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Humoral immune response suppresses reproductive physiology in male Brandt's voles (*Lasiopodomys brandtii*)

Xiao-Quan Cai[a,c], Ming Yang[b], Wen-Qin Zhong[a], De-Hua Wang[a,*]

[a]*State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Datun Lu, Chaoyang, Beijing 100101, China*
[b]*Life Science and Chemistry College, Shenyang Normal University, Shenyang 110034, China*
[c]*Graduate School of the Chinese Academy of Sciences, Beijing 100139, China*

Abstract

In order to evaluate the potential costs of humoral immune response, which is important for survival in small wild mammals, we studied the physiological function of adult male Brandt's voles (*Lasiopodomys brandtii*) challenged with human immunoglobulin G (IgG). Compared with controls, the immunochallenged voles showed significantly higher antibody levels 15 days after injection. Serum testosterone levels, and mass of testes and epididymides were lower in immunochallenged voles than in control animals. Body mass remained stable during the course of the experiment. Total and digestible energy intake showed a transient decrease following IgG injection, while resting metabolic rate (RMR) increased. Taken together, these data suggest a shift in metabolic priorities in response to immune challenge. Our results provide evidence that mounting a humoral immune response to an immunological challenge may have fitness costs in male Brandt's voles.
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Introduction

Immunity is one of the physiological key regulating host survival and has received much attention from behavioral ecologists, comparative physiologists and evolutionary ecologists in recent years (Sheldon and Verhulst, 1996; Demas, 2004; Lee and Klasing, 2004; Derting and Virk, 2005; Martin et al., 2006). Like other energetically demanding biological functions (e.g., growth, thermoregulation and reproduction), the immune system also requires energy to defend organisms against pathogens and parasites (Lochmiller and Deerenberg, 2000). Demas et al. (1997) first examined the energetic costs of mounting specific antibody responses in house mice and showed that metabolic rate increased approximately 20–30% when mounting an immune response. Derting and Compton (2003) stated that immunity upregulation can lead to the reallocation of energy between the immune system and other physiological functions in wild white-footed mice (*Peromyscus leucopus*). Demas (2004) reviewed the energetics of immunity and indicated that immune responses, like all other biological processes, require energy. Kester et al. (1977) stated that during an immune response accessory cells must process

the antigen to present it appropriately to lymphocytes, which transform into blasts and undergo clonal expansion after receiving this information. These and other immune processes such as phagocytosis, cell migration, and homing are mechanisms involving high metabolic demands.

The trade-off between immune function and reproduction eventually alters an animal's survival and reproductive success (Martin et al., 2006). Seasonally breeding animals generally bias their investment towards reproduction once conditions become suitable but shift their investment towards processes that promote survival when confronted with environmental stresses. Differential allocation of energy to host defense versus reproduction is assumed to drive the anti-phase relation between peak reproductive function and immunocompetence (Owens and Wilson, 1999; Lochmiller and Deerenberg, 2000). Further, Lee and Klasing (2004) point out that other aspects of the immune system such as innate and cell-mediated functions might be more energetically expensive. The relationship between immune and reproductive function has been documented in wild seasonally breeding small mammals (Nelson et al., 2002), but the direct energetic conflicts between reproduction and immune function remain largely unknown. The data for wild species is more ecologically significant but rare at present.

Brandt's voles (*Lasiopodomys brandtii*) live mainly on the grasslands of Inner Mongolia in China and the region of Baikal in Russia. In natural environments, the reproduction of Brandt's voles can last from early May to August (Wan et al., 1998). This species is non-hibernating, storing food and living in groups for winter survival (Li et al., 2003). Thus it is a good model to study the relationship between the immune and reproductive systems.

We have measured the relationship between social status and humoral immune function and suggest a trade-off between reproduction and immunocompetence in male Brandt's voles (Li et al., 2007). In captive conditions, density can affect the growth, reproduction and immune function, and the response to immunochallenge is gender-specific (Li et al., 2003). In this study, we addressed the energetic and potential breeding costs of the immune activation in male Brandt's voles. We hypothesized that mounting a humoral immune response to an immunological challenge has fitness costs in male Brandt's voles.

