Food Restriction and Refeeding Have No Effect on Cellular and Humoral Immunity in Mongolian Gerbils (*Meriones unguiculatus*)

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ABSTRACT

Small mammals in the temperate area often face fluctuations in food availability. Changes in food availability may have a great influence on an animals' immunity, which is important to their survival. We tested the hypothesis that cellular and humoral immunity would be suppressed by food restriction and restored to control levels by refeeding in Mongolian gerbils *Meriones unguiculatus*. Forty adult male gerbils were randomly divided into food-restricted (80% of baseline food intake) and food ad lib. groups. Similarly, another 40 adult male gerbils were also randomly assigned to two groups: a group for which food was restricted for 36 d and then provided ad lib. and a group that was continuously fed ad lib. Half of the gerbils in each group were injected with phytohemagglutinin (PHA) and keyhole limpet hemocyanin solution to assess cellular and humoral immunity, respectively; the others were injected with sterile saline as control groups. Food-restricted gerbils had significantly lower body mass, body fat mass, dry thymus mass, wet and dry spleen mass, and serum leptin levels than those of the controls, whereas refeeding restored these parameters to the controls. Both food restriction and refeeding had no significant effect on PHA response indicative of cellular immunity, immunoglobulin G and immunoglobulin M concentrations, and white blood cells. We also found that food restriction decreased corticosterone levels in food-restricted gerbils, while refeeding increased corticosterone levels in refed gerbils compared with the controls. Our results suggest that cellular and humoral immunity were not affected by food restriction and refeeding in gerbils.

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Introduction

The immune system protects animals against environmental pathogens, and hence it plays an important role in their survival and fitness (Sheldon and Verhulst 1996; Owens and Wilson 1999). However, immune function is influenced by many factors, such as food availability, which fluctuates seasonally in the temperate zone (Nelson and Demas 1996; Kaminogawa and Nanno 2004; Schaible and Kaufmann 2007). Many investigators have examined the effect of reduced food availability (i.e., food or caloric restriction) on immune function in small rodents, but their results are often inconsistent.

Some researchers have found that food restriction inhibits immunity. Cellular immunity was suppressed in food-restricted deer mice (*Peromyscus maniculatus*; Demas and Nelson 1998) and food-restricted short-day Siberian hamsters (*Phodopus sungorus*; Bilbo and Nelson 2004). Similarly, male rat-like hamsters (*Cricetulus triton*) whose mothers were food restricted during gestation had lower humoral immune responses than controls (Liang et al. 2004). Martin et al. (2007, 2008) also demonstrated that immunological memory was compromised and spleen-derived antibody-producing B cells were reduced by food restriction in deer mice. Additionally, food restriction impaired mitogen-induced T-cell proliferation and humoral immunity in mice (Pocino et al. 1987; Rogers et al. 2008; Ishikawa et al. 2009). Calorie-restricted hosts are more susceptible to infection by intact pathogens than their fed counterparts (Kristan 2008). On the contrary, other investigators have demonstrated that food or caloric restriction enhances immune function (Pahlavani et al. 2002; Jolly 2004; Ritz and Gardner 2006). Food restriction retarded the age-related decline of several immunological indexes (Pahlavani 2000), including the antibody production associated with influenza vaccination (Effros et al. 1991). Zysling et al. (2009) have also found that humoral immunity was enhanced in food-restricted short-day Siberian hamsters. Moreover, food restriction was able to restore impaired immune response in overweight rats (Lamas et al. 2004). Caloric restriction can also enhance cellular immune response in adult overweight men and women (Ahmed et al. 2009). Further research is required to clarify these discrepant results. There is also little information about the effect of food restriction and then refeeding on immune function.

Mongolian gerbils *Meriones unguiculatus* are small seasonally breeding, food-hoarding, nonhibernating, granivorous rodents living in the desert and semiarid regions of Mongolia and

Table 1: Effect of food restriction and refeeding on body mass and body composition in male Mongolian gerbils

	Fed-1/Saline	Fed-1/IC	FR/Saline	FR/IC	FR	IC	FR × IC
Sample size	10	10	10	9	...	...	...
Initial body mass (g)	69.2 ± 1.4	70.2 ± 1.4	70.2 ± 1.4	69.3 ± 1.5	...	...	...
Final body mass (g)	74.1 ± 2.6[a]	74.7 ± 2.6[a]	54.1 ± 2.6[b]	59.5 ± 2.7[b]	<.001	NS	NS
Wet carcass mass (g)	57.1 ± 2.1[a]	57.3 ± 2.1[a]	41.2 ± 2.1[b]	45.2 ± 2.3[b]	<.001	NS	NS
Dry carcass mass (g)	23.7 ± 1.6[a]	23.0 ± 1.6[ab]	16.9 ± 1.6[c]	16.9 ± 1.7[bc]	<.001	NS	NS
Body water mass (g)	33.4 ± 1.1[a]	34.3 ± 1.1[a]	24.4 ± 1.1[b]	28.3 ± 1.2[b]	<.001	<.05	NS
Water content (water/wet carcass; %)	58.7 ± 2.0	60.1 ± 2.0	60.3 ± 2.0	62.7 ± 2.1	NS	NS	NS
Fat-free dry carcass (g)	12.8 ± .56	13.0 ± .6	11.0 ± .6	11.0 ± .6	<.01	NS	NS
Body fat mass (g)	10.9 ± 1.2[a]	9.95 ± 1.2[ab]	5.86 ± 1.4[b]	5.87 ± .8[b]	<.001	NS	NS
Fat content (fat/wet carcass mass; %)	19 ± 2	17 ± 2	13 ± 2	13 ± 2	<.01	NS	NS
	Fed-2/Saline	Fed-2/IC	FR-r/Saline	FR-r/IC	FR-r	IC	FR-r × IC
Sample size	10	10	9	9	...	...	...
Initial body mass (g)	69.2 ± 1.4	71.4 ± 1.3	70.5 ± 1.6	72.0 ± 1.6	...	...	...
Body mass on RF_0 (g)	76.9 ± 3.5[a]	76.8 ± 1.8[a]	57.4 ± 4.2[b]	57.2 ± 2.8[b]	<.001	...	...
Final body mass (g)	81.2 ± 3.3	78.5 ± 3.3	81.8 ± 3.5	82.3 ± 3.5	NS	NS	NS
Wet carcass mass (g)	63.1 ± 2.8	60.9 ± 2.8	63.6 ± 2.9	63.6 ± 2.9	NS	NS	NS
Dry carcass mass (g)	32.7 ± 2.3	27.9 ± 2.3	30.7 ± 2.4	29.9 ± 2.4	NS	NS	NS
Body water mass (g)	30.4 ± 1.1	33.0 ± 1.1	32.9 ± 1.2	33.6 ± 1.2	NS	NS	NS
Water content (water/wet carcass; %)	49.6 ± 2.0	54.7 ± 2.0	52.1 ± 2.1	53.7 ± 2.1	NS	NS	NS
Fat-free dry carcass (g)	14.5 ± .6	14.2 ± .6	14.3 ± .6	14.6 ± .6	NS	NS	NS
Body fat mass (g)	18.3 ± 1.8	13.7 ± 1.8	16.4 ± 1.9	15.3 ± 1.9	NS	NS	NS
Fat content (fat/wet carcass mass; %)	27.4 ± 2.1	22.0 ± 2.1	25.3 ± 2.2	23.1 ± 2.2	NS	NS	NS

