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Class: Senior
Major: Biology

I came to Murray State University as a sophomore, a year after my twin sister had enrolled. When I arrived, I saw that my sister, who had just completed her freshman year, was already involved in research and very much enjoying her experience. I began my research on the cost of mounting an immune response in male, white-footed mice as a junior and continued to work on this project through my senior year. It has been a very worthwhile and rewarding experience. Not only did I gain hands-on experience working the laboratory of Dr. Derting, I learned how to work as a member of a team, I gained practical field experience, and I now see how one research question leads to another.

ABSTRACT

Energy Allocation in Immunochallenged and Testosterone-treated White-footed Mice (*Peromyscus leucopus*)

The cost of mounting an immune response was studied in control and testosterone-treated male, white-footed mice. Our null hypotheses assumed that there is no change in metabolic rate after stimulation of the immune system (i.e., immunochallenge), and that there is no change in energy allocation to other systems during an immunochallenge. Four test groups were established: control, testosterone-treated, immunochallenged and immunochallenged plus testosterone. Injections of testosterone propionate were given to elevate testosterone level. Animals were injected with sheep red blood cells and phytohemmaglutinin to challenge the humoral and cell-mediated branches of the immune system. The daily metabolic rate (DMR) and resting metabolic rate (RMR) of each animal was determined and body organ masses were measured. When compared with control animals, immunochallenged animals had a significantly higher RMR, but no difference in DMR. Thus, our first hypothesis was partially supported. Immunochallenge had no significant effect on intestinal and vital organ masses, but was associated with significantly heavier reproductive organ masses. Hence, our second hypothesis was rejected. We concluded that there was a significant cost associated with mounting an immune response, but there was also an increase in energy allocation to reproductive organs. Thus, our results suggested that when food is readily available, an increase in immune function does not necessarily detract from energy allocation to reproductive organs.

FACULTY MENTOR

Terry L. Derting, whose research training is in the area of physiological ecology, is a professor in the Department of Biological Sciences. Her current research interests include energetics, effects of habitat fragmentation on the physiology and fitness of small mammals, and curriculum development. As a faculty participant in Murray State's Howard Hughes Medical Institute award, Dr. Derting has mentored numerous undergraduate and graduate students in research in all her interest areas. She has also involved rising freshmen, whom she identified through her participation in the pre-college component of MSU's Howard Hughes award, in research.

Energy Allocation in Immunochallenged and Testosterone-treated White-footed Mice (*Peromyscus leucopus*)

Immunity plays an important role in maintaining the health of an organism and aids its survival during diverse conditions. The immune system protects animals from parasites and antigens with the help of cell-mediated and humoral immune responses. Recent studies in the field of immunity have shown that energy tradeoffs can occur between life history components (e.g. survival and reproduction) as animals maintain a competent immune system and mount immune responses under stressful conditions (Deerenberg, Apanius, Daan and Bos, 1997; Moreno, Sanz and Arriero, 1999; Nordling, Andersson, Zohari and Gustafsson, 1998; Richner, Christe and Oppliger, 1995; Saino, Calza, Ninni and Moller, 1999; Wiehn, Korpimaki and Pen, 1999). It is not known clearly however, if there is a significant energy cost associated with an immune response and if energy allocation to various body systems is altered when immune function is elevated.

The involvement of disease and immunocompetence in the regulation of small mammal populations is not well understood, but a strong relationship between physiological stress and immunity has been demonstrated (Lochmiller, Vestey and McMurray, 1993). Stressful stimuli like immunochallenges, anthropogenic disturbances, and limited resources tend to have an inhibitory effect on immune function (Nelson and Klein, 2000). During an environmental challenge, organisms can trade off different physiological functions, such as immune and endocrine functions (Wikelski and Ricklefs, 2001). For example, immunochallenged white-footed mice (*Peromyscus leucopus*) exhibited reduced testes and intestinal masses when mounting an immune response (Derting and Compton, 2003). Such changes in physiological functions are often reflected in energy expenditure, which can thus serve as a common currency to assess the outcome of life-history tradeoffs.