Materials and methods

Animals and humoral immunochallenge

All animal use procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

Twenty-two adult male Brandt's voles (about 120 days of age, body mass of 55–65 g) were randomly selected ($n = 10$ for controls, $n = 12$ for immunochallenged) from our laboratory colony established using animals trapped in the grasslands of Inner Mongolia, China. Animals were housed individually in cages (32 cm × 20 cm × 14 cm) with fresh sawdust as bedding. All animals were maintained at $23 \pm 1\,°C$ and a 14 L:10 D photoperiod with lights on at 0600 h. Food (rabbit chow; Beijing Ke Ao Fed Co., China) and water were provided *ad libitum*. The experiments were carried out in July 2003. The experimental voles were given a single injection of human-IgG (0.2 mg in 0.2 ml sterile saline, Li et al., 2007) and controls were injected with saline. The day of injection was regarded as day 0.

Energy intake

Energy intake for each animal was measured at 3 day intervals as described previously (Song and Wang, 2001, 2002; Zhao and Wang, 2006). Measurements were made before immunization and 2 weeks thereafter. The uneaten food and feces were collected, separated manually, and oven-dried to constant mass at $60\,°C$ before weighing (± 0.1 g). Caloric contents of food and feces were determined by oxygen bomb calorimetry (Parr1281, Parr Instruments, USA) according to the manual instruction.

Gross energy intake (GEI), digestible energy intake (DEI), and apparent digestibility of energy were calculated using the following equations (Grodzinski and Wunder, 1975; Liu et al., 2003):

$$\text{GEI (kJ day}^{-1}) = \text{dry matter intake (g day}^{-1})$$
$$\times \text{ energy content of food (kJ g}^{-1});$$
$$\text{DEI (kJ day}^{-1}) = \text{GEI} - \text{dry mass of feces (g day}^{-1})$$
$$\times \text{ energy content of feces (kJ g}^{-1});$$
$$\text{Digestibility (\%)} = \text{DEI/GEI} \times 100\%.$$

Resting metabolic rates

We determined resting metabolic rates (RMR) by measuring O_2-consumption using an established closed-circuit respirometer at $30\,°C$ within the animals' thermal neutral zone (Wang and Wang, 1996; Wang et al., 2000; Li and Wang, 2005). The metabolic chamber volume was 3.6 l and the temperature inside the chamber was maintained by a water bath ($\pm 0.5\,°C$). KOH and silica gel were used to absorb carbon dioxide and water in the metabolic chamber. The animals were in the chambers without bedding for about 60 min to stabilize and each test lasted for 60 min with oxygen consumption recordings at 5 min intervals. The two lowest consecutive

readings were used to calculate RMR and corrected to standard temperature and pressure (STP) conditions (Lu et al., 2007). Body mass (BM) of voles was measured before and after each test. Metabolic rates were measured on days – 2, 1, 7 and 14 post-injection. All metabolic measurements were performed between 10:00 and 17:00 h to minimize the circadian effects (Li and Wang, 2005).

Terminal sampling

After the last measuring of RMR on day 14, animals were sacrificed between 09:00 and 10:00 h on day 15. Trunk blood (1.5–3 ml) was collected and blood samples were centrifuged at 1680g and 4 °C for 30 min. Serum was stored at −80 °C until assayed for antibodies, testosterone and corticosterone levels. In addition, spleen, paired adrenal glands, testes, epididymides, and spermatophore were removed and weighed (±0.1 mg) without the connective tissues for each animal.

Antibody assay

Serum IgG concentration against human-IgG was determined using an ELISA as described in detail elsewhere (Li et al., 2003, 2007). Thawed serum samples were diluted 1:100 in PBS, and 0.1 ml of each serum dilution was added in duplicate to the wells of a 96-well immunoplate (Nunc MaxiSorp) coated with human IgG. All samples were run on a single plate. The plate was sealed, incubated, and washed before the addition of secondary antibody (horseradish peroxidase-conjugated anti-mouse IgG). The plate was again incubated and washed, and then treated with enzyme substrate (a mixture of 3, 3′, 5, 5′-tetramethylbenzidine and urea hydrogen peroxide). After 20 min, the enzyme reaction was stopped and the optical density (OD) of each well was determined using a plate reader equipped with a 405 nm wavelength filter. The average OD from the duplicates was used for data analysis (Li et al., 2003, 2007).