Note. Data are mean ± SE. 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. FR = food restriction; FR-r = food restriction and refeeding; RF_0 = initial day of refeeding; IC = immunochallenge; FR × IC = interaction of food restriction × immunochallenge; FR-r × IC = interaction of refeeding × immunochallenge; NS = not significant.

northern China (Walker 1968). Food resources fluctuate dramatically throughout the year, and periodic food shortage is common to wild gerbils. Such harsh habitats have also made these gerbils a good model to study special adaptive strategies to the environment (Xia et al. 1982; Zhong et al. 1985; Liu et al. 2007; Zhang and Wang 2007). Fieldwork has shown that humoral immunity in gerbils was higher in winter than in summer (Zhang and Wang 2006). Our laboratory studies have demonstrated that humoral immunity was unresponsive to low-protein diet (Chen et al. 2007), photoperiod, low temperature, and housing density (Li 2005), whereas cellular immunity was inhibited after a 3-d fasting in gerbils (Xu and Wang 2010). Additionally, gerbils decreased body mass, body fat mass, and leptin levels after food restriction (Zhang and Wang 2008). Understanding how immune function will vary in the face of fluctuations in food resources in Mongolian gerbils can help us understand their immune adaptive strategies to the environmental changes. It is also helpful for us to understand their distribution and population dynamics in the field from the immune perspective. In this study, we tested the hypothesis that food restriction would suppress and refeeding would restore the cellular and humoral immunity in Mongolian gerbils.

Material and Methods

Animals and Experimental Design

All animal procedures were licensed under the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Adult male gerbils used in this study were the offspring of Mongolian gerbils in our laboratory colony. After weaning, the animals were housed individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding under a constant photoperiod of 16L : 8D and temperature of 23° ± 1°C. Standard rat pellet chow (Beijing KeAo Feed, Beijing) and water were provided ad lib. The macronutrient composition of the diet was 6.2% crude fat, 18% crude protein, 23.1% neutral fiber, 5% crude fiber, 12.5% acid detergent fiber, and 10.0% ash, and the caloric value was 17.5 kJ/g. According to the preliminary experiment, one gerbil began to die after 10 d 60% food restriction (60% of baseline food intake fed ad lib.; S. H. Wu, unpublished data). Considering gerbils' welfare, we decided that gerbils would be subjected to an 80% food-restricted paradigm in our study. After body mass stabilized, 80 male gerbils (aged 7–12 mo, weighing 61.0–76.0 g) were selected. Forty male gerbils were randomly divided into food-

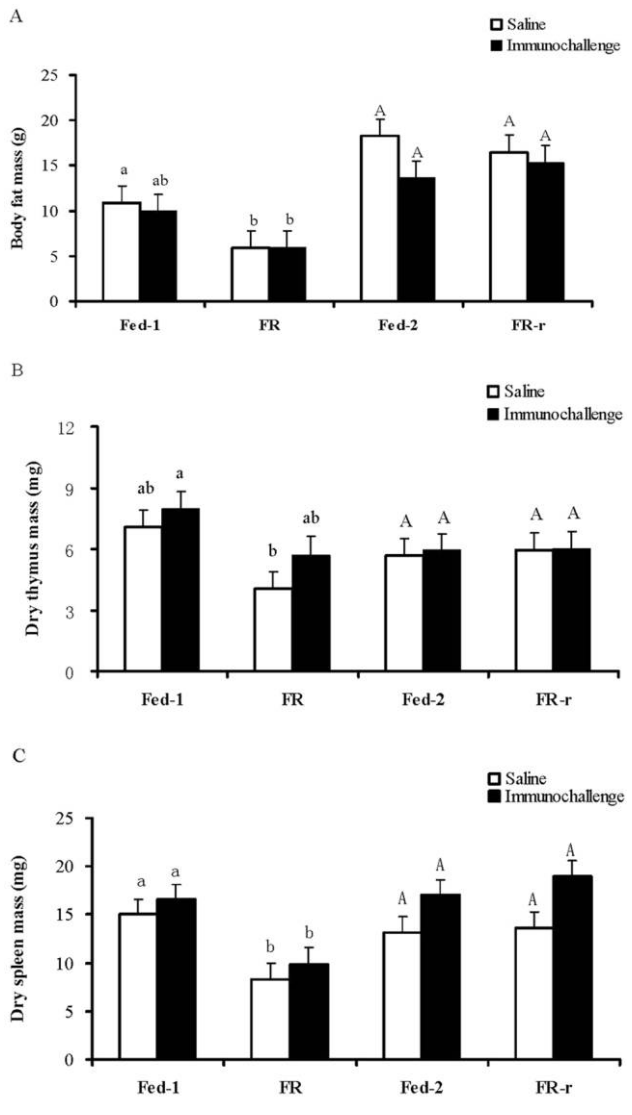

Figure 1. Effect of food restriction and refeeding on body fat mass (*A*), dry thymus mass (*B*), and dry spleen mass (*C*) in male Mongolian gerbils. Values are mean ± SE. Different letters (*a*,*b* or *A*,*B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of phytohemagglutinin and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding.

restricted (FR) for 35 d and fed ad lib. (Fed-1) groups. Similarly, another 40 gerbils were randomly assigned into two groups: food restricted for 36 d and then refed ad lib. (FR-r) and fed ad lib. (Fed-2). Half of the gerbils in each group were immunochallenged (IC) with keyhole limpet hemocyanin (KLH) solution and phytohemagglutinin (PHA; PHA-P, Sigma L-8754) to assess humoral and cellular immunity, respectively; the others were injected with sterile saline as control groups. All gerbils in this study were naive to KLH and PHA. Baseline food intake (g/d) of 40 gerbils used for food restriction was measured for 6 d (once every other day). Average food consumption per day was calculated for each individual, and the restricted food amount was 80% of the baseline food intake. FR_0 and FR_n represented initial day and *n* d of food restriction, respectively. Similarly, RF_0 and RF_n represented the initial day and *n* d of refeeding, respectively. One gerbil in the FR/IC group, one in the FR-r/saline group, and one in the FR-r/IC group died after 26, 27, 29 d of food restriction, respectively. These three animals were not included in the subsequent statistical analysis. All gerbils in FR/saline, FR/IC, FR-r/saline, and FR-r/IC groups consumed all the food that was provided during the food-restricted period.

