Leptin Effects on Immune Function and Energy Balance Are Photoperiod Dependent in Siberian Hamsters (*Phodopus sungorus*)

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**ABSTRACT**

Many adaptations have evolved in small mammals to maximize survival during winter. One such coping tactic in many species is an alteration of immune function in advance of the stressful conditions of winter. Leptin is a hormone produced by adipose tissue, and in addition to its central role in energy metabolism, leptin mediates the interactions among energy allocation, immune function, and reproduction. To examine this interaction further, exogenous leptin was administered for 2 weeks via osmotic minipumps to Siberian hamsters (*Phodopus sungorus*) housed in long or short days for a total of 12 weeks. Short-day hamsters displayed the expected reductions in humoral immune function, body mass, fat mass, and food intake. In Exp 1, exogenous leptin counteracted the reduction in food intake and the suppression of immune function in short days. In Exp 2, when the leptin-induced increase in food intake in short-day hamsters was prevented, leptin did not enhance immune function. In most of the measured fat pads and body mass, leptin had no effect in long days. In sum, leptin administered to short-day animals caused them to respond, in many cases, like long-day animals. Taken together, these data suggest that leptin acts indirectly to mediate energy allocation to humoral immune function. Additionally, leptin appears to act differentially, according to photoperiod, to regulate both immune and energetic parameters. *(Endocrinology 142: 2768–2775, 2001)*

Animals have evolved temporal strategies to coordinate energetically expensive activities, such as mating, migration, molting, and care of offspring, at different times of the year. In temperate and boreal zone rodents, seasonal breeding is part of a complex group of adaptations that serves to maximize survival, reproductive success, and survival of offspring (1–3). Winter is often a time of energetic crisis for nontropical rodents; energy availability is typically reduced during this time, whereas the energetic requirements for thermogenesis are high. Small animals have evolved to cope with this "energetic bottleneck" by reducing energy-demanding activities during the winter that are not essential for immediate survival (4). For example, reproductive activities, growth, and locomotor activity are often curtailed during energy shortages; during the winter, energy is allocated to thermoregulation, immune function, and cellular maintenance (reviewed in Refs. 4 and 5). If prolonged energy shortages continue to deplete energy stores, then survival may be compromised (6). Thus, trade-offs among competing energetic demands exist, and strategies for allocation of energy to competing needs vary according to an individual's life history strategy, age, sex, and other extrinsic and intrinsic factors (1, 2).

Mounting an immune response requires energy. The cascade of cellular events during the acute phase immune response and inflammation and the elevation of body temperature in response to cytokine activation presumably require substantial energy, although precise quantification is lacking (7, 8). Cytokine activation elevates body temperature, and the energy requirements of inflammation and acute phase immune responses may increase metabolic rates more than 10%/degree of centigrade body temperature elevation (reviewed in Ref. 9). The process of mounting a specific antibody response also appears to require energy. For example, house mice (*Mus musculus*) injected with a specific antigen, keyhole limpet hemocyanin (KLH), display an increase in both oxygen consumption and metabolic heat production compared with saline-injected controls (10). This result is not specific to small rodents; blue tits (*Parus caeruleus*) subjected to increased energy turnover have reduced antibody responses, and mounting an antibody response causes an increase in the basal metabolic rate in this avian species (11). In addition, when bumblebees (*Bombus terrestris*) are challenged with LPS or latex beads that mimic bacteria and activate phagocytosis and access to compensatory food intake is prevented, survival rates are reduced by 50–70% (12). Thus, a general energy deficit can increase the risk of infection and death because insufficient energy reserves may be available to sustain immunity.

