Integrative Zoology 2011; 6: 352-365 doi: 10.1111/j.1749-4877.2011.00259.x

ORIGINAL ARTICLE

Impairment of cellular and humoral immunity in overweight Mongolian gerbils (*Meriones unguiculatus*)

Deli XU,[1,2] Xinyu LIU[1] and Dehua WANG[1]

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China and [2]College of Life Sciences, Qufu Normal University, Qufu, China

Abstract

Animal immunity is usually impaired in obesity. We know little about the effect of being overweight or obese on the immune function of wild rodents. The present study is aimed to test the hypothesis that immunity is suppressed in overweight Mongolian gerbils (*Meriones unguiculatus*). In the study, 16 overweight (body mass: 90.8–127.6 g) and 16 lean gerbils (body mass: 60.5–77.7 g) were randomly selected from a total of 174 male gerbils (body mass range: 55.8–144.7 g). Half of the overweight and lean males were injected with sterile saline; the others were immunochallenged (IC) with phytohaemagglutinin and keyhole limpet hemocyanin to assess cellular and humoral immunity, respectively. Body fat mass, wet and dry spleen mass, leukocyte counts, blood glucose levels and serum leptin levels were significantly higher in the overweight gerbils than in the lean gerbils. However, phytohemagglutinin response indicative of cellular immunity and immunoglobulin G concentrations was significantly lower in the IC overweight gerbils than in the IC lean gerbils. These results indicate that cellular and humoral immunity are impaired in the overweight gerbils. Excessive body fat mass, higher leukocyte counts and serum leptin levels imply that overweight gerbils are in a low grade inflammatory state.

Key words: cellular immunity, humoral immunity, leptin, Mongolian gerbils (*Meriones unguiculatus*), overweight.

INTRODUCTION

Obesity is increasingly prevalent and is associated with a high risk of several chronic diseases (Conway & Rene 2004; Lazar 2005; Hotamisligil 2006; Irigaray *et al.* 2007; Speakman *et al.* 2008). It is also linked to increased susceptibility to infections (Samartín & Chandra 2001; Falagas & Kompoti 2006). The mechanism might be ascribed to altered immune function in obesity (Kumari & Chandra 1993; Marti *et al.* 2001; Lamas *et al.* 2002).

The impact of obesity on immunity has been investigated in several obese models (Matarese & Cava 2005). Cellular immunity is impaired in genetically obese rodents characterized by mutations in the leptin gene (*ob/ob* mice) (Chandra 1980; Busso *et al.* 2002; Lindström 2007) or the leptin receptor gene (*db/db* mice and *fa/fa* rats) (Mandel & Mahmoud 1978; Tanaka *et al.* 2000). Immune responses are also dampened in diet-induced obese mice (Mito *et al.* 2002; Amar *et al.* 2007; Smith

Correspondence: Dehua Wang, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, China.
Email: wangdh@ioz.ac.cn

et al. 2007) or rats (Moriguchi *et al.* 1998; Lamas *et al.* 2004). Likewise, capacity of lymphocyte proliferation (Nieman *et al.* 1999) and humoral immunity (Weber *et al.* 1986; Eliakim *et al.* 2006) are suppressed in obese humans. However, diet-induced obesity does not alter the immune responses in cats (Jaso-Friedmann *et al.* 2008).

Cellular and humoral immunity are 2 arms of the adaptive immune system that are responsible for controlling intracellular and extracellular pathogens (Marti *et al.* 2001). The former is usually assessed by phytohemagglutinin (PHA) response (Bellocq *et al.* 2006; Xu & Wang 2010) and the latter by immunoglobulin (Ig) production in response to specific antigen, such as keyhole limpet hemocyanin (KLH) (Demas *et al.* 2003; Zysling & Demas 2007). Lymphoid organs, such as thymus and spleen, are indirect parameters indicative of immune function (Savino & Dardenne 2000; Smith & Hunt 2004). Likewise, leukocytes are often used to estimate overall health (Calder & Kew 2002), and an elevated leukocyte count is associated with inflammation in obesity (Dixon & O'Brien 2006; Hotamisligil 2006).

Leptin is an adipocyte-derived proinflamatory cytokine and plays an important role in immunity, besides its regulatory role in energy homeostasis (Zhang *et al.* 1994; Matarese *et al.* 2005; Otero *et al.* 2006; Lam & Lu 2007). Adipose tissue is no longer regarded as simply a passive energy reserve, but an important endocrine and immune organ (Ahima & Flier 2000; Fantuzzi 2005; McGillis 2005; Schäffler *et al.* 2007). Glucose metabolism is required for normal survival and function in lymphocytes and T cells increase glucose uptake and glycolysis during an immune response (Matarese & Cava 2004; Maciver *et al.* 2008), whereas hyperglycemia depresses immunity (Black *et al.* 1990; Taylor & Beilmam 2005).

The major problem in understanding the influence of being overweight or obese on immunity in humans is that the obese population is too heterogeneous in various aspects, including dietary patterns, individual microbial and social environments (Lamas *et al.* 2002). Furthermore, the percentage of obesity caused by genetic deficiency is small (Matarese & Cava 2005). Therefore, wild overweight or obese rodents are potential models for examining the impact of obesity on immunity independent of the confounding heterogeneity in humans (Lamas *et al.* 2002). To date, however, little information is available about how obesity or being overweight affects immune function in wild rodents.

Mongolian gerbils (*Meriones unguiculatus* Milne-Edwards, 1867) are small, seasonal breeding, non-hibernating and granivorous rodents that mainly live in the desert and semi-arid regions of Mongolia and northern China (Walker 1968). Previous work has demonstrated that their body masses show seasonal changes in gerbils, body mass and humoral immunity are higher in winter than in summer (Li & Wang 2005; Zhang & Wang 2006, 2007). We have previously found that a 3-day fasting significantly decreases body mass and cellular immunity in gerbils (Xu & Wang 2010). These data suggest that immune function is positively related to body mass. Others have shown that immunity is also impaired in extremely lean subjects (i.e. anorexic and undernourished) (Faggioni *et al.* 2001; Schaible & Kaufmann 2007; Steiner & Romanovsky 2007). Therefore, it seems that body mass is a double-edged sword: being either too heavy or too thin is detrimental to immune function and overall health (Samartín & Chandra 2001; Lamas *et al.* 2002; Houston *et al.* 2007). If body mass is crucial to animals' immunity, then cellular and humoral immunity will be impaired in overweight gerbils.

