0013-7227/01/$03.00/0
Endocrinology

Vol. 142, No. 7
Printed in U.S.A.

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

DEBORAH L. DRAZEN, GREGORY E. DEMAS, AND RANDY J. NELSON

Departments of Psychology (D.L.D., R.J.N.), Neuroscience (R.J.N.), and Biochemistry and Molecular Biology (R.J.N.), Division of Reproductive Biology, The Johns Hopkins University, Baltimore, Maryland 21218-2686; and Center for Behavioral Neuroscience, Department of Biology, Georgia State University (G.E.D.), Atlanta, Georgia 30303

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 (1). 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

Received September 11, 2000.
Address all correspondence and requests for reprints to: Randy Nelson, Department of Psychology, 09 Townshend Hall, Ohio State University, Columbus, Ohio 43210. E-mail: rnelson+@osu.edu.
* This work was supported by USPHS Grant MH-57535 and NSF Grant IBN 00-80745 (to R.J.N.) and USPHS Grant NS-10596 (to G.E.D.).

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 ovariectomized 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 dark 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 M Tris buffer). Minipumps with leptin contained 2.6 μg/μl leptin (PeproTech, Inc., Rocky Hill, NJ) dissolved in 0.5 M 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 sealable 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 testes, 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), ^{125}I 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) ^{125}I 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-

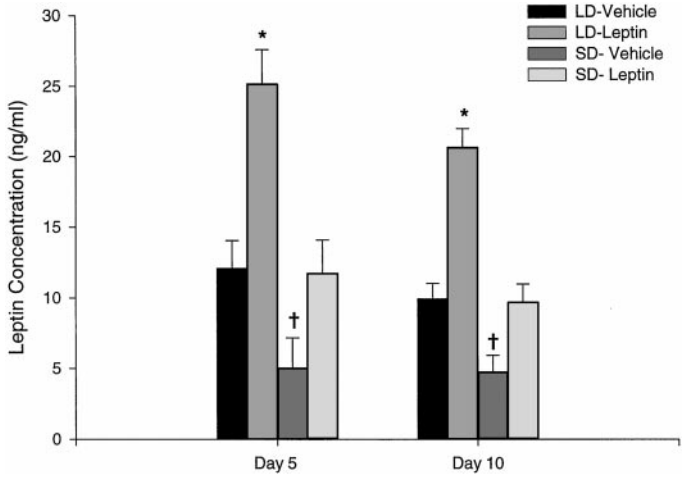

FIG. 1. Mean (±SEM) serum leptin concentrations in male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks starting at week 10 (split into 5 and 10 days post-KLH injection). *, Significantly greater than all other groups ($P < 0.05$); †, significantly less than all other groups ($P < 0.05$).

istration significantly reduced IBAT mass in short-day hamsters ($P < 0.05$), but had no effect on long-day hamsters ($P < 0.05$; 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 (Fig. 3). Leptin treatment significantly increased food intake in short-day hamsters after both 1 week (4.0%; data not shown) and 2 weeks (4.2%; $P < 0.05$ in both cases; Fig. 3). Leptin treatment did not affect food intake in long-day hamsters both after 1 week (data not shown) and 2 weeks (Fig. 3) of leptin treatment ($P > 0.05$ in both cases). There was a significant interaction of photoperiod and hormone treatment on food intake after both 1 and 2 weeks of treatment ($P < 0.05$ in both cases).

TABLE 1. Mean (±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

	Body mass (g)	Testes (mg)	Spleen (mg)
LD-vehicle	41.37 ± 1.41	887.0 ± 47.5	200.0 ± 6.35
LD-leptin	41.53 ± 0.97	838.0 ± 46.5	194.0 ± 7.70
SD-vehicle	36.95 ± 1.46[a]	103.2 ± 13.5[a]	255.0 ± 15.1[a]
SD-leptin	38.78 ± 1.69[a]	88.5 ± 11.3[a]	269.0 ± 18.5[a]

[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 ($P > 0.05$; 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 ($P < 0.05$; 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 ($P < 0.05$ in both cases; Fig. 5). Photoperiodic condition had no effect on cortisol concentrations ($P > 0.05$; Fig. 5). There were no significant interactions between photoperiod and hormone treatment for spleen mass and cortisol concentrations ($P > 0.05$ in both cases).