Cellular Immunity Assays

PHA response is a reliable tool to assess mammalian cellular immunity, which is one arm of the adaptive immune system and is generally responsible for intracellular pathogen control (Nelson and Demas 1996; Smits et al. 1999; Bellocq et al. 2006). PHA response was measured as described previously (Bellocq et al. 2006; Xu and Wang 2010). Specifically, gerbils in Fed-1/saline, Fed-1/IC, FR/saline, and FR/IC groups on FR_{33} and gerbils in Fed-2/saline, Fed-2/IC, FR-r/saline, and FR-r/IC groups on RF_{90} were caught. We then measured the footpad thickness of the left hind foot with a micrometer (Tesa Shopcal) to ±0.01 mm. Immediately thereafter, gerbils in the Fed-1/IC, FR/IC, Fed-2/IC, and FR-r/IC groups were injected subcutaneously with 0.1 mg of PHA dissolved in 0.03 mL of sterile saline (pH 7.4) in the middle of the footpad, while gerbils in the Fed-1/saline, FR/saline, Fed-2/saline, and FR-r/saline groups were injected subcutaneously with 0.03 mL of sterile saline (pH 7.4) in the middle of the footpad. Six hours, 24 h, and 48 h after injection, we measured footpad thickness. The PHA response (i.e., cellular immunity) was calculated as the difference between pre- and postinjection measurements divided by initial footpad thickness (PHA response = (post-PHA − pre-PHA)/pre-PHA). Six measures of footpad thickness were taken to obtain the value of each gerbil (Bellocq et al. 2006; Xu and Wang 2010). Only the 6 h data were included in the results because they were representative of the maximal response.

Humoral Immunity Assays

Humoral immunity is another arm of the adaptive immune system, and it primarily controls extracellular pathogens (Nelson and Demas 1996). Humoral immunity is usually evaluated by antibody production immunochallenged with a specific antigen such as KLH (Demas et al. 2003; Zysling and Demas 2007; Zysling et al. 2009). According to Duffy et al. (2000), it is viable to perform PHA and KLH injection at 1-wk intervals to assess cellular and humoral immunity. Specifically, gerbils in Fed-1/IC and FR/IC groups on FR_{25} and gerbils in Fed-2/IC and FR-r/IC groups on RF_{82} received a single subcutaneous injection of 100 μg of KLH suspended in 0.1 mL sterile saline in order to evaluate humoral immunity, while gerbils in Fed-1/saline, FR/saline, Fed-2/saline, and FR-r/saline groups received 0.1 mL sterile saline. After 5 d of KLH injection, animals

Table 2: Effect of food restriction on mean wet organ mass in male Mongolian gerbils

	Fed-1/Saline	Fed-1/IC	FR/Saline	FR/IC	FR	IC	FR × IC
Sample size	10	10	9	9	...	...	...
Brain (mg)	1,161 ± 20	1,118 ± 21	1,158 ± 22	1,156 ± 20	NS	NS	NS
Interscapular brown adipose tissue (mg)	170 ± 20	167 ± 20	164 ± 22	187 ± 20	NS	NS	NS
Heart (mg)	271 ± 7	274 ± 7	258 ± 8	261 ± 7	NS	NS	NS
Lungs (mg)	388 ± 24	390 ± 24	422 ± 26	421 ± 24	NS	NS	NS
Thymus (mg)	21 ± 3	23 ± 3	20 ± 4	22 ± 3	NS	NS	NS
Liver (mg)	2,082 ± 182	2,131 ± 185	1,913 ± 201	2,056 ± 180	NS	NS	NS
Spleen (mg)	52 ± 7	58 ± 7	34 ± 7	37 ± 6	<.05	NS	NS
Kidneys (mg)	560 ± 19	589 ± 19	550 ± 21	537 ± 19	NS	NS	NS
Adrenal glands (mg)	40 ± 2	41 ± 2	37 ± 3	38 ± 2	NS	NS	NS
Stomach with contents (mg)	1,071 ± 83	1,328 ± 84	1,167 ± 91	1,175 ± 82	NS	NS	NS
Stomach (mg)	427 ± 16[b]	423 ± 16[b]	568 ± 17[a]	588 ± 15[a]	<.001	NS	NS
Small intestine with contents (mg)	2,012 ± 95	2,111 ± 96	1,813 ± 105	1,974 ± 94	NS	NS	NS
Small intestine (mg)	783 ± 71	823 ± 72	882 ± 79	931 ± 71	NS	NS	NS
Cecum with contents (mg)	1,292 ± 87	1,235 ± 88	1,244 ± 96	1,294 ± 86	NS	NS	NS
Cecum (mg)	295 ± 19	300 ± 19	310 ± 21	336 ± 19	NS	NS	NS
Colon with contents (mg)	762 ± 74	828 ± 76	993 ± 82	981 ± 74	NS	NS	NS
Colon (mg)	390 ± 19[b]	413 ± 19[bc]	510 ± 21[a]	483 ± 19[ac]	<.01	NS	NS
Total alimentary tract (mg)	1,895 ± 98[b]	1,958 ± 99[ab]	2,270 ± 108[ab]	2,338 ± 97[a]	<.01	NS	NS
Epididymis (mg)	168 ± 19	190 ± 19	176 ± 21	133 ± 19	NS	NS	NS
Testes (mg)	781 ± 65	739 ± 66	900 ± 72	779 ± 65	NS	NS	NS
Seminal vesical (mg)	310 ± 37[ac]	349 ± 38[a]	138 ± 41[bc]	78 ± 37[b]	<.001	NS	NS

Note. Data are mean ± SE. 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. FR = food restriction; IC = immunochallenge; FR × IC = interaction of food restriction × immunochallenge; NS = not significant.

in all the groups were lightly anesthetized with isoflurane (Shandong LiNuo Pharmaceutical), and blood samples (~500 μL) were drawn from the retro-orbital sinus for later measurement of anti-KLH IgM. After another 5 d (i.e., after 10 d of KLH injection), each gerbil was euthanized by CO_2 asphyxiation, and trunk blood was collected for measurements of anti-KLH immunoglobulin G (IgG), WBC, leptin, and corticosterone. These sampling periods were chosen to capture peak immunoglobulin M (IgM; 5 d after KLH injection) and IgG (10 d after KLH injection) levels. 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 the samples were centrifuged at 4°C for 30 min at 4,000 rpm. Sera were collected and stored in polypropylene microcentrifuge tubes at −80°C until assayed.

Enzyme-linked immunosorbent assay (ELISA) was used to assess serum IgM and IgG concentrations (Demas et al. 2003; Zysling and 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 with 200 μL phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS-T, pH 7.4) three times, then blocked with 5% nonfat dry milk in PBS-T overnight at 4°C to reduce nonspecific binding, and then 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 repeatedly KLH-challenged gerbils, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naive 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 three times. Secondary antibody (alkaline-phosphatase-conjugated antimouse IgG diluted 1 : 2,000 with PBS-T; alkaline-phosphatase-conjugated antimouse 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; 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 adding 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- and intra-assay variability, the mean OD for each sample will be expressed as a ratio of its plate-positive-control OD for statistical analysis (Demas et al. 2003; Zysling and Demas 2007).

