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Fasting suppresses T cell-mediated immunity in female Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Immune defense is important for organisms' survival and fitness. Small mammals in temperate zone often face seasonal food shortages. Generally fasting can suppress immune function in laboratory rodents and little information is available for wild rodents. The present study tested the hypothesis that Mongolian gerbils (*Meriones unguiculatus*) could inhibit T cell-mediated immunity to adapt to acute fasting. Forty-two females were divided into the fed and fasted groups, in which the latter was deprived of food for 3 days. After 66 h fasting, half of the gerbils in each group were injected with phosphate buffered saline or phytohaemagglutinin (PHA) solution. T cell-mediated immunity assessed by PHA response was suppressed in the fasted gerbils compared with the fed gerbils. The fasted gerbils had lower body fat mass, wet and dry thymus mass, dry spleen mass, white blood cells, serum leptin and blood glucose concentrations, but higher corticosterone concentrations than those of the controls. Moreover, PHA response was positively correlated with body fat mass and serum leptin levels in the immunochallenged groups. Taken together, acute fasting leads to immunosuppression, which might be caused by low body fat mass and low serum leptin concentrations in female Mongolian gerbils.

1. Introduction

The immune system, which can protect organisms from infection and attack of pathogens, plays an important role in their survival and fitness (Sheldon and Verhulst, 1996; Owens and Wilson, 1999). However, immune function is affected by many factors such as food quality and quantity (Chandra, 1996; Calder and Kew, 2002; Kaminogawa and Nanno, 2004; Schaible and Kaufmann, 2007).

The impact of food shortage on immune function has been studied widely such as in human, cats, laboratory rodents, birds and invertebrates. Acute fasting has little effect on humoral and cell-mediated immunity in human (Holm and Palmblad, 1976; Palmblad et al., 1977; Neuvonen and Salo, 1984). However, 7 days of fasting leads to immunosuppression in cats (Freitag et al., 2000). Lord et al. (1998) have also demonstrated that fasting for 2 days suppresses T cell-mediated immunity in mice. Similarly, delayed type hypersensitivity indicative of T cell-mediated immune response is reduced in fasted rats or mice compared with controls (Wing and Young, 1980; Nohr et al., 1985; Nakamura et al., 2001, 2004), and two-day fasting increases susceptibility to endotoxic shock in fasted mice (Faggioni et al., 2000). Both T cell-mediated and humoral immune response are suppressed (Bourgeon et al., 2006a,b), while innate immunity does not vary significantly in female common eiders throughout the incubation fasting period (Bourgeon et al., 2007). And acute fasting compromises immunological activity in shrimps (Pascual et al., 2006), Sydney rock oysters (Butt et al., 2007), and scallops (Xu et al., 2008).

Phytohaemagglutinin (PHA) response, which involves a subcutaneous injection of PHA that induces local T cell stimulation and proliferation resulting in swelling (Smits et al., 1999), has been proved to be a reliable tool for assessing mammalian T cell-mediated immunity (Webb et al., 2003; Bellocq et al., 2006). Lymphoid organs such as thymus and spleen are indirect parameters which are indicative of immune function (Savino and Dardenne, 2000; Calder and Kew, 2002; Smith and Hunt, 2004). Specifically, the thymus is a central lymphoid organ which is important for primary T cell development (Savino and Dardenne, 2000), and a larger spleen is representative of a stronger immune system (Smith and Hunt, 2004). Total white blood cells (or leukocytes, WBC), which are fundamental to immune response against pathogens, are also useful to evaluate the overall health (Calder and Kew, 2002; Artacho et al., 2007).

Leptin, a cytokine-like peptide hormone synthesized and secreted almost exclusively by adipose tissues, plays an important role in immunity in addition to its regulatory role in energy homeostasis (Zhang et al., 1994; Faggioni et al., 2001; Matarese et al., 2005; Lam and Lu, 2007). It is proportional to adipose tissues, which are no longer regarded as simply passive energy reserves and have been

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considered as important endocrine and immune organs (Pond, 1996; Ahima and Flier, 2000a; Matarese and Cava, 2004; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al., 2007). Moreover, metabolic fuels such as blood glucose also play an important role in immunity. It is well documented that lymphocytes require glucose metabolism for normal survival and function (Maciver et al., 2008) and T cells increase glucose uptake and glycolysis during an immune response (Matarese and Cava, 2004). Glucocorticoids such as corticosterone, which can inhibit immune function, often increase during acute stress of fasting (Ahima et al., 1996; Sapolsky et al., 2000). To date, we still have no idea about how immune function responds to acute food deficiency in wild rodents.

Mongolian gerbils (*Meriones unguiculatus*) are small seasonally breeding, non-hibernating, and granivorous rodents which mainly distribute in the desert and semi-arid regions of Mongolia and Northern China (Walker, 1968). In these regions the annual average temperature is −0.4 °C, the average temperature in the coldest month is −22.3 °C and in the warmest month is 18.8 °C, with extreme minimum temperature below −40 °C (Chen, 1988; Zhao and Wang, 2006). Gerbils live in social groups of 2–17 year-round (Ågren et al., 1989) and their population is female-biased and is also biased toward older individuals (Liu et al., 2009). They prefer open habitats with short, sparse vegetation and dry, loose, and sandy soil (Zhong et al., 1985; Liu et al., 2007). In the wild, gerbils mainly select seeds of annual dicots and some foliage as food, and they hoard food communally for wintering (Zhong et al., 1985). Food resources fluctuate dramatically throughout a year indicating that periodic food shortage is common to wild gerbils, and such harsh habitats have also made them a good model to study the effect of food shortage on immune function (Xia et al., 1982; Zhong et al., 1985; Zhang and Wang, 2007; Liu et al., 2007). Field work has shown that humoral immunity is higher in winter gerbils than that in summer (Zhang and Wang, 2006), and it is not suppressed by a low protein diet (Chen et al., 2007). Moreover, humoral immunity in gerbils is irresponsive to photoperiod and low temperature and housing density (Li, 2005). Alternatively, Zhang (2007) has demonstrated that three-day fasting significantly reduces body mass, body fat mass, serum leptin concentrations and thermogenic capacity in gerbils. In order to further understand the immunological adaptive strategy of gerbils to acute food deficiency, we examined some immunological indices to test the hypothesis that Mongolian gerbils may down regulate immune function when facing food shortages. We predicted that T cell-mediated immune response, together with thymus, spleen and WBC, will decrease after a three-day fasting.

