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Leptin mediates seasonal variation in some but not all symptoms of sickness in Siberian hamsters

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ABSTRACT

Many seasonally breeding species, including Siberian hamsters (*Phodopus sungorus*), exhibit seasonal variation in sickness responses. One hypothesis regarding the mechanism of this variation is that sickness intensity tracks an animal's energetic state, such that sickness is attenuated in the season that an animal has the lowest fat stores. Energetic state may be signaled via leptin, an adipose hormone that provides a signal of fat stores. Siberian hamsters respond to extended housing in short, winter-like days by reducing fat stores and leptin levels, relative to those housed in long, summer-like days. Sickness responses are also attenuated in short-day hamsters as compared to long-day hamsters. We hypothesized that leptin provides a physiological signal by which seasonally breeding animals modulate sickness responses, such that animals with higher leptin levels show increased sickness intensity. To test this, we provided short-day hamsters with a long-day-like leptin signal and assessed their responses to lipopolysaccharide (LPS), a sickness-inducing antigen. We compared these responses to short-day vehicle-, long-day vehicle-, and long-day leptin-treated hamsters. Unexpectedly, LPS induced a hypothermic response (rather than fever) in all groups. Short-day vehicle-treated hamsters exhibited the greatest LPS-induced hypothermia, and leptin treatment attenuated this response, making hypothermia more long-day-like. Contrary to our hypothesis, short-day leptin-treated hamsters showed the least pronounced LPS-induced anorexia among all groups. These results suggest that leptin may mediate some but not all aspects of seasonal sickness variation in this species. Future studies should be targeted at determining roles of other energetic hormones in regulating seasonal sickness response variation.

Introduction

Seasonally breeding animals must respond to temporal changes in environmental factors like climate fluctuations, social interactions, and resource availability. While their abiotic and biotic environments are changing, seasonal breeders respond with appropriate morphological, physiological, and behavioral adaptations in order to maximize their chances of survival and reproductive success (Bronson, 1985). Such seasonal adaptations include changes in reproductive function and behavior, frequency and magnitude of agonistic behaviors, metabolism, and immune function (Demas et al., 2010). All of these processes require substantial energy, and as energetic resources may be less plentiful during certain times of the year (e.g., winter), changes in the expression of these traits occur when energy is shifted away from certain processes and toward those that will prioritize immediate survival (Nelson and Demas, 2004). In particular, immunity is quite sensitive to the energetic state of organism, as seasonal alterations in immune function can be best predicted by changes in an animal's energetic state rather than reproductive state or photoperiodic cues (Demas, 2004). Seasonal changes in immune responses have been documented in all three branches of the immune system (i.e., innate, cell-mediated, humoral) in several different species of mammals and birds (Martin et al., 2008b). Seasonal changes in immunity are commonly observed in inducible immune defenses because the energetic costs of mounting an immune response can be very high (e.g., can raise resting metabolic rate by as much as 50%) (Lochmiller and Deerenberg, 2000).

One of the initial and more energetically expensive immune responses is the acute phase response (APR), and the behavioral and physiological manifestations of sickness that accompany it. During the APR, pro-inflammatory cytokines are released from immune cells and act on the brain to generate the symptoms of a sickness response. Sickness responses are characterized by hyperthermia (i.e., fever) or hypothermia, anorexia, body mass loss, reductions in social, hedonic, and sexual behaviors, and hypothalamic–pituitary–adrenal (HPA) axis stimulation (Hart, 1988; Tizard, 2008). While these symptoms may appear to be a result of infection-induced weakness or malaise, these responses are actually a well-adapted mechanism to aid the host organism in clearance of the infectious agent (Hart, 1988). Blocking fever and anorexia during sickness can actually result in increased mortality via failure to eliminate the infection (Covert and Reynolds, 1977; Kluger

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et al., 1975; Kyriazakis et al., 1998; Vaughn et al., 1980), while blocking glucocorticoid production can cause mortality via sepsis (Bertini et al., 1988). Mounting an appropriate sickness response is clearly beneficial to an organism's survival, but being sick also carries significant energetic costs that can be detrimental to survival if too severe (Buchanan et al., 2003; Maier et al., 1994; Plata-Salaman, 1996). While variations in sickness response intensity at the extremes of the spectrum clearly negatively affect survival, the ability to modulate sickness intensity between these "mortality endpoints" may be critical for ensuring survival in environments with variable energetic resource availability.

