

# Food supplements modulate changes in leucocyte numbers in breeding male ground squirrels

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## Summary

Immunosuppression may be an important cost of reproduction in breeding males. It can result from elevated levels of testosterone or stress hormones and may serve to lower the energetic cost of maintaining immune function at a time of high demand. This suggests that greater access to energy resources could reduce immunosuppression as a cost of reproduction, minimizing the trade-off between energetic investment in current reproductive effort and survival. I examined the impact of food availability on immune function by provisioning male Belding's ground squirrels in the field from the time they emerged from hibernation to the start of breeding. Temporal changes in immune status, measured by leucocyte counts, differed between provisioned males and

un-provisioned controls. Provisioning advanced the increase in lymphocytes and neutrophils from after breeding to before. At the start of breeding, the leucocyte count was three times greater in provisioned males than in controls and was still nearly twice as great at the end of breeding. Control males increased all leucocyte numbers after breeding. This experiment demonstrates that variation in food intake can lead to individual variation in the extent of immunosuppression during breeding and therefore that reduced immune function may not be an obligatory cost of reproduction.

Key words: cost of breeding, immunosuppression, *Spermophilus beldingi*, ground squirrel, leucocyte, hibernation.

## Introduction

Immune suppression is thought to be a major cost of reproduction and sexually selected behavior. Immune suppression may be due to the inevitable increase in sex steroids during the breeding period and/or it may be stress induced (Boonstra et al., 2001b; Deerenberg et al., 1997; Gustafsson et al., 1994; Nordling et al., 1998; Sheldon and Verhulst, 1996). Because immune suppression during breeding appears to be inevitable, it has been used to explain the association of sexual size dimorphism with sex-based parasitism and mortality rates in mammals (Moore and Wilson, 2002). Immune suppression also has a fundamental role in handicap signaling models of mate choice. These models assume an androgen basis for both immune suppression and the development of a sexually selected trait, thus ornamentation reveals an immune suppression 'handicap' that can nevertheless be sustained by the male.

At least three hypotheses might explain suppressed immune function in breeding males. First, immune function might be depressed by elevated testosterone titers during breeding (Boonstra and Boag, 1992; Boonstra et al., 2001b; Bradley, 1987; Folstad and Karter, 1992; Grossman, 1985; Tang-Martinez and Taylor, 1998). Second, reproductive competition might impose stress on males, resulting in immune suppression through elevated glucocorticoid titers (Boonstra et al., 2001a,b; Bradley, 1987; Buchanan, 2000). Third, immunosuppression

might result from the diversion of energy or nutrients towards reproductive activities and away from immune function (Sheldon and Verhulst, 1996). Although this third hypothesis often implies intervening hormonal control and forms a basis of adaptive explanations for endocrine-based immunosuppression (Boonstra and Boag, 1992; Deerenberg et al., 1997; Gustafsson et al., 1994; Nordling et al., 1998; Wedekind and Folstad, 1994), energy limitations can also directly cause immunosuppression. Immune function is known to be depressed by specific nutrient deficits as well as by starvation (Cunningham-Rundles, 1993; Jain, 1993; Jose and Good, 1973; Lochmiller et al., 1993). Nutrient or energy limitation in breeding males is suggested by mass losses, which may reflect both increased energy expenditure on reproductive activities and reduced food intake (Andersson, 1994). However, the role of lowered food intake on changes in immune function during breeding is unknown.

If resource limitation is an important variable determining the extent of immunosuppression, the impact is most likely to be found in animals that mate when food availability is low. This situation is typical for male Belding's ground squirrels living at high elevations in western USA. Males emerging from hibernation are usually unable to forage due to extensive snow cover. As the snow melts and foraging opportunities increase, females emerge and, within a few days, come into estrus for a

few hours each. This presents males with a time and energy allocation conflict. Mate acquisition competes with foraging for time and uses energy needed to restore the complete function of physiological systems, including the immune system, which atrophied during hibernation (Lyman and Chatfield, 1955; Shivatcheva and Hadjioloff, 1987; Sidky et al., 1972; Spurrier and Dawe, 1973). Failure to maintain adequate immune function might be particularly costly because males acquire numerous wounds during the breeding period (Sherman and Morton, 1984). I investigated the effect of food availability on immune function by provisioning a set of males in the field just prior to breeding and following their subsequent performance through the breeding period relative to an unprovisioned control group. I assessed the status of the immune system from leucocyte (white cell) counts. While this is an indirect assessment of immune function, low leucocyte counts are predictive of a less effective immune system and have been used previously as a proxy for immune function (Boonstra et al., 2001a,b; Gustafsson et al., 1994; Voigt, 2000).