2. Materials and methods

2.1. Animals and experimental design

All animal procedures were performed in accordance with guidelines of the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Virgin adult female Mongolian gerbils used in this study were the offspring of gerbils in our laboratory colony. Before experiment, the gerbils were housed individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding under a constant photoperiod of 16L:8D (16 h:8 h light–dark cycle) and temperature of 23 ± 1 °C. Commercial standard rat pellets (Beijing KeAo Feed Co.) and water were provided *ad libitum*. After their body mass was stable, forty-two healthy female gerbils (age 7–8 months; mass 53.0–69.0 g) were selected and randomly divided into four groups: fed *ad libitum* and injected with sterile phosphate buffered saline (PBS, pH7.4) (Fed/PBS group, $n = 10$), fed *ad libitum* and injected with PHA solution (Fed/PHA group, $n = 10$), fasted and injected with sterile PBS (Fasted/PBS group, $n = 11$), and fasted and injected with PHA solution (Fasted/PHA group, $n = 11$). Previous work has shown that one adult gerbil died after 84 h fasting ($n = 10$) (Zhang, 2007). Considering gerbils' welfare, we decided that the fasting period was 3 days in the present study. All gerbils had access to enough water throughout the experiment. The initial day of the treatment was designated as day 0, and day 2 and day 3 represented fasting for two and 3 days, respectively. During the course of the experiment, one gerbil in the Fasted/PBS group and another in the Fasted/PHA group died after fasting for 66 h and 72 h, respectively, and these two gerbils were not included in the subsequent statistical analyses.

2.2. Organs and body composition

Organs were measured as described in Zhang and Wang (2007). In brief, after interscapular BAT (IBAT) was removed, the visceral organs, including heart, thymus, lung, liver, spleen, kidneys, paired adrenal glands, gonad (uterus and ovaries) and the digestive organs with contents (i.e., stomach, small intestine, caecum and colon) were dissected and weighed (±1 mg). The stomach, small intestine, caecum 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 dry carcass mass was the water mass of carcass. Total body fat was extracted from the dried carcass by petroleum ether extraction in a Soxhlet apparatus (Li and Wang, 2005), and body fat content was calculated as total body fat mass divided by wet carcass mass.

2.3. T cell-mediated immune response measurement

To determine how long the maximum PHA response (i.e., T cell-mediated immune response) would occur in female gerbils, we measured the footpad thickness of their left hind feet (aged 7–8 months, $n = 7$) with a micrometer (Tesa Shopcal, Swiss) to ±0.01 mm. Immediately thereafter, we injected subcutaneously 0.1 mg of PHA (PHA-P, Sigma L-8754) dissolved in 0.03 mL of sterile PBS (pH 7.4) in the middle of the footpad. After 6 h, 24 h, and 48 h of injection, we measured footpad thickness. The PHA response was calculated as the difference between pre- and post-injection measurements divided by initial footpad thickness (PHA response = (post PHA − pre PHA) / pre PHA). Each measurement of PHA response was replicated six times (Smits et al., 1999; Bellocq et al., 2006). The result shows that the maximum PHA response occurs after 6 h of PHA injection (Fig. 3A). Therefore, the injection time of PHA or PBS was after fasting for 66 h to get the maximum T cell-mediated immune response in the present experiment.

2.4. White blood cells measurement

At the end of the experiment, each gerbil was euthanized by CO_2 asphyxiation and trunk blood was collected around 15:00 h for the measurements of WBC, blood glucose, serum leptin and corticosterone to reduce the effect of circadian rhythm on these parameters. 20 μL whole blood was diluted immediately in 0.38 mL solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. At the same time, another 20 μL whole blood was obtained immediately for measuring blood glucose levels. The total number of WBC was determined by counting all leucocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9 cells/L) (Yang, 2004). The rest of the blood sample was allowed to clot for an hour on ice, and then it was centrifuged at 800 × *g* at 4 °C for 30 min. The serum was collected and then stored at −80 °C until the assay of leptin and corticosterone.

2.5. Blood glucose measurement

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

2.6. Serum leptin assays

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

2.7. Serum corticosterone assays

Serum corticosterone concentrations were determined by rat corticosterone ELISA (enzyme-linked immunosorbent assay) kit (Cat. No. HR083, RapidBio Lab. Calabasas, CA, USA). The lowest level of corticosterone that could be detected by this assay was 0.35 ng/mL when using a 125 µL sample. The detailed procedure followed the manufacturer's instructions of the rat corticosterone ELISA kit. Inter- and intra-assay variabilities for corticosterone ELISA were <7% and 5%, respectively (Kim et al., 2008).