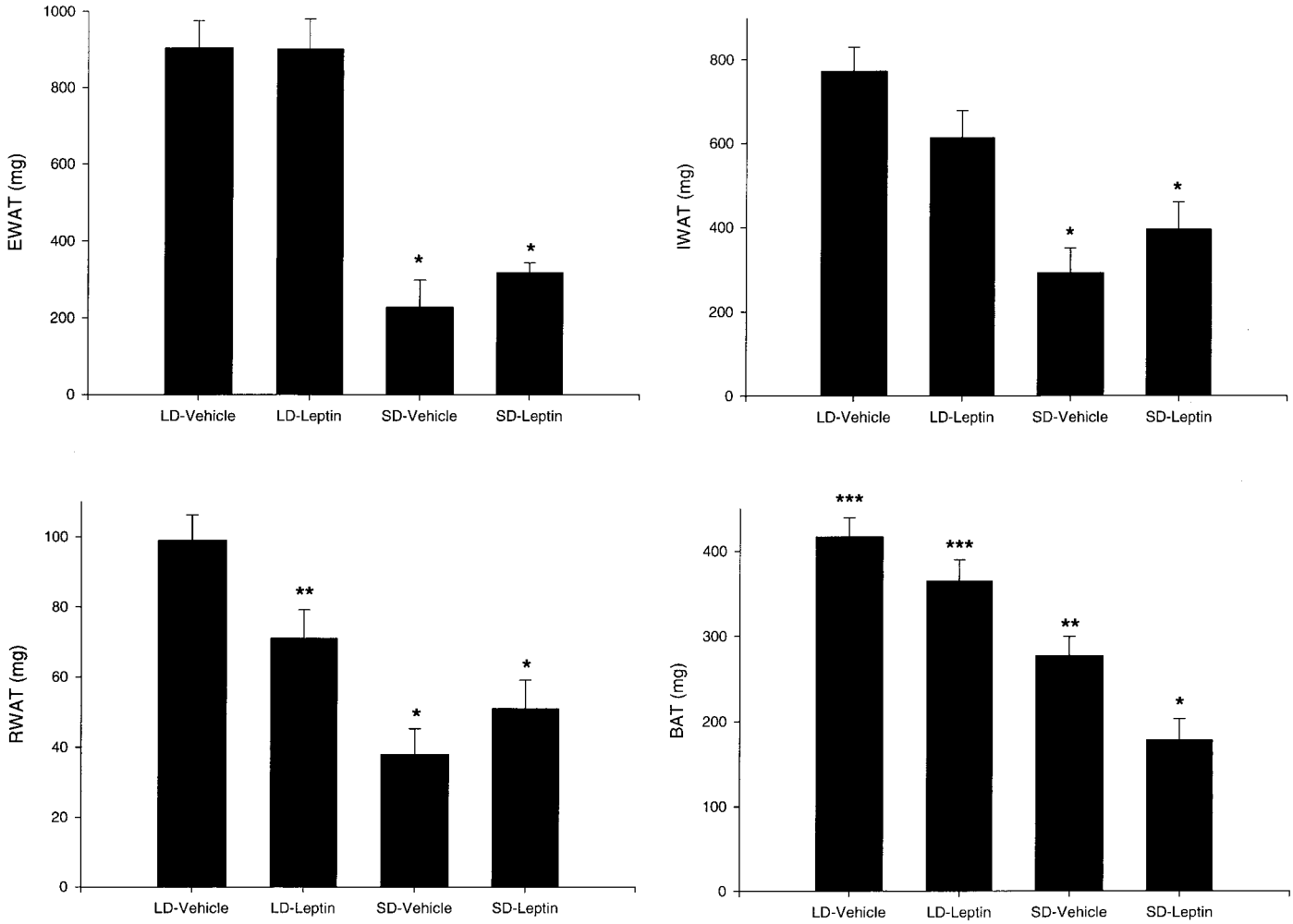

FIG. 2. Mean (±SEM) EWAT, IWAT, RWAT, and BAT mass in male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks starting at week 10. *Bars* that do not share *asterisks* are significantly different from one another ($P < 0.05$).