### Materials and methods

I studied a population of Belding's ground squirrels (*Spermophilus beldingi* Merriam 1888) at Dana Meadows, Yosemite National Park, CA, USA (elevation 3031 m) in the spring and summer of 1999. Over their range in western USA, Belding's ground squirrels are common in high alpine meadows (Jenkins and Eshelman, 1984), which are invariably covered with a meter or more of snow in early spring when they emerge from hibernation. Starting in early May, my assistants and I searched the study site daily for evidence of recently emerged squirrels. Live-traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) with a very small amount of bait (generic peanut butter, oatmeal and shredded carrot) were placed around a hole in the snow. Once captured, males were fitted with radio collars (Wildlife Materials Inc., Carbondale, IL, USA) and marked with hair dye to facilitate observations and re-capture. The first male emerged on 9 May. Females began to emerge 10 days later when snow cover was thin or had disappeared from burrow sites. Matings began on 30 May and ended on 12 June.

### Provisioning

I began provisioning on 12 May, by which time four males had been located. The first male was assigned a treatment (provisioned or control) based on a coin toss, and subsequent treatment assignments were alternated. The last two males were added to the experiment on 18 May. One pair of males who were always found sharing a burrow or swapping burrows were assigned the same treatment (control). By otherwise alternating the assignment of provisioned and control treatments according to capture sequence, I hoped to evenly distribute time since emergence between treatments and, by doing so, to evenly distribute any variable, such as age, that co-varied with emergence. Only adult males were used in this study; yearling males were distinguished and eliminated by

size and the lack of scrotal testes (Morton and Gallup, 1975). The first yearling male was captured on 26 May. I studied 12 control and 11 provisioned males, although all are not represented in every sample. To provision a male, I placed approximately 20 g of the bait mixture into a burrow after using radiotracking to both confirm his location and establish that no other study male was in the same burrow. Provisioning ended on 25 May after male activity and ranging increased prior to the first mating.

### Data collection

I weighed each male to the nearest 0.1 g (Ohaus Scout, 400/.1, Pine Grove, NJ, USA) and collected a blood sample soon after his first appearance above ground (Sample 1, 9–18 May). Subsequent samples were collected as follows: Sample 2, 26 May (four days prior to the first known mating); Sample 3, 10 June (two days prior to the last known mating); Sample 4, 26 June; Sample 5, 15 July (when summer fattening began); and Sample 6, 31 July (Figs 1, 2A). On sample days, males were captured in the morning at or near their burrows. Within 15 min of capture, the trap was covered and moved to a protected location off the snow and out of the wind. Squirrels were transferred from the trap to a mesh bag and anesthetized with an inhalant anesthetic (isoflurane; Abbot Laboratories, North Chicago, IL, USA) to facilitate handling. Within 5 min of removal from the trap, we weighed each squirrel, checked for wounds and collected a thin film blood smear from toenails that were clipped short enough to obtain a small droplet from the blood supply in the nail. Previously clipped nails were easily identifiable and were not clipped again. We preferentially used the 1st and 5th digit of the hind feet as these were the smallest. When needed, bleeding was stopped with direct pressure to the cut together with Kwik-stop styptic powder (Gimborn Pet Specialities, Atlanta, GA, USA). Up to 2 h elapsed between trapping and sampling, but there was no consistent bias in trapping or handling times across individuals or treatments. Behavioral data were recorded from 09.00 h to

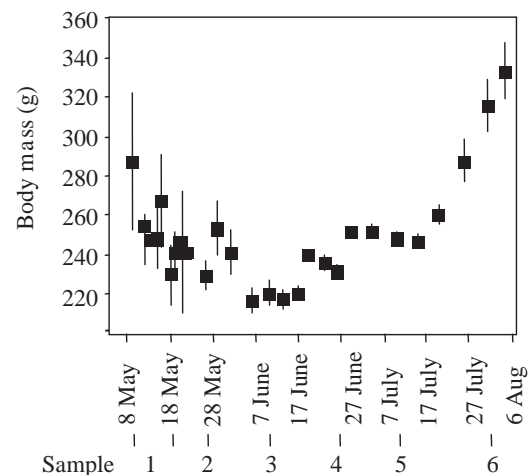


Fig. 1. Total body mass (means  $\pm$  S.E.M.) of un-provisioned (control) males.