MATERIALS AND METHODS

Animals and experimental design

All animal procedures were licensed under the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Adult male Mongolian gerbils used in this study were the offspring of gerbils in our laboratory colony. The gerbils were housed individually after weaning in plastic cages (30 × 15 × 20 cm), with sawdust as bedding under a constant long-day photoperiod of 16 L:8 D (16 h:8 h light–dark cycle) and temperature of 23 ± 1 °C. Because our gerbil laboratory colony was raised under a long-day photoperiod, and gerbils were not particularly sensitive to photoperiod (Zhao & Wang 2006), we selected a long-day photoperiod for carrying out the experiment. Commercial standard rat pellets (Beijing KeAo Feed) and water were provided *ad libitum* throughout the experiment. We selected 26 heavier (15%, weight: 98.0–144.7 g) and 26 lighter (15%, weight: 55.8–64.2 g) gerbils from 174 males (age: 9–18 months, weight: 55.8–144.7 g). After 3 months of individual housing, 16 overweight males (weight: 90.8–127.6 g) were selected randomly from the heavier group; similarly, 16 lean males (weight: 60.5–77.7 g) were selected randomly from the lighter group. According to the Pearson correlation analysis, body mass was

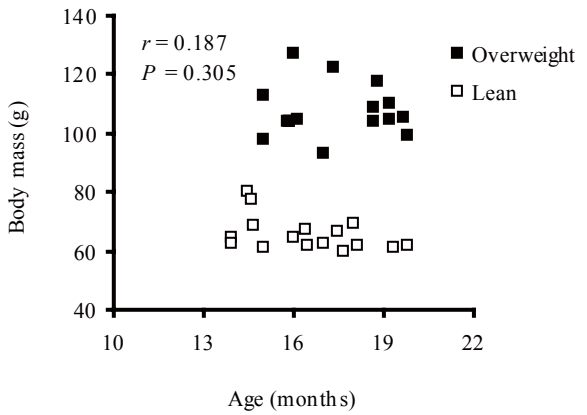

Figure 1 The correlation between body mass and age in the overweight and lean gerbils. Body mass was not correlated with age ($r = 0.187$, $P = 0.305$) in the overweight and lean gerbils ($n = 32$).

not correlated with age ($r = 0.187$, $P = 0.305$) in both the heavier and lighter gerbils ($n = 32$) (Fig. 1). Thereafter, the overweight males were randomly divided into 2 groups: the overweight/saline group ($n = 8$), which were injected with sterile saline, and the overweight/IC group ($n = 8$), which were immunochallenged (IC) with PHA (PHA-P, Sigma L-8754) and KLH (Sigma H-7017) to assess cellular and humoral immunity, respectively. Likewise, the lean gerbils were randomly assigned to the lean/saline ($n = 8$) and lean/IC ($n = 8$) groups.

Cellular immunity assays

Phytohaemagglutinin response (i.e. cellular immunity) was measured as described previously (Bellocq *et al.* 2006; Xu & Wang 2010). Briefly, on day 0, we measured the footpad thickness of gerbils' left hind foot with a micrometer (Tesa Shopcal, Swiss) to ±0.01 mm. Immediately thereafter, gerbils in the IC groups (overweigh/IC and lean/IC groups) were injected subcutaneously with 0.1 mg of PHA dissolved in 0.03 mL sterile saline in the middle of the footpad, whereas gerbils in the control groups were injected with 0.03 mL sterile saline. We measured footpad thickness 6 hours later. The PHA response was calculated as the difference between pre-injection and post-injection measurements divided by initial footpad thickness (PHA response = (post PHA – pre PHA)/pre PHA). Each measurement of PHA response was replicated 6 times on the same gerbil (Bellocq *et al.* 2006; Xu & Wang 2010).

Humoral immunity assays

On day 6, gerbils in the control and IC groups received a single subcutaneous injection of 0.1 mL sterile saline and 100 μg KLH suspended in 0.1 mL saline, respectively. After 5 days (i.e. day 11), they were anesthetized lightly with isoflurane, and around 15.00 hours, blood samples (approximately 500 μL) were drawn from the retro-orbital sinus for assessing anti-KLH IgM, with 20 μL whole blood used immediately for measuring blood glucose levels. At the end of the experiment (i.e. day 16), each gerbil was killed by CO_2 asphyxiation and trunk blood was collected around 15.00 hours for measurements of anti-KLH IgG, leukocyte count and serum leptin levels (Zysling & Demas 2007; Xu & Wang 2010). Blood samples were allowed to clot on ice for 1 h and were then centrifuged at 4 °C for 30 min at 1829 × g. Serum was collected and stored in polypropylene microcentrifuge tubes at –80 °C until antibody and leptin were assayed.

Enzyme-linked immunosorbent assay was used to assess serum IgM and IgG concentrations (Demas *et al.* 2003; Zysling & Demas 2007). Specifically, microtiter plates were coated with 100 μL 0.5 mg/mL KLH in sodium bicarbonate buffer (pH 9.6) overnight at 4 °C. Plates were washed 3 times with 200 μL phosphate buffered saline containing 0.05% Tween 20 (PBS-T, pH 7.4), then blocked with 5% non-fat dry milk in PBS-T overnight at 4 °C to reduce non-specific binding, and washed again 3 times 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 KLH repeatedly challenged gerbils, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naïve gerbils, 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 3 times. Secondary antibody (alkaline phosphatase-conjugated-anti mouse IgG diluted 1:2000 with PBS-T [Sigma Chemical, St Louis, MO]; alkaline phosphatase-conjugated anti-mouse IgM diluted 1:500 with PBS-T [Sigma Chemical, St Louis, MO]) 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 Chemical, St Louis, MO; 1 mg/mL 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 add-

ing 50 μL of 1.5 mol/L NaOH solution to each well. The optical density (OD) of each well was determined using a plate reader (Bio-Rad, Benchmark, Richmond, CA) equipped with a 405 nm wavelength filter, and the mean OD for each set of duplicate wells was calculated. To minimize inter-assay and intra-assay variability, the mean OD for each sample is expressed as a ratio of its plate positive control OD for statistical analysis (Demas *et al.* 2003; Zysling & Demas 2007).

Organs and body composition

Body composition was measured as described previously (Li & Wang 2005; Xu & Wang 2010). In brief, after interscapular brown adipose tissue was removed, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, adrenal glands, testes, epididymis, seminal vesicals and the digestive organs and contents (i.e. stomach, small intestine, cecum and colon) were dissected and weighed (±1mg). The stomach, small intestine, cecum and colon were rinsed with saline to eliminate all the gut contents, before being dried and weighed. The remaining carcass and all the organs were dried in an oven at 60 °C to constant mass, and then weighed again to obtain the dry mass. The difference between the wet carcass mass and the dry carcass mass determined the carcass water mass. Total body fat was extracted from the dried carcass by petroleum ether extraction in a Soxhlet apparatus (Li & Wang 2005), and body fat content was calculated as total body fat mass divided by wet carcass mass (Xu & Wang 2010).

Leukocytes assays

At the end of the experiment, after collecting trunk blood, 20 μL whole blood was diluted immediately in 0.38 mL solution containing 1.5% glacial acetic acid and 1% crystal violet (Sigma), and the leukocytes were counted in an improved Neubauer chamber using a microscope. The total number of leukocytes was determined by counting all leucocytes in the four large corner squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9cells/L) (Yang 2004).

Blood glucose assays

Blood glucose levels were measured with a FreeStyle Mini Blood Meter (Abbott Diabetes Care, Alameda, USA) according to the manufacture's instructions. The blood glucose range tested was 1.1–27.8 mmol/L. The within-lot and within-vial variabilities were <5.6 and <4.1%, respectively (Xu & Wang 2010).

Serum leptin assays

Serum leptin concentrations were determined by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. No. XL-85K, Linco Research, Missouri, USA). The range detected by this assay was 1.0–50 ng/mL when using a 100 μL sample (see manufacturer's instructions for multi-species leptin RIA Kit). Inter-assay and intra-assay variabilities for leptin RIA were <8.7 and 3.6%, respectively (Zhao & Wang 2006).

Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Prior to all statistical analysis, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. All the ratios, including PHA response, water and body fat contents, were subjected to arcsine transformation. The differences in body mass after IC were analyzed by 2-way analysis of variance (ANOVA) (body mass [classification of the overweight and lean groups] × IC), followed by least significant difference (LSD) *post hoc* tests. Group differences in wet organ mass with body mass as the covariate and dry organ mass with dry body mass (total mass of dry carcass mass and all the dry organ mass) as the covariate were analyzed by a 2-way analysis of covariance, followed by LSD *post hoc* tests. Group differences in other parameters (body composition, PHA response, IgM and IgG concentrations, leukocyte count, blood glucose and leptin levels) were analyzed by a 2-way ANOVA followed by LSD *post hoc* tests. Significant group differences were further evaluated using general linear model multivariate analysis followed by LSD *post hoc* tests. Pearson correlation analysis was performed to determine the relationship of PHA response, IgG and IgM concentrations with body mass, body fat mass, leptin and glucose levels in the IC groups. The correlations of serum leptin levels with body mass and body fat mass were also detected for all the gerbils. Results were presented as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass

On day 0, body mass in the overweight/saline group (108.0 ± 3.4 g) was 63.5% (41.9 g) heavier than that of the lean/saline group (66.1 ± 2.2 g); similarly, body mass in the overweight/IC group (107.0 ± 3.1 g) was 63.7% (41.6 g) heavier than that of the lean/IC group

(65.3 ± 2.1 g). At the end of the experiment, body mass in the overweight/saline group (103.7 ± 2.8 g) was 57.4% (37.8 g) higher than that of the lean/saline group (65.9 ± 2.8 g). Likewise, body mass in the overweight/IC group (108.2 ± 2.8 g) was 65.0% (42.6 g) higher than that of the lean/IC group (65.6 ± 2.8 g). There was no significant effect of IC and the interaction of body mass × IC on body mass at any time point after IC.

Organs and body composition

The overweight gerbils had significantly higher body fat mass ($F_{1,28}$ = 58.389, P < 0.001) by 105.4% and body fat content ($F_{1,28}$ = 30.628, P < 0.001) than the lean gerbils (Table 1).

As for the immune organs, wet ($F_{1,27}$ = 5.241, P = 0.030) and dry ($F_{1,27}$ = 7.145, P = 0.013) spleen mass were higher in the overweight groups than in the lean groups, whereas wet ($F_{1,27}$ = 0.701, P = 0.410) and dry ($F_{1,27}$ = 0.252, P = 0.620) thymus mass did not differ between the overweight and lean groups (Tables 2 and 3). Overweight gerbils had heavier wet heart mass ($F_{1,27}$ = 10.021, P = 0.004) and wet lung mass ($F_{1,27}$ = 9.897, P = 0.004) than the lean gerbils, whereas other wet organ mass did not differ between overweight and lean gerbils (Table 2). All dry organ mass, except adrenal gland, cecum, epididymis and seminal vesical, were higher in overweigh gerbils than in lean gerbils (Table 3).

Cellular immune response

Immunochallenge had a significant effect on PHA response ($F_{1,28}$ = 84.715, P < 0.001), and the effect of body mass ($F_{1,28}$ = 4.036, P = 0.054) and the interaction of body mass × IC ($F_{1,28}$ = 3.734, P = 0.063) on PHA response were close to significant in our experiment. Further analysis showed that PHA response in the lean/IC group was significantly higher, by 34.1%, than that of the overweight/IC group (Fig. 2). PHA response was negatively correlated with body mass (r = –0.542, P = 0.030), body fat mass (r = – 0.545, P = 0.029) in the IC groups. However, it was no longer correlated with body mass (r = –0.187, P = 0.658) and body fat mass (r = 0.051, P = 0.905) in the overweight/IC group, and, likewise, it was not correlated with body mass (r = –0.590, P = 0.123) and body fat mass (r = –0.369, P = 0.369) in the lean/IC group.

Humoral immunity

Immunochallenge had a significant effect on IgG concentrations ($F_{1,28}$ = 424.070, P < 0.001), and the effect of body mass ($F_{1,28}$ = 3.875, P = 0.059) and the in-

Table 1 Body composition in the overweight and lean Mongolian gerbils

Parameters	Overweight		Lean		Statistical summary		
	Saline	IC	Saline	IC	Body mass	IC	Body mass × IC
Final body mass (g)	103.7 ± 2.8^a	108.2 ± 2.8^a	65.9 ± 2.8^b	65.6 ± 2.8^b	<0.001	ns	ns
Wet carcass mass (g)	66.9 ± 1.5^a	66.7 ± 1.5^a	46.2 ± 1.5^b	45.2 ± 1.5^b	<0.001	ns	ns
Dry carcass mass (g)	38.6 ± 2.3^a	45.1 ± 2.3^a	19.8 ± 2.3^b	20.9 ± 2.3^b	<0.001	ns	ns
Water mass (g)	28.3 ± 2.0	21.6 ± 2.0	26.3 ± 2.0	24.3 ± 2.0	ns	<0.05	ns
Water content (water mass/wet carcass mass) (%)	42.3 ± 3.4^b	32.5 ± 3.4^b	57.2 ± 3.4^a	54.0 ± 3.4^a	<0.001	ns	ns
Fat free dry carcass (g)	17.7 ± 0.5^a	18.2 ± 0.5^a	12.6 ± 0.5^b	12.5 ± 0.5^b	<0.001	ns	ns
Body fat mass (g)	21.0 ± 2.1^a	26.9 ± 2.1^a	7.2 ± 2.109^b	8.4 ± 2.1^b	<0.001	ns	ns
Body fat content (body fat mass/wet carcass mass) (%)	31.7 ± 3.4^a	40.8 ± 3.4^a	15.8 ± 3.4^b	18.7 ± 3.4^b	<0.001	ns	ns

Values are means ±SE (n = 8). Values for a specific parameter that share different superscripts are significantly different at P < 0.05, determined by a 2-way analysis of variance and least significant difference *post hoc* tests. IC, immunochallege; body mass, classification of the overweight and lean gerbils; body mass × IC, interaction of body mass × immunuchallenge; ns, not significant.