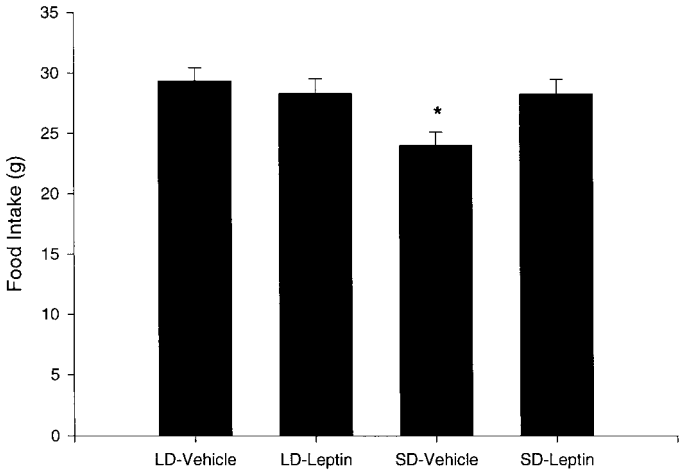

FIG. 3. Mean (±SEM) food intake of male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks starting at week 10. *, Significantly less than all other groups ($P < 0.05$).

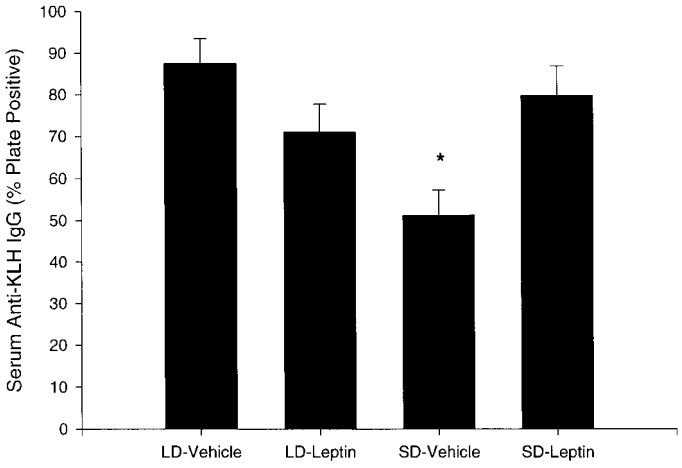

FIG. 4. Mean (±SEM) serum anti-KLH IgG concentrations (represented as the percentage of plate positive) in male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks, starting at week 10. *, Significantly less than all other groups ($P < 0.05$).

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 pad 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).

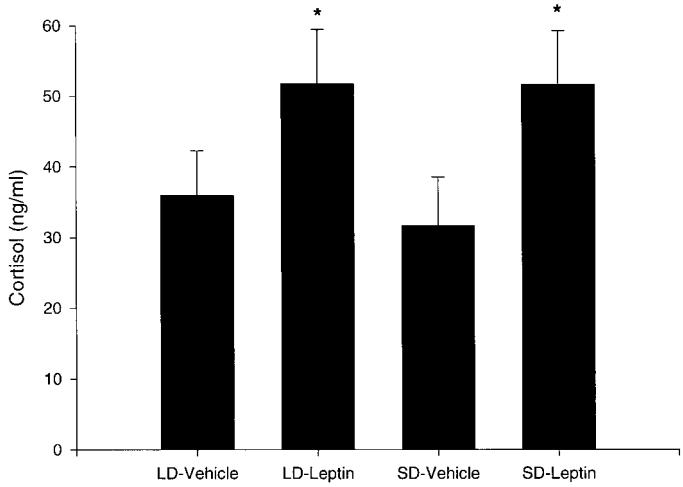

FIG. 5. Mean (±SEM) serum cortisol concentrations in male Siberian hamsters housed for 12 weeks in long (LD 16:8) or short (LD 8:16) days that received infusions of vehicle or leptin for 2 continuous weeks starting at week 10. *, Significantly greater than long- and short-day vehicle-treated hamsters ($P < 0.05$).

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

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

[a] Significant differences between means.