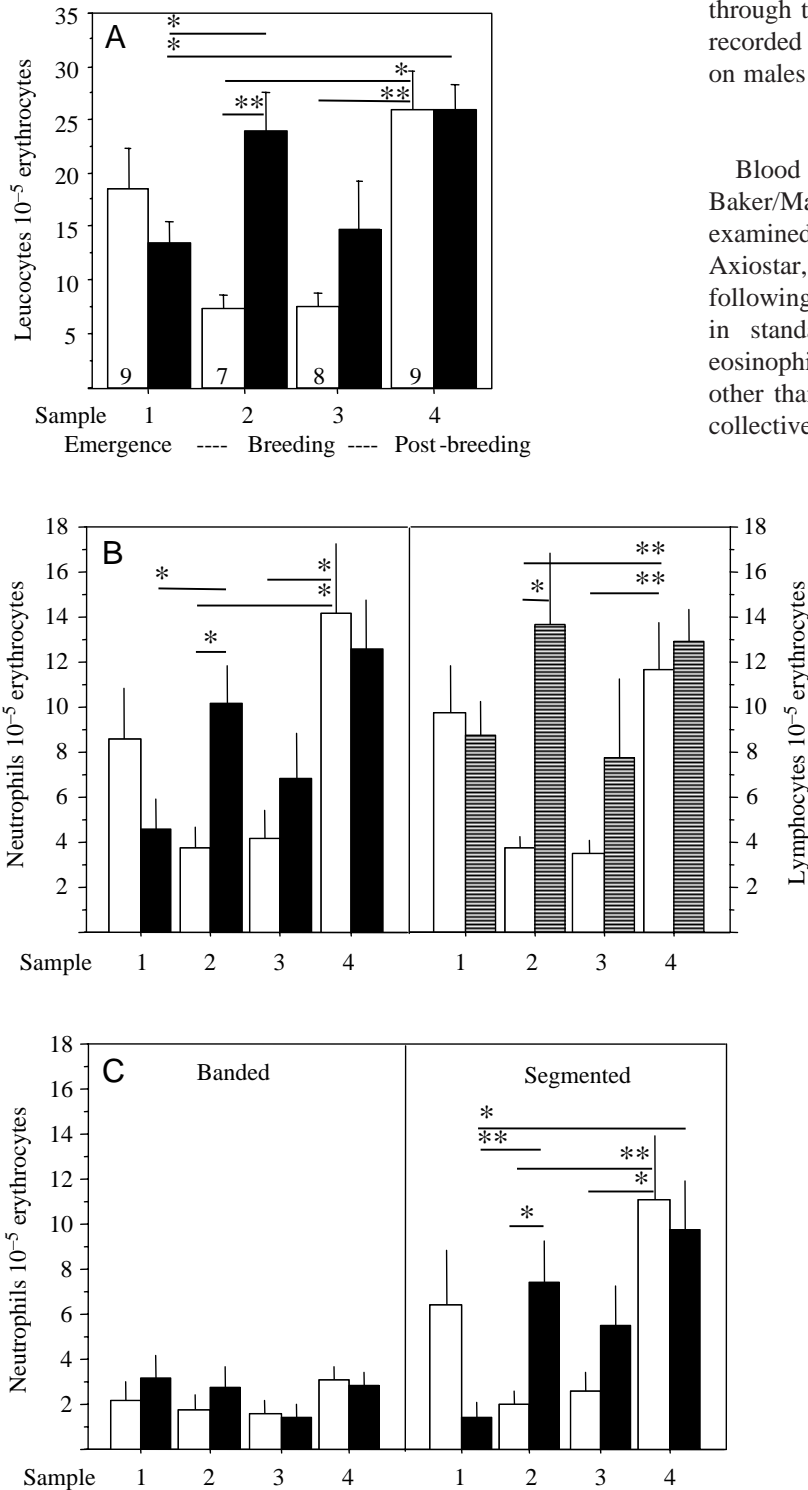


Fig. 2. (A) The number of leucocytes in blood smears from provisioned (filled bars) and control (open bars) male Belding's ground squirrels. (B) Total neutrophil (filled black bars) and lymphocyte (striped bars) counts from provisioned and control (open bars) squirrels. (C) Banded and segmented neutrophil counts from provisioned (solid bars) and control (open bars) squirrels. Sample sizes are shown at the base of each bar, and overhead bars indicate significant differences from Bonferroni-adjusted *t*-tests. \*Bonferroni  $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . Values are means  $\pm$  S.E.M.

as late as 18.30 h on 20 days from the start of provisioning through the breeding period. Male location and activity were recorded at least every 15 min, with a closer watch being kept on males near an estrous female.

*Analysis of blood smears*

Blood smears were stained with Geimsa solution (J. T. Baker/Mallinkrodt, Baker Inc., Phillipsburg, NJ, USA) and examined under oil immersion at 100 $\times$  magnification (Zeiss Axiostar, Thornwood, NY, USA). I was able to identify the following leucocytes based on the morphology of these cells in standard laboratory rodents (Voigt, 2000): basophil, eosinophil, lymphocyte, monocyte and neutrophil. Leucocytes other than lymphocytes and neutrophils were extremely rare collectively, averaging  $1.4 \pm 0.2\%$  across all samples, and were therefore excluded from the analysis. Most lymphocytes and neutrophils are in storage, with relatively fewer in circulation at any given time (Goldsby et al., 2000; Jain, 1993). Lymphocytes are responsible for the specific immune response, while the phagocytic neutrophils form a 'first line of defense', being associated with non-specific immune responses. I distinguished between immature (banded) neutrophils, which have an elongated, band-shaped nucleus, and mature (segmented) forms, in which the nucleus is segmented into multiple lobes; banded neutrophils are