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# Glucose supplement reverses the fasting-induced suppression of cellular immunity in Mongolian gerbils (*Meriones unguiculatus*)

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ABSTRACT

Glucose plays an important role in immunity. Three day fasting will decrease cellular immunity and blood glucose levels in Mongolian gerbils (*Meriones unguiculatus*). In the present study, we tested the hypothesis that glucose supplement can reverse the fasting-induced suppression in cellular immunity in gerbils. Twenty-eight male gerbils were selected and randomly divided into fed and fasting groups. Half of the gerbils in each group were then provided with either 10% glucose water or pure water. After 66 h, each gerbil was injected with phytohaemagglutinin (PHA) solution to challenge cellular immunity. Results showed that glucose supplement restored blood glucose levels in fasted gerbils to those of the fed controls. It also recovered cellular immunity, body fat mass and serum leptin levels in fasted gerbils to the values of the fed controls. Blood glucose levels were positively correlated with body fat mass, leptin levels and cellular immune responses. Thymus and spleen masses, and white blood cells in fasted gerbils were not affected by glucose supplement. In general, our data demonstrate that glucose supplement could reverse fasting-induced suppression of cellular immunity in Mongolian gerbils.


## 1. Introduction

Most small mammals in temperate zones are faced with fluctuations in environmental conditions (e.g., temperature, rainfall, food availability) and must adapt to large fluctuations in energy availability (Nelson and Demas, 1996; Martin et al., 2008a). Periodic food shortage such as fasting or starvation, which leads to immunosuppression and reduced blood glucose levels, is common among them and poses a great threat to their survival (Ahima et al., 1996; Lord et al., 1998; Xu and Wang, 2010). Energy availability, including energy reserves and metabolic fuels such as glucose, is crucial to sustaining costly immune responses (Sheldon and Verhulst, 1996; Moret and Schmid-Hempel, 2000; Demas, 2004; Lee, 2006). Glucose is required for normal survival and functioning of lymphocytes and its metabolism is critical to T-cell activation and proliferation (Gregory et al., 1993; Maciver et al., 2008). Decreased glucose availability induced by 2-deoxy-D-glucose leads to immunosuppression in mice (Miller et al., 1994; Dréau et al., 1997, 1998, 2000), rats (Chou et al., 1996), deer mice (*Peromyscus maniculatus*) (Lysle et al., 1988; Demas et al., 1997) and Siberian hamsters (*Phodopus sungorus*) (Zysling and Demas, 2007; Martin et al., 2008b). Humoral immunity in food-restricted poults can be significantly improved by drinking glucose water (Hadri et al., 2004). However, there is still little information about the role of glucose in immunity in wild rodents.

Phytohaemagglutinin (PHA) response is a reliable tool for assessing mammalian cellular immunity, which is responsible for intracellular pathogen control (Smits et al., 1999; Bellocq et al., 2006; Martin et al., 2008a). Immune organs such as thymus and spleen are indirect immunological parameters which are indicative of immune function (Calder and Kew, 2002). Specifically, thymus is a central immune organ which is crucial for primary T cell development (Savino and Dardenne, 2000), and a larger spleen represented stronger immunity in a carefully designed study in which disease condition was controlled (Smith and Hunt, 2004). Moreover, total leukocytes (white blood cells, WBC), which are fundamental to immune responses against pathogens, are also useful for evaluating animals' overall health (Calder and Kew, 2002).

Leptin is secreted mainly by adipocytes and 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). It can directly regulate T cell-mediated immune response (Lam and Lu, 2007). Leptin is positively correlated with adipose tissues, which today are no longer regarded as simply passive energy depots, but as important endocrine and immune organs (Ahima and Flier, 2000; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al.,

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2007). Reductions in total body fat decrease humoral immunity in Siberian hamsters and prairie voles (*Microtus ochrogaster*) (Demas et al., 2003).

Mongolian gerbils (*Meriones unguiculatus*) are small, seasonally breeding, non-hibernating, granivorous rodents living in the desert and semi-arid regions of Mongolia and Northern China (Walker, 1968). Our previous work has shown that a 3-day fasting period leads to suppressed cellular immunity and lower blood glucose levels in fasted gerbils compared with the fed controls (Xu and Wang, 2010). The mechanism of immunosuppression still remains unclear. In the present study, we tested the hypothesis that blood glucose plays an important role in immunity in gerbils. We predicted that glucose supplement would restore suppressed cellular immunity to the level of the fed control.

## 2. Materials and methods

### 2.1. Subjects 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. Male gerbils used in this study were the offspring of gerbils in our laboratory colony. Before the experiment, the males were housed individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding under a constant photoperiod of a 16 h:8 h light–dark cycle and a temperature of 23 ± 1 °C. Standard rat chow (Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum*. After body mass stabilized, 28 males (aged 10–11 months, weighing 56.1–70.8 g) were selected and randomly divided into fed and fasting groups. Half of the gerbils in each group were provided with either pure water or 10% glucose water. Thus, there were four groups ($n = 7$ in each group): fed/water group, fed/glucose group, fasting/water group and fasting/glucose group. The fasting period was three days and 10% glucose water was provided *ad libitum* to the glucose groups during the entire course of the experiment. The initial day of the treatment was designated as day 0; day 2 and day 3 represented fasting for two and three days, respectively.