The proximate mechanisms by which energy availability is translated into a physiological signal that an animal can use to adjust energy allocation to specific physiological processes remain unspecified. One potential candidate that may act as an endogenous signal of energy availability is the peptide hormone, leptin (Ob protein). Leptin is produced primarily by adipose tissue, and circulating leptin concentrations are...
positively correlated with the percentage of body fat in a variety of mammals (13, 14). In addition, fasting decreases circulating leptin concentrations (15, 16), and exogenous leptin administration generally reduces food intake (17). Leptin appears to act as a signal mediating physiological functions such as reproduction both directly and indirectly by reflecting energy availability. For example, leptin fully reverses the effects of fasting-delayed puberty in rats restricted to 80% of their *ad libitum* food intake (18). Exogenous leptin administered during fasting also maintains high LH secretion in ovariecetomized adult rats (*Rattus norvegicus*) (19). Fasting-induced infertility can be reversed in Syrian hamsters (*Mesocricetus auratus*) through exogenous leptin treatment; this reversal appears to be due to the indirect effects of leptin on metabolic fuel oxidation (20).

Recently, a link has been established between leptin and immune function; mice that are deficient in leptin (*ob/ob*) or in functional leptin receptors (*db/db*) are obese and also display impaired T cell immunity despite excessive energy stored as fat (21). Leptin has a specific effect on T lymphocyte responses, differentially regulating the proliferation of naive and memory T cells. Leptin also regulates the actions of various cytokines in proinflammatory immune responses (22–25). Importantly, treatment with leptin counteracts the immunosuppressive effects of starvation (21). Thus, leptin is a likely candidate to mediate the interactions among energy allocation, immune function, and reproduction.

One possible explanation for the observation that leptin replacement counters the immunosuppressive effects of starvation is that the initial decrease in immunity is due to a stress response associated with starvation (21), rather than to reduced energy availability. To address this issue, Siberian hamsters were used in the present study because they are an ideal animal model in which leptin concentrations can be significantly reduced merely through manipulating photoperiod, which does not elicit a stress response. Short-day hamsters consistently reduce body mass (reflected primarily as a decrease in fat) compared with long-day-housed hamsters and therefore display a dramatic reduction in serum leptin concentrations (26). Consistent with the short-day decrease in fat and leptin concentrations, leptin gene expression is reduced in epididymal white adipose tissue (EWAT) and intrascapular brown adipose tissue (IBAT) during winter acclimatization or short photoperiods (27). In addition, leptin receptor gene expression is reduced in the hypothalamic arcuate nucleus in short days (28). Furthermore, maintenance in short days suppresses the ability of Siberian hamsters to mount a specific antibody response (26, 29). The goal of the present study was to examine the role of leptin during photoperiodic changes in immune function in Siberian hamsters. Specifically, if leptin acts as a signal of energy availability, then short-day-housed hamsters should demonstrate reduced body mass, fat mass, leptin concentrations, and humoral immunity. Exogenous leptin administration, however, should counteract the suppression of immune function by providing a false signal of energy availability. Alternatively, if the suppression of immune function in short days is independent of the reduction in leptin, then exogenous leptin should have no effect on immune function.

**Materials and Methods**

**Exp 1**

Forty adult male Siberian hamsters (*Phodopus sungorus*) (>60 days of age) were obtained from our laboratory breeding colony or from the colony at Georgia State University. Both of these colonies are derived from animals from a colony maintained by Dr. Bruce Goldman (University of Connecticut, Storrs, CT). Hamsters were weaned at 21 days of age and housed with same sex siblings. Two weeks before the onset of the experiment, all animals were individually housed in polypropylene cages (27.8 × 7.5 × 13 cm) in colony rooms. They were maintained on a 24-h cycle of 16 h of light and 8 h of darkness per day (LD 16:8; lights illuminated at 0600 h Eastern Standard Time) in rooms with an ambient temperature of 21 ± 2°C and relative humidity at 50 ± 5%. Food (LabDiet 5001, PMI Nutrition, Brentwood, MO) and tap water were provided *ad libitum* throughout the study.