Table 3: Effect of food restriction on mean dry organ mass in male Mongolian gerbils

	Fed-1/Saline	Fed-1/IC	FR/Saline	FR/IC	FR	IC	FR × IC
Sample size	10	10	10	9	...	...	...
Heart (mg)	68 ± 3^{ab}	75 ± 3^{a}	57 ± 3^{b}	61 ± 3^{b}	<.01	NS	NS
Lungs (mg)	91 ± 5	89 ± 5	81 ± 5	92 ± 5	NS	NS	NS
Thymus (mg)	6 ± 1^{ab}	7 ± 1^{a}	4 ± 1^{b}	5 ± 1^{ab}	<.05	NS	NS
Liver (mg)	653 ± 39^{ab}	736 ± 38^{a}	510 ± 39^{b}	608 ± 41^{ab}	<.01	<.05	NS
Spleen (mg)	15 ± 1^{a}	16 ± 1^{a}	7 ± 1^{b}	9 ± 1^{b}	<.001	NS	NS
Kidneys (mg)	138 ± 5^{ab}	151 ± 5^{a}	130 ± 5^{b}	137 ± 5^{ab}	NS	<.05	NS
Adrenal glands (mg)	16 ± 1	16 ± 1	13 ± 1	13 ± 1	<.05	NS	NS
Stomach (mg)	102 ± 5^{b}	108 ± 5^{ab}	110 ± 5^{ab}	122 ± 5^{a}	<.05	<.05	NS
Small intestine (mg)	117 ± 15	119 ± 14	130 ± 15	146 ± 15	NS	NS	NS
Cecum (mg)	53 ± 5	48 ± 4	48 ± 5	54 ± 5	NS	NS	NS
Colon (mg)	90 ± 5	94 ± 5	99 ± 5	101 ± 5	NS	NS	NS
Total alimentary tract (mg)	360 ± 20	370 ± 20	387 ± 20	423 ± 21	NS	NS	NS
Epididymis (mg)	39 ± 4	45 ± 4	35 ± 4	30 ± 4	<.05	NS	NS
Testes (mg)	138 ± 10	136 ± 10	122 ± 10	121 ± 11	NS	NS	NS
Seminal vesical (mg)	83 ± 10^{a}	98 ± 10^{a}	36 ± 10^{b}	22 ± 11^{b}	<.001	NS	NS

Note. Data are mean ± SE. 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 as the covariate and Bonferroni post hoc tests. FR = food restriction; IC = immunochallenge; FR × IC = interaction of food restriction × immunochallenge; NS = not significant.

Body Composition

Immune organs such as thymus and spleen are indirect immunological parameters indicative of immune function (Savino and Dardenne 2000; Calder and Kew 2002; Smith and Hunt 2004). Thymus is a central immune organ that is crucial for primary T-cell development (Savino and Dardenne 2000), and a larger spleen represents stronger immunity (Smith and Hunt 2004). In addition, adipose tissue is now no longer regarded as inert energy depots but has been recently considered as an important endocrine and immune organ (Fantuzzi 2005; Trayhurn 2005; Schäffler et al. 2007). Body composition was measured as described previously (Li and Wang 2005; Xu and 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 with contents (i.e., stomach, small intestine, cecum, and colon)—were dissected and weighed (±1 mg). 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 dry carcass mass was the water mass of the 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 (Xu and Wang 2010).

White Blood Cell Assays

Total white blood cells (WBCs; or leukocytes), which are fundamental to immune responses against pathogens, are useful to estimate overall health (Calder and Kew 2002). 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 WBCs 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, pp. 91–94).

Serum Leptin Assays

Leptin is an adipocyte-derived cytokine-like hormone and is proportional to adipose tissue (Zhang et al. 1994; Pond 1996; Ahima and Flier 2000). Apart from its regulatory role in energy homeostasis, leptin also plays an important role in immunity, such as its direct regulatory role in T-cell immune response (Fantuzzi and Faggioni 2000; Faggioni et al. 2001; Matarase et al. 2005; Lago et al. 2007; Lam and Lu 2007). Serum leptin concentrations were determined by radioimmunoassay (RIA) with a ^{125}I multispecies kit (XL-85K, Linco Research, St. Charles, MO). The range detected by this assay was 1.0–50 ng/mL when using a 100-μL sample (see manufacturer's instructions for multispecies leptin RIA kit). Inter- and intra-assay variabilities for leptin RIA were <8.7% and 3.6%, respectively (Zhao and Wang 2006).

Serum Corticosterone Assays

Stressful conditions such as food restriction usually stimulate the hypothalamic-pituitary-adrenal axis, and hence secretion of glucocorticoids such as corticosterone increases, which can sup-

Table 4: Effect of refeeding on mean wet organ mass in male Mongolian gerbils

	Fed-2/Saline	Fed-2/IC	FR-r/Saline	FR-r/IC	FR-r	IC	FR-r × IC
Sample size	10	10	10	9	...	...	...
Brain (mg)	1,177 ± 18	1,139 ± 18	1,178 ± 19	1,157 ± 19	NS	NS	NS
Interscapular brown adipose tissue (mg)	280 ± 35	229 ± 35	277 ± 37	231 ± 37	NS	NS	NS
Heart (mg)	313 ± 9^{ab}	347 ± 9^{a}	303 ± 9^{b}	316 ± 9^{ab}	<.05	<.05	NS
Lungs (mg)	411 ± 17	459 ± 18	421 ± 18	393 ± 18	NS	NS	<.05
Thymus (mg)	25 ± 4	25 ± 4	25 ± 4	26 ± 4	NS	NS	NS
Liver (mg)	2,902 ± 85	3,076 ± 86	2,904 ± 90	3,028 ± 90	NS	NS	NS
Spleen (mg)	54 ± 7	70 ± 7	55 ± 8	80 ± 8	NS	<.01	NS
Kidneys (mg)	633 ± 15	668 ± 15	620 ± 16	643 ± 16	NS	NS	NS
Adrenal glands (mg)	41 ± 2	49 ± 2	42 ± 2	44 ± 2	NS	<.05	NS
Stomach with contents (mg)	1,749 ± 165	1,528 ± 166	1,929 ± 174	1,702 ± 174	NS	NS	NS
Stomach (mg)	481 ± 20	477 ± 20	485 ± 21	524 ± 21	NS	NS	NS
Small intestine with contents (mg)	2,317 ± 99	2,281 ± 100	2,162 ± 105	2,252 ± 105	NS	NS	NS
Small intestine (mg)	723 ± 53^{ab}	683 ± 54^{b}	798 ± 56^{ab}	907 ± 56^{a}	<.05	NS	NS
Cecum with contents (mg)	1,237 ± 78	1,196 ± 78	1,333 ± 82	1,284 ± 82	NS	NS	NS
Cecum (mg)	276 ± 17	303 ± 17	299 ± 18	312 ± 18	NS	NS	NS
Colon with contents (mg)	953 ± 50	940 ± 50	1,017 ± 52	951 ± 52	NS	NS	NS
Colon (mg)	435 ± 23	443 ± 23	456 ± 24	468 ± 24	NS	NS	NS
Total alimentary tract (mg)	1,915 ± 83	1,906 ± 83	2,038 ± 87	2,210 ± 87	<.05	NS	NS
Epididymis (mg)	220 ± 13	230 ± 13	252 ± 14	244 ± 14	NS	NS	NS
Testes (mg)	921 ± 37	990 ± 38	1,036 ± 39	1,015 ± 39	NS	NS	NS
Seminal vesical (mg)	327 ± 36	291 ± 36	415 ± 38	435 ± 38	<.01	NS	NS