not as phagocytically active as the segmented forms (Jain, 1993). Leucocyte counts were obtained by recording the number and kind of each cell type as well as the number of erythrocytes encountered in a single pass across the slide, then standardizing the leucocyte numbers to 100 000 erythrocytes. To reduce biases in counts due to uneven distribution of cell types across the smears, I began searching for cells off the ends of the smear, and the search path moved from one long axis of the slide to another (Voigt, 2000). Counts were made by eight observers who were trained and tested on standards. Each datum is the average of counts from two observers.

*Statistical analysis*

The primary focus of the analysis was four samples taken from near-emergence from hibernation to approximately two weeks after breeding in late June. Cell counts for Samples 5 and 6 were not significantly different from those of Sample 4. I evaluated the effects of treatment and sample time on the leucocyte counts using mixed models repeated-measures ANOVAs (SAS) that included an autoregressive term to remove any dependence in leucocyte scores between successive samples. For analyses with significant interactions, I used *t*-tests to evaluate

the effect of treatment within sample periods (unpaired) and differences between sample periods within treatments (paired), and the resulting  $P$  values were Bonferroni-adjusted. All leucocyte measures were square-root transformed to remove right skew prior to analysis, but the figures plot untransformed values. Descriptive statistics are reported as means  $\pm$  S.E.M.

### Results

Repeated-measures ANOVAs revealed significant interactions between treatment and time for total leucocytes ( $F_{3,41}=5.82$ ,  $P=0.002$ ; Fig. 2A), lymphocyte numbers ( $F_{3,41}=3.20$ ,  $P=0.03$ ; Fig. 2B), total neutrophils ( $F_{3,41}=3.31$ ,  $P=0.03$ ; Fig. 2B) and segmented neutrophil count ( $F_{3,41}=3.61$ ,  $P=0.02$ ; Fig. 2C). These relationships are examined further below.