At the onset of the experiment, animals were weighed to the nearest 0.1 g to establish baseline body mass. Twenty of the animals were randomly assigned to 10 weeks of long photoperiod (LD 16:8), and the remaining (n = 20) animals were assigned to 10 weeks of short photoperiod (LD 8:16). Because body mass did not differ among groups at the onset of the experiment, it was assumed that initial leptin concentrations did not differ among groups (26). After 10 weeks, half of the long-day animals (n = 10) and half of the short-day animals (n = 10) were randomly assigned to receive surgically implanted osmotic minipumps (200 μl volume; 0.5 μl/h delivery rate; Alzet 2002, Alza Corp., Mountain View, CA) containing leptin. The rest of the animals received minipumps containing vehicle (0.5 μl Tris buffer). Minipumps with leptin contained 2.6 μg/μl leptin (Peprotech, Inc., Rocky Hill, NJ) dissolved in 0.5 μl Tris buffer. Minipumps were implanted sc in the intrascapular region of the animals. Animals were allowed to recover from surgery for 3 days before further treatment. After this time, daily food intake was measured to the nearest 0.1 g until the end of the experiment.

After the 3-day recovery period, animals received a single sc injection of 100 μg of the novel antigen KLH suspended in 0.1 ml sterile saline (day 0) and were then returned to the colony room. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*). KLH was used because it generates a robust antigenic response in rodents, but does not make the animals ill (e.g., inflammation or fever) (30). This particular assessment was chosen because it was previously reported that generating specific antibodies, including those against KLH, raises oxygen consumption and heat production (10, 11). Blood was drawn from the retroorbital sinus at two different sampling periods (days 5 and 10 postimmunization). These sampling periods were chosen to capture peak IgG production during the course of the immune response to KLH (26). On each sampling day, animals were brought into the surgery room individually and lightly anesthetized with methoxyflurane vapors (Metofane, Medical Developments, Melbourne, Australia) and blood samples (500 μl) were drawn between 1000 and 1200 h Eastern Standard Time. Samples were allowed to clot for 1 h, the clots were removed, and the samples were centrifuged (at 4°C) for 30 min at 2500 rpm. Serum aliquots were aspirated and stored in sealed polypropylene microcentrifuge tubes at −80°C until assayed for IgG. On the last day of sampling (day 10) animals were killed by cervical dislocation. Paired tests, epididymal, inguinal, and retroperitoneal white adipose tissue (EWAT, IWAT, and RWAT, respectively), intrascapular brown adipose tissue (IBAT) (31), and spleens were removed and cleaned of connective tissue at autopsy. All tissue was weighed to the nearest 0.001 g by laboratory assistants blinded to the experimental hypotheses and treatment assignments.

**Exp 2**

Thirty adult male Siberian hamsters (*Phodopus sungorus*) (>60 days of age) were obtained from the colony at Georgia State University and handled as described in Exp 1. At the onset of the experiment, animals were weighed to the nearest 0.1 g to establish baseline body mass. Ten of the animals were randomly assigned to 10 weeks of long photoperiod, and the remaining animals (n = 20) were assigned to 10 weeks of short photoperiod. Body mass and food intake were measured weekly. After 10 weeks, half of the short-day animals (n = 10) were randomly selected to receive surgically implanted osmotic minipumps containing leptin as described in Exp 1. The rest of the animals received minipumps con-
taining Tris buffer vehicle. Food intake was controlled in all animals by providing them with a preset amount of food equal to their mean weekly food intake immediately before minipump implantation for each animal. This was done to prevent the increased food intake in short-day leptin-treated hamsters seen in Exp 1. Three days after minipump implantation, animals received injections of KLH, and blood samples (500 μl) were drawn on days 5 and 10 postinjection as described in Exp 1. Animals were then killed by cervical dislocation, and paired testes, EWAT, IWAT, RWAT, IBAT, and spleens were removed, cleaned of connective tissue, and weighed to the nearest 0.001 g.

