4	69.8±3.3	73.9±1.5	71.4±2.6	0.148	0.703	1.063	0.310	0.026	0.872
MID (10^{9} l^{-1})	0.52±0.10	0.55±0.05	0.38±0.06	0.36±0.04	3.475	0.071	0.400	0.531	0.090	0.766
% MID	8.1±0.8	8.1±0.6	9.5±0.7	8.7±0.7	1.968	0.170	0.262	0.612	0.401	0.531
NG (10^{9} l^{-1})	1.1±0.3	1.1±0.1	0.5±0.1	0.7±0.1	5.811	0.022	0.274	0.604	0.152	0.699
% NG	18.7±2.7	22.0±3.0	16.7±1.2	19.9±2.5	0.695	0.410	1.739	0.196	<0.001	0.992

RBCs, red blood cells; WBCs, white blood cells; MID, intermediate granulocytes (eosinophil and basophil granulocytes); NG, neutrophil granulocytes. Groups are as described in Table 1.

Data are means±s.e.m. (sample size is given in parentheses). Values were first analysed by a two-way ANOVA and Bonferroni *post hoc* tests. Group differences were further examined by one-way ANOVA followed by Tukey's *post hoc* tests; different superscript letters indicate a significant difference (P<0.05).

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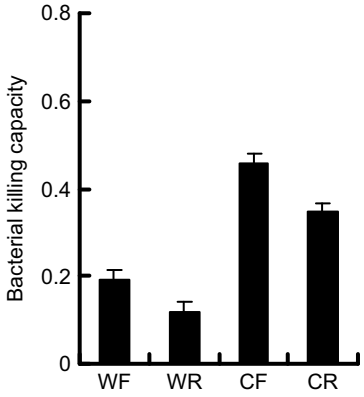

Fig. 3. Effect of cold and food restriction on innate immunity in striped hamsters. Innate immunity was assessed as bacterial killing capacity relative to the positive control. Groups are as described in Fig. 1.

DISCUSSION

Our data show that cold stress or food restriction do not affect body mass in striped hamsters. As expected, cold stress reduced IgM titre and haematological parameters including white blood cells, lymphocytes and neutrophilic granulocytes, suggesting a suppressive effect of cold stress on humoral immunity in hamsters, which was consistent with other studies (Cichoń et al., 2002; Demas and Nelson, 1996; Kusumoto and Saitoh, 2008). However, cold stress boosted innate immunity and did not affect cellular immunity in hamsters, compatible with other findings (Demas and Nelson, 1998). Food restriction decreased thymus mass, implying that primary T-cell development might be impaired in food-restricted hamsters. Nevertheless, food restriction had no effect on spleen mass, haematological parameters (i.e. white blood cells, lymphocytes, neutrophilic granulocytes and intermediate granulocytes), or innate, cellular and humoral immunity in striped hamsters, consistent with our previous research in Mongolian gerbils (Xu et al., 2011). These results conflict with other findings in which cellular or humoral immunity was suppressed (Demas and Nelson, 1998; Bilbo and Nelson, 2004; Liang et al., 2004; Martin et al., 2007, 2008) or enhanced (Effros et al., 1991; Jolly, 2004; Zysling et al., 2009) by food restriction. We obtained different results from previous studies for the effects of cold stress and food restriction on immunological parameters in striped hamsters. Different experimental paradigms, and differences in the degree or duration of food restriction, species used and experimental conditions might account for these discrepancies. For instance, cellular immunity was suppressed in short-day but not in long-day food-restricted deer mice (Bilbo and Nelson, 2004). Our previous findings showed that cellular immunity was not affected by fasting (100% deprivation of food intake) for 1 day but was suppressed by fasting for 2 or 3 days in Mongolian gerbils (Xu and Wang, 2010, 2015). However, 20% food restriction (i.e. fed 80% of baseline food intake) for 36 days had no effect on cellular and humoral immunity in this species (Xu et al., 2011). These results show that differences in the degree or duration of food restriction are important in determining animal immunity. A 10% restriction level was minimal in our study and had no influence on most immunological parameters including innate, cellular and humoral immunity in striped hamsters; however, the same degree of food restriction suppressed humoral immunity in grey red-backed voles (*Myodes rufocanus*; Kusumoto, 2009), indicating the importance of the study species.