### 2.2. Cellular immunity assay

Cellular immune response was measured as described previously (Bellocq et al., 2006; Xu and Wang, 2010). According to our preliminary experiment, the maximum PHA response occurs 6 h after PHA injection in male gerbils. Thus, after 66 h treatment, we measured footpad thickness of the left hindfoot of all gerbils with a micrometer (Tesa Shop-Cal; TESA SA, Renens, Switzerland) to ± 0.01 mm. Immediately thereafter, 0.1 mg of PHA (Sigma L-8754; Sigma–Aldrich) dissolved in 0.03 ml of sterile phosphate buffered saline (PBS, pH 7.4) were subcutaneously injected in the middle of the footpad. 6 h after injection, we measured footpad thickness again. The PHA response (i.e., cellular immunity) was calculated as the difference between pre- and post-injection measurements divided by initial footpad thickness (PHA response = (post PHA − pre PHA)/pre PHA). Six measures of footpad thickness were taken to obtain an average value for each gerbil (Bellocq et al., 2006; Xu and Wang, 2010).

### 2.3. White blood cell measurement

At the end of the experiment, each gerbil was euthanized by $CO_2$ asphyxiation and trunk blood was collected around 3:00 pm (lights on at 04:00 h, and lights off at 20:00 h) for the measurement of WBC, blood glucose, and serum leptin to reduce the effect of the circadian rhythm on these parameters. 20 μl whole blood was diluted immediately in 0.38 ml solution containing 1.5% glacial acetic acid, and 1% crystal violet (Sigma–Aldrich, St. Louis, MO, USA). The leukocytes were counted in an improved Neubauer chamber using a microscope. The total number of WBC was determined by counting all leucocytes in the four large corner squares of the Neubauer chamber and multiplying the raw data by $5 \times 10^7$ 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 4000 rpm at 4 °C for 30 min. The serum was collected and then stored at −80 °C for the leptin assay.

### 2.4. Blood glucose measurement

20 μl whole blood was obtained immediately after euthanization. Blood glucose levels were measured with the FreeStyle Mini blood glucose meter (Abbott Diabetes Care Inc., Alameda, CA, USA) according to the manufacturer's instructions. The range of blood glucose tested was 1.1–27.8 mmol/l. Within-lot and within-vial precision of this test are <5.6% and <4.1%, respectively (Rivers et al., 2006).

### 2.5. Serum leptin assay

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

### 2.6. Body and organ composition

Organs were measured as described in Zhang and Wang (2007). In brief, after interscapular brown adipose tissue was removed, the visceral organs including heart, thymus, lungs, liver, spleen, kidneys, paired adrenal glands, epididymis, testes, seminal vesicle 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. Water mass of the carcass was calculated as the difference between the wet and dry carcass mass. 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).

### 2.7. Statistical analyses

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. All the ratios including PHA response, water and body fat content were subjected to arcsine transformation. The differences in body mass among the four groups on day 0 were analyzed by a one-way analysis of variance (ANOVA), while the differences of body mass among the four groups on day 1, day 2 and day 3 were analyzed by a two-way ANOVA (fasting × glucose supplement) followed by Bonferroni post hoc tests. Group differences in wet organ mass, with body mass as the covariate, and in dry organ mass, with dry carcass mass as the covariate, were analyzed by a two-way analysis of covariance (ANCOVA), followed by Bonferroni post hoc tests. Group differences in other parameters (body composition, PHA response, WBC, blood glucose and leptin levels) were analyzed by a two-way ANOVA followed

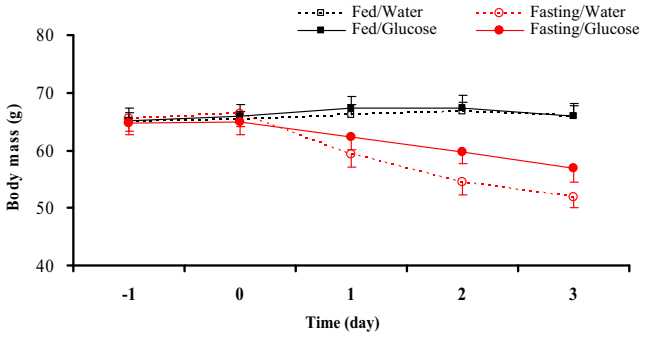


**Fig. 1.** Effects of fasting and glucose supplement on body mass in Mongolian gerbils. Values are means ± SE ($n$ = 7).

by Bonferroni post hoc tests. Significant group differences were further evaluated by a general linear model multivariate analysis followed by Bonferroni post hoc tests. Pearson's correlation analysis was performed to determine the correlations of blood glucose with body fat mass, PHA response and leptin levels. The correlations of the PHA response with body fat mass and leptin levels were also calculated. Results were presented as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Body mass

On day 0, body mass did not differ significantly between the fed/water, fed/glucose, fasting/water and fasting/glucose groups ($F_{3,24} = 0.11$, $P > 0.05$; Fig. 1). Body mass of the fasting gerbils decreased significantly compared with the fed gerbils on day 1 ($F_{1,24} = 8.33$, $P < 0.01$), day 2 ($F_{1,24} = 23.39$, $P < 0.001$), and day 3 ($F_{1,24} = 32.81$, $P < 0.001$). Compared with body mass on day 0 (fasting/water group: 66.4 ± 2.3 g; fasting/glucose group: 64.9 ± 2.2 g), gerbils in the fasting/water group lost 7.1 g, 11.8 g, and 14.5 g, while gerbils in the fasting/glucose group lost 2.6 g, 5.2 g, and 8.0 g after fasting for 1, 2, and 3 days, respectively.

### 3.2. Cellular immunity

The PHA response was significantly reduced by fasting ($F_{1,24} = 11.86$, $P < 0.01$) as compared with the fed gerbils, while it was not influenced by glucose supplement ($F_{1,24} = 2.36$, $P > 0.05$) and the interaction of fasting × glucose supplement ($F_{1,24} = 0.52$, $P > 0.05$; Fig. 2A). Further analysis showed that gerbils in the fasting/water group had a significantly lower PHA response than the fed gerbils; however, it did not differ between the fasting/glucose and the fed groups (Fig. 2A). The PHA response was positively correlated with blood glucose levels ($r = 0.525$, $P < 0.01$; Fig. 2B) and body fat mass ($r = 0.513$, $P < 0.01$; Fig. 2C).