Humoral immunity
To assess humoral immunity, serum anti-KLH IgG concentrations were assayed using an enzyme-linked immunosorbant assay. Microtiter plates were coated with antigen by incubating them overnight at 4 C with 0.5 mg/ml KLH in sodium bicarbonate buffer (pH 9.6), washed with PBS (pH 7.4) containing 0.05% Tween 20 (PBS-T; pH 7.4), then blocked with 5% nonfat dry milk in PBS-T overnight at 4 C to reduce nonspecific binding and washed again with PBS-T. Thawed serum samples were diluted 1:20 with PBS-T, and 150 μl of each serum dilution were added in duplicate to the wells of the antigen-coated plates. Positive control samples (pooled sera from hamsters previously determined to have high levels of anti-KLH antibody, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naive hamsters, similarly diluted with PBS-T) were also added in duplicate to each plate: plates were sealed, incubated at 37 C for 3 h, then washed with PBS-T. Secondary antibody (alkaline phosphatase-conjugated antimouse IgG diluted 1:2000 with PBS-T; Cappel, Durham, NC) was added to the wells, and the plates were sealed and incubated for 1 h at 37 C. Plates were washed again with PBS-T, and 150 μl of the enzyme substrate p-nitrophenyl phosphate (Sigma, St. Louis, MO; 1 mg/ml in diethanolamine substrate buffer) were added to each well. Plates were protected from light during the enzyme-substrate reaction, which was terminated after 20 min by adding 50 μl 1.5 m NaOH to each well. The OD of each well was determined using a plate reader (Benchmark, Bio-Rad Laboratories, Inc., Richmond, CA) equipped with a 405-nm wavelength filter, and the mean OD for each set of duplicate wells was calculated. To minimize intraassay variability, the mean OD for each sample was expressed as a percentage of its plate positive control OD for statistical analyses.

Leptin RIA
Blood serum leptin concentrations were assayed by RIA using the Linco Research, Inc. (St. Charles, MO) 125I Multispecies Kit. This assay has been previously validated for Siberian hamsters (26). The leptin assay was highly specific, cross-reacting at less than 1% with other hormones. Serum leptin values were determined in a single RIA. The coefficients of variation were all less than 10%, and intraassay variation was less than 2%.

Cortisol RIA
Serum cortisol concentrations were determined by RIA using the Diagnostics Products (InterMedico, Markham, Canada) 125I double antibody kit. Previous studies have validated this kit for measuring cortisol in Siberian hamsters, the primary glucocorticoid in this species (32). The procedures recommended in the kit were followed, except that half the volume of all reagents was used, and the volume of standards and samples was reduced from 25 to 10 μl (32). The cortisol assay was highly specific, cross-reacting at less than 1% with other hormones. Serum cortisol values were determined in a single RIA. The coefficients of variation were all less than 10%.

Statistical analyses
All data for Exp 1 were analyzed using 2 (minipump) × 2 (photoperiod) between-subjects ANOVA. All data for Exp 2 were analyzed using a one-way ANOVA. All pairwise comparisons of mean differences or both experiments were conducted using Tukey's honestly significant difference post-hoc comparisons. Differences between group means were considered statistically significant at P < 0.05.

Results
Exp 1
Serum leptin. Serum leptin was significantly reduced in short- compared with long-day hamsters regardless of hormonal treatment after both 8 and 13 days (i.e., blood sample days 5 and 10) of treatment (P < 0.05 in all cases). Leptin infusion significantly increased serum leptin in both long- and short-day hamsters after both 8 and 13 days of treatment (P < 0.05 in all cases; Fig. 1).

Body and tissue masses and food intake. Paired testes mass was significantly smaller in short- compared with long-day hamsters (P < 0.05); leptin infusion had no effect on testes mass in either photoperiod (P > 0.05 in both cases; Table 1). Short days significantly reduced body mass in vehicle-treated hamsters (−10.7%; P < 0.05), but not in leptin-treated hamsters (P > 0.05; Table 1). Leptin had no effect on body mass of long- or short-day hamsters (P > 0.05 in both cases; Table 1). There were no significant interactions of photoperiod and hormone treatment on testes mass or body mass (P > 0.05 in both cases). Spleen mass was significantly increased in short-day hamsters (P < 0.05; Table 1). Leptin treatment had no effect on spleen mass in either photoperiodic condition (P > 0.05; Table 1).