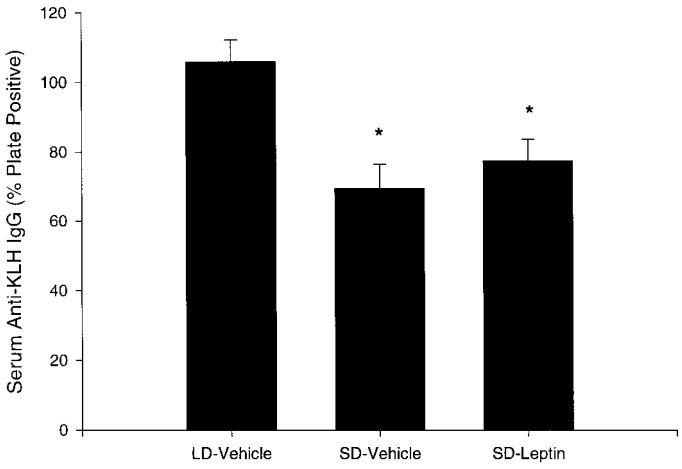

FIG. 6. Mean (±SEM) serum anti-KLH IgG concentrations (the percentage of plate positive) in male Siberian hamsters housed for 12 weeks in long days (LD 16:8), receiving infusions of vehicle, or short days (LD 8:16), receiving infusions of vehicle or leptin for 2 continuous weeks, starting at week 10. Food intake was maintained at prehormone infusion levels for each individual for the course of the 2-week treatment. *, Significantly less than long-day vehicle-treated hamsters ($P < 0.05$).

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 immunosuppression 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 reproductively 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.

Acknowledgments

The authors thank Brian Spar, Michelle Dumler, Lara Blakey, and Nancy Edwards for technical assistance, and Isaac Osie-Bobie for expert animal care.

References

1. **Bronson FH** 1998 Energy balance and ovulation: small cages versus natural habitats. Reprod Fertil Dev 10:127–137
2. **Kumar V** 1997 Photoperiodism in higher vertebrates: an adaptive strategy in temporal environments. Indian J Exp Biol 35:427–437
3. **Nelson RJ, Demas GE, Klein SL** 1998 Photoperiodic mediation of seasonal breeding and immune function in rodents: a multi-factorial approach. Am Zool 38:226–237
4. **Wade GN, Schneider JE** 1992 Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 16:235–272
5. **Klein SL, Nelson RJ** 1999 Influence of social factors on immune function and reproduction. Rev Reprod 4:1168–178
6. **Sheldon BC, Verhulst S** 1996 Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–321
7. **Henken AM, Brandsma HA** 1982 The effect of environmental temperature on immune response and metabolism of the young chicken. II. Effect of the immune response to sheep red blood cells on energy metabolism. Poultry Sci 61:1667–1677
8. **Maier SF, Watkins LR, Fleshner M** 1994 Psychoneuroimmunology: the interface between behavior, brain, and immunity. Am Psychol 49:1004–1017