2.8. Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc., 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 difference of the PHA response at 6 h, 24 h and 48 h after injection of PHA was analyzed by repeated measures, followed by Bonferroni *post hoc* tests. The difference of body mass on days 0, 1, 2 among the four groups was analyzed by a one-way analysis of variance (ANOVA), while the difference of body mass on day 3 among the four groups was analyzed by a two-way ANOVA (fasting and immunochallenge) followed by Bonferroni *post hoc* tests. Group difference in wet organ mass with body mass as the covariate and dry organ mass with dry carcass mass as the covariate was analyzed by a two-way analysis of covariance (ANCOVA) followed by Bonferroni *post hoc* tests. Group difference in other parameters (body composition, PHA response, WBC, blood glucose, leptin and corticosterone) was analyzed by a two-way ANOVA followed by Bonferroni *post hoc* tests. Significant group difference was further evaluated by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests. Pearson correlation analysis was performed to determine the correlations of PHA response with body mass, body fat mass, WBC, blood glucose, leptin and corticosterone concentrations. The correlations of leptin with body fat mass, corticosterone; the correlation of body fat mass with corticosterone, blood glucose, WBC; and the correlation of blood glucose and WBC were also detected. Results were presented as means ± SE, and $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Body mass

Body mass among the four groups was stable at the beginning of the experiment and showed no significant difference on day 0 ($F_{3,36}=0.404$, $P>0.05$). However, body mass in the Fasted/PBS or the Fasted/PHA groups was much lower than in the Fed/PBS or the Fed/PHA groups ($F_{1,36}=147.434$, $P<0.001$) after fasting for 3 days (Fig. 1). There was no significant effect of immunochallenge with injection of PHA ($F_{1,36}=0.019$, $P>0.05$), and the interaction of fasting × immunochallenge ($F_{1,36}=0.401$, $P>0.05$) on body mass on day 3 for both the fed and fasted gerbils. Compared with body mass on day 0 (Fasted/PBS group: 59.2 ± 1.4 g; Fasted/PHA group: 60.0 ± 1.4 g), gerbils in the Fasted/PBS and Fasted/PHA groups lost 13.8 g (23.4%) and 12.9 g (21.5%) body mass on day 3, respectively.

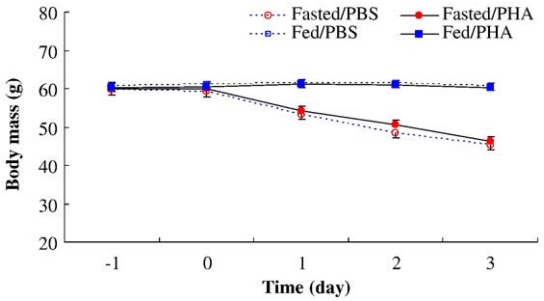

Fig. 1. Changes of body mass in female Mongolian gerbils during three-day fasting. Values are means ± SE ($n=10$). Body mass on day 0 among the four groups did not differ significantly, however body mass on days 1, 2, and 3 was much lower in the fasted groups than in the fed groups. Note: ○ = Fasted/PBS group; ● = Fasted/PHA group; □ = Fed/PBS group, ■ = Fed/PHA group.

3.2. Organs and body composition

The fasted gerbils had lower body fat mass ($F_{1,36}=68.019$, $P<0.001$), wet ($F_{1,35}=14.112$, $P<0.01$) and dry ($F_{1,35}=10.483$, $P<0.01$) thymus mass (Fig. 2A), dry spleen mass ($F_{1,35}=17.623$, $P<0.001$) (Fig. 2B), stomach with contents ($F_{1,35}=4.191$, $P<0.05$), dry stomach mass ($F_{1,35}=6.020$, $P<0.05$) and dry caecum mass

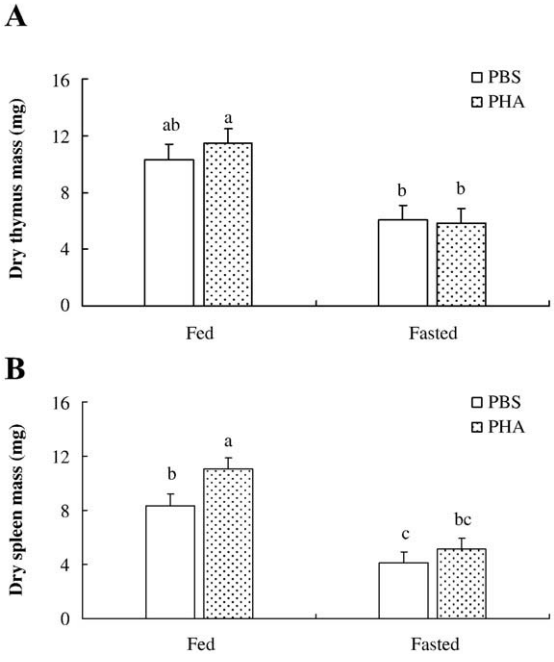

Fig. 2. Effect of three-day fasting on dry spleen mass (A), dry thymus mass (B) in female Mongolian gerbils. Values are means ± SE ($n=10$). Spleen and thymus dry mass were lower in the fasted gerbils than in the fed groups. Different letters (a or b) above hatched bars and solid bars indicate significant differences ($P<0.05$). PBS, injection of PBS saline; PHA, injection of PHA solution.

Table 1
Changes of body composition in the fed and fasted female Mongolian gerbils after three-day fasting.