Both EWAT and IWAT mass were significantly reduced in short days compared with long days (P < 0.05); leptin administration had no effect on EWAT or IWAT mass in either photoperiod (P > 0.05 in both cases; Fig. 2). There was a significant interaction between photoperiod and hormone treatment on IWAT mass (P < 0.05). RWAT mass was significantly smaller in short- compared with long-day hamsters (P < 0.05; Fig. 2). Leptin treatment significantly reduced RWAT mass in long days (P < 0.05), but not in short days (P > 0.05; Fig. 2). There was a significant interaction of photoperiod and hormone treatment on RWAT mass (P < 0.05). IBAT mass was significantly reduced in short days compared with long days (P < 0.05; Fig. 2). Leptin admin-
istration significantly reduced IBAT mass in short-day hamsters \((P < 0.05)\), but had no effect on long-day hamsters \((P > 0.05; \text{Fig. 2})\). There was no interaction of photoperiod and hormone treatment on IBAT mass \((P > 0.05)\).

Short days significantly reduced food intake in vehicle-treated animals \((P < 0.05)\), but not in leptin-treated animals \((P > 0.05)\), both after 1 week \((-5.2\%)\) and 2 weeks \((-5.4\%)\) of leptin or vehicle treatment \((\text{Fig. 3})\). Leptin treatment significantly increased food intake in short-day hamsters after both 1 week \((4.0\%; \text{data not shown})\) and 2 weeks \((4.2\%; P < 0.05 \text{ in both cases; \text{Fig. 3}})\). Leptin treatment did not affect food intake in long-day hamsters both after 1 week \((\text{data not shown})\) and 2 weeks \((\text{Fig. 3})\) of leptin treatment \((P > 0.05 \text{ in both cases})\).

**TABLE 1.** Mean \((\pm \text{SEM})\) body, paired testes, and splenic masses of long day control (LD-vehicle), long day leptin-treated (LD-leptin), short day control (SD-vehicle), and short day leptin-treated (SD-leptin) hamsters in Exp 1.

<table>
<thead>
<tr>
<th></th>
<th>Body mass (g)</th>
<th>Testes (mg)</th>
<th>Spleen (mg)</th>
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<tbody>
<tr>
<td>LD-vehicle</td>
<td>41.37 ± 1.41</td>
<td>887.0 ± 47.5</td>
<td>200.0 ± 6.35</td>
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<tr>
<td>LD-leptin</td>
<td>41.53 ± 0.97</td>
<td>838.0 ± 46.5</td>
<td>194.0 ± 7.70</td>
</tr>
<tr>
<td>SD-vehicle</td>
<td>36.95 ± 1.46(^a)</td>
<td>103.2 ± 13.5(^a)</td>
<td>255.0 ± 15.1(^a)</td>
</tr>
<tr>
<td>SD-leptin</td>
<td>38.78 ± 1.69(^a)</td>
<td>88.5 ± 11.3(^a)</td>
<td>269.0 ± 18.5(^a)</td>
</tr>
</tbody>
</table>

\(^{a}\) Significant differences between means.

**Immunological measures and cortisol.** Serum anti-KLH IgG concentrations were significantly reduced in short-day hamsters treated with saline \((P < 0.05)\), but not in short-day hamsters treated with leptin \((P > 0.05; \text{Fig. 4})\). Leptin treatment had no effect on IgG in long-day hamsters \((P > 0.05)\), but in short days, leptin-treated animals displayed significantly greater IgG than vehicle-treated animals \((P < 0.05; \text{Fig. 4})\). There was a significant interaction of photoperiod and hormone treatment on serum anti-KLH IgG concentrations \((P < 0.05)\).

Serum cortisol concentrations were elevated in leptin-treated animals in both long- and short-day animals \((P < 0.05 \text{ in both cases; \text{Fig. 5}})\). Photoperiodic condition had no effect on cortisol concentrations \((P > 0.05; \text{Fig. 5})\). There were no significant interactions between photoperiod and hormone treatment for spleen mass and cortisol concentrations \((P > 0.05 \text{ in both cases})\).