#### *Control males*

During breeding (Samples 2 and 3), control males had fewer circulating white blood cells than at any other time, suggesting suppressed immune function at this time. The total leucocyte count dropped by half from emergence (Sample 1) to the beginning of the breeding period (Sample 2), although not significantly, remained low through the breeding period (Samples 2–3) and increased significantly, more than trebling, after breeding was over (Sample 4; Fig. 2A). Counts remained elevated for the rest of the summer (see Materials and methods). This pattern was matched by changes in the lymphocytes and neutrophils (Fig. 2B). Changes in total neutrophil numbers were mainly due to changes in the number of mature, segmented neutrophils (Fig. 2C), which more than quadrupled after the end of breeding (Samples 3–4). The mean proportion of segmented (mature) neutrophils increased steadily over time (Kendall's tau=0.79, d.f.=5,  $P=0.003$ ).

#### *Provisioned males*

There were substantial differences between control and provisioned groups in the numbers and kinds of circulating white blood cells over time. Provisioned males were fed from emergence (Sample 1) until four days before the first mating (Sample 2). During this time, their total leucocyte counts increased significantly while controls decreased (Fig. 2A; change from Sample 1 to 2,  $t_9=3.532$ ,  $P=0.006$ ). As a result, provisioned males entered breeding (Sample 2) with three times as many leucocytes as controls (Fig. 2A). Leucocyte numbers in provisioned males decreased slightly during breeding but were still nearly twice as high as controls at the end of breeding (Sample 3). As with controls, counts tended to increase after breeding (Samples 3–4; Fig. 2A) and the two groups had similar leucocyte counts by Sample 4, two weeks after the last mating.

The provisioned and control males also differed in how the sub-populations of leucocytes changed. In provisioned males, trends in lymphocyte numbers over time followed the pattern for total leucocytes although no differences were significant. However, due to the pre-breeding decline of lymphocyte

numbers in control males (see above), provisioned males began breeding with over three times as many lymphocytes as controls (Sample 2; Fig. 2B), reflecting the increase in provisioned lymphocyte numbers relative to controls (change from Sample 1 to 2,  $t_9=2.605$ ,  $P=0.03$ ). The neutrophil count in provisioned males increased significantly from emergence to the onset of breeding (change from Sample 1 to 2,  $t_9=3.162$ ,  $P=0.01$ ), when it was significantly higher than that of controls (Fig. 2B). Subsequent changes in neutrophil counts in provisioned males were not significant. As with controls, provisioned males showed little change in the total number of immature, banded neutrophils over time (Fig. 2C). Therefore, the significant increase in the total number of neutrophils as breeding began (Sample 2) appears to be due to an increase in the number of segmented neutrophils. Provisioned males began breeding with significantly more segmented neutrophils than did controls and maintained these numbers throughout breeding (Fig. 2C). In short, provisioning abolished the trend for a pre-breeding decline in lymphocyte numbers seen in control males and advanced the increase in lymphocytes and neutrophils from after breeding to before.

#### *Mass effects*

Mean male mass decreased from emergence through breeding, increased slowly for approximately a month and then increased rapidly in preparation for hibernation (Fig. 1; Checker, 2001). Temporal changes in leucocyte counts from emergence through breeding generally followed changes in mass (Figs 1, 2); however, body mass was not significantly correlated with leucocyte numbers in any sample period.

Provisioned males gained mass from Sample 1 to 2 relative to controls as both absolute mass (provisioned mean gain =  $21.9\pm 8.7$  g; control mean loss =  $-23.0\pm 5.8$  g;  $t_{10}=3.903$ ,  $P=0.003$ ,  $N=12$ ) and as a proportion of initial mass (provisioned proportional gain =  $0.1\pm 0.04$  g; control proportional loss =  $-0.09\pm 0.02$  g;  $t_{10}=3.810$ ,  $P=0.003$ ,  $N=12$ ). While leucocyte numbers were not related to mass at Sample 2 ( $P=0.26$ ), there was a positive correlation between the change in mass since the first sample and the change in neutrophil numbers (Fig. 3), which reflects the impact of the provisioning treatment on both mass and cell numbers (see below). While the changes in total leucocyte numbers and lymphocytes both exhibited a positive trend with the change in mass, the relationships were not significant (change in mass vs change in leucocyte count, Sample 1 to 2,  $P=0.11$ ,  $r=0.484$ ,  $N=12$ ; change in mass vs change in lymphocyte count, Sample 1 to 2,  $P=0.25$ ,  $r=0.368$ ,  $N=12$ ). During the breeding period (Samples 2–3), provisioned males tended to lose more mass than did controls ( $40\pm 9$  g vs  $8.9\pm 11.3$  g;  $t_{11}=2.183$ ,  $P=0.052$ ,  $N=13$ ), and after breeding both groups gained mass at similar rates (Samples 3–4; provisioned =  $8.9\pm 8.1$  g day<sup>-1</sup>; control =  $8.9\pm 5.4$  g day<sup>-1</sup>). During breeding, cell numbers were not related to mass change.