During an infection or environmental challenge, the host's immune system may be given maximum preference over other bodily functions. The various symptoms of an immune response are increase in the utilization of glucose, increase in the degradation and decrease in the synthesis of proteins in the skeletal muscles, anorexia, increase in metabolic rate, alteration of the composition of trace minerals, decrease in skeletal development, and increase in hepatic lipogenesis (Klasing and Johnston, 1991; Tsigos, Papanicolaou, Defensor, Mitsiadis, Kyrou and Chrousos, 1997). Also, vaccinations with mild immune challenges like typhoid vaccine (Cooper, Horan, Little and Rothwell, 1992) and protein antigens like keyhole limpet hemocyanin (Demas, Chefer, Talan and Nelson, 1997) can result in a 15-30 percent increase in the metabolic rate of a host. Accordingly, some studies suggest that mounting an immune response is an energetically costly process for an organism. However, Derting and Compton (2003) found no change in metabolic rates of white-footed mice when immunochallenged or immunosuppressed.

Changes in food intake and food assimilation often take place during an immune response. This may be due to an increased demand for energy. Klasing and his co-workers have shown that a stimulated immune system can adversely affect the performance of growing chicks (Klasing, Laurin, Peng and Fry, 1987). For example, the growth of 3-week-old chicks challenged with sheep red blood cells (SRBC) decreased by approximately 10 percent (Klasing et al., 1987). Similarly, Henken and Brandsman (1982) found that chickens injected with SRBC consumed more food but gained less weight as compared with control chickens injected with saline. These studies showed that although there was an increase in food consumption during an immune response, there was no significant growth. So, it is possible that the efficiency of the digestive system is reduced during an immune response due to energy trade offs. Energy trade-offs between immunity and reproductive functions are also likely to occur.

Immunity and Testosterone

Steroid hormones can affect immune function (Grossman, 1984). Testosterone is responsible for the development of secondary sexual characters, plumage, brightness (Moss et al., 1979), and testosterone-dependent ornaments like the peacock's tail, bright feathers and sharp voice in males during the mating season.

Experimental studies on mammals have also shown that testosterone suppresses the immune system (Grossman, 1985). Many researchers have stated that raised testosterone levels suppress the immune response in males, that makes them more prone to infection (Klein and Nelson, 1998; Zuk, 1996). Specifically, testosterone can reduce both cell-mediated and humoral immune responses (reviewed in Schuurs and Verheul, 1990). Wedekind and Folstad (1994) suggested that testosterone suppresses the immune system because essential resources are allocated instead to produce secondary sexual characteristics such as horns, songs or stamina when repeatedly performing a display (Enstorm, Ketterson and Nolan, 1997). Another hypothesis related to elevated testosterone level and immunosuppression is that of protection of haploid spermatozoa (Hillgarth, Ramennofsky and Wingfield, 1997). Testosterone may suppress antibody production in order to protect the antigenic sperm.

On the other hand, there is a wide array of research on enhancement of immunity when testosterone level is increased. For example, Dunlap and Schall (1995) found that uninfected male fence lizards had unexpectedly higher levels of testosterone than infected males. Hillgarth and Wingfield (1997) suggested, however, that during allocation of essential resources for production of secondary sexual characteristics, the metabolic resources saved by suppressing immunity would be trivial compared to the associated risk of infection. Hence, high levels of testosterone may not be related to immunosuppression. Also, if high-testosterone males have higher parasite loads, then those males may be of such high quality overall that they can display and attract females despite higher infection due to immunosuppression (Weatherhead, Metz, Bennett and Irwin, 1993).