**FIG. 2.** Mean \((\pm \text{SEM})\) EWAT, IWAT, RWAT, and BAT mass in male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks starting at week 10. 

Bars that do not share asterisks are significantly different from one another \((P < 0.05)\).
Exp 2

Body and tissue masses. Paired testes mass was significantly smaller in both short-day vehicle-treated and short-day leptin-treated hamsters compared with that in long-day hamsters (P < 0.05 in both cases); leptin infusion had no effect on testes mass (P > 0.05; Table 2). Short days significantly reduced body mass compared with that in long-day hamsters (P < 0.05), but leptin-treated hamsters did not differ from vehicle-treated hamsters in body mass (P > 0.05; Table 2). EWAT, IWAT, RWAT, and IBAT masses were significantly reduced in short days compared with long days (P < 0.05 in all cases); leptin had no effect on any of the WAT or IBAT masses (P > 0.05 in both cases; Table 2). Spleen mass was significantly increased in short-day vehicle-treated hamsters compared with long-day animals (P < 0.05); short-day leptin-treated hamsters did not differ in spleen mass compared with either short-day vehicle-treated animals or long-day animals (P > 0.05; Table 2).

Immunological measures. Serum anti-KLH IgG concentrations were significantly reduced in both short-day vehicle-treated and short-day leptin-treated hamsters compared with those in long-day hamsters (P < 0.05 in both cases; Fig. 6). Leptin treatment had no effect on anti-KLH IgG concentrations (P > 0.05).

Discussion

Consistent with previous findings (26, 29), Siberian hamsters maintained in short days displayed reduced humoral immune function compared with long-day-housed hamsters. Leptin did not affect immune function in long days, but in short days, hamsters treated with exogenous leptin displayed IgG concentrations comparable to long-day controls (all short-day hamsters responded reproductively to photoperiod). In accord with numerous studies (reviewed in Ref. 33), Siberian hamsters reduced body mass in short days. In contrast, short-day-housed hamsters treated with leptin did not significantly reduce body mass. Consistent with previous findings, hamsters reduced food intake in short days (34). Short-day animals treated with leptin, however, did not reduce food intake; food intake of leptin-treated short-day hamsters was comparable with that of long-day animals. When short-day animals given leptin were limited in their food intake to the level they maintained before leptin administration, leptin did not enhance immune function. As predicted of control hamsters, maintenance in short days led to a dramatic reduction in all fat pads measured compared with those in long-day controls. EWAT and IWAT masses were not significantly affected by leptin administration in long days; RWAT, however, was reduced by leptin in long days. Leptin administration had no significant effect on EWAT, IWAT, or RWAT in short days. In contrast, IBAT was significantly reduced in short-day hamsters given leptin.

In agreement with recent in vitro and in vivo studies (21, 24), the present results demonstrate the immunoenhancing
TABLE 2. Mean (±SEM) body, paired testes, and splenic masses of long day control (LD-vehicle), short day control (SD-vehicle), and short day leptin-treated (SD-leptin) hamsters in Exp 2

<table>
<thead>
<tr>
<th></th>
<th>Body mass (g)</th>
<th>Testes (mg)</th>
<th>Spleen (mg)</th>
<th>BAT (mg)</th>
<th>EWAT (mg)</th>
<th>IWAT (mg)</th>
<th>RWAT (mg)</th>
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<tr>
<td>LD-vehicle</td>
<td>42.53 ± 1.18a</td>
<td>0.904 ± 0.023</td>
<td>210.0 ± 9.1</td>
<td>441.0 ± 35.7</td>
<td>937.0 ± 62.4a</td>
<td>751.0 ± 42.4</td>
<td>151.0 ± 16.1</td>
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<tr>
<td>SD-vehicle</td>
<td>34.85 ± 1.88a</td>
<td>0.102 ± 0.026a</td>
<td>266.0 ± 8.4a</td>
<td>319.0 ± 35.6a</td>
<td>243.0 ± 22.1a</td>
<td>178.0 ± 30.7a</td>
<td>24.4 ± 3.1a</td>
</tr>
<tr>
<td>SD-leptin</td>
<td>33.48 ± 1.63a</td>
<td>0.077 ± 0.019a</td>
<td>247.0 ± 16.0a</td>
<td>278.0 ± 23.8a</td>
<td>255.0 ± 33.1a</td>
<td>258.0 ± 28.7a</td>
<td>46.5 ± 3.5a</td>
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</table>