#### *Male activity*

Before matings began, males were active for a mean of

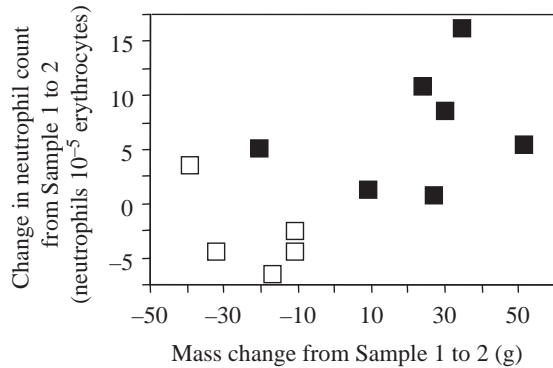


Fig. 3. Change in mean neutrophil numbers in relation to mass from shortly after emergence from hibernation (Sample 1) to the start of breeding (Sample 2). Provisioned males (solid) and control males (open).  $P=0.035$ ,  $r=0.611$ ,  $N=12$ .

$4.4 \pm 0.3$  h, increasing to  $7.2 \pm 0.4$  h early in the breeding period (paired  $t_{17}=8.783$ ,  $P=0.0001$ ,  $N=18$ ), with no difference between control and provisioned groups. Similarly, foraging time did not differ between control and provisioned groups. Overall, foraging increased significantly during the breeding period, from a mean of  $0.54 \pm 0.1$  h  $\text{day}^{-1}$  before breeding to  $2.4 \pm 0.2$  h  $\text{day}^{-1}$  during breeding ( $t_{17}=11.014$ ,  $P=0.0001$ ,  $N=18$ ). During the breeding period, provisioned males were involved in significantly more agonistic encounters, spending a mean of  $51 \pm 7$  min in fights or chases, in contrast to  $33 \pm 2$  min for control males ( $t_{16}=2.124$ ,  $P=0.05$ ,  $N=18$ ). Mating success, however, did not differ between the treatment groups (provisioned,  $3.9 \pm 0.4$  copulations; control,  $3.7 \pm 0.6$  copulations; range 1–5). All males had received numerous wounds by the end of the breeding period.

### Discussion

Male Belding's ground squirrels decreased circulating lymphocyte and neutrophil numbers from the time they emerged from hibernation to the onset of breeding. These cell populations did not increase until after breeding was over (Fig. 2A). Provisioning males prior to breeding resulted in significant increases in both cell types. As these are general indicators of immune function (Goldsby et al., 2000; Jain, 1993), the increase in cell numbers seen in provisioned males prior to breeding, particularly the increase in the number of mature, segmented neutrophils, is predictive of an immune system that is better prepared to respond to infections from wounds inflicted by other males as they fight for access to females and from females during copulation (G.C.B., personal observation).

The allocation of limited energy and nutrients during breeding may be particularly important for mammals, such as ground squirrels, that breed shortly after emerging from hibernation. The size and function of many organs, including organs associated with immune function, are reduced during hibernation (Lyman and Chatfield, 1955; Morton and Parmer,

1975; Shivatcheva and Hadjioloff, 1987; Sidky et al., 1972). The limited food available in early spring (Morton, 1975; Morton and Sherman, 1975) must be directed to the recovery of organ function as well as breeding activity. Like most ground squirrels, Belding's do not cache food. Of the >20 *Spermophilus* species, only four are thought to cache when suitable food items are available (*S. saturatus*, *S. richardsonii*, *S. columbianus* and *S. parryii*; Wilson and Ruff, 1999; Michener and Locklear, 1990) and, with the exception of *S. parryii* (Buck and Barnes, 1999), only the males do so, using the stores primarily to support testes development and spermatogenesis and possibly to maintain condition in early spring. Whether these caches are sufficient in energy or nutrient content to also support the development of immune function raises another dimension to the costs and benefits of caching in hibernating sciurids.