Our first objective was to determine the cost associated with mounting an immune response. We determined whether there was any change in metabolic rates, both daily and resting metabolic rate, during an immunochallenge. Our second objective was to determine if energy trade-offs were involved with immunochallenge. We tested two null hypotheses: (1) there is no change in metabolic rate during an immunochallenge, and (2) there is no change in energy allocation to other systems during an immunochallenge. A third objective of our research was to evaluate the cost of responding to an immune challenge with and without high levels of testosterone. If energy is limiting, then high testosterone levels may be associated with reduced immunocompetence and no change in the total daily energy budget. Alternatively, immunocompetence may be maintained when testosterone levels are high, resulting in increased daily energy expenditure.

Methods

Subjects

Our test species was the white-footed mouse (*Peromyscus leucopus*). Adult males were captured in the wild and housed in the College of Science Animal Care Facility for one month prior to testing. All animals were cared for in accordance with United States Department of Agriculture animal care guidelines. The Murray State University Institutional Animal Care and Use Committee approved all experimental protocols. We used a two by two experimental design with testosterone and immunochallenge as the independent variables. We established a testosterone and an immunochallenge treatment, resulting in four groups of animals: 1) control (CC), 2) testosterone-treated (CT), 3) immunochallenged (IC), and 4) immunochallenged plus testosterone (IT). The CC group had normal energy demand, the IC group had moderate energy demand, and the IT group had maximum potential for energy demand. The sample size for each experimental group was eight mice.

Immunochallenge Procedure

To test our first hypothesis, we estimated the daily metabolic rate (DMR) and resting metabolic rate (RMR) of each animal. To test our second hypothesis regarding energy allocation to other physiological systems during an immunochallenge, we estimated the humoral immune response, cell-mediated immune response, and organ masses of each experimental group.

Daily Metabolic Rates: Daily metabolic rate (DMR) was measured at the beginning of the experiment by placing each animal in a cage with a wire bottom to collect the feces. DMR was calculated by measuring daily food intake and fecal production (Grodzinski et al., 1975). The energy of the food (Purina Rodent Diet 5001, gross energy equivalent is approximately 16.75 kJ/g) and the dry mass of food ingested was used to calculate ingested energy in kJ/day. The feces were collected, dried at 55 ºC, and weighed. Dry mass absorption of the ingested food was determined by

subtracting the dry mass of the feces from the dry mass of the ingested food per day. The digestibility of dry matter was calculated by dividing ingested dry mass into absorbed mass. Estimated DMR was found by calculating the product of dry matter digestibility and ingested energy (Derting and Austin, 1998). Comparisons between the groups were made to determine differences in DMR, energy ingested, and digestibility. DMR was estimated on days one, 11 and 13 of the experiment.

Resting Metabolic Rates: Resting metabolic rate (RMR) was estimated by measuring oxygen consumption of both the experimental and control mice. Each mouse was tested using a Columbus Instruments O_2 ECO oxygen analyzer that had been calibrated with a primary standard gas (20.501 $\pm$ 0.005 percent oxygen). Each animal was put in a temperature-controlled chamber that was kept at 30 $\pm$ 1 ºC using a circulating water bath. The temperature was within the thermoneutral zone of the white-footed mouse (Hayward, 1965). The amount of oxygen consumed was converted into kJ/hour, and results were recorded for each animal. RMR was estimated on days three and 11 of the experiment.

Testosterone Treatments: Injections of testosterone propionate were given daily for 10 days to elevate testosterone level. All of the CT and IT animals were given 0.1 ml of a solution of 2.1 mg of testosterone proprionate (Sigma) in 3 ml of peanut oil subcutaneously. Testosterone injections were given from day four onwards until the end of the experiment. Control animals were injected with 0.1 ml of 100 percent peanut oil. All testosterone and oil injections were administered between 1230 and 1500 hr CST.