* Significant differences between means.

properties of leptin. The present study confirms and extends previous findings that leptin replacement can counteract a reduction in immune function by increasing energy availability (i.e. food intake) (21). In our study, however, the naturalistic reduction in leptin by manipulation of photoperiod was not associated with a stress response. Importantly, photoperiod had no effect on cortisol concentrations in the present study. These results suggest that the actions of reduced leptin on humoral immune function are based on reduced energy stores and not on stress-induced immune suppression via the HPA axis.

The present results suggest that leptin can act differentially based on the photoperiod to which animals have been exposed (35). In Exp 1, in long-day animals leptin had no effect on the specific antibody response, whereas in short-day animals leptin caused the antibody response to be restored to levels comparable with those in long-day animals. In Exp 2, when the leptin-induced increase in food intake was not permitted in short-day animals, the antibody response remained at the level of short-day control animals. These results suggest that typical long-day leptin concentrations increase energy availability to short-day animals. In short days, body mass and, more specifically, fat mass are greatly reduced, so that exogenous leptin might provide a meaningful signal to an animal that is in a state of reduced energy availability. Basal levels of leptin are already increased in long-day animals (relative to those in short-day animals), potentially preventing exogenous leptin from having any further effects (i.e. a ceiling effect). The lack of an effect of leptin on humoral immunity in long days is not inconsistent with previous studies of leptin and immune function. Studies that have tested the relation between leptin and immune function in vivo have first manipulated endogenous leptin concentrations (e.g. via food restriction) and then replaced the hormone (21). In the present study animals in which leptin administration had no effect on immune function were long-day, vehicle-treated hamsters, in which energy balance remained undisturbed. Thus, leptin treatment appears to restore the reduction in immune function via increased energy availability, rather than by directly enhancing it. It appears that humoral immune function is responsive to energy availability, and restoration of energy availability increases immune function to long-day levels. Taken together, these results suggest that leptin mediates energy allocation to immune function.

The present nonimmune data contrast with the results of two previous studies in hamsters. In one recent study leptin treatment of hamsters reduced body and fat mass to a greater extent in short- compared with long-day animals and reduced food intake similarly in both photoperiods (35). In this study, however, leptin was administered via twice daily injections. In another study that found a decrease in food intake in both long- and short-day hamsters after a single leptin injection, the data were collected at single time point (6 h postinjection) (36). It is possible that these differences in leptin responses are a result of the different methods of administration; the chronic infusion via osmotic minipumps used in the present study might lead to different physiological effects than more acute injections. It is possible that the total amount of leptin administered differed over the course of the studies. One route of administration leads to a bolus of hormone, followed by a drop, and the other results in constant hormone concentrations. Thus, there might be a difference in the sensitivity to leptin according to these different methods. Additionally, in the study that administered twice daily leptin injections (35), when serum leptin was assayed, serum leptin values were lower in leptin-treated hamsters as compared with saline-treated animals in both photoperiods. In the present study the leptin minipumps lead to significantly increased serum leptin values in both photoperiods. It is important to note that the increase in leptin in short days was clearly in the physiological range for long-day animals. Alternatively, it is possible that some of the differences in the response to leptin between previous studies and the present study are due to differences in the photoperiod used. In one of the injection studies (35) a photoperiod of LD 16:8 was used for long days, and LD 10:14 was used for short days. In the present study a photoperiod of 16:8 was used for long days, and 8:16 for long days.
In long-day animals, leptin did not alter food intake, body mass, or white fat (except for RWAT). This is in contrast to the results of other studies in nonseasonal breeders, which suggest that leptin administration leads to a decrease in body mass due to hypophagia (13, 14). It is possible that in seasonally breeding animals such as hamsters, a supraphysiological signal of leptin is ignored; exogenous leptin might only signal to metabolic functions during times of energy crisis. If leptin is an indicator of photoperiodic status, then the addition of leptin when an animal is already in long days might provide only a superfluous signal. There are also species differences with regard to food intake in general between rats and mice, and Siberian hamsters that could account for the differences in leptin effects observed in this species. It is possible that changes caused by leptin administration in Siberian hamsters are reflected in external energy stores (i.e., food caches) rather that in the internal stores, which were indirectly measured in the present study. There are several other cases in which seemingly contradictory effects in Siberian hamsters can be explained by differences in means of energy storage. For example, Siberian hamsters do not increase food intake after a fast, but, rather, they increase food hoarding (37).