Table 2 Wet organ mass in the overweight and lean Mongolian gerbils

Parameters	Overweight		Lean		Statistical summary		
	Saline	IC	Saline	IC	Bodymass	IC	Body mass × IC
Brain (mg)	1212 ± 38	1195 ± 44	1112 ± 41	1153 ± 41	ns	ns	ns
IBAT (mg)	345 ± 47	433 ± 54	269 ± 50	287 ± 50	ns	ns	ns
Heart (mg)	447 ± 22^{a}	417 ± 25^{ab}	308 ± 23^{b}	299 ± 24^{b}	<0.01	ns	ns
Lungs (mg)	615 ± 35^{a}	608 ± 41^{ac}	387 ± 38^{b}	424 ± 38^{bc}	<0.01	ns	ns
Thymus (mg)	18 ± 6	21 ± 6	28 ± 6	28 ± 6	ns	ns	ns
Liver (mg)	3557 ± 458	3278 ± 530	3243 ± 491	3186 ± 496	ns	ns	ns
Spleen (mg)	124 ± 19^{a}	98 ± 22^{ab}	27 ± 20^{b}	34 ± 21^{b}	<0.05	ns	ns
Kidneys (mg)	924 ± 54	837 ± 62	714 ± 57	696 ± 58	ns	ns	ns
Adrenal glands (mg)	57 ± 4	52 ± 4	50 ± 4	47 ± 4	ns	ns	ns
Stomach with contents (mg)	1898 ± 225	1559 ± 260	1802 ± 240	1589 ± 243	ns	ns	ns
Stomach (mg)	557 ± 53	562 ± 61	529 ± 57	556 ± 57	ns	ns	ns
Small intestine with contents (mg)	2435 ± 183	2433 ± 212	2654 ± 196	2391 ± 198	ns	ns	ns
Small intestine (mg)	1100 ± 113	1087 ± 130	1090 ± 121	997 ± 122	ns	ns	ns
Cecum with contents (mg)	1578 ± 122^{a}	1335 ± 141^{b}	1338 ± 130^{ab}	1220 ± 132^{ab}	ns	<0.05	ns
Cecum (mg)	344 ± 30	332 ± 35	404 ± 32	387 ± 32	ns	ns	ns
Colon with contents (mg)	1208 ± 94	1121 ± 108	935 ± 100	794 ± 101	ns	ns	ns
Colon (mg)	561 ± 39	548 ± 45	546 ± 42	497 ± 42	ns	ns	ns
Total digetive tract (mg)	2563 ± 193	2529 ± 223	2569 ± 207	2437 ± 209	ns	ns	ns
Epididymis (mg)	292 ± 35	278 ± 40	241 ± 37	214 ± 37	ns	ns	ns
Testes (mg)	1191 ± 100	1111 ± 115	932 ± 107	967 ± 108	ns	ns	ns
Seminal vesical (mg)	412 ± 84	423 ± 97	345 ± 90	270 ± 91	ns	ns	ns

Values are means ±SE (n = 8). Values for a specific parameter that share different superscripts are significantly different at $P < 0.05$, determined by a 2-way analysis of covariance with body mass as the covariate and least significant difference *post hoc* tests. IBAT, interscapular brown adipose tissue; IC, immunochallege; body mass, classification of the overweight and lean gerbils; body mass × IC, interaction of body mass × immunuchallenge; ns, not significant.

Table 3 Dry organ mass in the overweight and lean Mongolian gerbils

Parameters	Overweight		Lean		Statistical summary		
	Saline	IC	Saline	IC	Body mass	IC	Body mass×IC
Heart (mg)	108 ± 4^{a}	108 ± 5^{a}	69 ± 5^{b}	66 ± 5^{b}	<0.001	ns	ns
Lungs (mg)	148 ± 6^{b}	169 ± 8^{a}	78 ± 7^{c}	89 ± 7^{c}	<0.001	<0.01	ns
Thymus (mg)	8 ± 2	9 ± 2	7 ± 2	7 ± 2	ns	ns	ns
Liver (mg)	1418 ± 145^{a}	1431 ± 189^{a}	715 ± 168^{b}	669 ± 162^{b}	<0.01	ns	ns
Spleen (mg)	27 ± 3^{a}	23 ± 4^{ac}	8 ± 4^{b}	11 ± 4^{bc}	<0.05	ns	ns
Kidneys (mg)	233 ± 11^{a}	227 ± 15^{a}	153 ± 13^{b}	152 ± 13^{b}	<0.01	ns	ns
Adrenal glands (mg)	19 ± 1	17 ± 2	19 ± 1	16 ± 1	ns	ns	ns
Stomach (mg)	148 ± 8^{a}	158 ± 1^{a}	110 ± 9^{b}	116 ± 9^{b}	<0.05	ns	ns
Small intestine (mg)	265 ± 20^{a}	285 ± 26^{a}	212 ± 24^{ab}	173 ± 23^{b}	<0.05	ns	ns
Cecum (mg)	78 ± 4	72 ± 5	63 ± 5	58 ± 5	ns	ns	ns
Colon (mg)	135 ± 6^{a}	139 ± 8^{ab}	114 ± 7^{ab}	111 ± 7^{b}	<0.05	ns	ns
Total digestive tract (mg)	627 ± 29^{a}	654 ± 38^{a}	499 ± 34^{b}	458 ± 33^{b}	<0.01	ns	ns
Epididymis (mg)	79 ± 7	69 ± 10	60 ± 9	51 ± 8	ns	ns	ns
Testes (mg)	210 ± 13^{a}	200 ± 17^{ab}	140 ± 15^{c}	145 ± 14^{bc}	<0.05	ns	ns
Seminal vesical (mg)	114 ± 17	116 ± 23	85 ± 20	62 ± 19	ns	ns	ns

Values are means ±SE ($n = 8$). Values for a specific parameter that share different superscripts are significantly different at $P < 0.05$, determined by a 2-way analysis of covariance with dry body mass as the covariate and least significant difference post hoc tests. IC, immunochallege; body mass, classification of the overweight and lean gerbils; body mass × IC, interaction of body mass × immunuchallenge; ns, not significant.

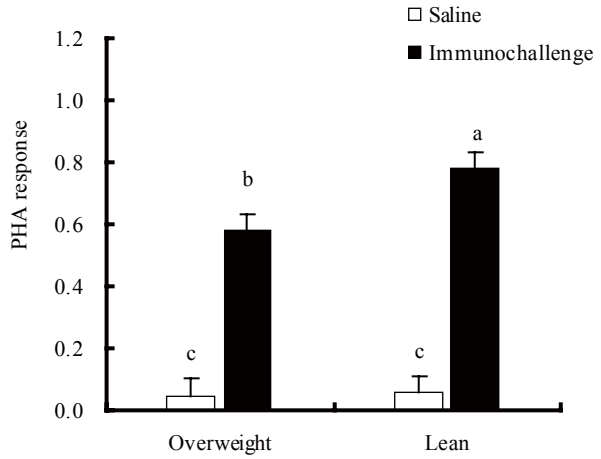

Figure 2 PHA response in the overweight and lean Mongolian gerbils. PHA response was significantly lower in the Overweight/IC group than in the Lean/IC group. Different letters (a or b) above white and black bars indicate significant differences ($P < 0.05$).

teraction of body mass × IC ($F_{1,28} = 4.045$, $P = 0.054$) on IgG concentrations were close to significant. Further analysis showed that IgG concentrations in the lean/IC group were significantly higher, by 18.7%, than those of the overweight/IC group (Fig. 3a). IC had a significant effect on IgM concentrations ($F_{1,28} = 91.225$, $P < 0.001$), whereas body mass ($F_{1,28} = 0.172$, $P = 0.682$) and the interaction of body mass × IC ($F_{1,28} = 2.224$, $P = 0.147$) had no significant effect on IgM concentrations (Fig. 3b).