White Blood Cell Counts: Initial peripheral leukocyte counts were performed on day one of the experiment. Blood samples were obtained from the retro-orbital sinus of each animal while anesthetized. White blood cell counts were prepared in a micro centrifuge tube by mixing 2 µl of blood with 40 µl of white blood cell stain (2 percent glacial acetic acid and violet). After vortexing the mixture, 10 µl of the solution was placed onto a hemacytometer and allowed to settle for two minutes. Initial leukocytes were counted from five grids in a hemacytometer. We then multiplied the counted number by 80 to obtain the number of WBC per mm^3 of blood. Similarly, final peripheral leukocyte counts were made on day 15. Mice were given two weeks to recover from the pre-treatment blood sample.

Humoral and Cell-Mediated Immunity Challenge Procedure

The IC and IT animals were injected with SRBC and phytohemoglutination (PHA) to challenge the humoral and cell-mediated branches of the immune system, respectively. 0.1 ml of a 10 percent suspension of SRBC in physiologically buffered saline (PBS, pH=7.4) was given intraperitoneally. 0.25 ml of PHA was given subcutaneously in a randomly chosen hind foot of each animal, along with 0.25 ml of 0.9 percent sterile PBS in the other foot as a control. For PHA measurements, the thickness of each foot was measured before injection and 24 hour post injection using a pressure sensitive micrometer (Mitutoyo Corp., Calif.). The SRBC injections were given on days five and 10. The PHA and PBS injections were given on day 13.

In-Vitro Humoral Immunity: The in-vitro humoral immune response was estimated by measuring the hemagglutinating antibody titers with a microhemagglutination assay. Peripheral blood samples were obtained on day 10. The samples were centrifuged, the serum was removed, and they were then incubated at 57 ºC for 15 minutes. In a 96-well microtiter plate, serial two-fold dilutions were made of 25 µl of serum in 25 µl of PBS. Twenty-five µl of a 0.75 percent SRBC solution in PBS was added to each well. Plates were lightly vortexed and incubated at 37 ºC for an hour. Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution of serum showing positive hemagglutination (Lochmiller et al., 1993).

Hormone Concentration: Plasma levels of testosterone were measured using a radio-immuno assay kit obtained from Diagnostic Products Corporation.

Hematocrit: Heparinized hematocrit tubes were directly filled with blood from the retro-orbital sinus of the test species and sealed with critoseal. The tubes were centrifuged using a TRIAC centrifuge on the setting of “Blood” for 5 minutes. The packed cell volume (PCV) and total volume lengths were recorded.

Organ Masses: After the 15-day testing period, each mouse was sacrificed by cervical dislocation or anesthetic. Each animal was then cut ventrally and all of the major organs of the body were removed. Organs were placed in aluminum weighing dishes containing 0.9 percent PBS. Each organ (heart, spleen, liver, stomach, small intestine, caecum and colon) or organ pair (testes,

seminal vesicles, kidneys, lungs and adrenal glands) was then blotted dry with a paper towel and weighed. The stomach, caecum, small intestine and large intestine (colon) were weighed both with and without their contents. The length of these organs was also recorded after they were emptied of their contents.

Mucosa and Serosa Masses: After weighing, the small intestine was divided into three equal sections called the proximal, middle and distal sections. To measure the mucosa and serosa of the small intestine, 3 cm was removed from each intestinal section. Each 3 cm section was then spread on a slide to expose the mucosa. The mucosa was then scraped off of a 2 cm piece of each section and placed on a slide. The 3 cm of each proximal, middle and distal section was discarded. The remaining portion of each intestinal section was again weighed. Masses of all the organs and slides (with intestinal mucosa) were measured after drying in an oven (54 ºC) to a constant mass.

Results

To analyze our data, we used both one-way and two-way analyses of variance (ANOVA) and covariance (ANCOVA). For all statistical tests, differences were considered statistically significant at $p \leq 0.05$. Differences in the body masses, foot thickness, hormone concentration, hemagglutination titer, WBC counts and hematocrit measurements were analyzed using ANOVAs to determine main effects of immunochallenge and testosterone between and within subjects. Differences among means in one-way ANOVA's were determined using Tukey's Studentized Range test. To determine the differences between groups, organ masses and metabolic rates were compared using ANCOVA with body mass as the covariate. All statistical analyses were performed using SAS version 8.02.