Note. Data are mean ± SE. 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. FR-r = food restriction and refeeding; IC = immunochallenge; FR-r × IC = interaction of refeeding × immunochallenge; NS = not significant.

press immune function (Sapolsky et al. 2000; Marketon and Glaser 2008). Serum corticosterone concentrations were determined by rat corticosterone ELISA kit (HR083, RapidBio Lab, Calabasas, CA). The lowest level of corticosterone that could be detected by this assay was 1.0 nmol/L when using a 125-μL sample. The detailed procedure followed the manufacturer's instructions of the rat corticosterone ELISA kit (Xu and Wang 2010).

Statistical Analysis

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL). Before all statistical analyses, data were examined for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. The ratio values, including PHA response and body fat content, were subjected to arcsine transformation. The differences of body mass before and after immune challenge were analyzed by one-way ANOVA and two-way ANOVA (food treatment × immunochallenge) followed by Bonferroni post hoc tests, respectively. Group differences in wet organ mass with body mass as the covariate and dry organ mass with dry carcass mass as the covariate were analyzed by a two-way ANCOVA followed by Bonferroni post hoc tests. Group differences in other parameters (body compositions, PHA response, IgM and IgG concentrations, WBCs, leptin and corticosterone concentrations) were analyzed by a two-way ANOVA followed by Bonferroni post hoc tests. Significant group differences were 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, IgG and IgM concentrations with leptin and corticosterone in the IC groups (i.e., Fed-1/IC, FR/IC, Fed-2/IC, and FR-r/IC groups). The correlation of serum leptin levels with body fat mass was also calculated for all the gerbils. Results were expressed as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

Results

Body Mass

On FR_0, body mass among the eight groups did not differ significantly ($F_{7,69} = 0.32$, $P = 0.943$). Body mass in the FR/saline and FR/IC groups was significantly lower than that of the Fed-1/saline and Fed-1/IC groups from FR_4 ($F_{3,35} = 4.05$, $P < 0.05$) to FR_{35} ($F_{3,35} = 16.03$, $P < 0.001$). Compared with body mass on FR_0 (FR/saline: 70.2 ± 1.5 g; FR/IC: 69.3 ± 1.8 g), gerbils in FR/saline (54.1 ± 2.6) and FR/IC (59.5 ± 2.7) groups lost 16.2 ± 2.8 g and 9.8 ± 1.3 g after 35 d of food restriction, respectively (Table 1). Body mass in the FR-r/saline and FR-r/IC groups was also significantly lighter than in the Fed-2/saline and Fed-2/IC groups from FR_3 ($F_{3,34} = 2.95$, $P = 0.046$) to FR_{36} ($F_{3,34} = 12.83$, $P < 0.001$). Compared with

Table 5: Effect of refeeding on mean dry organ mass in male Mongolian gerbils

	Fed-2/Saline	Fed-2/IC	FR-r/Saline	FR-r/IC	FR-r	IC	FR-r × IC
Sample size	10	10	10	9	...	...	...
Heart (mg)	74 ± 3	84 ± 3	73 ± 3	76 ± 3	NS	<.05	NS
Lungs (mg)	92 ± 5	113 ± 5	99 ± 6	94 ± 6	NS	NS	<.05
Thymus (mg)	6 ± 1	6 ± 1	7 ± 1	7 ± 1	NS	NS	NS
Liver (mg)	838 ± 43	929 ± 43	945 ± 45	977 ± 45	NS	NS	NS
Spleen (mg)	14 ± 2	18 ± 2	14 ± 2	20 ± 2	NS	<.05	NS
Kidneys (mg)	154 ± 5	163 ± 5	158 ± 5	165 ± 5	NS	NS	NS
Adrenal glands (mg)	14 ± 1[b]	18 ± 1[a]	16 ± 1[ab]	15 ± 1[ab]	NS	NS	<.05
Stomach (mg)	115 ± 5	113 ± 5	118 ± 5	125 ± 5	NS	NS	NS
Small intestine (mg)	135 ± 19[ab]	122 ± 19[b]	199 ± 19[a]	181 ± 19[ab]	<.01	NS	NS
Cecum (mg)	42 ± 5	52 ± 5	57 ± 5	57 ± 5	<.05	NS	NS
Colon (mg)	96 ± 8	102 ± 8	94 ± 8	107 ± 8	NS	NS	NS
Total alimentary tract (mg)	388 ± 24	389 ± 24	467 ± 25	471 ± 25	<.01	NS	NS
Epididymis (mg)	48 ± 3	51 ± 3	57 ± 3	55 ± 3	<.05	NS	NS
Testes (mg)	153 ± 7	168 ± 7	174 ± 7	169 ± 7	NS	NS	NS
Seminal vesical (mg)	85 ± 12	81 ± 12	116 ± 12	127 ± 12	<.01	NS	NS

Note. Data are mean ± SE. 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 as the covariate and Bonferroni post hoc tests. FR = food restriction; FR-r = food restriction and refeeding; IC = immunochallenge; FR × IC = interaction of food restriction × immunochallenge; FR-r × IC = interaction of refeeding × immunochallenge; NS = not significant.

body mass on RF_0 (FR-r/saline: 70.5 ± 1.6 g; FR-r/IC: 72.0 ± 1.6 g), body mass in the FR-r/saline (57.4 ± 4.2 g) and FR-r/IC (57.2 ± 2.8 g) groups lost 13.1 ± 2.9 g and 14.8 ± 2.5 g after 36 d of food restriction, respectively (Table 1). Body mass in the FR-r/saline and FR-r/IC groups was still lower than that in the Fed-2/saline and Fed-2/IC groups on RF_4 ($F_{3,34} = 3.20$, $P < 0.05$), RF_5 ($F_{3,34} = 3.14$, $P < 0.05$), RF_6 ($F_{3,34} = 2.95$, $P < 0.05$), RF_7 ($F_{3,34} = 3.48$, $P < 0.05$), and RF_8 ($F_{3,34} = 3.27$, $P < 0.05$), whereas body mass among the four groups was no longer different at other time points during the refeeding period. Compared with body mass on RF_0 (FR-r/saline: 57.4 ± 4.2 g, FR-r/IC: 57.2 ± 2.8 g), gerbils in the FR-r/saline (RF_{92}: 81.8 ± 3.5 g) and FR-r/IC (RF_{92}: 82.3 ± 3.5 g) groups gained 25.1 ± 5.0 g and 24.4 ± 5.5 g after 92 d of refeeding, respectively (Table 1).