Parameters	Fed		Fasted		Statistical summary		
	PBS	PHA	PBS	PHA	F	IC	F×IC
Body mass on day 3 (g)	60.8±1.2[a]	60.2±1.2[a]	45.3±1.2[b]	46.3±1.2[b]	<0.001	ns	ns
Wet carcass mass (g)	47.7±1.1[a]	47.1±1.1[a]	36.9±1.1[b]	37.7±1.1[b]	<0.001	ns	ns
Dry carcass mass (g)	20.7±0.7 [a]	19.6±0.7 [a]	13.4±0.7 [b]	13.3±0.7 [b]	<0.001	ns	ns
Water of carcass (g)	27.0±0.9[a]	27.5±0.9[a]	23.5±0.9[b]	24.5±0.9[ab]	<0.01	ns	ns
Water content (water mass/wet carcass mass) (%)	56.8±1.3[a]	58.4±1.3[a]	63.8±1.3[b]	64.7±1.3[b]	<0.001	ns	ns
Fat free dry carcass (g)	12.1±0.3 [a]	12.4±0.3 [a]	10.7±0.3 [b]	10.9±0.3 [b]	<0.001	ns	ns
Body fat mass (g)	8.6±0.7 [a]	7.2±0.7 [a]	2.6±0.7 [b]	2.3±0.7 [b]	<0.001	ns	ns
Body fat content (body fat mass/wet carcass mass) (%)	17.9±1.5[a]	15.3±1.5[a]	7.1±1.5[b]	6.2±1.5[b]	<0.001	ns	ns

Values are means±SE ($n=10$).
Values for a specific parameter that share different superscripts are significantly different at $P<0.05$, determined by a two-way ANOVA and Bonferroni *post hoc* tests.
F, fasting; IC, immunochallenge; F×IC, interaction of fasting×immunochallenge; ns, not significant.

($F_{1,35}=7.472$, $P<0.05$), but higher wet gonad mass ($F_{1,35}=9.285$, $P<0.01$) than those of the fed gerbils, whereas other organ masses were not affected by fasting (Tables 1–3). In addition, immunochallenge increased wet ($F_{1,35}=11.327$, $P<0.01$) and dry ($F_{1,35}=8.383$, $P<0.01$) spleen mass, decreased dry colon mass ($F_{1,35}=4.319$, $P<0.05$), while it had no significant effect on other organ masses

Table 2
Mean wet organ masses in the fed and fasted female Mongolian gerbils after three-day fasting.

Parameters	Fed		Fasted		Statistical summary		
	PBS	PHA	PBS	PHA	F	IC	F×IC
IBAT (mg)	136±16	134±15	93±16	89±15	ns	ns	ns
Heart (mg)	221±8	226±8	242±8	244±8	ns	ns	ns
Thymus (mg)	47±5[a]	54±5[a]	23±5[b]	22±5[b]	<0.01	ns	ns
Lungs (mg)	318±38	328±36	429±38	422±36	ns	ns	ns
Liver (mg)	1870±132	1832±127	1584±134	1595±126	ns	ns	ns
Spleen (mg)	27±4[b]	40±3[a]	24±4[b]	27±3[b]	ns	<0.01	ns
Kidneys (mg)	506±19	502±18	501±19	508±18	ns	ns	ns
Adrenal gland (mg)	40±5	44±5	33±5	36±5	ns	ns	ns
Gonad (uterus and ovaries)(mg)	37±6[b]	43±6[b]	70±6[a]	69±6[a]	<0.01	ns	ns
Stomach with contents (mg)	1038±129	1050±124	632±130	637±122	<0.05	ns	ns
Stomach (mg)	401±21	397±20	416±21	404±20	ns	ns	ns
Small intestine with contents (mg)	1850±122[ab]	1966±117[a]	1618±123[ab]	1488±116[b]	ns	ns	ns
Small intestine (mg)	595±70	588±67	569±71	534±66	ns	ns	ns
Caecum with contents (mg)	1141±97	1109±93	1014±98	1091±92	ns	ns	ns
Caecum (mg)	321±25[ab]	339±24[a]	248±25[b]	257±23[ab]	ns	ns	ns
Colon with contents(mg)	798±75	717±72	567±76	546±71	ns	ns	ns
Colon (mg)	294±27	291±26	344±27	306±25	ns	ns	ns
Total digestive tract (mg)	1610±101	1614±96	1578±102	1502±95	ns	ns	ns

Values are means±SE ($n=10$).
Values for a specific parameter that share different superscripts are significantly different at $P<0.05$, determined by a two-way ANCOVA with body mass as the covariate and Bonferroni *post hoc* tests.
F, fasting; IC, immunochallenge; F×IC, interaction of fasting×immunuchallenge; ns, not significant.

Table 3
Mean dry organ masses in the fed and fasted female Mongolian gerbils after three-day fasting.