In squirrels, reactivation of lymphoid tissue begins before above-ground activity commences (Shivatcheva and Hadjioloff, 1987; Sidky, 1972), but this study shows that a mature complement of leucocytes has not been established at this time. The subsequent development of the leucocyte population is dependent upon access to food. Although control males foraged more during breeding, they appeared to allocate their resources to fueling reproductive activities and did not increase mass or leucocyte numbers until after breeding. The provisioning experiment demonstrates that males have the capacity to improve one aspect of immune function, circulating white cell numbers, nearly one month earlier than in controls. An important implication from this study is that immunosuppression may not be an obligatory cost of reproduction for males. In good habitats or with sufficient time to forage, males can potentially maintain both immune function and breeding effort by increasing net energy intake. A key allocation problem for males may therefore be whether to spend time acquiring mates vs nutrients, and the cost of insufficient intake may be reduced immune function. This provides an alternative, or at least a contributing, mechanism for reduced immune function in breeding male mammals. While all mammals do not face conditions as extreme as those encountered by these squirrels, many lose mass during breeding (Andersson, 1994), suggesting that energy allocation to immune function could be at risk.

Previous studies have proposed that immune suppression in male mammals during breeding is a consequence of elevated titers of either stress hormones, particularly glucocorticoids, or testosterone (Boonstra and Boag, 1992; Boonstra et al., 2001a,b; Buchanan, 2000; Grossman, 1985; Nelson and Demas, 1996). While there is little question that endocrine changes during breeding are immunosuppressive, these effects alone are unlikely to account for the different leucocyte profiles in the provisioned and control males in this study.

Stress associated with breeding can lead to endocrine changes that reduce the number of circulating leucocytes (Buchanan, 2000; Jain, 1993; Voigt, 2000). Endocrine changes associated with acute stress remove lymphocytes from circulation by moving them to marginal pools and tissues, which can enhance

immune responses (Dhabhar and McEwen, 1997, 1999), while chronic stress leads to increased lympholysis, as well as increasing the retention of neutrophils in circulation, and is ultimately immunosuppressive (Baxter and Forsham, 1972; Dhabhar and McEwen, 1997, 1999). In the present study, differential exposure to acute stressors such as trapping and handling or aggressive social interactions cannot account for the lower breeding leucocyte counts in control males (see Materials and methods; Boonstra and Singleton, 1993; Kenagy and Place, 1999; Moberg, 1985). Similarly, all males were active in breeding, which has been shown to be a source of chronic stress leading to higher cortisol titers and lower white cell counts in male Arctic ground squirrels (*S. parryi*; Boonstra et al., 2001a,b). While the drop in lymphocyte numbers seen in control males at the onset of breeding is consistent with changes induced by chronic stress, an increase in neutrophil numbers did not occur (Fig. 2B,C). Similarly, although provisioned males reduced total leucocyte numbers during the breeding period, the trend was to decrease both lymphocytes and neutrophils, and neither decline was significant on its own (Fig. 2B,C). Therefore, stress responses are insufficient to explain both the higher leucocyte counts in provisioned males and the pattern of changes in leucocyte numbers during breeding.

The immunosuppressive effect of testosterone has attracted attention as a potential mediator of a trade-off between immune function and dominance in male competition (Tang-Martinez and Taylor, 1998; Wedekind and Folstad, 1994). Not surprisingly, testosterone levels are elevated in male ground squirrels during the mating period (Barnes, 1986; Boonstra et al., 2001a). As with the stress hormones, the greater nutritional status of provisioned males at the start of breeding may ameliorate much of the immunosuppressive effects of testosterone. Provisioned males, on average, were involved in more agonistic encounters than were control males and they tended to be among the first males to mate with a female, suggesting that they were either dominant or that they were better able to locate an estrous female (G.C.B., unpublished data). How their behavior relates to androgen levels is unknown, but if androgen levels correlate with these indicators of dominance, provisioned males would be predicted to have a lower white cell count than controls. This is clearly not the case (Fig. 2A) and, although the provisioned males tended to reduce leucocyte counts, the controls still maintained the lowest counts during breeding.