### 3.3. White blood cells

Fasting ($F_{1,24} = 1.035$, $P > 0.05$), glucose supplement ($F_{1,24} = 3.51$, $P > 0.05$) and the interaction of fasting × glucose supplement ($F_{1,24} = 1.515$, $P > 0.05$) had no significant effects on WBC (Fig. 3).

### 3.4. Blood glucose levels

Glucose supplement kept blood glucose levels in the fasting/glucose group at those of the fed control groups ($F_{1,24} = 5.33$, $P < 0.05$), whereas blood glucose levels in the fasting/water group

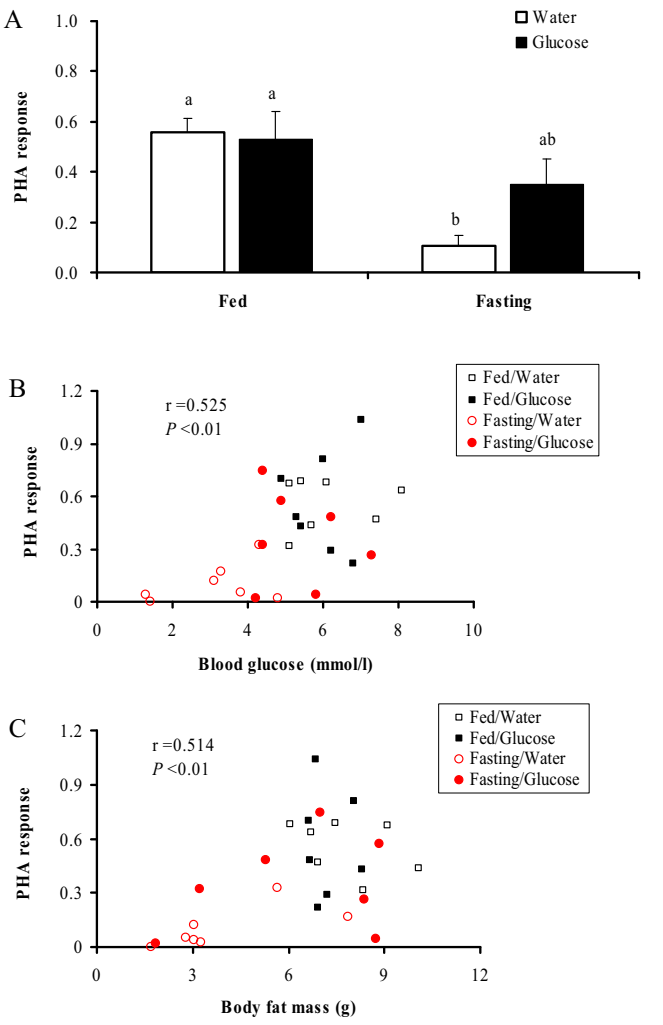


**Fig. 2.** Effects of fasting and glucose supplement on (A) PHA response, and the correlation of PHA response with (B) blood glucose levels and (C) body fat mass in Mongolian gerbils. Values are means ± SE ($n$ = 7). Different letters (a or b) above bars indicate significant differences ($P < 0.05$).

were significantly lower than those in the fed control groups (Fig. 4). Blood glucose levels were affected significantly by fasting ($F_{1,24} = 17.65$, $P < 0.001$) and by the interaction of fasting × glucose supplement ($F_{1,24} = 7.51$, $P < 0.05$).

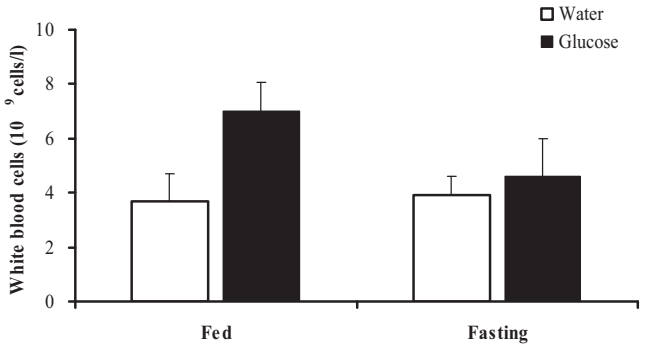


**Fig. 3.** Effects of fasting and glucose supplement on white blood cells in Mongolian gerbils. Values are means ± SE ($n$ = 7). Different letters (a or b) above bars indicate significant differences ($P < 0.05$).

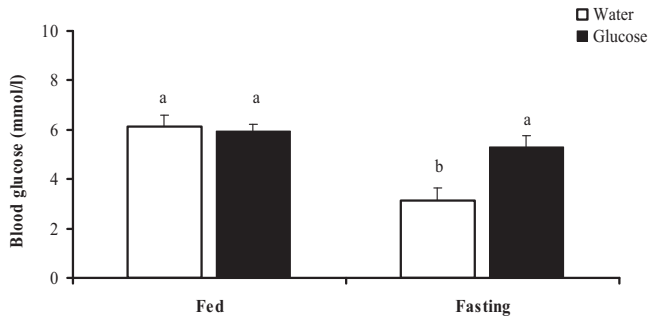


**Fig. 4.** Effects of fasting and glucose supplement on blood glucose levels in Mongolian gerbils. Values are means ± SE ($n$ = 7).