Seasonally breeding animals live in environments where energetic resources vary across the annual cycle (i.e., resources are more plentiful in summer than winter), and studies of sickness responses in several seasonally breeding species have revealed that sickness response intensity can also vary with the seasons (reviewed in Ashley et al., 2012; Ashley and Wingfield, 2012). Collectively, the patterns of sickness response variation in these species reveal that there is not one critical season in which animals display a weak or strong sickness response, suggesting that seasonal photoperiodic cues or reproductive status may not drive variation in sickness intensity. Rather, the common predictor of sickness response intensity across these studies is the current energetic state of the animal—sickness responses are attenuated in the season in which the organism has the lowest energy reserves (i.e., lowest body mass and fat stores) (Bilbo et al., 2002; Owen-Ashley et al., 2006, 2008; Owen-Ashley and Wingfield, 2006; Prendergast et al., 2008). In further support of the hypothesis that energetic state is a predictor of sickness response intensity in seasonally breeding animals, pre-sickness body mass and body fat levels are correlated with infection-induced anorexia and body mass loss, such that animals with higher initial body masses and fat stores show greater percent decreases in food intake and body mass after experimental infection (Owen-Ashley et al., 2006, 2008). These observations suggest that the magnitudes of the energetically expensive components of a sickness response are constrained by a minimum body mass that an animal can reach before it risks its survival (Ashley and Wingfield, 2012; Owen-Ashley and Wingfield, 2007).

If energetic state is the critical predictor of seasonal variation in sickness response intensity, then the adipose hormone leptin is a promising candidate for a neuroendocrine mediator of this variation. Leptin is not only tightly coupled with the energetic state of an organism, but it also interacts with the immune system (Carlton et al., 2012; La Cava and Matarese, 2004). Leptin levels are directly proportional to the mass of adipose tissue in several mammalian species (Johnson et al., 2004; Maffei et al., 1995), and as such, high levels of leptin indicate adequate energy stores, whereas low levels are consistent with energy deficit. Leptin levels change across seasons in seasonally breeding animals, and these seasonal changes track seasonal changes in body mass and body fat (Concannon et al., 2001; Gaspar-Lopez et al., 2009; Horton et al., 2000). Immune function can be restored via leptin treatment in animals that have been food deprived or have had body fat experimentally reduced (Demas and Sakaria, 2005; Lord et al., 1998), and there is also evidence that leptin may modulate seasonal changes in immunity (Drazen et al., 2001). Although there is no yet established role of leptin in mediating seasonal variation in sickness responses, there is considerable evidence that leptin does influence sickness responses (Harden et al., 2006; Sachot et al., 2004), although the direct mechanisms and their effects are not entirely understood (Carlton et al., 2012).