In addition, IgG concentrations were negatively correlated with body mass ($r = –0.506$, $P = 0.046$); however, no correlation with body fat mass was detected ($r = –0.391$, $P = 0.135$) in the IC groups. There was no correlation between IgG concentrations and body mass in the overweight/IC ($r = –0.097$, $P = 0.820$) and lean/IC ($r = –0.434$, $P = 0.282$) groups. IgG concentrations were not correlated with body fat mass in the overweight/IC ($r = 0.059$, $P = 0.889$) and lean/IC ($r = –0.035$, $P = 0.935$) groups. IgM concentrations were not correlated with body mass ($r = –0.165$, $P = 0.542$) and body fat mass ($r = –0.069$, $P = 0.798$) in the IC groups. There was no correlation between IgM concentrations and body mass in the overweight/IC ($r = 0.327$, $P = 0.430$) and lean/IC ($r = –0.557$, $P = 0.152$) groups. IgM concentrations were not correlated with body fat mass in the overweight/IC ($r = 0.323$, $P = 0.436$) and lean/IC ($r = –0.084$, $P = 0.843$) groups.

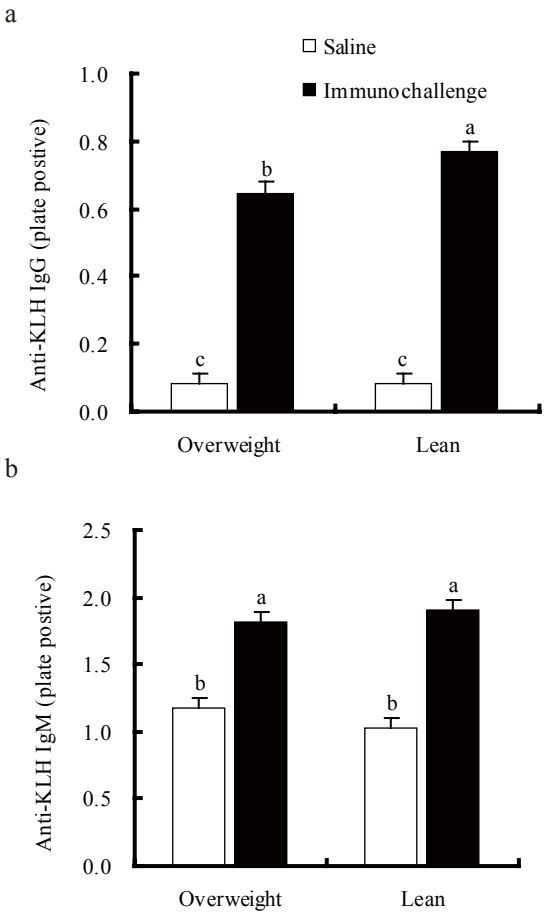

Figure 3 Serum IgG and IgM concentrations in the overweight and lean Mongolian gerbils. IgG concentrations were significantly lower in the Overweight/IC group than that in the Lean/IC group. Different letters (a,b or c) above white and black bars indicate significant differences ($P < 0.05$).

Leukocytes

Leukocyte count was higher in the overweight gerbils than in the lean gerbils ($F_{1,28} = 9.615$, $P = 0.004$), and it was not affected by IC ($F_{1,28} = 3.044$, $P = 0.092$), but was somewhat affected by the interaction of body mass × IC ($F_{1,28} = 3.692$, $P = 0.065$) (Fig. 4). Further analysis showed that IC significantly decreased leukocyte count in the overweight gerbils but not in the lean gerbils. Leukocyte count was positively correlated with body mass for all the gerbils ($r = 0.364$, $P = 0.040$); however, it was not correlated with body mass in the overweight ($r = –0.424$, $P = 0.101$) and the lean ($r = 0.450$, $P = 0.080$) gerbils.

Blood glucose levels

The overweight gerbils had higher blood glucose levels than the lean gerbils ($F_{1,28}$ = 4.273, P = 0.048), whereas IC ($F_{1,28}$ = 1.377, P = 0.251) and the interaction of body mass × IC ($F_{1,28}$ = 0.05, P = 0.825) had no significant effect on glucose levels (Fig. 5).

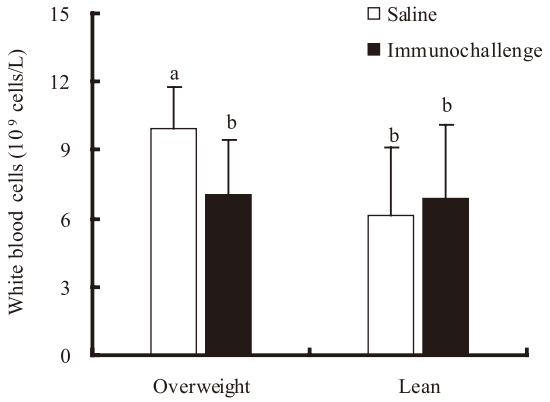

Figure 4 Leukocyte count in the overweight and lean Mongolian gerbils. Overweight gerbils had higher leukocyte count than that of the leans. Different letters (a or b) above white and black bars indicate significant differences (P < 0.05).

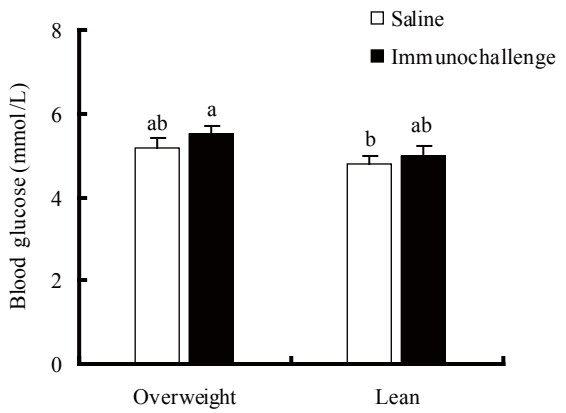

Figure 5 Blood glucose levels in the overweight and lean Mongolian gerbils. Overweigh gerbils had higher blood glucose levels than those of the leans. Different letters (a or b) above white and black bars indicate significant differences (P < 0.05).

Body mass was positively correlated with blood glucose levels for all the gerbils (r = 0.414, P = 0.018); however, it was not correlated with blood glucose levels in the overweight (r = 0.279, P = 0.295) and the lean (r = 0.185, P = 0.492) gerbils. In addition, glucose levels were negatively correlated with PHA response (r = –0.505, P = 0.046), but not with IgG (r = –0.084, P = 0.757) or IgM (r = 0.144, P = 0.596) concentrations in the IC groups. Glucose levels were still negatively correlated with PHA response in the overweight/IC group (r = –0.711, P = 0.048), but not in the lean/IC group (r = –0.185, P = 0.661). There was no correlation between glucose levels and IgG concentrations in the overweight/IC (r = 0.168, P = 0.691) and lean/IC (r = –0.024, P = 0.954) groups. Glucose levels were not correlated with IgM concentrations in the overweight/IC (r = 0.140, P = 0.742) and lean/IC (r = 0.319, P = 0.441) groups.

Serum leptin concentrations

Serum leptin concentrations in the overweight gerbils were significantly higher than in the lean gerbils ($F_{1,28}$ = 8.542, P = 0.007), and were not affected by IC ($F_{1,28}$ = 3.002, P = 0.094) or by the interaction of body mass × IC ($F_{1,28}$ = 0.975, P = 0.332) (Fig. 6).