### 3.5. Serum leptin levels

Serum leptin levels were decreased significantly by fasting ($F_{1,24}$ = 10.132, $P$ < 0.01; Fig. 5A), whereas they were not affected by glucose supplement ($F_{1,24}$ = 0.793, $P$ > 0.05) and the interaction of fasting × glucose supplement ($F_{1,24}$ = 2.530, $P$ > 0.05). Serum leptin levels were positively correlated with blood glucose levels ($r$ = 0.493, $P$ < 0.01; Fig. 5B) and PHA response ($r$ = 0.539, $P$ < 0.01; Fig. 5C).

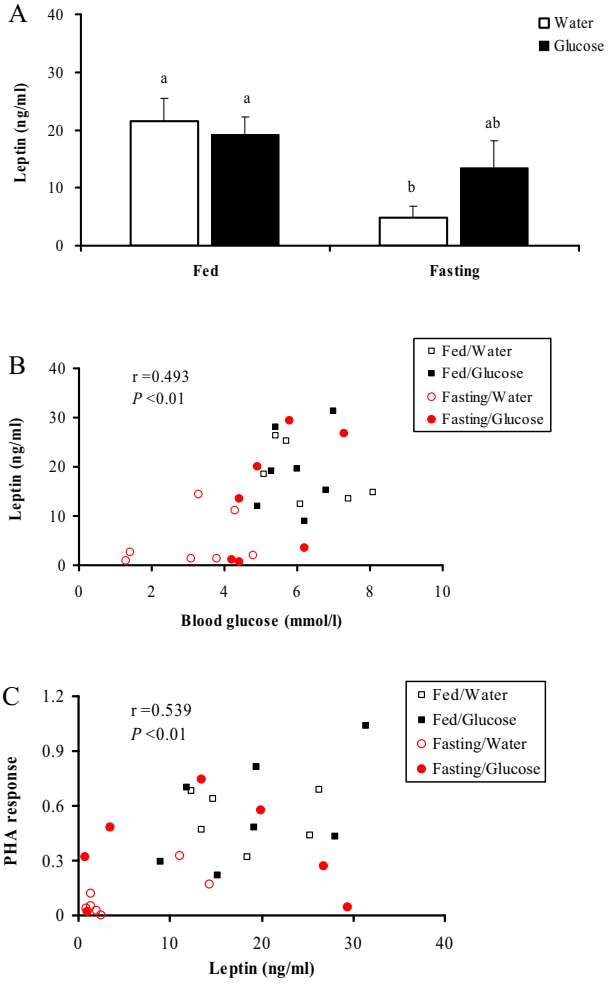


**Fig. 5.** Effects of fasting and glucose supplement on (A) serum leptin level, and the correlation of serum leptin with (B) blood glucose levels and (C) PHA response in Mongolian gerbils. Values are means ± SE ($n$ = 7). Different letters (a or b) above bars indicate significant differences ($P$ < 0.05).

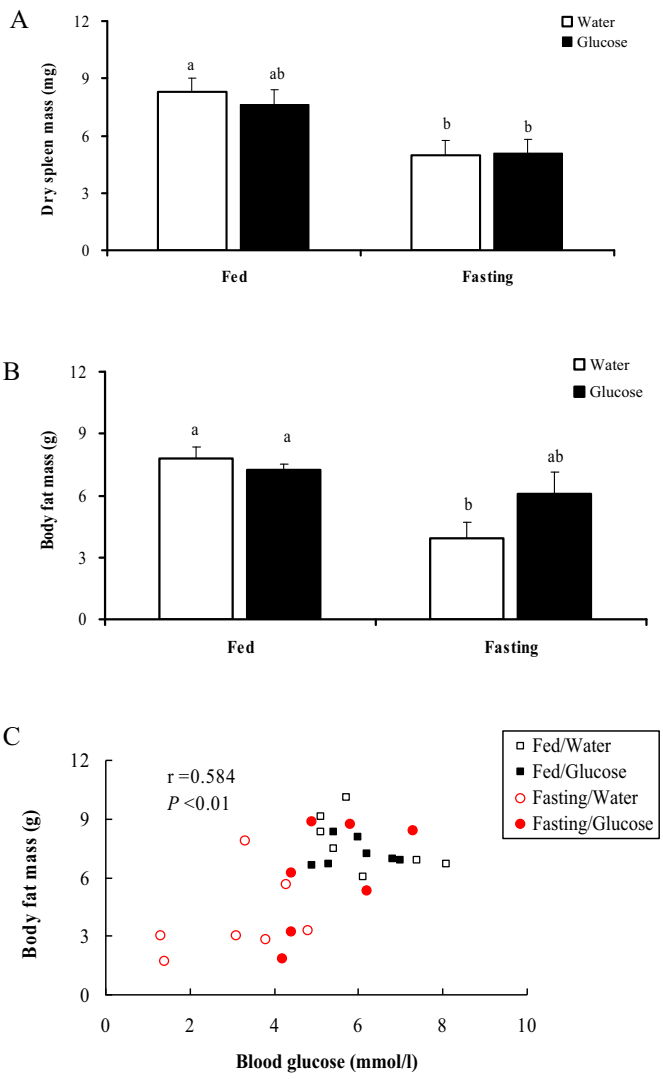


**Fig. 6.** Effects of fasting and glucose supplement on (A) dry spleen mass, (B) body fat mass and (C) the correlation of body fat mass with blood glucose levels in Mongolian gerbils. Values are means ± SE ($n$ = 7). Different letters (a or b) above bars indicate significant differences ($P$ < 0.05).