Testosterone and corticosterone radioimmunoassay

Serum testosterone concentration was determined using the ^{125}I RIA kit provided by the Beimian Dongya Institute of Biotechnology (Beijing, China). This kit has previously been validated for use in Brandt's voles (Li et al., 2007). Thawed serum samples were diluted 1:30 in assay buffer, pipetted into duplicate assay tubes and incubated with ^{125}I radio-labeled testosterone tracer and rabbit anti-testosterone antibody for 1 h at 37 °C. Pellets were precipitated with goat anti-rabbit gamma globulin serum and polyethylene glycol and were centrifuged at room temperature for 20 min at 1500g.

The supernatant was then decanted and aspirated, and gamma emissions from each tube were recorded for 1 min using an automated gamma counter (Beckman Instruments, USA). All testosterone values were determined in a single radioimmunoassay for which the intra-assay coefficient of variation was 4.4%.

Serum corticosterone concentration was determined using a double-antibody ^{125}I radioimmunoassay kit, according to the manufacturer's instructions (DSL-80100; Diagnostic Systems Laboratories, Webster, TX, USA). The RIA is highly specific, cross-reacting at less than 1% with other hormones and with a lower detection limit of 2.7 ng/ml. The intra-assay and inter-assay coefficients of variation were <5% and <10%, respectively.

Statistical analyses

All statistical analyses were performed using SPSS version 10.0 for Windows. Prior to performing statistical comparisons, all data were tested for normality and equality of variances using Kolmogorov–Smirnow and Levene's tests. We used independent sample t-tests to detect the differences for the measured variables between the immunochallenged and control animals. Differences in measurements that were repeated over time were analyzed using repeated measures two-way ANOVA (time and treatment) followed by Tukey's HSD test. Because no changes in BM were detected between the two groups and during the course of the experiment, the variables were not adjusted for BM and were not compared among groups using ANCOVA with BM as the covariate. For all statistical tests, differences were considered statistically significant at $P < 0.05$. Values in the text are presented as means ± SE.

Results

BM kept stable during the course of the experiment for both immunochallenged and control voles (Fig. 1). There was no significant effect of time on BM (repeated measures ANOVA, $P > 0.05$) and no interaction between time and treatment ($P > 0.05$).

Specific antibody in immunochallenged voles was 97.5% higher than in controls at 15 days post-injection ($t = 5.11$, $P < 0.01$, Fig. 2).

GEI and DEI tended to be lower for immunochallenged voles compared with controls and showed significant differences from day 4 to day 6 after injection (GEI, $t = -2.51$, $P = 0.021$; DEI, $t = -2.31$, $P = 0.032$, Fig. 3a and b). There was no difference in digestibility between immunochallenged and control voles before and after injection ($P > 0.05$ in all cases). There were no significant effects of time on GEI, DEI and digestibility
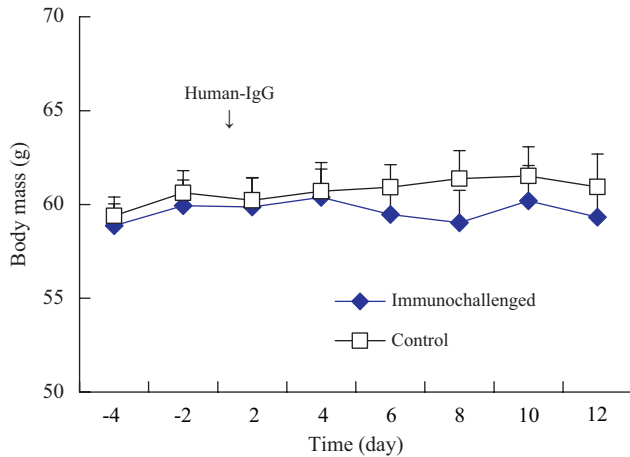
Fig. 1. Body mass of immunochallenged and control male Brandt's voles (*Lasiopodomys brandtii*). The arrow indicates the day of injection of human IgG.
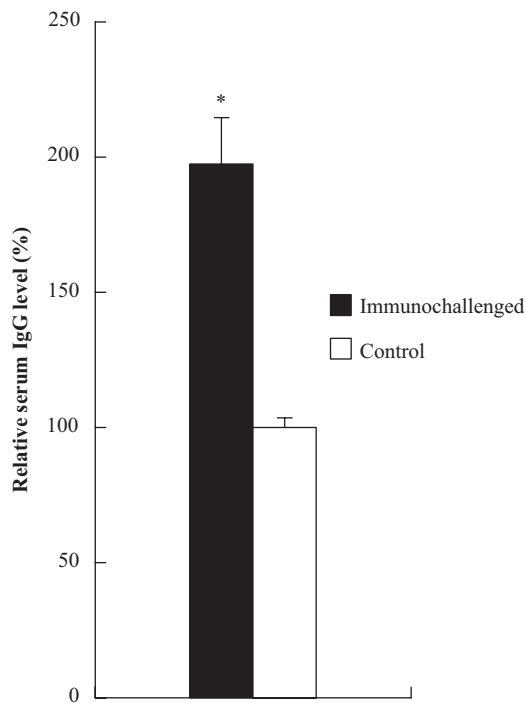