The goal of the present study was to test the hypothesis that leptin serves as a neuroendocrine signal mediating seasonal variation in sickness responses. To accomplish this, we housed male Siberian hamsters (*Phodopus sungorus*) in long and short days to induce two photoperiodic morphs, experimentally elevated leptin levels in a subset of hamsters in each morph, and then measured sickness response variables (e.g., body temperature, anorexia, body mass loss, anhedonia, nest building behavior, HPA axis activation) in response to inoculation with lipopolysaccharide (LPS), a sickness-inducing bacterial mimetic. When housed in short days, Siberian hamsters regress their gonads to a non-reproductive state and decrease food intake, body mass, and fat stores. In addition, Siberian hamsters have lower leptin levels in short days as compared to long days (Horton et al., 2000) and display less intense sickness responses (i.e., lower fever amplitude, shorter durations of and lesser decreases in food intake and body mass loss, lesser decreases in hedonic and nest shredding behaviors, higher cortisol secretion) (Bilbo et al., 2002, 2003; Wen et al., 2007). We predicted that if leptin mediates seasonal variation in sickness responses, then short-day leptin-treated hamsters would display sickness responses similar to long-day vehicle-treated hamsters and would display more intense sickness responses than short-day vehicle-treated hamsters. Leptin treatment, however, should have no effect on the sickness responses of long-day housed hamsters because previous studies in this species have shown that leptin does not enhance other measures of immunity in long-day animals even though it enhances them in short-day animals (Demas, 2002; Drazen et al., 2001).

Methods

Animals and housing conditions

Adult male (>60 days of age) Siberian hamsters (n = 117) were obtained from our breeding colony at Indiana University. The progenitors of these animals were generously provided by Dr. Randy Nelson (Ohio State University) and Dr. Timothy Bartness (Georgia State University). In order to minimize the effects of inbreeding, our animals are outbred approximately every 10 generations. All animals were initially group housed (2–5 with same sex siblings on weaning at 17–18 days of age) in long-day photoperiods (light:dark (L:D) 16:8), and then individually housed in polypropylene cages (27.8 × 17.5 × 13.0 cm) for one week prior to the start of the experiment. Food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and tap water were available ad libitum during the entire course of the experiment. Temperature (20 ± 2 °C) and humidity (50 ± 10%) were maintained at constant levels. Animals were then randomly assigned to either long (L:D 16:8) (n = 40) or short days (L:D 8:16) (n = 74) for the remainder of the study. A greater number of hamsters were housed in short days to account for reproductive non-responders (described below). All animal methods were reviewed and approved by the Institutional Animal Care and Use Committee at Indiana University Bloomington (protocol no. 10-038).

A subset of hamsters within the short-day group often fails to show reproductive responsiveness to photoperiod (i.e., do not display gonadal regression, reductions in body mass and fat stores, or changes in pelage coloration and thickness) despite prolonged exposure to short days. These individuals are referred to as photoperiodic non-responders (Puchalski and Lynch, 1986). After ten weeks of exposure to short-day photoperiods, 36 animals were determined to be non-responders (defined by a reduction in body mass less than or equal to 10% of their mass at the beginning of the experiment) and were removed from the experiment. At the conclusion of the experiment, paired testes mass was collected to confirm short-day responsiveness (defined as a paired testes mass < 0.15 g) (Greives et al., 2008). At the end of the study, we were left with 40 hamsters exhibiting the long-day phenotype (referred to as LD from here forward) and 38 hamsters exhibiting the short-day responder phenotype (referred to as SD from here forward).

Experimental methods

During the first 10 weeks of photoperiodic treatment, hamsters were weighed weekly to the nearest 0.1 g to track photoperiodic responsiveness. After these ten weeks and when the photoperiodic non-responders were removed from the study, body mass (to the

nearest 0.1 g) and food consumption (to the nearest 0.1 g) were assessed daily to establish pre-leptin treatment body mass and food intake baseline values. Food consumption was assessed by weighing the food pellets remaining in the hopper each day.

After five days, animals were surgically implanted with an osmotic mini-pump subcutaneously in the intra-scapular region under isoflurane anesthesia (Alzet 1002; 100 μl volume; 0.25 μl/h delivery rate; 14 days; Durect Corp., Cupertino, CA, USA). Half of the animals from each photoperiodic group were randomly assigned to receive mini-pumps containing recombinant murine leptin (n = 39; 2.67 μg/μl leptin; Peprotech Inc., Rocky Hill, NJ, USA) dissolved in 0.5 M Tris buffer. The remaining animals received mini-pumps containing vehicle (n = 39; 0.5 M Tris buffer) (Drazen et al., 2001). Murine leptin has been used in several studies in Siberian hamsters and has elicited responses consistent to those reported for mice, suggesting that murine leptin can bind the Siberian hamster leptin receptor (Demas and Sakaria, 2005; Drazen et al., 2001; French et al., 2009; Klingenspor et al., 2000). For the eight days after mini-pump implantation, body mass and food consumption were collected daily to assess surgery recovery and leptin effects on these measures.