### 3.6. Body and organ composition

Fasted gerbils had lower body fat mass ($F_{1,24}$ = 12.21, $P$ < 0.01) and lower wet and dry spleen mass (wet mass: $F_{1,23}$ = 6.17, $P$ < 0.05; dry mass: $F_{1,23}$ = 12.54, $P$ < 0.01) than the fed gerbils (Fig. 6A and B), while fasting had no significant effect on wet and dry thymus mass (wet mass: $F_{1,23}$ = 1.05, $P$ > 0.05; dry mass: $F_{1,23}$ = 2.41, $P$ > 0.05). Body fat mass in the fasting/glucose group was 56.4% higher than in the fasting/water group, though glucose supplement had no significant effect on body fat mass ($F_{1,24}$ = 1.20, $P$ > 0.05; Table 1). Body fat mass was positively correlated with blood glucose levels ($r$ = 0.584, $P$ < 0.01; Fig. 6C). Glucose supplement had no significant effect on wet and dry thymus mass (wet mass: $F_{1,23}$ = 3.48, $P$ > 0.05; dry mass: $F_{1,23}$ = 3.38, $P$ > 0.05), nor on wet and dry spleen mass (wet mass: $F_{1,23}$ = 2.64, $P$ > 0.05; dry mass: $F_{1,23}$ = 0.11, $P$ > 0.05; Tables 2 and 3). Fasting decreased wet colon mass and dry kidney mass, while glucose supplement increased wet and dry small intestine mass, and total dry alimentary tract mass (see Tables 2 and 3).

**Table 1**
Effects of fasting and glucose supplement on body composition in Mongolian gerbils. Values are means ± SE ($n$ = 7). Values for a specific parameter that have different superscripts are significantly different at $P < 0.05$, determined by two-way ANOVA and Bonferroni post hoc tests.

| | Fed/water | Fed/glucose | Fasting/water | Fasting/glucose | F | G | F × G |
|---|---|---|---|---|---|---|---|
| Body mass on day 3 (g) | 66.0 ± 2.0$^{a}$ | 66.0 ± 2.0$^{a}$ | 51.9 ± 2.0$^{b}$ | 56.8 ± 2.0$^{b}$ | <0.001 | ns | ns |
| Wet carcass mass (g) | 50.5 ± 1.5$^{a}$ | 51.1 ± 1.5$^{a}$ | 40.9 ± 1.5$^{b}$ | 45.3 ± 1.5$^{ab}$ | <0.001 | ns | ns |
| Dry carcass mass (g) | 20.0 ± 1.0$^{a}$ | 19.4 ± 1.0$^{a}$ | 14.4 ± 1.0$^{b}$ | 17.3 ± 1.0$^{ab}$ | <0.01 | ns | ns |
| Body water mass (g) | 30.5 ± 1.0$^{a}$ | 31.8 ± 1.0$^{a}$ | 26.5 ± 1.0$^{b}$ | 28.1 ± 1.0$^{ab}$ | <0.01 | ns | ns |
| Water content (water mass/wet carcass mass) (%) | 60.5 ± 1.4 | 62.0 ± 1.4 | 65.1 ± 1.4 | 62.1 ± 1.4 | ns | ns | ns |
| Fat-free dry carcass (g) | 12.2 ± 0.3$^{a}$ | 12.1 ± 0.3$^{a}$ | 10.4 ± 0.3$^{b}$ | 11.2 ± 0.3$^{ab}$ | <0.01 | ns | ns |
| Body fat mass (g) | 7.8 ± 0.7$^{a}$ | 7.2 ± 0.7$^{a}$ | 3.9 ± 0.7$^{b}$ | 6.1 ± 0.7$^{ab}$ | <0.01 | ns | ns |
| Body fat content (body fat mass/wet carcass mass) (%) | 15.4 ± 1.4$^{a}$ | 14.2 ± 1.4$^{ab}$ | 9.3 ± 1.4$^{b}$ | 13.0 ± 1.4$^{ab}$ | <0.05 | ns | ns |

F, fasting; G, glucose supplement; F × G, interaction of fasting × glucose supplement; ns, not significant.

**Table 2**
Effects of fasting and glucose supplement on mean wet organ masses in Mongolian gerbils. Values are means ± SE ($n$ = 7). Values for a specific parameter that have 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.

| | Fed/water | Fed/glucose | Fasting/water | Fasting/glucose | F | G | F × G |
|---|---|---|---|---|---|---|---|
| IBAT (mg) | 141 ± 17 | 148 ± 17 | 141 ± 20 | 172 ± 16 | ns | ns | ns |
| Heart (mg) | 231 ± 9 | 249 ± 9 | 257 ± 10 | 251 ± 8 | ns | ns | ns |
| Thymus (mg) | 21 ± 4 | 26 ± 4 | 13 ± 5 | 22 ± 4 | ns | ns | ns |
| Lung (mg) | 319 ± 27 | 353 ± 27 | 341 ± 30 | 366 ± 24 | ns | ns | ns |
| Liver (mg) | 1947 ± 108 | 1856 ± 107 | 1988 ± 121 | 2074 ± 98 | ns | ns | ns |
| Spleen (mg) | 38 ± 3$^{a}$ | 32 ± 3$^{ab}$ | 27 ± 3$^{ab}$ | 25 ± 3$^{b}$ | <0.05 | ns | ns |
| Kidneys (mg) | 539 ± 18 | 534 ± 18 | 541 ± 21 | 516 ± 17 | ns | ns | ns |
| Adrenal gland (mg) | 44 ± 3 | 44 ± 3 | 38 ± 3 | 37 ± 2 | ns | ns | ns |
| Stomach with content (mg) | 1142 ± 152 | 1054 ± 152 | 852 ± 171 | 688 ± 139 | ns | ns | ns |
| Stomach (mg) | 344 ± 15 | 356 ± 15 | 380 ± 17 | 348 ± 14 | ns | ns | ns |
| Small intestine with content (mg) | 1892 ± 138 | 1850 ± 138 | 1566 ± 155 | 1509 ± 125 | ns | ns | ns |
| Small intestine (mg) | 364 ± 51 | 525 ± 51 | 502 ± 58 | 557 ± 47 | ns | <0.05 | ns |
| Caecum with content (mg) | 1171 ± 117 | 1074 ± 117 | 1106 ± 131 | 971 ± 107 | ns | ns | ns |
| Caecum (mg) | 205 ± 12 | 225 ± 12 | 216 ± 13 | 194 ± 11 | ns | ns | ns |
| Colon with content (mg) | 918 ± 87 | 787 ± 87 | 651 ± 98 | 575 ± 79 | ns | ns | ns |
| Colon (mg) | 314 ± 17$^{ab}$ | 349 ± 17$^{a}$ | 277 ± 19$^{ab}$ | 274 ± 15$^{b}$ | <0.05 | ns | ns |
| Total alimentary tract (mg) | 1227 ± 71 | 1455 ± 71 | 1375 ± 79 | 1373 ± 64 | ns | ns | ns |
| Epididymis (mg) | 126 ± 19 | 135 ± 19 | 206 ± 21 | 140 ± 17 | ns | ns | <0.05 |
| Testes (mg) | 696 ± 55 | 677 ± 55 | 891 ± 62 | 728 ± 50 | ns | ns | ns |
| Seminal vesicle (mg) | 122 ± 53 | 155 ± 53 | 234 ± 60 | 85 ± 48 | ns | ns | ns |