Serum leptin concentrations were pos itively correlated with body mass (r = 0.577, P = 0.001) and body fat mass (r = 0.683, P < 0.001) for all the gerbils. Likewise, body mass was positively correlated with leptin levels in the overweight (r = 0.714, P = 0.002) and the lean (r = 0.663, P = 0.005) gerbils. Body fat mass was still positively correlated with leptin levels in the overweight (r = 0.777, P < 0.001) and lean (r = 0.888, P < 0.001) gerbils. In addition, leptin levels were not correlated with PHA response (r = –0.383, P = 0.143), IgG (r = –0.118, P = 0.664) or IgM concentrations (r = –0.156, P = 0.563) in the IC groups. Leptin levels were negatively correlated with PHA response in the overweight/IC group (r = –0.723, P = 0.043), but not in the lean/IC group (r = –0.286, P = 0.492). There was no correlation between leptin levels and IgG concentrations in the overweight/IC (r = –0.105, P = 0.804) and the lean/IC (r = 0.064, P = 0.881) groups. Leptin levels were not correlated with IgM concentrations in the overweight/IC (r = 0.208, P = 0.620) and lean/IC (r = –0.189, P = 0.654) groups.

DISCUSSION

Our main results showed that PHA response and IgG concentrations were significantly lower in the overweight/IC gerbils than in the lean/IC gerbils, indicating

that both cellular and humoral immunity were impaired in the overweight gerbils (Fig. 7).

Higher body mass, body fat mass, blood glucose and leptin levels might contribute to the immunological impairments in the overweight gerbils. Spleen mass was higher in overweight gerbils than in lean gerbils, and thymus mass was not different between overweight and lean gerbils, suggesting that immune organs were not impaired in overweight gerbils. However, both thymus and spleen mass were reduced in *ob/ob* mice; the reason might be ascribed to their leptin deficiency (Matarese 2000). Higher leukocyte count and hyperleptinemia implied that overweight gerbils might be in a low grade inflammatory status, which might be caused by excessive body fat mass.

Excessive body mass or fat loss during starvation (Lord *et al.* 1998; Flier 1998; Xu & Wang 2010) or experimental reductions in body fat (Demas *et al.* 2003) can compromise cellular or humoral immunity. Excessive body mass or fat gain, such as in obesity, can also harm immunity (Chandra & Sarchielli 1996; Samartín & Chandra 2001; Lamas *et al.* 2002). In the present experiment, we observed that both cellular and humoral immunity were impaired in the overweight gerbils in contrast with the lean gerbils. For wild gerbils, their body mass and population density fluctuate seasonally (Zhang & Wang 2007; Liu *et al.* 2007, 2009). Our results might contribute to understanding the relationship between body mass and population dynamics in this species in light of immunity. These results might also help in the comprehension of the immunological consequences of body mass changes in other research areas. For instance, body mass of primates and wild rodents, such as Norway rats (*Rattus norvegicus* [Berkenhout, 1769]), have increased over the past several decades (Klimentidis *et al.* 2010), and obesity occurs seasonally in some rodents, such as the Syrian hamster (*Mesocricetus auratus*) (Cincotta *et al.* 1991). According to previous study, the body mass of wild gerbils under natural conditions is lighter than that of the gerbils raised in a laboratory. For example, mean body mass of wild male gerbils in summer and winter was 46.2 ± 2.1 and 65.2 ± 1.9 g, respectively (Zhang 2005). The maximum body mass of wild gerbils is more than 80 g (W Liu, pers. comm. Wei Liu's focus is wild gerbil population research). The results for our research group showed that the body mass of the offspring of wild gerbils tends to increase when wild gerbils are raised in a laboratory. This result concurs with previous published studies (Stuermer *et al.* 2003), therefore, the effect of laboratory-raising on immunity in gerbils needs to be further investigated.

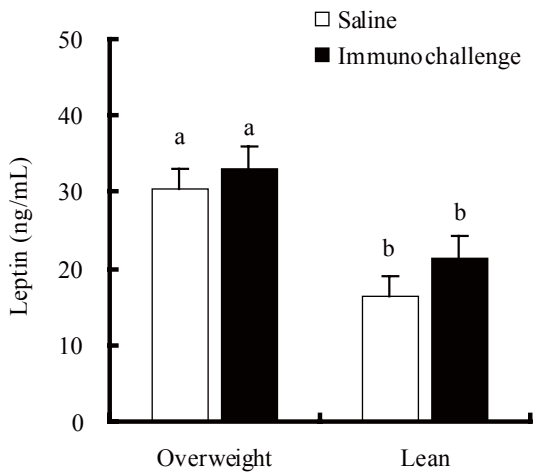

Figure 6 Leptin concentrations in the overweight and lean Mongolian gerbils. Leptin concentrations were higher in the overweight groups than in the lean groups. Different letters (a or b) above white and black bars indicate significant differences ($P < 0.05$).

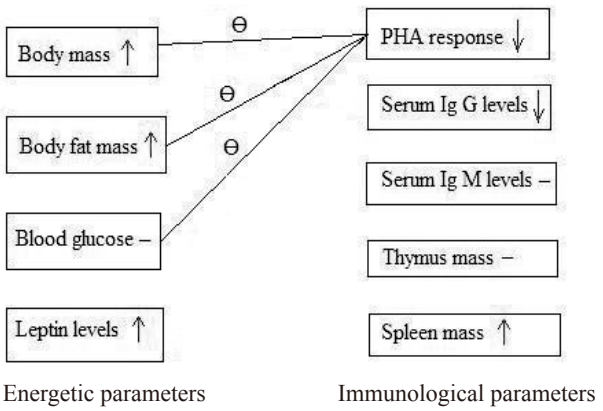

Figure 7 Energetic and immunological parameters of the Overweight/IC group compared with those of the Lean /IC group. Note: The symbols (↑, ↓ and -) stand for increase, decrease and stable respectively. The symbol (Θ) on the linking solid line indicates negative correlation in the immunochallenged groups.

Glucose is the major energy source for immune cells (Matarese & Cava 2004; Maciver *et al.* 2008). However, hyperglycemia might suppress immune function through non-enzymatic glycosylation of circulating immunoglobulins (Black *et al.* 1990; Taylor & Beilmam 2005). In our study, overweight gerbils had higher blood glucose levels than lean gerbils, implying a possible role for their immunosuppression.

Leptin plays an important role in immunity (Fantuzzi & Faggioni 2000; Matarese *et al.* 2005; Lam & Lu 2007). Like body fat, leptin is a double-edged sword (Matarese *et al.* 2002). Appropriate leptin concentrations are crucial to sustain optimal immune response, and both lower and higher leptin levels can depress immune function (Flier 1998; Lord *et al.* 1998; Matarese *et al.* 2002). Moreover, elevated circulating leptin levels commonly found in obesity are a marker of leptin resistance, which can lead to immune dysfunction in a similar manner to malnutrition (Friedman & Halaas 1998; Matarese *et al.* 2002; Schaible & Kaufmann 2007; Myers *et al.* 2008; Friedman 2009). Taken together, hyperleptinemia in the overweight gerbils might be another reason for the immunosuppression (Matarese *et al.* 2002).