Parameters	Fed		Fasted		Statistical summary		
	PBS	PHA	PBS	PHA	F	IC	F×IC
Heart (mg)	55±3	57±2	54±2	55±2	ns	ns	ns
Thymus (mg)	10±1[ab]	11±1[a]	6±1[b]	6±1[b]	<0.01	ns	ns
Lungs (mg)	108±18	114±16	90±17	96±17	ns	ns	ns
Liver (mg)	528±59	524±52	566±55	545±56	ns	ns	ns
Spleen (mg)	8±1[b]	11±1[a]	4±1[c]	5±1[bc]	<0.001	<0.01	ns
Kidneys (mg)	129±6	130±5	117±5	120±5	ns	ns	ns
Adrenal glands (mg)	13±2	15±2	10±2	11±2	ns	ns	ns
Gonad (uterus and ovaries)	15±2	17±2	19±2	20±2	ns	ns	ns
Stomach (mg)	99±4	96±3	85±3	85±3	<0.05	ns	ns
Small intestine (mg)	105±14	86±12	82±13	79±13	ns	ns	ns
Caecum (mg)	63±7	58±6	32±7	36±7	<0.05	ns	ns
Colon (mg)	74±7	58±6	65±7	59±7	ns	<0.05	ns
Total digestive tract (mg)	342±21	298±19	264±20	259±20	ns	ns	ns

Values are means±SE ($n=10$).
Values for a specific parameter that share different superscripts are significantly different at $P<0.05$, determined by a two-way ANCOVA with dry carcass mass as the covariate and Bonferroni *post hoc* tests.
F, fasting; IC, immunochallenge; F×IC, interaction of fasting×immunuchallenge; ns, not significant.

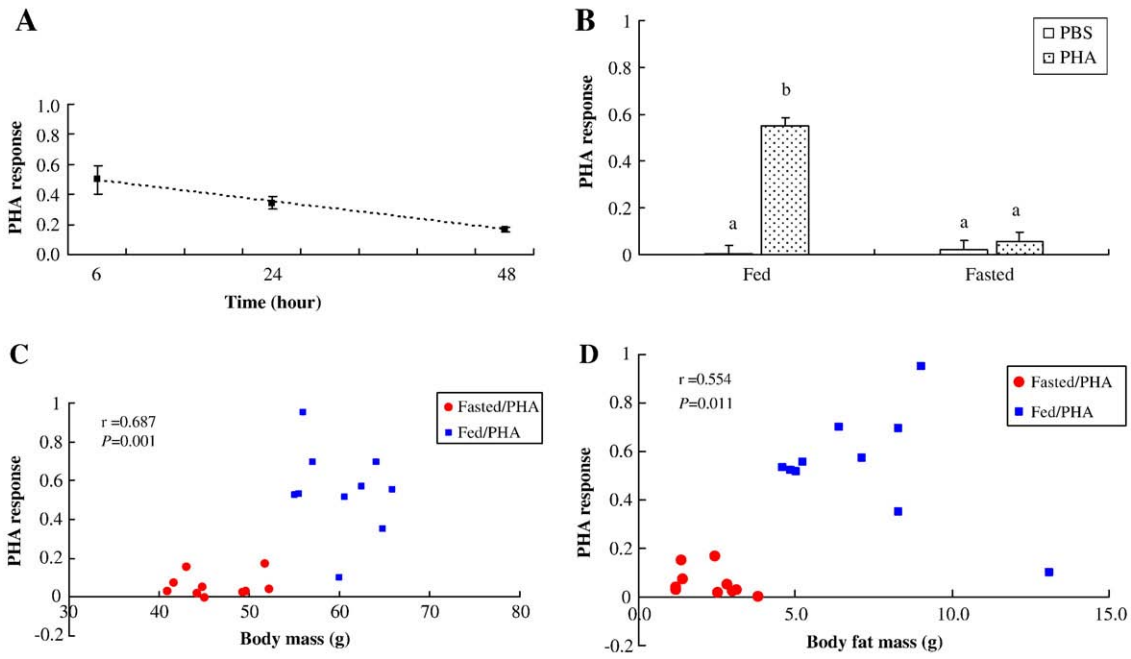

Fig. 3. PHA response at 6 h, 24 h and 48 h after PHA injection ($n=7$) (A), effect of three-day fasting on PHA response ($n=10$) (B), and the correlation of PHA response with body mass (C), body fat mass (D) in female Mongolian gerbils. Values are means ± SE. The maximum PHA response occurred after 6 h of PHA injection. Additionally, PHA response was significantly lower in the Fasted/PHA group than in the Fed/PHA group, and it was positively correlated with body mass and body fat mass. Different letters (a or b) above hatched bars and solid bars indicate significant differences ($P<0.05$). PBS, injection of PBS saline; PHA, injection of PHA solution. Note: ● = Fasted/PHA group; ■ = Fed/PHA group.

(Tables 2 and 3). Furthermore, all the organ masses were not affected by the interaction of fasting × immunochallenge (Tables 2 and 3).

3.3. T cell-mediated immune response

The maximum PHA response occurred after 6 h of PHA injection in Mongolian gerbils ($F_{1,6}=84.677$, $P<0.001$) (Fig. 3A). After fasting for 3 days, PHA response decreased significantly in the fasted gerbils compared with the fed gerbils ($F_{1,36}=29.702$, $P<0.001$) (Fig. 3B). In addition, both immunochallenge ($F_{1,36}=43.581$, $P<0.001$) and the interaction of fasting × immunochallenge ($F_{1,36}=34.838$, $P<0.001$) had significant effect on PHA response. Furthermore, PHA response was positively correlated with body mass ($r=0.687$, $P<0.01$) (Fig. 3C), body fat mass ($r=0.554$, $P<0.05$) (Fig. 3D) in the immunochallenged groups (the Fed/PHA and Fasted/PHA groups). However, PHA response was no longer correlated with body mass ($r=-0.293$, $P>0.05$), body fat mass ($r=-0.227$, $P>0.05$) in the Fed/PHA group, and it was also not correlated with body mass ($r=-0.121$, $P>0.05$), body fat mass ($r=-0.371$, $P>0.05$) in the Fasted/PHA group.