Although the immunosuppressive effects of glucocorticoids and androgens do not readily explain the relatively low leucocyte numbers in the control males, the immuno-enhancing effect of leptin (Fantuzzi and Faggioni, 2000) may have a role in the differences between control and provisioned groups. The effects of leptin on the immune system include the proliferation of T cells (Lord et al., 1998) and possibly neutrophils (Fantuzzi and Faggioni, 2000; Laharrague et al., 2000). Leptin is produced in a number of tissues including gastric mucosa and adipose (Bado et al., 1998). As the increase in body mass observed in the provisioned males was mostly in lean tissue (G.C.B., unpublished data), an increase in leptin from adipose

tissue is unlikely. However, feeding is a stimulus that can lead to an increase in leptin secretion by the gastric mucosa (Bado et al., 1998) as well as an increase in the growth of the digestive tract in early spring. Increased leptin in peripheral circulation together with the growth of lymphoid tissue in the digestive tract (Cunningham-Rundles, 1993; Shivatcheva and Hadjioloff, 1987) are possible important mediators of the increased leucocyte numbers observed in the provisioned males.

A net fitness benefit could result from immune suppression if mounting an immune response or maintaining an immune system during breeding uses resources that could otherwise be allocated to reproduction (Buchanan, 2000; Raberg et al., 1998; Sheldon and Verhulst, 1996; Wedekind and Folstad, 1994). The potential for a trade-off between immune function and reproduction is indicated by studies that document reduced immune function during breeding seasons in contrast to non-breeding periods (Boonstra et al., 2001b; Festa-Bianchet, 1989; Nelson and Demas, 1996) or those that demonstrate a negative correlation between reproductive effort and immune status across individuals during the same breeding period (Deerenberg et al., 1997; Gustafsson et al., 1994; Nordling et al., 1998), although all studies do not show this trade-off (Lozano and Ydenberg, 2002). While much work emphasizes the role of energy, non-energy nutrients are also important for the development and maintenance of immune function. Restriction of protein and carotenoids can lead to a reduction in the size of lymphoid organs and the number and function of leucocytes and can affect the immune response (Cunningham-Rundles, 1993; Gonzalez et al., 1999; Jose and Good, 1973; Lochmiller et al., 1993; Nordling et al., 1998). Therefore, it is reasonable to anticipate co-variation between energy and nutritional state and immune function, particularly under conditions of limited resource availability.

If limited resources can be a proximate cause of an inability to maintain immune function during breeding, then greater access to resources could allow an individual to avoid the trade-off. The implication of glucocorticoid- and testosterone-induced immunosuppression during breeding is that immunosuppression will covary with the endocrine titers. In this study, provisioned males prepared for and participated in breeding alongside control males but, because of the provisioning, they were able to increase their peripheral leucocyte numbers. Similarly, access to food may in part explain the results of field studies that do not find notable reductions in immune function (Boonstra and Singleton, 1993; Hasselquist et al., 1999; Nelson and Demas, 1996) and in laboratory studies with *ad libitum* food where the resulting ability of animals to maintain mass or condition has been cited as a factor contributing to a lack of immune suppression (Svensson et al., 1998).

The lower leucocyte count in control males was not related to mating success. Control males foraged more and engaged in fewer agonistic behaviors but, in this study, the degree to which this affected the ability of males to acquire mates was not significant. If the lower cell numbers reflect an important reduction in immune capacity then males may be making a

trade-off during breeding, favoring reproductive effort over survival, as has been proposed for a variety of animals (Boonstra and Boag, 1992; Boonstra et al., 2001b; Bradley, 1987; Deerenberg et al., 1997; Festa-Bianchet, 1989; Gustafsson et al., 1994; Nordling et al., 1998; Sheldon and Verhulst, 1996). The extent to which the low leucocyte counts in breeding male Belding's ground squirrels reflect impaired immune function to the kind of injuries that males face during breeding and how this affects survival or future reproductive success await additional work.

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