Body Composition

Food restriction significantly reduced body fat mass by 43.7% ($F_{1,35} = 14.75$, $P < 0.001$; Table 1), dry thymus mass ($F_{1,34} = 5.67$, $P < 0.05$), wet ($F_{1,34} = 5.08$, $P < 0.05$), and dry ($F_{1,34} = 25.12$, $P < 0.001$) spleen mass in the FR groups compared with Fed-1 groups (Fig. 1*A*–1*C*; Tables 2, 3). Wet and dry seminal vesical mass and other dry organ mass—including heart, liver, kidneys, adrenal gland, and epididymis—were also decreased by food restriction; however, food restriction increased wet and dry stomach mass, wet colon mass, and wet total alimentary tract mass (Tables 2, 3). Additionally, body fat mass and all wet and dry organ masses were not affected by the interaction of food restriction × immunochallenge (Tables 1–3). After refeeding, body fat mass ($F_{1,34} = 0.01$, $P > 0.05$; Table 1), wet ($F_{1,33} = 0.04$, $P > 0.05$) and dry ($F_{1,33} = 0.04$, $P > 0.05$) thymus mass, and wet ($F_{1,33} = 0.50$, $P > 0.05$) and dry ($F_{1,33} = 0.42$, $P > 0.05$) spleen mass were no longer different between Fed-2 and FR-r groups (Fig. 1*A*–1*C*; Tables 4, 5). In addition, wet and dry small intestine mass, dry cecum mass, wet and dry total alimentary tract mass, dry epididymis mass, and wet and dry seminal vesical mass were heavier, while wet heart mass was lighter in the FR-r groups than in the Fed-2 groups (Tables 4, 5).

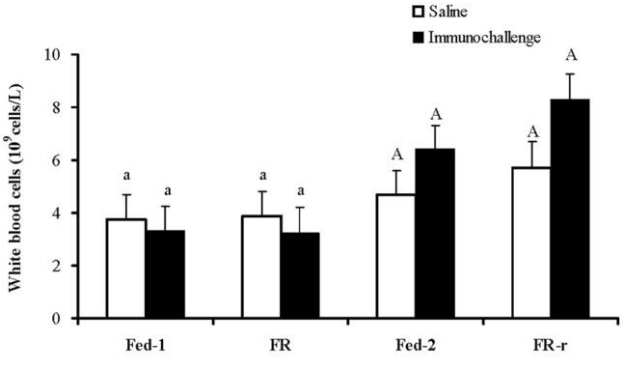

Figure 2. Effect of food restriction and refeeding on white blood cells in male Mongolian gerbils. Different letters (*a*,*b* or *A*,*B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of phytohemagglutinin and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding.

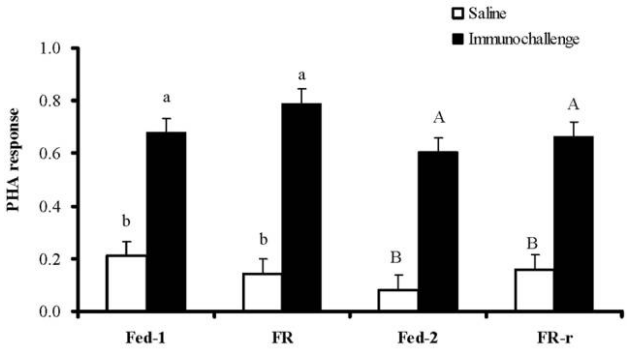

Figure 3. Effect of food restriction and refeeding on phytohemagglutinin (PHA) response in Mongolian gerbils. Values are mean ± SE. Different letters (*a*,*b* or *A*,*B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of PHA and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding.

White Blood Cells

WBCs were not influenced by food restriction ($F_{1,35} < 0.01$, $P > 0.05$), immunochallenge ($F_{1,35} = 0.44$, $P > 0.05$), and the interaction of food restriction × immunochallenge ($F_{1,35} = 0.02$, $P > 0.05$) in the Fed-1/saline, Fed-1/IC, FR/saline, and FR/IC groups (Fig. 3). WBCs in the Fed-2/IC and FR-r/IC groups was significantly increased compared with Fed-2/saline and FR-r/saline groups ($F_{1,34} = 4.39$, $P < 0.05$), whereas it was not affected by refeeding ($F_{1,34} = 2.03$, $P > 0.05$) and the interaction of refeeding × immunochallenge ($F_{1,34} = 0.18$, $P > 0.05$; Fig. 2).

Cellular Immune Response

PHA response in the FR/saline and FR/IC groups was not suppressed by food restriction compared with the Fed-1/saline and Fed-1/IC groups ($F_{1,35} = 0.13$, $P > 0.05$; Fig. 3). Similarly, refeeding did not influence PHA response in the FR-r/saline and FR-r/IC groups compared with the Fed-2/saline and Fed-2/IC groups ($F_{1,34} = 1.86$, $P > 0.05$; Fig. 3).

Humoral Immunity

Food restriction had no significant effect on IgG ($F_{1,35} < 0.001$, $P > 0.05$) and IgM ($F_{1,35} = 4.03$, $P > 0.05$) concentrations in the FR/saline and FR/IC groups compared with the Fed-1/saline and Fed-1/IC groups (Fig. 4). Additionally, refeeding did not affect IgG ($F_{1,34} = 0.27$, $P > 0.05$) and IgM ($F_{1,34} = 1.78$, $P > 0.05$) concentrations in the FR-r/saline and FR-r/IC groups in contrast to the Fed-2/saline and Fed-2/IC groups (Fig. 4).