Leptin administration increased food intake in short-day-housed hamsters in Exp 1. Leptin administration to animals that are generally considered nonphotoperiodic, such as rats and mice, usually leads to a decrease in food intake (38). Elevated leptin receptor gene expression contributes to an increase in sensitivity to leptin (39). Given that hypothalamic leptin receptor gene expression is reduced in short days (28), it is possible that this reduction might dramatically reduce the sensitivity to leptin in short-day animals. It is also possible that the constant infusion of leptin via minipumps even further down-regulates leptin receptor expression, causing further diminished sensitivity to leptin, potentially leading to the effects on food intake observed in the present study. Reduced receptor expression might also alter leptin negative feedback in short-day animals (28). These potential mechanisms remain to be determined.

The immune function of Siberian hamsters is suppressed in short days in both reproductive responders and nonresponders (26). Importantly, however, leptin concentrations are dramatically reduced in reproductive responders, but are unchanged in nonresponders (26). Given that nonresponders are able to reduce immune function in short days without a decrease in leptin, it appears that nonresponders might be interpreting the leptin signal differently from responders. Presumably, if nonresponders were included in the present study, they would not have enhanced immune function in response to exogenous leptin, because, as in long-day animals, leptin would already be at a high, potentially ceiling concentration, consistent with the results of the present study. We did not test this hypothesis, because all short-day hamsters in the present study were reproducively responsive to photoperiod; future studies are necessary to determine whether nonresponders fail to use leptin as a signal for immune function changes and, if so, what signal might be used that allows nonresponders to show the same degree of immunosuppression as responders. Alternatively, it is possible that nonresponders reduce the number of leptin receptors or reduce the affinity of leptin receptors for circulating leptin, leading to a decreased immune response to the leptin signal.

In the present study photoperiod had no effect on serum cortisol concentrations. This is consistent with data from deer mice, in which photoperiod did not have an effect on circulating corticosterone concentrations (40). In both photoperiods, however, leptin administration resulted in a significant increase in cortisol concentrations. In one previous study central leptin administration leads to a rise in corticosterone secretion at the onset of the dark phase (41). Other studies that show a stimulatory relationship between glucocorticoids and leptin have reported the reverse relationship; glucocorticoids stimulate leptin secretion (42). In many other studies, however, it appears that leptin inhibits glucocorticoid release (43, 44). In contrast to the present study, previous studies did not use seasonally breeding rodents. In general, glucocorticoids have a suppressive effect on immune function (reviewed in Ref. 45). In the present study, however, the leptin-induced enhancement in short days of humoral immune function was accompanied by increased cortisol concentrations. It remains possible that sampling at multiple time points would reveal a difference in the pattern of cortisol secretion observed.

Taken together, the results of the present study suggest that leptin is acting to mediate energy allocation to humoral immune function. When leptin concentrations in reproductive responders are reduced by short photoperiods, immune function is also reduced; exogenous leptin is able to enhance immune function back to the level of long-day animals that have not experienced any loss of leptin. The effects of leptin on immune function in short-day animals appear to be indirect, acting via increased energy availability by increasing food intake. The data indicate that exogenous leptin acts differentially based on photoperiod, because leptin has immune and energetic effects in short, but not in long, days, when leptin concentrations are already high, and energy balance is unperturbed.

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