9. **Mahmoud I, Salman SS, Al-Khateeb A** 1994 Continuous darkness and continuous light induced structural changes in the rat thymus. J Anat 185:143–149
10. **Demas GE, Chefer V, Talan MC, Nelson RJ** 1997 Metabolic costs of an antigen-stimulated immune response in adult and aged C57BL/6J mice. Am J Physiol 273:R1631–R1637
11. **Svensson E, Raberg L, Koch C, Hasselquist D** 1998 Energetic stress, immunosuppression and the cost of an antibody response. Func Ecol 12:912–919
12. **Moret Y, Schmid-Hempel P** 2000 Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1166–1168
13. **Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P** 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549
14. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F** 1995 Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540–543
15. **Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS** 1995 Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1:1311–1314
16. **Ahima RS, Prabakaran D, Flier JS** 1998 Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J Clin Invest 101:1020–1027
17. **Luheshi GN, Gardner JD, Rushforth DA, Louden AS, Rothwell NJ** 1999 Leptin actions on food intake and body temperature are mediated by IL-1. Proc Natl Acad Sci USA 96:7047–7052
18. **Cheung CC, Thorton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA** 1997 Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 138:855–858
19. **Nagatani S, Guthikonda P, Thompson R, Tsukamura H, Maeda K, Foster D** 1998 Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology 67:370–376
20. **Schneider JE, Goldman MD, Tang S, Bean B, Ji H, Friedman MI** 1998 Leptin indirectly affects estrous cycles by increasing metabolic fuel oxidation. Horm Behav 33:217–228
21. **Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI** 1998 Leptin modulates the T-cell immune responses and reverses starvation-induced immunosuppression. Nature 394:897–901
22. **Faggioni R Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR, Grunfeld C** 1999 Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am J Physiol 276:R136–R142
23. **Finck BN, Kelley KW, Dantzer R, Johnson RW** 1998 *In vivo* and *in vitro* evidence for the involvement of tumor necrosis factor in the induction of leptin by lipopolysaccharide. Endocrinology 139:2278–2283
24. **Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM** 1998 Leptin regulates proinflammatory immune responses. FASEB J 12:57–65
25. **Takahashi N, Waelput W, Guisez Y** 1999 Leptin is an endogenous protective protein against the toxicity exerted by tumor necrosis factor. J Exp Med 189:207–212
26. **Drazen DL, Kriegsfeld LJ, Nelson RJ** 2000 Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod Am J Physiol 278:R1401–R1407
27. **Yellon SM, Teasley LA, Fagoaga OR, Nguyen HC, Truong HN, Nehlson-Cannerella L** 1999 Role of photoperiod and the pineal gland in T cell-dependent humoral immune reactivity in the Siberian hamster. J Pineal Res 27:243–248
28. **Mercer JG, Moar KM, Ross AW, Hoggard N, Morgan PJ** 2000 Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. Am J Physiol 278:R271–R281
29. **Klingenspor MA, Dickopp A, Heldmaier G, Klaus S** 1996 Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters. FEBS Lett 399:290–294
30. **Dixon F, Jacot-Guillarmod H, McConahey PJ** 1966 The antibody responses of rabbits and rats to hemocyanin. J Immunol 97:350–355
31. **Bartness TJ** 1987 Animal and human body fat changes: measurement and interpretation. In: Toates FM, Roland NE (eds) Feeding and Drinking. Elsevier, Amsterdam, pp 463–498
32. **Reburn CJ, Wynne-Edwards KE** 1999 Hormonal changes in males of a naturally biparental and a uniparental mammal. Horm Behav 35:163–176
33. **Bartness TJ, Wade GN** 1985 Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci Biobehav Rev 9:599–612
34. **Bartness TJ** 1996 Photoperiod, sex, gonadal steroids, and housing density affect body fat in hamsters. Physiol Behav 60:517–529
35. **Klingenspor M, Niggeman H, Heldmaier** 2000 Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. J Comp Physiol B 170:37–43
36. **Reddy AB, Cronin AS, Ford H, Ebling FJP** 1999 Seasonal regulation of food intake and body weight in the male Siberian hamster: studies of hypothalamic orexin (hypocretin), neuropeptide Y (NPY) and pro-opiomelanocortin (POMC). Eur J Neurosci 11:3255–3264
37. **Bartness TJ, Clein MR** 1994 Effects of food deprivation and restriction, and metabolic blockers on food hoarding in Siberian hamsters. Am J Physiol 266:R1111–R1117
38. **Ahima RS** 2000 Leptin and the neuroendocrinology of fasting. Front Horm Res 26:42–56
39. **Baskin DG, Seeley RJ, Kuijper JL, Lok S, Weogle DS, Erickson JC, Palmiter RD, Schwartz MW** 1998 Increased expression of mRNA for the long form of the leptin receptor in the hypothalamus is associated with leptin hypersensitivity and fasting. Diabetes 47:538–543
40. **Demas GE, DeVries AC, Nelson RJ** 1997 Effects of photoperiod and 2-deoxy-D-glucose-induced metabolic stress on immune function in female deer mice. Am J Physiol 272:R1762–R1767
41. **Vandijk G, Donahey JCK, Thiele TE, Scheurink AJW, Steffens AB, Wilkinson CW, Tenenbaum R, Campfield LA, Burn P, Seeley RJ, Woods SC** 1997 Central leptin stimulates corticosterone secretion at the onset of the dark phase. Diabetes 46:1911–1914
42. **Chautard T, Spinedi E, Voirol M, Pralong FP, Gaillard RC** 1999 Role of glucocorticoids in the response of the hypothalamo-corticotrope, immune and adipose systems to repeated endotoxin administration. Neuroendocrinology 69:360–369
43. **Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA** 1997 Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. Diabetes 46:1235–1238
44. **Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, Gaillard RC** 1998 Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. Endocrinology 139:4264–4268
45. **McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spencer RL, Weiss JM** 1997 The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. Brain Res Rev 23:79–133