Serum Leptin Concentrations

Food restriction significantly decreased serum leptin concentrations in the FR/saline and FR/IC groups compared with the Fed-1/saline and Fed-1/IC groups ($F_{1,35} = 30.64$, $P < 0.001$); after refeeding, serum leptin concentrations among the Fed-2/saline, Fed-2/IC, FR-r/saline, and FR-r/IC groups were no longer significantly different ($F_{1,34} = 0.34$, $P > 0.05$; Fig. 5*A*). Leptin concentrations were not affected by immunochallenge ($F_{1,34} = 0.01$, $P > 0.05$) and the interaction of refeeding × immunochallenge ($F_{1,34} = 1.55$, $P > 0.05$). Leptin concentrations were positively correlated with body fat mass ($r = 0.66$, $P < 0.001$) among the Fed-1/saline, Fed-1/IC, FR/saline, and FR/IC groups (Fig. 5*B*) but were not correlated with PHA response ($r = 0.27$, $P > 0.05$) and IgG ($r = 0.06$, $P > 0.05$) and IgM ($r = 0.05$, $P > 0.05$) concentrations in the Fed-1/IC and FR/IC groups. Similarly, Leptin concentrations were positively correlated with body fat mass ($r = 0.40$, $P < 0.05$) in the Fed-2/saline, Fed-2/IC, FR-r/saline, and FR-r/IC groups (Fig. 5*C*) but were not correlated with PHA response ($r = -0.45$, $P = 0.052$) and IgG ($r = 0.34$, $P = 0.149$) and IgM ($r = 0.18$, $P = 0.459$) concentrations in the Fed-2/ICand FR-r/IC groups.

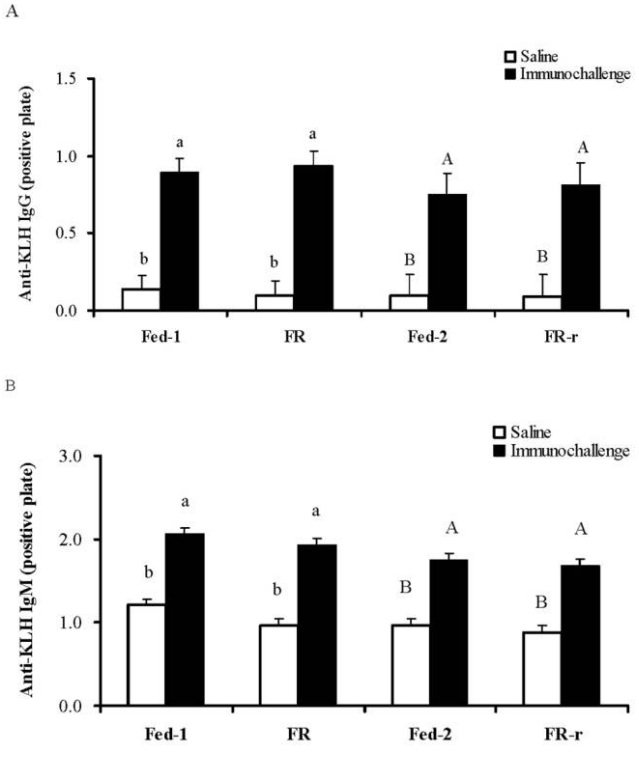

Figure 4. Effect of food restriction and refeeding on serum immunoglobulin G concentrations (*A*) and serum immunoglobulin M concentrations (*B*) in male Mongolian gerbils. Values are mean ± SE. Different letters (*a*,*b* or *A*,*B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of phytohemagglutinin and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding.

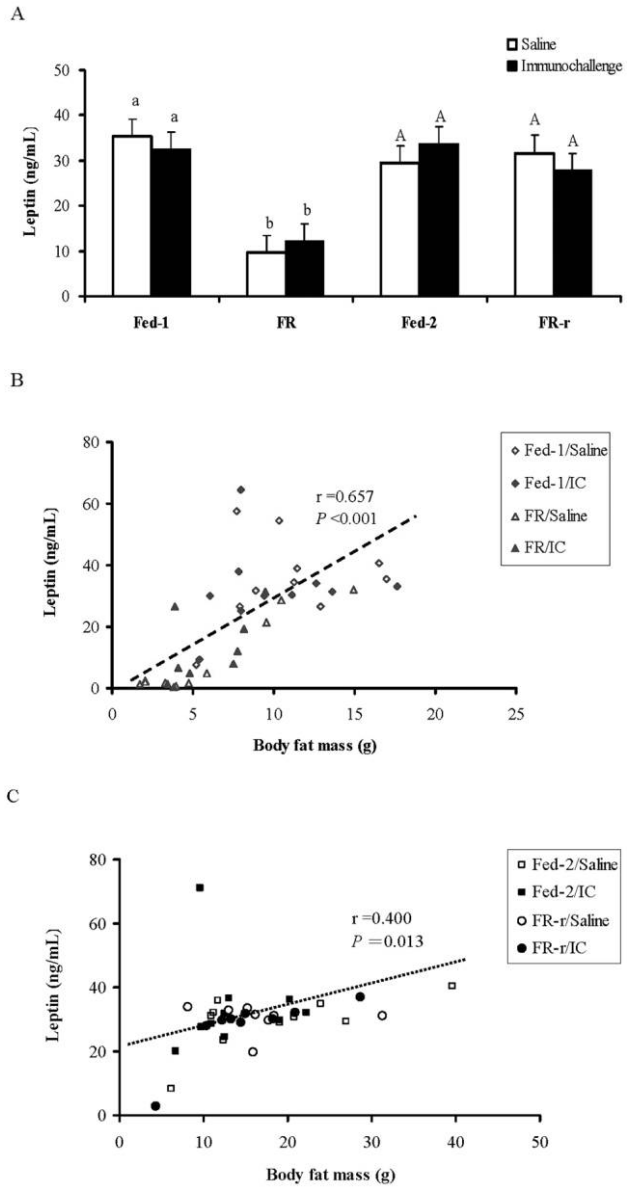

Figure 5. Effect of food restriction and refeeding on leptin concentrations (*A*) and its correlation with body fat mass in the food restriction (FR) and fed ad lib. 1 (Fed-1) groups (*B*) and in the food restricted for 36 d and then refed ad lib. (FR-r) and fed ad lib. 2 (Fed-2) groups (*C*) in Mongolian gerbils. Values are mean ± SE. Different letters (*a,b* or *A,B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of phytohemagglutinin and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding; *IC* = immunochallenged. The dashed line indicates the correlation of body fat mass and leptin levels in the FR and Fed-1 groups, and the dotted line indicates the correlation of body fat mass and leptin levels in the FR-r and Fed-2 groups. *Unfilled diamonds* = Fed-l/saline group; *filled diamonds* = Fed-l/IC group; *unfilled triangles* = FR/saline group; *filled triangles* = FR/IC group; *unfilled squares* = Fed-2/saline group; *filled squares* = Fed-2/IC group; *unfilled circles* = FR-r/saline group; *filled circles* = FR-r/IC group.

Serum Corticosterone Concentrations

Food restriction decreased corticosterone concentrations in the FR/saline and FR/IC groups compared with the Fed-1/saline and Fed-1/IC groups ($F_{1,35} = 19.64$, $P < 0.001$). However, refeeding increased corticosterone concentrations ($F_{1,34} = 8.78$, $P < 0.01$) in FR-r/saline and FR-r/IC groups compared with the Fed-2/saline and Fed-2/IC groups (Fig. 6). Corticosterone concentrations were not correlated with PHA response ($r = 0.05$, $P > 0.05$) and IgG ($r = 0.18$, $P > 0.05$) and IgM ($r = 0.11$, $P > 0.05$) concentrations in the Fed-1/IC and FR/IC groups, and they were also not correlated with PHA response ($r = 0.36$, $P > 0.05$) and IgG ($r = 0.15$, $P > 0.05$) and IgM ($r = 0.14$, $P > 0.05$) concentrations in the Fed-2/ICand FR-r/IC groups.