F, fasting; G, glucose supplement; F × G, interaction of fasting × glucose supplement; ns, not significant.

**Table 3**
Effects of fasting and glucose supplement on mean dry organ masses in Mongolian gerbils. Values are means ± SE ($n$ = 7). Values for a specific parameter that have 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.

| | Fed/water | Fed/glucose | Fasting/water | Fasting/glucose | F | G | F × G |
|---|---|---|---|---|---|---|---|
| Heart (mg) | 58 ± 3 | 62 ± 3 | 58 ± 3 | 55 ± 2 | ns | ns | ns |
| Thymus (mg) | 4 ± 1 | 5 ± 1 | 2 ± 1 | 4 ± 1 | ns | ns | ns |
| Lung (mg) | 91 ± 8 | 98 ± 8 | 80 ± 9 | 89 ± 7 | ns | ns | ns |
| Liver (mg) | 541 ± 40 | 552 ± 39 | 623 ± 45 | 529 ± 37 | ns | ns | ns |
| Spleen (mg) | 8 ± 1$^{a}$ | 8 ± 1$^{ab}$ | 5 ± 1$^{b}$ | 5 ± 1$^{b}$ | <0.01 | ns | ns |
| Kidneys (mg) | 138 ± 5$^{a}$ | 134 ± 5$^{ab}$ | 124 ± 6$^{ab}$ | 114 ± 5$^{b}$ | <0.05 | ns | ns |
| Adrenal gland (mg) | 15 ± 2 | 12 ± 2 | 10 ± 3 | 10 ± 2 | ns | ns | ns |
| Stomach (mg) | 87 ± 4 | 93 ± 4 | 88 ± 4 | 82 ± 4 | ns | ns | ns |
| Small intestine (mg) | 57 ± 9 | 79 ± 8 | 72 ± 10 | 91 ± 8 | ns | <0.05 | ns |
| Caecum (mg) | 29 ± 2 | 35 ± 2 | 32 ± 2 | 28 ± 2 | ns | ns | <0.05 |
| Colon (mg) | 55 ± 5 | 66 ± 4 | 53 ± 5 | 53 ± 4 | ns | ns | ns |
| Total alimentary tract (mg) | 229 ± 13 | 274 ± 13 | 245 ± 15 | 254 ± 12 | ns | <0.05 | ns |
| Epididymis (mg) | 27 ± 4 | 34 ± 4 | 38 ± 4 | 25 ± 4 | ns | ns | <0.05 |
| Testes (mg) | 123 ± 10 | 121 ± 9 | 135 ± 11 | 104 ± 9 | ns | ns | ns |
| Seminal vesicle (mg) | 39 ± 14 | 52 ± 13 | 44 ± 15 | 13 ± 13 | ns | ns | ns |

F, fasting; G, glucose supplement; F × G, interaction of fasting × glucose supplement; ns, not significant.

## 4. Discussion

As expected, glucose supplement kept fasting-induced suppressed cellular immunity in the fasting/glucose group at the level of the fed controls, which demonstrated that blood glucose plays an important role in the cellular immune response in Mongolian gerbils. Glucose is an important metabolic fuel and its metabolism provides energy for many biological processes including costly immune responses (Demas, 2004; Lee, 2006; Maciver et al., 2008). Glucose uptake and glycolysis increase during an immune response (Matarese and Cava, 2004). Gerbils in the fasting/glucose group were no longer short of blood glucose due to the glucose supplement, indicating that they did not lack energy. However, gerbils in the fasting/water group were deficient in blood glucose.

According to the glucostatic theory, glucose itself is the signal that indicates the storage and use of carbohydrates to the brain (Seeley and Woods, 2003; Levin et al., 2004). In the face of an energy crisis, the fasted gerbils might sense the reduction in circulating glucose levels and divert energy from less critical physiological functions such as immunity to the systems most important for

survival such as brain and heart (Lord et al., 1998) so that their cellular immune responses were downregulated. Another possible signal may be insulin that is secreted in response to changes in blood glucose levels. It serves as an important indicator of current energy availability within an animal (Benoit et al., 2004). Fasting also reduces the circulating levels of insulin (Ahima et al., 1996), and recent research has shown that insulin is an important peripheral signal linking energy availability and immunity in Siberian hamsters (*Phodopus sungorus*) (Garcia et al., 2010). Although we did not measure the circulating levels of insulin in the present study, it might act as the endocrine signal of blood glucose availability and might be the actual cue utilized by the immune system to alter its functioning.