3.4. White blood cells

WBC was reduced significantly in the fasted gerbils by three-day fasting ($F_{1,36}=16.379$, $P<0.001$), compared with the fed gerbils (Fig. 4A). Moreover, both immunochallenge ($F_{1,36}=21.131$, $P<0.001$) and the interaction of fasting × immunochallenge ($F_{1,36}=15.899$, $P<0.001$) had significant effect on WBC. Additionally, WBC was positively correlated with PHA response ($r=0.729$, $P<0.001$) and body fat mass ($r=0.585$, $P<0.01$) in the immunochallenged groups (Fig. 4B). However, as for the Fed/PHA group, WBC was no longer correlated with PHA response ($r=0.267$, $P>0.05$) and body fat mass ($r=-0.025$, $P>0.05$); likewise it was also not correlated with PHA response ($r=0.220$, $P>0.05$) and body fat mass ($r=0.008$, $P>0.05$) in the Fasted/PHA group.

3.5. Blood glucose levels

The fasted gerbils had significantly lower blood glucose levels than those of the fed controls ($F_{1,36}=391.420$, $P<0.001$) (Fig. 5A). There was no significant effect of immunochallenge ($F_{1,36}=0.298$, $P>0.05$)

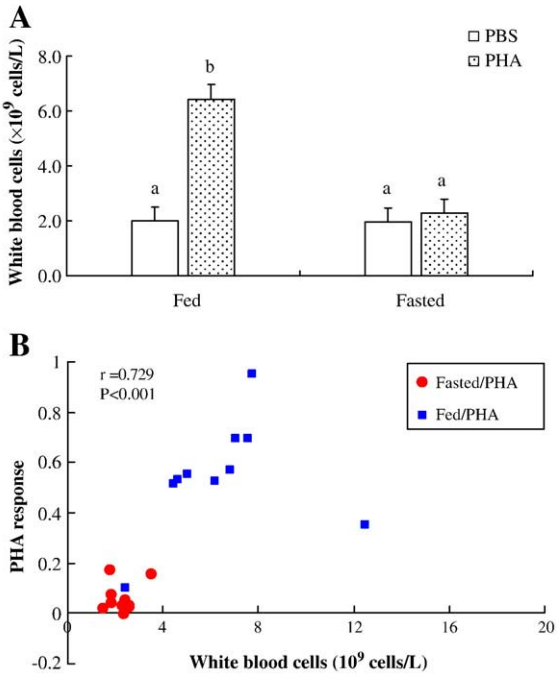

Fig. 4. Effect of three-day fasting on white blood cells (A) and the correlation of white blood cells and PHA response (B) in female Mongolian gerbils. Values are means ± SE ($n=10$). WBC was lower in the Fasted/PHA group than in the Fed/PHA group, and was positively correlated with PHA response in immunochallenged groups. PBS, injection of PBS saline; PHA, injection of PHA solution. Note: ● = Fasted/PHA group; ■ = Fed/PHA group.

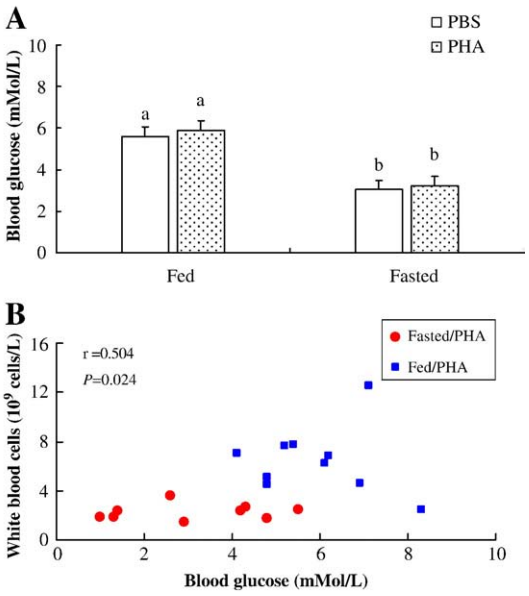

Fig. 5. Effect of three-day fasting on blood glucose levels (A) and the correlation with WBC (B) in female Mongolian gerbils. Values are means ± SE ($n = 10$). Blood glucose levels in the fasted gerbils were lower than in the fed gerbils, and were positively correlated with WBC. Different letters (a or b) above hatched bars and solid bars indicate significant differences ($P<0.05$). PBS, injection of PBS saline; PHA, injection of PHA solution. Note: ● = Fasted/PHA group; ■ = Fed/PHA group.