Fig. 2. Relative serum IgG levels of immunochallenged and control male Brandt's voles at 15 days post-injection. Relative serum IgG levels are expressed as percentage (%) of the mean value of the control group. Asterisk indicates $P < 0.05$.

Fig. 3. Gross energy intake (a) and digestible energy intake (b) of immunochallenged and control male Brandt's voles (*Lasiopodomys brandtii*). The arrow indicates the day of injection of human IgG. Asterisks indicate $P < 0.05$.

Masses of paired testes and paired epididymides were lower (by 74%, $t = -6.81$, $P < 0.01$ and by 85%, $t = -3.17$, $P < 0.01$, respectively) in the immunochallenged voles than in the controls (Fig. 4). Spermatophore mass tended to be lower in the immunochallenged males ($t = -1.88$, $P = 0.075$, Fig. 5). No significant differences were detected in the masses of spleen and adrenal glands ($P > 0.05$, Fig. 5).

Serum testosterone concentrations were lower in immunochallenged voles than in controls (*t*-test, $t = -6.06$, $P < 0.01$, Fig. 6). No difference in serum corticosterone concentration was detected between immunochallenged and control voles ($P > 0.05$, Fig. 7).

Discussion

Our data show that Brandt's voles challenged with human IgG increased serum antibody levels within 2 weeks. Generally the peak antibody levels are reached after around 10 days. Demas et al. (1997) challenged C57BL/6J mice with keyhole limpet hemocyanin (KLH)

(repeated measures ANOVA, $P > 0.05$) and no interaction between time and treatment ($P > 0.05$).

RMR (ml O_2 g^{-1} h^{-1}) in immunochallenged voles was higher than in controls on day 7 ($t = 3.33$, $P < 0.01$) and day 14 ($t = 2.86$, $P < 0.05$) (Fig. 4). There was no significant effect of time on measures of RMR (repeated measures ANOVA, $P > 0.05$) and no interaction between time and treatment ($P > 0.05$).
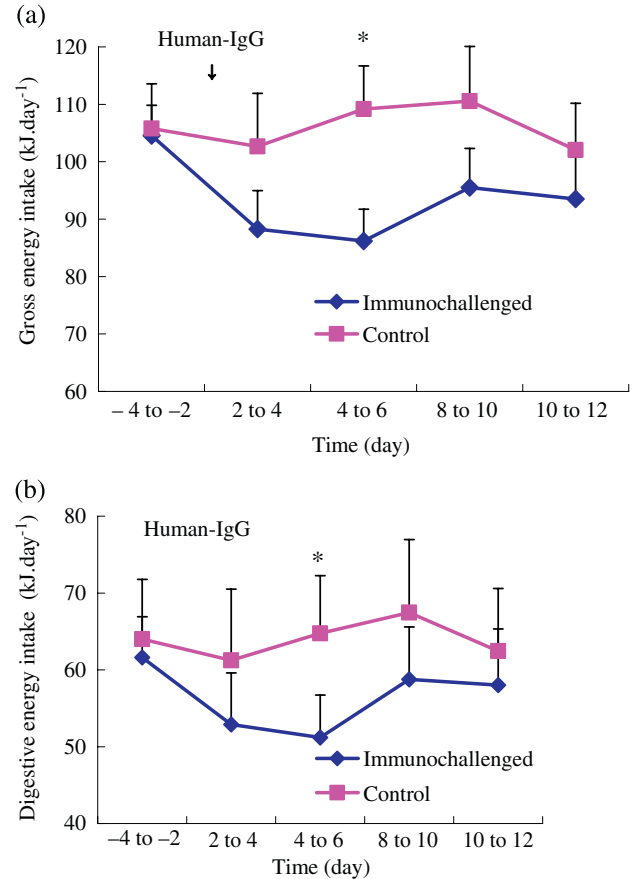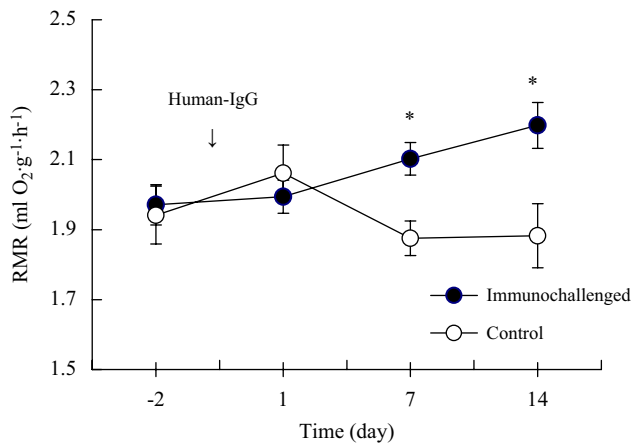
Fig. 4. Resting metabolic rates (RMR) of immunochallenged and control male Brandt's voles (*Lasiopodomys brandtii*). The arrow indicates the day of injection of human IgG. Asterisks indicate $P < 0.05$.