The positive correlation between blood glucose levels and cellular immunity suggests the importance of glucose for maintaining optimal immune response. These results also imply that maintaining normal cellular immunity is costly in terms of energy. Glucose supplement failed to restore the immune organs (thymus, spleen) and WBC to the level of the controls, suggesting that other factors besides glucose might also play an important role in immunity (Chandra, 1996; Kaminogawa and Nanno, 2004).

Energy reserves (body fat mass) play an important role in immune homeostasis (Matarese and Cava, 2004; Fantuzzi, 2005; Schäffler et al., 2007). Houston et al. (2007) have shown that animals with low energy reserves choose to allocate less energy to immune defense than animals with higher reserves. In the present study, we observed that glucose supplement blunted the decrease of body fat mass in fasted gerbils. Body fat mass was proportional to blood glucose levels and cellular immunity, which indicates that the glucose supplement protected body fat mass against overmobilization and sustained optimal cellular immune responses in fasting gerbils.

Leptin also plays an important role in immunity and is a link between energy reserves and immunity (Ahima et al., 1996; Flier, 1998; Matarese et al., 2005; Lam and Lu, 2007; Steiner and Romanovsky, 2007). Lord et al. (1998) have demonstrated that exogenous leptin administration reverses fasting-induced immunosuppression in fasted mice. Serum leptin levels were also reduced in gerbils in a previous study (Xu and Wang, 2010) and in the present study. According to the lipostatic theory, the brain monitors the storage and metabolism of fat, and leptin is the main adiposity signal to the brain (Seeley and Woods, 2003). A falling leptin concentration functions as a peripheral signal of starvation which serves to conserve energy in the face of limited energy reserves (Ahima et al., 1996; Lord et al., 1998). Moreover, leptin acts as a neuroendocrine signal communicating information about total body fat to the immune system (Ahima et al., 1996; Lord et al., 1998; Demas and Sakaria, 2005). Its levels in the fasting gerbils rose to those of the fed controls after glucose supplement. It might be the prevention of the fall in leptin levels that made cellular immunity in the fasting/glucose group recover, at least in part, to the level of the fed controls.

In summary, glucose supplement could reverse fasting-induced immunosuppresssion in Mongolian gerbils through its attenuating role in body fat mass and leptin levels. Our data also demonstrated that blood glucose played an important role in immunity and that mounting cellular immune responses was costly in terms of energy.

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## References

Ahima, R.S., Flier, J.S., 2000. Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327–332.
Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., Flier, J.S., 1996. Role of leptin in the neuroendocrine response to fasting. Nature 18, 250–252.
Bellocq, J.G., Krasnov, B.R., Khokhlova, I.S., Pinshow, B., 2006. Temporal dynamics of a T-cell mediated immune response in desert rodents. Comp. Biochem. Physiol. A 145, 554–559.
Benoit, S.C., Clegg, D.J., Seeley, R.J., Woods, S.C., 2004. Insulin and leptin as adiposity signals. Recent Prog. Horm. Res. 59, 267–285.
Calder, P.C., Kew, S., 2002. The immune system: a target for functional foods? Br. J. Nutr. 88, S165–S176.
Chandra, R.K., 1996. Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. Proc. Natl. Acad. Sci. 93, 14304–14307.
Chou, S., Kojic, L.D., Messingham, K.N., Cunnic, J.E., 1996. Characterization of the effect of 2-deoxy-D-glucose (2-DG) on the immune system. Brain Behav. Immun. 10, 399–416.
Demas, G.E., 2004. The energetics of immunity: a neuroendocrine link between energy balance and immune function. Horm. Behav. 45, 173–180.
Demas, G.E., Sakaria, S., 2005. Leptin regulates energetic tradeoffs between body fat and humoral immunity. Proc. R. Soc. Lond. B 272, 1845–1850.
Demas, G.E., DeVries, A.C., Nelson, R.J., 1997. Effects of photoperiod and 2-deoxy-D-glucose-induced metabolic stress on immune function in female deer mice. Am. J. Physiol. 272, 1762–1767.
Demas, G.E., Drazen, D.L., Nelson, R.J., 2003. Reductions in total body fat decreases humoral immunity. Proc. R. Soc. Lond. B 270, 905–911.
Dréau, D., Morton, D.S., Foster, M., Swiggett, J.P., Sonnenfeld, G., 1997. Immune alterations in male and female mice after 2-deoxy-D-glucose administration. Physiol. Behav. 62, 1325–1331.
Dréau, D., Morton, D.S., Foster, M., Fowler, N., Sonnenfeld, G., 1998. Effects of 2-deoxy-D-glucose administration on immune parameters in mice. Immunopharmacology 39, 201–213.
Dréau, D., Foster, M., Morton, D.S., Fowler, N., Kinney, K., Sonnenfeld, G., 2000. Immune alterations in three mouse strains following 2-deoxy-D-glucose administration. Physiol. Behav. 70, 513–520.
Faggioni, R., Feingold, K.R., Grunfeld, C., 2001. Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB J. 15, 2565–2571.
Fantuzzi, G., 2005. Adipose tissue, adipokines, and inflammation. J. Allergy Clin. Immunol. 115, 911–919.
Flier, J.S., 1998. Lowered leptin slims immune response. Nat. Med. 4, 1124–1125.
Garcia, N.W., Greives, T.J., Zysling, D.A., French, S.S., Chester, E.M., Demas, G.E., 2010. Exogenous insulin enhances humoural immune responses in short-day, but not long-day, Siberian hamsters (*Phodopus sungorus*). Proc. R. Soc. Lond. B 277, 2211–2218.
Gregory, R., McElveen, J., Tattersall, R.B., Todd, I., 1993. The effects of 3-hydroxybutyrate and glucose on human T-cell responses to *Candida albicans*. Immunol. Med. Microbiol. 7, 315–320.
Hadri, L.E., Garlich, J.D., Qureshi, M.A., Ferket, P.R., Odetallah, N.H., 2004. Glucose and electrolyte supplementation of drinking water improve the immune responses of poults with inanition. Poult. Sci. 83, 803–809.
Houston, A.I., McNamara, J.M., Barta, Z., Klasing, K.C., 2007. The effect of energy reserves and food availability on optimal immune defence. Proc. R. Soc. Lond. B 274, 2835–2842.
Kaminogawa, S., Nanno, M., 2004. Modulation of immune functions by foods. eCAM 1, 241–250.
Lam, Q.L.K., Lu, L., 2007. Role of leptin in immunity. Cell. Mol. Immunol. 4, 1–13.
Lee, K.A., 2006. Linking immune defenses and life history at the level of the individual and species. Integr. Comp. Biol. 46, 1000–1015.
Levin, B.E., Routh, V.H., Kang, L., Sanders, N.M., Dunn-Meynell, A.A., 2004. Neuronal glucosensing: what do we know after 50 years? Diabetes 53, 2521–2528.
Li, X.S., Wang, D.H., 2005. Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils: the roles of photoperiod and cold. J. Comp. Physiol. B 175, 593–600.
Lord, G.M., Matarese, G., Howard, J.K., Baker, R.J., Bloom, S.R., Lechler, R.I., 1998. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394, 897–901.
Lysle, D.T., Cunnick, J.E., Wu, R., Caggiula, A.R., Wood, P.G., Rabin, B.S., 1988. 2-deoxy-D-glucose modulation of T-lymphocyte reactivity: differential effects on lymphoid compartments. Brain Behav. Immun. 2, 212–221.
Maciver, N.J., Jacobs, S.R., Wieman, H.L., Wofford, J.A., Coloff, J.L., Rathmell, J.C., 2008. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. J. Leukoc. Biol. 84, 949–957.
Martin, L.B., Weil, Z.M., Nelson, R.J., 2008a. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philos. Trans. R. Soc. Lond. B 363, 321–339.