Effects of Treatments

Testosterone Injections: Animals injected with testosterone (CT and IT) had significantly greater testosterone levels compared with control mice (CC and IC) (one-way ANOVA, $p < 0.05$; Figure 1).

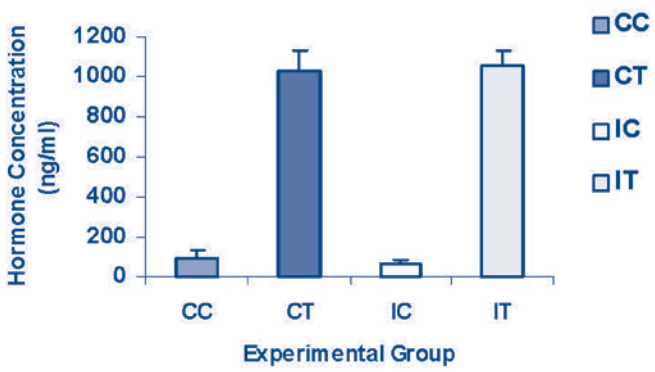

Figure 1. Testosterone level ($\overline{X} \pm 1$ SE) among the testosterone-treated groups (CT, IT) compared with the controls (CC, IC).

Immunochallenge Injections: SRBC injections caused a significant increase in the production of antibodies in the IC and IT groups (ANOVA $p = 0.029$; Figure 2). Therefore, our test of humoral immunity showed that there were antibodies in the immunochallenged groups that were treated with SRBC. The IC group had more antibodies in peripheral blood than the IT group (Figure 2). Mice injected with SRBC and testosterone (IT) exhibited a smaller humoral response.

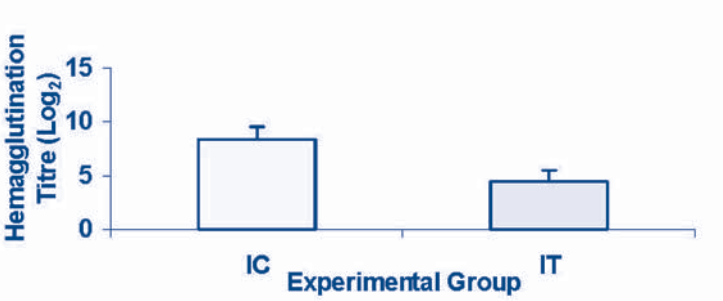

Figure 2. Hemagglutination titer ($\overline{X} \pm 1$ SE) after injection with SRBC among the immunochallenged groups.

Injections of PHA caused a significant increase in foot thickness in the IC and IT groups compared with their control groups (one-way ANOVA, p = 0.018; Figure 3). The initial measurements of foot thickness showed no significant difference among the groups. But, measurement after PHA injections showed almost a 10-fold increase in foot thickness in IC mice as compared with CC mice. There was a small increase in foot thickness in the non-immunochallenged groups (CC and CT), but it was not significant (one-way ANOVA, $p > 0.05$). The IC group tended to have a higher response to PHA than the IT group (one-way ANOVA, $p < 0.0001$; Figure 3). Thus, testosterone may have contributed to a reduced cell-mediated response in the IT mice.

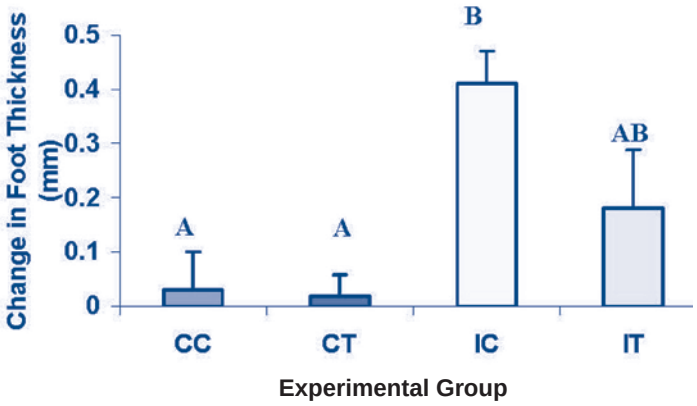

Figure 3. Change in foot thickness ($\overline{X}$ ± 1 SE) as a result of injection with PHA (IC and IT) or saline (CC and CT). Means with different letters were significantly different from each other (one-way ANOVA).