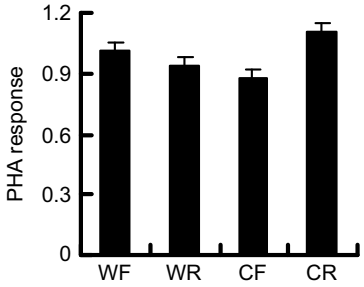

Fig. 4. Effect of cold and food restriction on cellular immunity in striped hamsters. Cellular immunity was assessed as the phytohaemagglutinin (PHA) response. Groups are as described in Fig. 1.

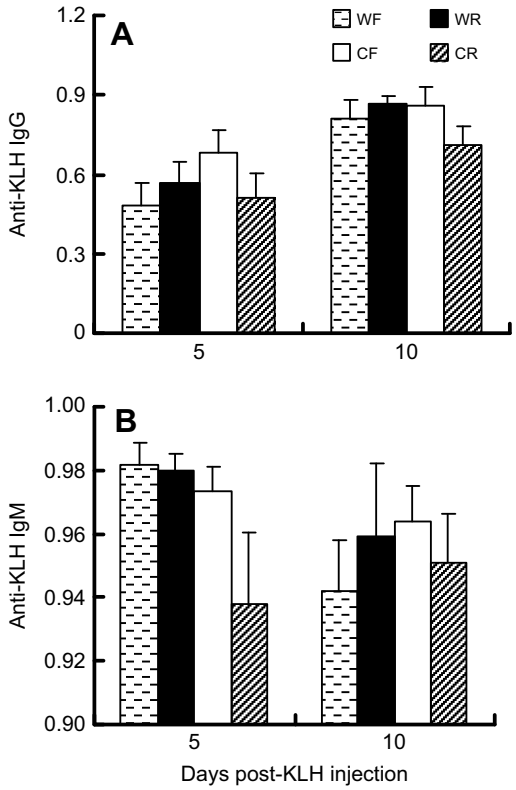

Fig. 5. Effect of cold and food restriction on humoral immunity in striped hamsters. Levels of serum anti-KLH IgG (A) and IgM (B) relative to the positive control. Groups are as described in Fig. 1.

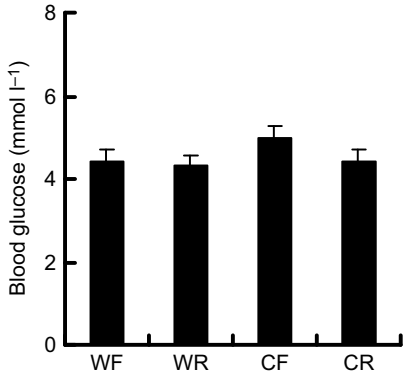

Fig. 6. Effect of cold and food restriction on blood glucose levels in striped hamsters. Groups are as described in Fig. 1.

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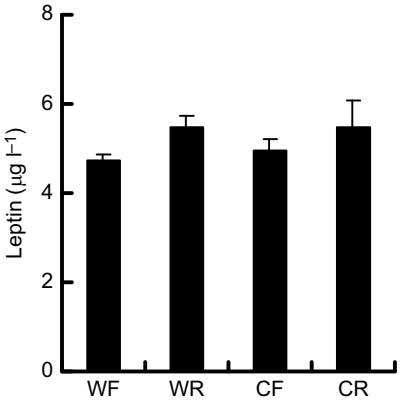

Fig. 7. Effect of cold and food restriction on serum leptin levels in striped hamsters. Groups are as described in Fig. 1.