Martin, L.B., Weil, Z.M., Bowers, S.L., Nelson, R.J., 2008b. Sex-specific effects of glucose deprivation on cell-mediated immunity and reproduction in Siberian hamsters (*Phodopus sungorus*). J. Comp. Physiol. B 178, 623–628.
Matarese, G., Cava, A.L., 2004. The intricate interface between immune system and metabolism. Trends Immunol. 5, 193–200.
Matarese, G., Moschos, S., Mantzoros, C.S., 2005. Leptin in immunology. J. Immunol. 173, 3137–3142.
Miller, E.S., Klinger, J.C., Akin, C., Koebel, D.A., Sonnenfeld, G., 1994. Inhibition of murine splenic T lymphocyte proliferation by 2-deoxy-D-glucose-induced metabolic stress. J. Neuroimmunol. 52, 165–173.
Moret, Y., Schmid-Hempel, P., 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290, 1166–1168.
Nelson, R.J., Demas, G.E., 1996. Seasonal changes in immune functions. Q. Rev. Biol. 71, 511–549.
Rivers, S.M., Kane, M.P., Bakst, G., Busch, R.S., Hamilton, R.A., 2006. Precision and accuracy of two blood glucose meters: FreeStyle Flash versus One Touch Ultra. Am. J. Health Syst. Pharm. 63, 1411–1416.
Savino, W., Dardenne, M., 2000. Neuroendocrine control of thymus physiology. Endocr. Rev. 21, 412–443.
Schäffler, A., Schölmerich, J., Salzberger, B., 2007. Adipose tissue as an immunological organ: toll-like receptors, C1q/TNFs and CTRPs. Trends Immunol. 28, 393–400.
Seeley, R.J., Woods, S.C., 2003. Monitoring of stored and available fuel by the CNS: implications for obesity. Nat. Rev. Neurosci. 4, 901–909.
Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11, 317–321.
Smith, K.G., Hunt, J.L., 2004. On the use of spleen mass as a measure of avian immune system strength. Oecologia 138, 28–31.
Smits, J.E., Bortolotti, G.R., Tella, J.L., 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. Funct. Ecol. 13, 567–572.
Steiner, A.A., Romanovsky, A.A., 2007. Leptin: at the crossroads of energy balance and systemic inflammation. Prog. Lip. Res. 46, 89–107.
Trayhurn, P., 2005. Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiol. Scand. 184, 285–293.
Walker, E.P., 1968. Mammals of the World, 2nd ed. Johns Hopkins Press, Baltimore.
Xu, D.L., Wang, D.H., 2010. Fasting suppresses T cell-mediated immunity in female Mongolian gerbils (*Meriones unguiculatus*). Comp. Biochem. Physiol. A 155, 25–33.
Yang, X.P., 2004. Laboratory Manual in Animal Physiology, 9. Higher Education Press, Beijing, China, 1–94.
Zhang, Z.Q., Wang, D.H., 2007. Seasonal changes in thermogenesis and body mass in wild Mongolian gerbils (*Meriones unguiculatus*). Comp. Biochem. Physiol. A 148, 346–353.
Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.
Zhao, Z.J., Wang, D.H., 2006. Effects of photoperiod on energy budgets and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*). J. Therm. Biol. 31, 323–331.
Zysling, D.A., Demas, G.E., 2007. Metabolic stress suppresses humoral immune function in long-day, but not short-day, Siberian hamsters (*Phodopus sungorus*). J. Comp. Physiol. B 177, 339–347.