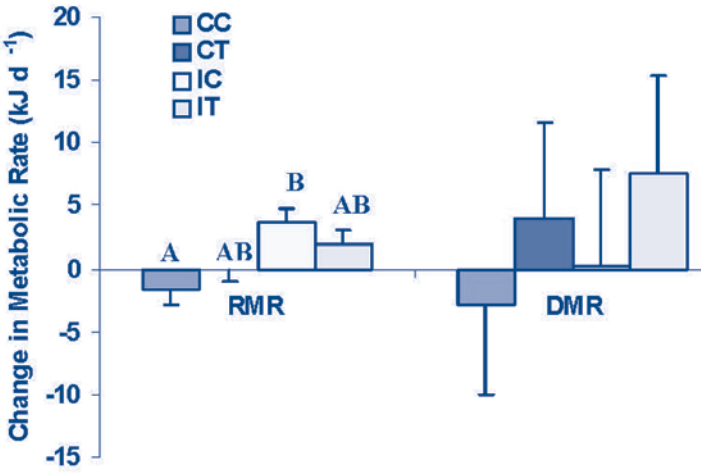

Figure 4. Changes ($\overline{X}$ ± 1 SE) in DMR and RMR among the four groups of animals. Means with different letters were significantly different from each other (one-way ANCOVA, $p < 0.05$). Results for two-way ANCOVA's are given in text.

Metabolic Rates: There was no main effect of testosterone on ingestion, digestion, RMR or DMR (two-way ANCOVA, $p > 0.05$). Immunochallenge, however, had a significant main effect on RMR (two-way ANCOVA, $p = 0.006$; Figure 4). Although DMR varied, there was no significant effect of immunochallenge treatments on the DMR of groups.

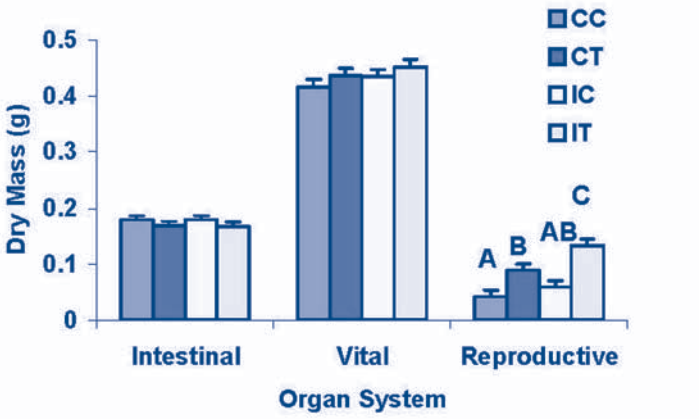

Figure 5. The dry masses ($\overline{X}$ ± 1 SE) of the intestinal organs (stomach, small intestine, caecum and colon), vital organs (heart, lungs, kidneys, liver, adrenals and liver), and reproductive organs (seminal vesicles and testes) among the groups. Means with different letters were significantly different from each other (one-way ANCOVA, $p < 0.05$). Results for two-way ANCOVA's are given in text.