Fig. 6. Serum testosterone concentrations of immunochallenged and control male Brandt's voles at 15 days post-injection. Asterisk indicates $P < 0.05$.

and immunity function was increased within 10–15 days. As a humoral immune organ, the spleen mass of immunochallenged Brandt's voles tended to increase, but it did not reach significant levels, perhaps because of the mild immunochallenge (Smith and Hunt, 2004). Derting and Compton (2003) also found that only in high-intensity-immunochallenged white-footed mice spleen mass was increased.

Induction of immune activity increases energy expenditure, depresses reproductive behavior and decreases tissue growth in rodents (Martin et al., 2006). Furthermore, Martin et al. (2007) found that the secondary antibody responses and the benefits of immunological memory are energetically costly in deer mice (*Peromyscus maniculatus*). Immunochallenged Brandt's voles showed a trend to decrease GEI and
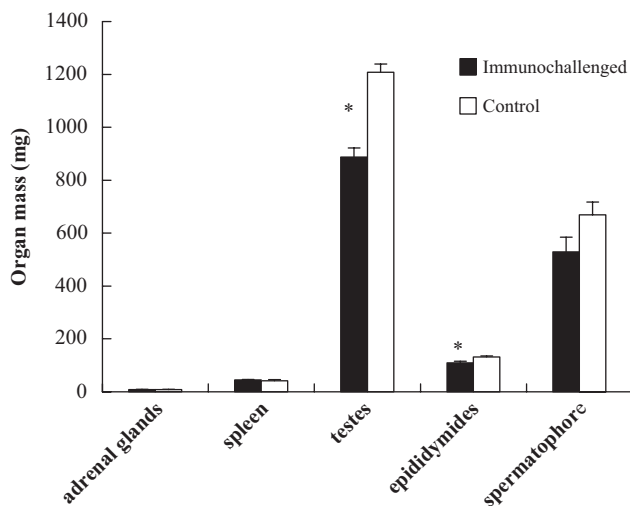
DEI. Some researchers also reported reduced food intake as a consequence of fighting an infection (Wagland et al., 1984; Scrimshaw, 1991). Derting and Compton (2003) suggested that instead of ingesting more food, animals can compensate for the energetic costs of the immune response by reducing the energy allocation to other physiological systems, such as the reproductive and digestive systems. While GEI and DEI showed a transient decrease following IgG injection,