Discussion

As expected, food restriction decreased body mass, body fat mass, thymus and spleen mass, and leptin levels in Mongolian gerbils, and refeeding restored these parameters to the control levels. However, cellular immunity, humoral immunity, and WBCs were not responsive to food restriction and refeeding. Surprisingly, corticosterone levels decreased in FR groups and increased in FR-r groups compared with the controls.

Food restriction led to thymus and spleen atrophy, suggesting immunosuppression in food-restricted gerbils compared with fed gerbils, and refeeding could recover these immune organs to control levels. However, immune organs are insufficient, and other immunological indexes are required to explain changes of immunity (Calder and Kew 2002; Zhang and Wang 2006). In this study, both cellular and humoral immunity did not vary during food restriction and refeeding in gerbils. Gerbils demonstrated a completely different immune adaptive strategy from other animals whose cellular or humoral immunity was suppressed (Demas and Nelson 1998; Bilbo and Nelson 2004; Liang

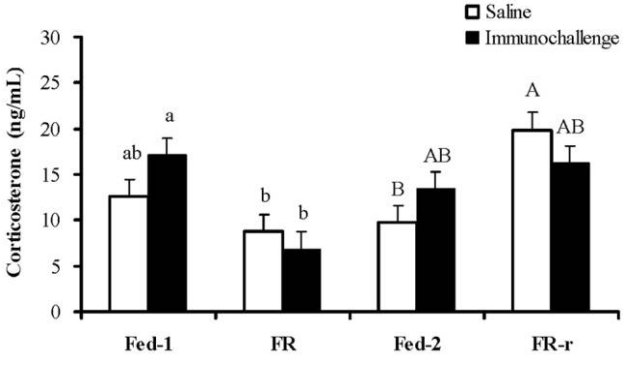

Figure 6. Effect of food restriction and refeeding on corticosterone concentrations in Mongolian gerbils. Values are mean ± SE. Different letters (*a,b* or *A,B*) above white bars and black bars indicate significant differences ($P < 0.05$). *Saline* = injection of phosphate buffered saline and saline; *Immunochallenge* = injection of phytohemagglutinin and keyhole limpet hemocyanin solution; *FR* = food restriction; *FR-r* = food restriction and refeeding.

et al. 2004; Martin et al. 2007, 2008) or enhanced (Effros et al. 1991; Jolly 2004; Zysling et al. 2009) in face of reduced food availability. The reasons may be ascribed to different food-restricted paradigms, the species used, and experimental conditions. For example, Bilbo and Nelson (2004) have shown that cellular immunity was suppressed in short-day but not in long-day food-restricted deer mice, which suggests that photoperiod has an important effect on cellular immunity. In our experiment, all the gerbils were raised in long-day photoperiod. Therefore, further research is required to clarify the role of photoperiod on immunity in gerbils.

It is known that both severe (i.e., fasting) and moderate reductions in energy reserves can suppress immune function (Lord et al. 1998; Demas et al. 2003; Xu and Wang 2010). Houston et al. (2007) have demonstrated that immune function increases steeply with energy reserves when they are very low; however, it tends to level off when energy reserves are higher. Although FR gerbils had significantly lower body fat mass than the controls, the residue energy reserves might still be able to sustain optimal humoral and cellular immunity in food restricted gerbils.

It seems that leptin is a double-edged sword; being either too high or too low are both detrimental to immune function (Flier 1998; Lord et al. 1998; Matarase et al. 2002; Lam and Lu 2007). Although serum leptin levels were reduced in food-restricted gerbils and were restored to control levels in refed gerbils, they might still belonged to the appropriate range to maintain optimal immune responses.

Stress hormones, such as cortisol or corticosterone, usually increase during reduced food availability (Murphy and Wideman 1992; Demas and Nelson 1998; Bilbo and Nelson 2004), which can suppress immune function (Sapolsky et al. 2000; Marketon and Glaser 2008). However, Zysling et al. (2009) found that serum cortisol levels were reduced in food-restricted Siberian hamsters compared with fed controls. We also observed that serum corticosterone concentrations decreased in food-restricted gerbils compared with fed controls. Surprisingly, refeeding increased corticosterone concentrations in FR-r groups compared with Fed-2 groups, which was inconsistent with other researches. For example, corticosterone levels in rabbits decreased significantly after food restriction for 4–5 wk compared with the fed controls, and they were still lower in refed rabbits than in fed rabbits after refeeding for 1–3 wk, but they returned to the fed controls after refeeding for 3–4 wk (Rommers et al. 2004). Corticosterone levels increased after 48 h fasting and recovered to the fed control levels after 6 h (Jahng et al. 2005) or 48 h (Djordjević et al. 2003) refeeding in rats. The reason for this discrepancy might be ascribed to a different food-restricted regimen, the refeeding time, and the species used. Given that corticosterone levels had no correlation with cellular immunity or IgG and IgM concentrations, changes in corticosterone levels may not fully interpret the influence of food restriction and refeeding on immunity, and other mechanisms may be involved.

The ultimate explanation for our results may be that some animals may have evolved to survive harsh conditions such as low resource availability. As for gerbils, they may adopt many energy-saving strategies, such as reducing body mass, decreasing thermogenic capability, suppressing reproductive organs to decrease energy requirement, and increasing alimentary tract mass to enhance digestive capability when confronted with reduced food availability (Wunder et al. 1977; Zhang and Wang 2008). In this study, we also found that food restriction reduced body mass, suppressed reproductive organs, and increased the mass of digestive organs in FR gerbils. Many physiological parameters of gerbils are not responsive to food quality, photoperiod, and temperature; hence, the Mongolian gerbil is a stable species (Zhao and Wang 2006; Li and Wang 2007; Liu and Wang 2007). For instance, humoral immunity was unresponsive to low-protein diet, photoperiod, low temperature, and housing density (Li 2005; Chen et al. 2007). Our results also showed that gerbils may maintain stable cellular and humoral immunity even in the face of decreased food availability, which is important to their survival.

In summary, different components of the immune system respond differently to food restriction and refeeding in Mongolian gerbils. Immune organs were suppressed in FR gerbils while WBCs and cellular and humoral immunity were not responsive to food restriction and refeeding. These data suggest that gerbils could sustain optimal immune response even with reduced food availability, which may partially explain their distribution in harsh desert and semiarid environments. Our data also provide a special physiological adaptive strategy for fluctuations in food availability for small rodents in temperate areas.

Acknowledgments

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