Gastrointestinal Organs: Immunochallenge had no significant effect on masses of the gastrointestinal organs (two-way ANCOVA, $p > 0.05$; Figure 5). Testosterone, however, had a significant negative main effect on wet masses of the stomach and caecum (two-way ANCOVA, $p = 0.045$ and $p = 0.040$, respectively). Testosterone also had a significant negative main effect on the dry mass of stomach (two-way ANCOVA, $p = 0.015$). As a result, testosterone had a significant negative main effect on the total mass of gastrointestinal organs (two-way ANCOVA, $p = 0.04$; Figure 5).

Vital Organs: Immunochallenge had a positive main effect on wet liver mass (two-way ANCOVA, $p = 0.029$). Testosterone had a positive main effect on wet and dry kidney masses (two-way ANCOVA, $p = 0.002$ and $p = 0.019$, respectively). After summing the masses of the vital organs (heart, lungs, spleen, liver and kidneys), both immunochallenge and testosterone treatments had no significant effect on the total wet and dry vital organ masses (two-way ANCOVA, $p > 0.05$; Figure 5).

Reproductive Organs: Both immunochallenge and testosterone had significant main effects on the reproductive organs. Immunochallenge had a positive main effect on the wet and dry masses of seminal vesicles and testes (two-way ANCOVA, seminal

vesicles wet mass $p = 0.034$, seminal vesicles dry mass $p = 0.035$, testes wet mass $p = 0.009$, testes dry mass $p = 0.005$, respectively). Testosterone treatment had a significant positive main effect on the wet and dry masses of the seminal vesicles (two-way ANCOVA, $p < 0.0001$; Figure 5).

Discussion

The main objectives of our research were to understand how animals pay for the cost of immunity and to determine patterns of energy allocation in immunochallenged and testosterone-treated white-footed mice. Our experiment showed several significant results. DMR and RMR tests were used to test our first hypothesis. We saw that an immunochallenge had a significant main effect on RMR (Figure 4), although there was no effect on DMR. Thus, our first null hypothesis was partially supported. The masses of the intestinal, vital and reproductive organs were used to test our second hypothesis. Immunochallenge had no significant effect on the masses of the intestinal organs, but was associated with a significant increase in the masses of the reproductive organs (Figure 5). We, therefore, rejected our second null hypothesis. During an immunochallenge energy tradeoffs might have occurred that resulted in energy allocation to some other system. Additional experiments are required to understand the relationship between increased RMR, immunity, and reproductive organ masses and how they interact with each other.

There were several possible outcomes of our research. It was possible that during mounting an immune response, the amount of energy required for immunity increased, which might have reduced the amount of energy available for use for other physiological activities. Several studies have shown that under energetically challenging conditions animals tend to trade-off energy from some energetically costly activities for the activities that are critical to survival (Nelson and Klein, 2000). Nelson and Demas et al., (1996) showed that environmental, social and intrinsic factors can affect both immune and reproductive functions. Bronson and Heideman (1994) showed that whenever confronted with a negative energy balance, mammals tended to inhibit their reproduction. But our results contradicted these results because we saw that immunochallenged animals were able to maintain high masses of reproductive organs under good conditions. This suggests that both survival and protection of its genes are vital to an organism.

Another potential result of our research was that when energy use for immunity increased during an immunochallenge an animal might simply have eaten more food, resulting in no decrease in energy use for other activities. Our findings showed that there was a significant cost associated with mounting an immune response, as indicated by the increase in RMR in the IC and IT mice. There have been various other studies done using more indirect measures that showed similar results (e.g., Ilmonen, Taarna and Hasselquist, 2000; Norris and Evans, 2000). Cooper et al. (1992) and Demas et al. (1997) showed that there was a 15 percent to 30 percent increase in metabolic rate during immunochallenge in response to typhoid vaccine or injection of protein antigens like keyhole limpet hemocyanin during vaccination, respectively. But on the other hand, immunochallenge had no main effect on the DMR, and there was no significant increase in total energy expenditure (i.e., DMR). Thus, to fuel the increase in immune activity, some energy must have been taken away from activities (e.g., locomotor activity, endocrine function) that we did not measure. Our results indicated that animals do pay a price for immunity by reducing energy expenditure on other activities. Because there was an increase in energy allocation to the reproductive organs in IT animals, however, we found no trade-off in energy use between immunity and reproduction. Thus, animals were able to mount an immune response and maintain their reproductive organs when maintained under favorable conditions (i.e., *ad libitum* food, warm temperature), even when immunochallenged. Maintenance of both increased immune function and reproductive organ masses indicates that immunity and reproduction are high priority processes. This result was not surprising because health and reproduction are likely to be essential for maximizing fitness in multiparous species. Our results contradicted data collected from a similar study on white-footed mice (Derting and Compton, 2003), where trade-off in energy allocation occurred between immune function and the reproductive and digestive organs. The cost of mounting an immune response was met through reduced energy allocation to the reproductive and digestive systems (Derting and Compton).