Fig. 5. Selected organ masses of immunochallenged and control male Brandt's voles (*Lasiopodomys brandtii*) at 15 days post-injection. Asterisks indicate $P < 0.05$.
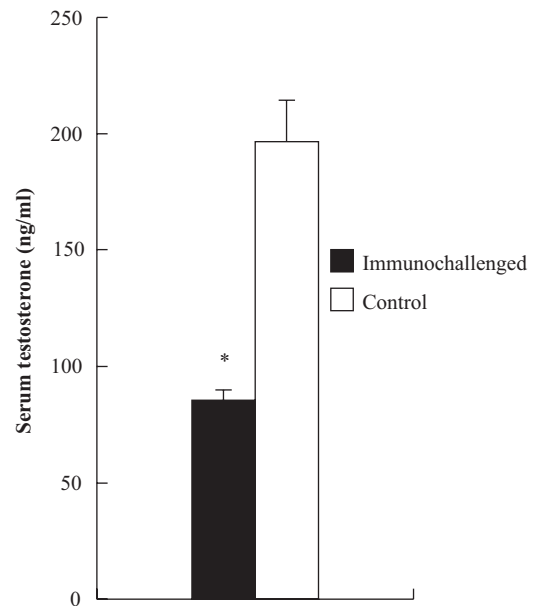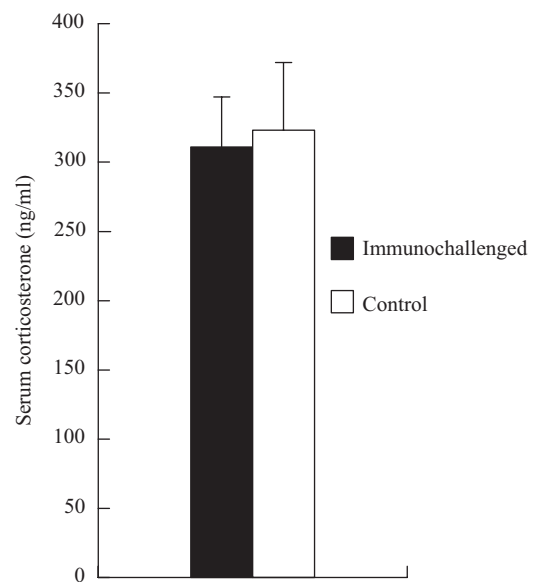

Fig. 7. Serum corticosterone concentrations of immunochallenged and control male Brandt's voles (*Lasiopodomys brandtii*) at 15 days post-injection. Asterisk indicates $P < 0.05$.

RMR increased in Brandt's voles. This means that Brandt's voles must show a behavioral compensation for the changes in energy balance or obtain additional energy from catabolizing the reproductive organs. Demas et al. (1997) observed similar responses in laboratory mice (*Mus musculus*) when challenged with KLH. Lochmiller and Deerenberg (2000) stated that a complete shift in metabolic priorities would be a more efficient way to obtain energy for the immune response rather than increasing food intake.

Reduction of energy allocation to the reproductive system when confronted with energy limitations is a fundamental component in the evolution of life history strategies in many species (Schneider and Wade, 2000). We found that the masses of paired testes and epididymides decreased by 74% and 85%, respectively, in immunochallenged Brandt's voles compared with controls. Immunochallenged voles also decreased serum testosterone concentrations. Testes and testosterone are associated with reproductive status and females may use these immunologically induced changes to distinguish the infected and uninfected males during mate choice (Nelson, 1995). For example, male rats exposed to lipopolysaccharide (LPS) or IL-1β engaged in less anogenital sniffing and sexual behavior and exhibited reduced sexual motivation toward females than did saline-injected males (Avitsur et al., 1998). LPS injection can modify the physiology and behavior in male meadow voles (*Microtus pennsylvanicus*) and prairie voles (*Microtus ocharogaster*) and females may use these changes to discriminate healthy from potentially infected males (Klein and Nelson, 1999). Taken together, our data indicate that mounting an immune response may potentially reduce the individual fitness due to the suppression of reproductive function.

Immunochallenged Brandt's voles exhibited a decrease in serum testosterone concentration and no changes were found in corticosterone levels. Klein and Nelson (1999) found that meadow voles and prairie voles can exhibit an increase in corticosterone and a decrease in testosterone concentrations within 3 h when injected with LPS. We measured corticosterone at the end of the experiment and the immune challenge did not result in a sustained increase in corticosterone levels.

In conclusion, our data suggest a shift in metabolic priorities in response to an immune challenge and provide new evidence that mounting a humoral immune response to an immunological challenge may have fitness costs for male Brandt's voles.

Acknowledgements

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