Obesity is characterized by a chronic, systemic low-grade state of inflammation, suggesting a link between metabolism and immune function (Matarese & Cava 2004; Hotamisligil 2006; Trayhurn 2007). Leukocyte count is a biomarker of inflammation (Lee & Pratley 2005; Dixon & O'Brien 2006). Womack *et al.* (2007) show an elevated leukocyte count in overweight or obese humans, compared with those who are of a normal weight. These results are consistent with the present study in which the leukocyte count was higher in the overweight gerbils as compared to the lean gerbils. IC significantly decreased leukocyte counts in the overweight gerbils, but had an insignificant effect on leukocyte count in the lean gerbils, suggesting that overall health might be compromised in overweight gerbils. Additionally, leptin is a proinflammatory cytokine that is regarded as an inflammatory marker (Fantuzzi 2005; Otero *et al.* 2006; Lago *et al.* 2007; Iikuni *et al.* 2008; Martin *et al.* 2008). It is also believed that excess fat mass overstimulates the immune system, creating a state of chronic inflammation (Trayhurn & Wood 2004; Trayhurn 2007). In our study, we found that overweight gerbils had excessive body fat mass, and higher leukocyte counts and leptin levels than lean gerbils, suggesting that overweight gerbils might be in a low grade inflammatory state.

In summary, overweight gerbils had higher body fat mass and leukocyte counts, as well as higher blood glucose and serum leptin levels than lean gerbils. Both cellular and humoral immunity are impaired in overweight gerbils. These results imply that obesity has great influence on immune function. Maintaining normal body mass is important for gerbils to sustain optimal immune responses, to forage and to escape predation. The evidence from laboratory animals indicates the appearance of several health impairments associated with weight is important, given the prevalence of obesity in many countries. Developing animal models that can assist in the understanding of the implications to wild animal populations, and even to human populations, is highly desirable. Our data show that Mongolian gerbils are ideal for use in such research.

ACKNOWLEDGMENTS

We are grateful to all the members of Animal Physiological Ecology Group for their discussions on the data and help on the experiment. This study was supported by grants to DHW from the National Science and Technology Ministry Project (No. 2007BC109103) and the National Natural Science Foundation of China (30625009).

REFERENCES

Ahima RS, Flier JS (2000). Adipose tissue as an endocrine organ. *Trends in Endocrinology and Metabolism* **11**, 327–32.

Amar S, Zhou Q, Shaik-Dasthagirisaheb Y, Leeman S (2007). Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. *Proceedings of the National Academy of Sciences* **104**, 20466–71.

Bellocq JG, Krasnov BR, Khokhlova IS, Pinshow B (2006). Temporal dynamics of a T cell mediated immune response in desert rodents. *Comparative and Biochemical Physiology A* **145**, 554–9.

Black CT, Hennessey PJ, Andrassy RJ (1990). Short-term hyperglycemia depresses immunity through nonenzymatic glycosylation of circulating immunoglobulin. *Journal of Trauma* **30**, 830–32.

Busso N, So A, Chobaz-Péclat V *et al.* (2002). Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis. *Journal of Immunology* **168**, 875–82.

Calder PC, Kew S (2002). The immune system: a target for functional foods? *British Journal of Nutriton* **88**, S165–76.

Chandra RK (1980). Cell-mediated immunity in genetically obese C57BL/6J *ob/ob* mice. *Amemrican Journal of Clinical Nutrition* **33**, 13–6.

Chandra RK, Sarchielli P (1996). Body size and immune responses. *Nutrition Research* **16**, 1813–9.

Cincotta AH, Schiller BC, Albert H, Meier AH (1991). Bromocriptine inhibits the seasonally occurring obesity, hyperinsulinemia, insulin resistance, and impaired glucose tolerance in the Syrian hamster, *Mesocricetus auratus. Metabolism* **40**, 639–44.

Conway B, Rene A (2004). Obesity as a disease: no lightweight matter. *Obesity Reviews* **5**, 145–51.

Demas GE, Drazen DL, Nelson RJ (2003). Reductions in total body fat decrease humoral immunity. *Proceedings of the Royal Society B* **270**, 905–11.

Dixon JB, O'Brien PE (2006). Obesity and the white blood cell count: changes with sustained weight loss. *Obesity Surgery* **16**, 251–7.

Eliakim A, Swindt C , Zaldivar F, Casali P, Cooper DM (2006). Reduced tetanus antibody titers in overweight children. *Autoimmunity* **39**, 137–41.

Faggioni R, Feingold KR, Grunfeld C (2001). Leptin regulation of the immune response and the immunodeficiency of malnutrition. *The FASEB Journal* **15**, 2565–71.

Falagas ME, Kompoti M (2006). Obesity and infection. *Lancet Infectious Diseases* **6**, 438–46.

Fantuzzi G, Faggioni R (2000). Leptin in the regulation of immunity, inflammation, and hematopoiesis. *Journal of Leukocyte Biology* **68**, 437–46.

Fantuzzi G (2005). Adipose tissue, adipokines, and inflammation. *Journal of Allergy Clinical Immunology* **115**, 911–9.

Flier JS (1998). Lowered leptin slims immune response. *Nature Medicine* **4**, 1124–5.

Friedman JM (2009). Leptin at 14 yrs of age: an ongoing story. *Americal Journal of Clinical Nutrition* **89**, S973–9.

Friedman JM, Halaas JL (1998). Leptin and the regulation of body weight in mammals. *Nature* **395**, 763–70.

Hotamisligil GS (2006). Inflammation and metabolic disorders. *Nature* **444**, 860–67.

Houston AI, McNamara JM, Barta Z, Klasing KC (2007). The effect of energy reserves and food availability on optimal immune defense. *Proceedings of the Royal Society B* **274**, 2835–42.

Iikuni N, Lam QLK, Lu L, Matarese G, Cava AL (2008). Leptin and inflammation. *Current Immunology Reviews* **4**, 70–79.

Irigaray P, Newby JA, Lacomme S, Belpomme D (2007). Overweight/obesity and cancer genesis: more than a biological link. *Biomedicine Pharmacotherapy* **61**, 665–78.

Jaso-Friedmann L, Leary JH, Praveen K, Waldron M, Hoenig M (2008). The effects of obesity and fatty acids on the feline immune system. *Veterinary Immunology and Immunopathology* **122**, 146–52.

Klimentidis YC, Beasley TM, Lin HY *et al.* (2010). Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics. *Proceedings of Royal Society B: Biological Sciences* 1–8.

Kumari BS, Chandra RK (1993). Overnutrition and immune responses. *Nutrition Research* **13**, S3–18.

Lago F, Dieguez C, Gómez-Reino J, Gualillo O (2007). Adipokines as emerging mediators of immune response and inflammation. *Nature Clinical Practice Rheumatology* **3**, 716–24.

Lam QLK, Lu LW (2007). Role of leptin in immunity. *Cellular and Molecular Immunology* **4**, 1–13.

Lamas O, Marti A, Martínez JA (2002). Obesity and immunocompetence. *European Journal of Clinical Nutriton* **56**, S42–5.

Lamas O, Martínez JA, Marti A (2004). Energy restriction restores the impaired immune response in overweight (cafeteria) rats. *Journal of Nutrtional Biochemistry* **15**, 418–25.

Lazar MA (2005). How obesity causes diabetes: not a tall tale. *Science* **307**, 373–5.

Lee YH, Pratley RE (2005). The evolving role of inflammation in obesity and the metabolic syndrome. *Current Diabetes Reports* **5**, 70–75.