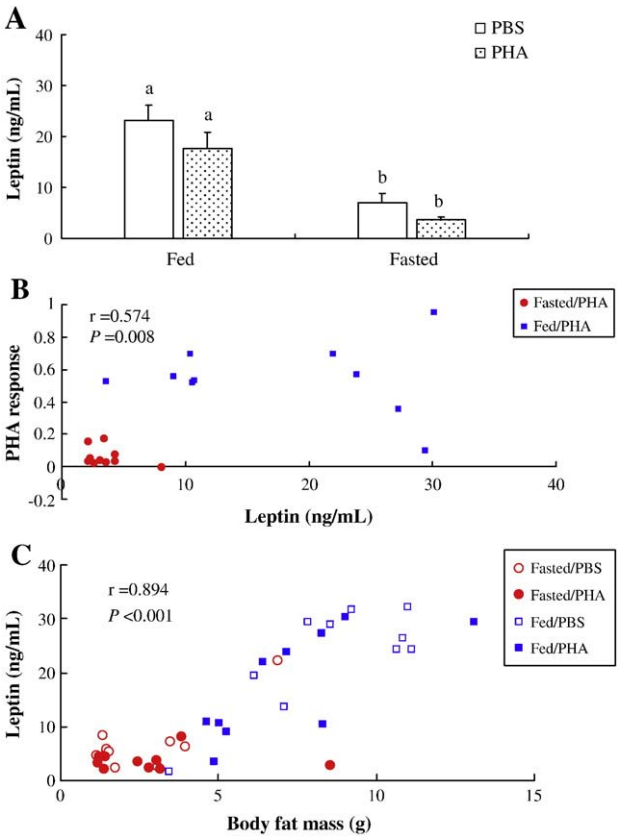

Fig. 6. Effect of three-day fasting on leptin concentrations (A), and the correlation of leptin concentrations with PHA response (B), body fat mass (C) in female Mongolian gerbils. Values are means ± SE ($n = 10$). Serum leptin concentrations were lower in the fasted groups than in the fed groups, and were positively correlated with PHA response and body fat mass. Different letters (a or b) above hatched bars and solid bars indicate significant differences ($P<0.05$). Note: ○ = Fasted/PBS group; ● = Fasted/PHA group; □ = Fed/PBS group, ■ = Fed/PHA group.

and the interaction of fasting × immunochallenge ($F_{1,36} = 0.015$, $P>0.05$) on blood glucose levels for both the fed and fasted groups. In addition, blood glucose levels were positively correlated with body fat mass ($r = 0.763$, $P<0.001$) and WBC ($r = 0.504$, $P<0.05$) (Fig. 5B) for both the fasted and fed gerbils, and nearly positively correlated with PHA response in the immunochallenged groups ($r = 0.421$, $P = 0.064$). However, blood glucose levels were no longer correlated with body fat mass ($r = 0.387$, $P>0.05$), WBC ($r = 0.041$, $P>0.05$) in the fed groups, while blood glucose levels were still positively correlated with body fat mass ($r = 0.480$, $P<0.05$) but not correlated with WBC ($r = -0.188$, $P>0.05$) in the fasted groups.

3.6. Serum leptin concentrations

Serum leptin concentrations in the Fasted/PBS or the Fasted/PHA group were significantly lower than in the Fed/PBS or the Fed/PHA groups ($F_{1,36} = 41.528$, $P<0.001$) (Fig. 6A). However, both immunochallenge ($F_{1,36} = 3.501$, $P>0.05$) and the interaction of fasting × immunochallenge ($F_{1,36} = 0.203$, $P>0.05$) did not affect serum leptin concentrations. Furthermore, serum leptin concentrations were positively correlated with PHA response ($r = 0.574$, $P<0.01$) (Fig. 6B) in the immunochallenged groups, while this positive correlation disappeared both in the Fed/PHA ($r = 0.037$, $P>0.05$) and Fasted/PHA ($r = -0.358$, $P>0.05$) groups. Moreover, serum leptin concentrations were positively correlated with body fat mass ($r = 0.894$, $P<0.001$) (Fig. 6C) for both the fasted and fed groups, and they were still positively correlated with body fat mass in the fed groups ($r = 0.787$, $P<0.001$) and the fasted groups ($r = 0.703$, $P<0.01$).

3.7. Serum corticosterone concentrations

The fasted gerbils had significantly higher serum corticosterone concentrations than those of the fed controls after three-day fasting ($F_{1,36} = 17.525$, $P<0.001$) (Fig. 7A). Alternatively, there was no significant effect of immunochallenge ($F_{1,36} = 0.386$, $P>0.05$) and the interaction of fasting × immunochallenge ($F_{1,36} = 0.395$, $P>0.05$) on serum corticosterone concentrations. In addition, corticosterone concentrations were negatively correlated with body fat mass ($r = -0.644$, $P<0.001$), blood glucose ($r = -0.709$, $P<0.001$), and serum leptin concentrations ($r = -0.471$, $P<0.01$) (Fig. 7B) for both the fasted and fed gerbils, and were nearly negatively correlated with PHA response ($r = -0.409$, $P = 0.073$) in the immunochallenged groups. However, corticosterone concentrations were no longer correlated with body fat mass ($r = -0.388$, $P>0.05$) and blood glucose levels ($r = 0.248$, $P>0.05$), and nearly negatively correlated with leptin concentrations ($r = -0.415$, $P = 0.069$) in the fed groups, while as for the fasted groups, corticosterone concentrations were still negatively correlated with body fat mass ($r = -0.701$, $P<0.01$) and blood glucose levels ($r = -0.647$, $P<0.01$), but were not correlated with leptin concentrations ($r = -0.129$, $P>0.05$).

4. Discussion

Our data clearly showed that three-day fasting suppressed T cell-mediated immunity in female Mongolian gerbils. Immune organs such as thymus and spleen were also reduced in the fasted gerbils compared with the fed controls. WBC in the fed but not the fasted gerbils increased significantly after PHA injection, which suggested the inability of WBC to proliferate upon immunochallenge in fasted gerbils. Additionally, lower serum leptin and higher corticorsterone concentrations might contribute to immunosuppression in fasted gerbils.