In general, cold stress or food restriction can modulate immune responses via bioenergetic and endocrine pathways. Energy reserves such as fat mass and metabolic fuels including blood glucose function to provide energy for expensive physiological processes including immune responses (Demas et al., 1997; Moret and Schmid-Hempel, 2000; Martin et al., 2002; Demas, 2004; Matarese and Cava, 2004; Trayhurn, 2005; Maciver et al., 2008). Moreover, adipose tissues are also endocrine and immune organs (Ahima and Flier, 2000; Matarese and Cava, 2004; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al., 2007). Houston et al. (2007) have shown that animals with low energy reserves allocate less energy to immune defence than animals with higher reserves. Therefore, reductions in body fat mass can impair immunity (Demas et al., 2003). Perigonadal, subcutaneus and total body fat were all reduced by cold stress in striped hamsters, which might cause suppression of humoral immunity in hamsters. Glucose is another metabolic fuel that plays an important role in mounting immune responses (Matarese and Cava, 2004; Maciver et al., 2008). It was not affected by cold stress or food restriction, and it was also not correlated with all the immunological parameters detected, implying glucose was not the reason for the changes of immune function in striped hamsters. Cold exposure increases thermogenic capacity and metabolic rate across many species, including striped hamsters (Li et al., 2001; Liu et al., 2009; Chi and Wang, 2011; Zhao et al., 2010; Zhao, 2011; Zhou et al., 2015). Although thermogenic capacity and metabolic rate were not measured in the present study, the competition for energy resources between immune responses and thermogenesis might also account for the suppression of humoral immunity in striped hamsters (Yang et al., 2011; Odegaard and Chawla, 2013; Evans et al., 2015).

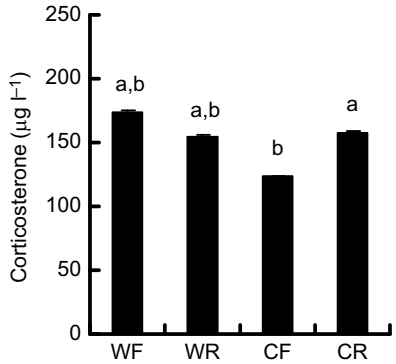

Fig. 8. Effect of cold and food restriction on corticosterone levels in striped hamsters. Different letters indicate a significant difference at $P<0.05$ (two-way ANOVA and Bonferroni *post hoc* tests). Groups are as described in Fig. 1.

Leptin and corticosterone are two important hormones regulating immune responses by endocrine pathways. Leptin can regulate immune responses directly (Matarese et al., 2005; Lam and Lu, 2007; Steiner and Romanovsky, 2007), and lower leptin levels impair immune function (Lord et al., 1998; Flier, 1998; Ahima and Flier, 2000). The positive correlation between leptin levels and bacterial killing capacity implies an enhancing effect of leptin on innate immunity. Serum leptin concentration was not affected by cold stress or food restriction, and it also showed no correlation with other immunological parameters, suggesting that leptin could not explain the changes of most immunological indices in striped hamsters. Corticosterone often increases in response to stressful conditions such as cold (Bligh-Tynan et al., 1993) or food restriction (Murphy and Wideman, 1992; Demas and Nelson, 1998; Bilbo and Nelson, 2004). It often has a suppressive effect on immune function (Sapolsky et al., 2000; Webster Marketon and Glaser, 2008). In the present study, corticosterone concentration was affected significantly by the interaction cold stress×food restriction, but not by cold stress or food restriction, which is incompatible with other research in which corticosterone levels increased under conditions of cold stress (Adels et al., 1986; Shu et al., 1993) or food restriction (Murphy and Wideman, 1992; Demas and Nelson, 1998; Bilbo and Nelson, 2004). Furthermore, other studies found that food restriction decreased the levels of cortisol (Zysling et al., 2009) or corticosterone (Xu et al., 2011). No significant correlation was detected between corticosterone levels and innate and cellular immunity, IgG and IgM titres; therefore, changes in corticosterone levels may also not account for the influence of cold stress or food restriction on immune function and other mechanisms may be involved.

In summary, cold stress and food restriction exert different effects on different components of the immune system in striped hamsters. Cold stress decreased haematological parameters such as white blood cells, lymphocytes, neutrophilic granulocytes and humoral immunity but enhanced innate immunity and had no effect on thymus and spleen mass, intermediate granulocytes and cellular immunity. Food restriction reduced thymus mass but did not influence spleen mass, haematological parameters (i.e. white blood cells, lymphocytes, neutrophilic granulocytes and intermediate granulocytes) or innate, humoral and cellular immunity. Changes of fat mass might account for the suppressive effect of cold stress on humoral immunity, whereas blood glucose, serum leptin and corticosterone could not explain the influence of cold stress or food restriction on immune responses in hamsters.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.-L.X.; Methodology: D.-L.X.; Validation: Y.-F.T.; Investigation: X.-L.H.; Resources: Y.-F.T.; Writing - original draft: D.-L.X.; Writing - review & editing: D.-L.X., Y.-F.T.; Supervision: D.-L.X.; Funding acquisition: D.-L.X.

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