Li XS, Wang DH (2005). Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*): the roles of short photoperiod and cold. *Journal of Comparative Physiology* B **175**, 593–600.

Lindström P (2007). The physiology of obese-hyperglycemic mice [*ob/ob* mice]. *The ScientificWorld Journal* **7**, 666–85.

Liu W, Wan XR, Zhong WQ (2007). Population dynamics of the Mongolian gerbils: seasonal patterns and interactions among density, reproduction and climate. *Journal of Arid Environments* **68**, 383–97.

Liu W, Wang GM, Wang YN, Zhong WQ, Wan XR (2009). Population ecology of wild Mongolian gerbils. *Journal of Mammals* **90**, 832–40.

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998). Leptin modulates the T cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897–901.

MacIver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC (2008). Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *Journal of Leukocyte Biology* **84**, 949–57.

Mandel MA, Mahmoud AAF (1978). Impairment of cell mediated immunity in mutation diabetic mice (*db/db*). *Journal of Immunology* **120**, 1375–7.

Marti A, Marcos A, Martinez JA (2001). Obesity and immune function relationships. *Obesity Reviews* **2**, 131–40.

Martin SS, Qasim BA, Reilly MP (2008). Leptin resistance: a possible interface of inflammation and Metabolism in obesity-related cardiovascular disease. *Journal of the American College of Cardiology* **52**, 1201–10.

Matarese G (2000). Leptin and the immune system: how nutritional status influences the immune response. *European Cytokine Network* **11**, 7–14.

Matarese G, Cava AL (2004). The intricate interface between immune system and metabolism. *Trends in Immunology* **5**, 193–200.

Matarese G, Cava AL (2005). Immune responses in obesity models. *Drug Discovery Today: Diseases Model* **2**, 177–81.

Matarese G, Cava AL, Sanna V *et al*. (2002). Balancing susceptibility to infection and autoimmunity: a role for leptin? *Trends in Immunology* **23**, 182–7.

Matarese G, Moschos S, Mantzoros CS (2005). Leptin in immunology. *Journal of Immunology* **173**, 3137–42.

McGillis JP (2005). White adipose tissue, inert no more! *Endocrinology* **146**, 2154–6.

Mito N, Kitada C, Hosoda T, Sato K (2002). Effect of diet-induced obesity on ovalbumin-specific immune response in a murine asthma model. *Metabolism* **51**, 1241–6.

Moriguchi S, Kato M, Sakai K, Yamamoto S, Shimizu E (1998). Exercise training restores decreased cellular immune functions in obese Zucker rats. *Journal of Applied Physiology* **84**, 311–7.

Myers MG, Cowley MA, Münzberg H (2008). Mechanisms of leptin action and leptin resistance. *Annual Reviews of Physiology* **70**, 537–56.

Nieman DC, Henson DA, Nehlsen-Cannarella SL *et al*. (1999). Influence of obesity on immune function. *Journal of American Dietetic Association* **99**, 294–9.

Otero M, Lago R, Gomez R *et al*. (2006). Towards a pro-inflammatory and immunomodulatory emerging role of leptin. *Rheumatology* **45**, 944–50.

Samartín S, Chandra RK (2001). Obesity, overnutrition and the immune system. *Nutrition Research* **21**, 243–62.

Savino W, Dardenne M (2000). Neuroendocrine control of thymus physiology. *Endocrine Reviews* **21**, 412–43.

Schäffler A, Schölmerich J, Salzberger B (2007). Adipose tissue as an immunological organ: toll-like receptors, C1q/TNFs and CTRPs. *Trends in Immunology* **28**, 393–9.

Schaible UE, Kaufmann SHE (2007). Malnutrition and infection: complex mechanisms and global impacts. *Plos Medicine* **4**, 806–12.

Smith KG, Hunt JL (2004). On the use of spleen mass as a measure of avian immune system strength. *Oecologia* **138**, 28–31.

Smith AG, Sheridan PA, Harp JB, Beck MA (2007). Diet-induced obese mice have increased mortality and altered immune responses when infected with influenza virus. *Journal of Nutrtion* **137**, 1236–43.

Speakman J, Hambly C, Mitchell S, Król E (2008). The contribution of animal models to the study of obesity. *Laboratory Animal* **42**, 413–32.

Steiner AA, Romanovsky AA (2007). Leptin: at the crossroads of energy balance and systemic inflammation. *Progress in Lipid Research* **46**, 89–107.

Stuermer IW, Plotz K, Leybold A *et al.* (2003). Intraspecific allometric comparison of laboratory gerbils with Mongolian gerbils trapped in the wild indicates domestication in *Meriones unguiculatus* (Milne–Edwards, 1867) (Rodentia: gerbillinae). *Zoologischer Anzeiger* **242**, 249–66.

Tanaka S, Isoda F, Kiuchi Y, Ikeda H, Mobbs CV, Yamakawa T (2000). T lymphopenia in genetically obese-diabetic Wistar fatty rats: effects of body weight reduction on T cells. *Metabolism* **49**, 1261–6.

Taylor JH, Beilmam GJ (2005). Hyperglycemia in the intensive care unit: no longer just a marker of illness severity. *Surgery Infection* **6**, 233–45.

Trayhurn P (2007). Adipocyte biology. *Obesity Reviews* **8**, S41–4.

Trayhurn P, Wood IS (2004). Adipokines: inflammation and the pleiotropic role of white adipose tissue. *British Journal of Nutriton* **92**, 347–55.

Walker EP (1968). *Mammals of the World*, 2nd edn. Johns Hopkins Press, Baltimore, Maryland.

Weber DJ, Rutala WA, Samsa GP, Bradshaw SE, Lemon SM (1986). Impaired immunogenicity of hepatitis B vaccine in obese persons. *The Journal of American Medical Association* **254**, 3187–9.

Womack J, Tien PC, Feldma J *et al.* (2007). Obesity and immune cell counts in women. *Metabolism Clinical and Experimental* **56**, 998–1004.

Xu DL, Wang DH (2010). Fasting suppresses T cell-mediated immunity in female Mongolian gerbils (*Meriones unguiculatus*). *Comparative and Biochemical Physiology A* **155**, 25–33.

Yang XP (2004). *Laboratory Manual in Animal Physiology*, 1st edn. Higher Education Press, Beijing, China, pp. 91–4.

Zhang YY, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–32.

Zhang ZQ (2005). Seasonal changes in thermogenesis, energy budgets and immune function in Mongolian gerbils (*Meriones unguiculatus*) (PhD thesis). Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Zhang ZQ, Wang DH (2006). Seasonal changes in immune function, body fat mass and organ mass in Mongolian gerbils (*Meriones unguiculatus*). *Acta Theriologica Sinica* **26**, 338–45 (In Chinese with English summary).

Zhang ZQ, Wang DH (2007). Seasonal changes in thermogenesis and body mass in wild Mongolian gerbils (*Meriones unguiculatus*). *Comparative and Biochemical Physiology A* **148**, 346–53.

Zhao ZJ, Wang DH (2006). Effects of photoperiod on energy budgets and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*). *Journal of Thermal Biology* **31**, 323–31.

Zysling DA, Demas GE (2007). Metabolic stress suppresses humoral immune function in long-day, but not short-day, Siberian hamsters (*Phodopus sungorus*). *Journal of Comparative Physiology* B **177**, 339–47.