The testosterone treatment had a suppressive effect on the immune system of the white-footed mice. Testosterone-treated mice (IT) had significantly lower hemagglutination titers as compared to control mice (IC) (Figure 2). This suggests that testosterone contributed to reduced humoral immune response. Similarly, testosterone was associated with a reduced hypersensitivity

response resulting from the stimulation of the cell-mediated branch of the immune system. The cell-mediated immune responses were reduced in the IT mice as compared with the CT mice (Figure 3). These results were consistent with the finding of numerous previous researchers. In the case of reduced humoral immunity responses, primary antibody responses *in vivo* were suppressed when testosterone concentrations were increased within the high physiological ranges (Duffy, Bentley, Drazen and Ball, 2000). Similarly, Lehmann, Siebold, Emmons and Muller (1988) showed that testosterone and dihydrotestosterone had inhibitory effects on the proliferation of peripheral blood white blood cells in response to a T-cell-B-cell dependent mitogen *in vitro*. Also, various synthetic derivatives of testosterone, like 19-nortestosterone and stanazol, have been associated with specific immuno-suppressive effects, reduced lymphocyte proliferation, and chemotaxis of spleen and thymus derived cells that help in fighting infection (Ferr·dez, de la Fuente, Ferr·ndez and Manso, 1996). In the case of reduced cell-mediated immune response, Wan, Haw and Blackburn (1989) suggested that since testosterone contributed to suppression or inhibition of specific aspects of macrophage functions, it might have resulted in an overall reduction in immune function of the cell.

Testosterone was also related to reduced body mass, reduced masses of gastrointestinal organs, and increased mass of reproductive organs (Figure 5). This suggests that during trade-offs in energy use, animals preferentially allocate their energy to reproductive organs at the cost of digestive organs and body mass. Maintenance of a moderate increase in immune function and marked increase in reproductive organ masses is consistent with other studies. For example, Dunlap and Schall (1995) showed that male lizards (*Sceloporus occidentalis*) were able to maintain immunocompetence concurrently with high levels of reproductive function. Also, recently it has been suggested that the hormones that were traditionally considered as reproductive hormones can modulate immune function (Kalra and Kalra, 1996; Ober and van der Ven, 1997; Priddy, 1997).

Our results indicated that animals do pay a price of immunity by reducing energy expenditure on other energetically costly physiological process(es). The interesting fact was that the increase in immune function did not detract energy from reproduction, which is an energetically expensive activity. Thus, immune and reproductive functions appear to be high priority processes for maintaining fitness of the animal. It is not possible for animals to maximize the functioning of some systems without imposing costs on other systems (Nelson and Klein 2000). Therefore, there must have been tradeoffs of energy involved during immunochallenge and testosterone treatments. Further research is needed to identify the specific source(s) of energy used to fuel simultaneous increases in RMR, immune functions and reproductive organ masses. It is safe to say that during the course of evolution which might have involved various energetic trade-offs, animals have been successful in their attempt to allocate energy to various physiological process(es) under energetically challenging conditions while maintaining maximum lifetime reproductive success at the same time (Nelson and Klein 2000).

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