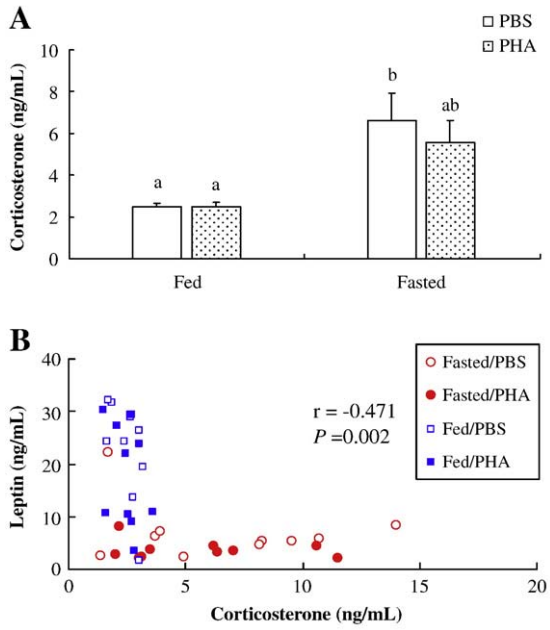

Fig. 7. Corticosterone concentrations (A) and the correlations of corticosterone with serum leptin concentrations (B) in female Mongolian gerbils after three-day fasting. Values are means ± SE ($n = 10$). Serum corticosterone concentrations were higher in the fasted groups than in the fed groups, and were negatively correlated with leptin concentrations. Different letters (a or b) above hatched bars and solid bars indicate significant differences ($P<0.05$). Note: ○ = Fasted/PBS group; ● = Fasted/PHA group; □ = Fed/PBS group, ■ = Fed/PHA group.

4.1. Body fat mass, blood glucose and immune function

Energy availability including body fat mass and blood glucose was greatly reduced after three-day fasting, which might be responsible for immunosuppression in the fasted gerbils. Adipose tissues not only function as endocrine and immune organs (Ahima and Flier, 2000a; Matarese and Cava, 2004; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al., 2007), but also provide energy for expensive physiological processes including immune function (Demas et al., 1997; Moret and Schmid-Hempel, 2000; Martin et al., 2003; Demas, 2004; Trayhurn, 2005). Additionally, Houston et al. (2007) have shown that animals with low energy reserves would choose to allocate less energy to immune defense than animals with higher reserves. Therefore, reductions in body fat can impair immunity (Chandra, 1996; Demas et al., 2003). In face of energy crisis, small mammals must divert energy from less critical physiological functions such as immunity to the most important system such as the brain and heart for survival (Lochmiller and Deerenberg, 2000). Suppression of T cell-mediated immunity in fasted gerbils in the present experiment is not contradictory with previous field results that humoral immunity is higher in winter than in summer (Zhang and Wang, 2006). In fact, body mass and body fat mass of gerbils are higher in winter than in summer, and the effect of acute fasting and chronic decreased food availability on immune function may be different. Generally, acute fasting suppresses immune function (Wing and Young, 1980; Lord et al., 1998; Nakamura et al., 2001), while chronic decreased food availability can enhance immune function (Effros et al., 1991; Jolly, 2004; Zysling et al., 2009). Taken together, immune function might track body fat rather than food availability.

Glucose is required for mounting an immune response (Matarese and Cava, 2004; Maciver et al., 2008). Thus, decreased glucose availability induced by 2-deoxy-glucose can suppress immunity (Zysling and Demas, 2007; Martin et al., 2008), and humoral immunity can be significantly improved by drinking glucose water in food restricted poults (Hadri et al., 2004). Similarly, T cell-mediated immune response in the fasted gerbils can be partially reversed by drinking 10% glucose water (unpublished data). Therefore, low glucose levels might also contribute to immunosuppression in the fasted gerbils.

4.2. Leptin and immune function

Leptin can regulate T cell-mediated immunity directly (Matarese et al., 2005; Lam and Lu, 2007; Steiner and Romanovsky, 2007), and it also acts as a trophic factor for T lymphocytes (Fantuzzi, 2006). Previously, Lord et al. (1998) have shown that exogenous leptin administration can reverse starvation-induced immunosuppression in fasted mice. Likewise, leptin can also protect mice from starvation-induced lymphoid atrophy and increase thymic cellularity (Howard et al., 1999). All these studies suggest that low leptin concentrations impair immune response (Flier, 1998). Moreover, leptin is also a starvation signal and mediates the adaptation to fasting (Ahima et al., 1996; Ahima and Flier, 2000b; Zhan et al., 2009). In the present study, we observed that the fasted gerbils had significantly lower leptin levels than those of the fed controls. Taken together, the reduced levels of leptin during fasting might be a starvation signal indicating that the body has depleted its energy reserves, and hence it appears to be a signal to shut down all the non-essential physiological functions including immune response (Fantuzzi, 2006).

4.3. Corticosterone and immune function

The major response to stress such as fasting is the increase of corticosterone (Ahima et al., 1996; Sapolsky et al., 2000). Similarly, we also observed that corticosterone increased significantly in the fasted gerbils compared with the fed gerbils, which indicated the activation of the hypothalamic–pituitary–adrenal (HPA) axis during fasting. The nearly negative correlation between PHA response and corticosterone implied that it might have a suppressive role on T cell-mediated immunity in fasted gerbils. Additionally, corticosterone concentrations were negatively correlated with body fat mass and glucose levels for both the fed and fasted gerbils. Interestingly this negative relationship disappeared in the fed groups but not in the fasted groups. This result suggests that corticosterone participates in mobilizing energy reserves to meet the energy requirement during fasting.

5. Conclusion

Three-day fasting leads to activation of the HPA axis, and energy reserves are mobilized to meet energy demands. Wild gerbils may use low leptin levels as a starvation signal to adapt to acute fasting. Suppression of T cell-mediated immunity during fasting might be an energy-saving strategy for gerbils to increase survival in face of food shortage. The interaction between immunity and leptin or other hormones still remains to be elucidated in Mongolian gerbils.

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