On the eighth day after mini-pump implantation and ~15 min before the onset of darkness (long days: ~1945 h; short days: ~1545 h), a portion of the animals in each group (LD-Vehicle, n = 10; LD-Leptin, n = 10; SD-Vehicle, n = 11; SD-Leptin, n = 11) were injected intraperitoneally (i.p.) with 25 μg LPS (LPS from *Salmonella enterica* serotype typhimurium, Sigma-Aldrich, St. Louis, MO, USA; Durazzo et al., 2008; French et al., 2013) suspended in 0.1 ml sterile 0.9% saline. The remaining animals were injected i.p. with 0.1 ml sterile 0.9% saline (LD-Vehicle, n = 10; LD-Leptin, n = 10; SD-Vehicle, n = 8; SD-Leptin, n = 8). Sickness responses and behaviors (i.e., fever, anorexia, body mass loss, anhedonia, nest building) were assessed throughout the four days following injections.

Blood sampling and necropsies

Blood samples were drawn from each animal 4 h after the onset of darkness (long days: 2400 h; short days: 2000 h) 3 days before injection and on the day of injection in order to assess circulating leptin and cortisol concentrations. We chose this time point (~4 h after LPS injection) because it has previously been shown to be the point where there is the greatest difference in LPS-induced cortisol secretion between LD- and SD-housed Siberian hamsters (Bilbo et al., 2003). Briefly, animals were lightly anesthetized with isoflurane vapors, and blood samples were drawn from the retro-orbital sinus. Blood samples were allowed to clot at room temperature for 1 h, the clots were removed, and samples were centrifuged at 4 °C for 30 min at 2500 rpm. Serum aliquots were aspirated and stored in sealable polypropylene microcentrifuge tubes at −20 °C until assayed. All blood samples were collected within 3 min of initial handling. Animals were euthanized 5 days after LPS injection and necropsies were performed. Testes were removed, cleaned of connective tissues, and weighed to the nearest 0.1 mg in order to assess reproductive responsiveness.

Sickness response measurements

Fever, anorexia, and body mass

On the day of injection, colonic temperatures (T_c; to the nearest 0.1 °C) were collected immediately before injection and 2, 4, 6, 8, 10, 12, 16, 20, and 24 h after injection using a MicroTherma 2T thermometer (ThermoWorks, Alpine, UT, USA) and a lubricated RET-3-ISO thermocouple probe (Physitemp Instruments, Inc., Clifton, NJ, USA) inserted ~12 mm into the rectum. To assess anorexia and body mass loss, daily body mass and food consumption measurements continued until the end of the study.

Anhedonic behavior

To assess the effects of our treatments on hedonic behavior, we provided hamsters with a highly palatable sodium saccharin solution (Baillie and Prendergast, 2008). Beginning 5 days before LPS and saline injections, for the first 6 h of the dark phase (long days: 2000 h to 200 h; short days: 1600 h to 2200 h) hamsters were provided with a fluid bottle containing a solution of 0.1% sodium saccharin (saccharin sodium salt hydrate, Sigma-Aldrich, St. Louis, MO, USA) dissolved in tap water (Baillie and Prendergast, 2008). The saccharin solution bottles were weighed (to the nearest 0.1 g) before they were given and after they were collected from the hamsters each day. Presentation of saccharin solution continued daily through day 3 post-injection.

Nest building behavior

To assess the effects of our treatments on thermoregulatory behavior, beginning 5 days before LPS or saline injection, each hamster was provided with a compressed cotton nestlet weighing ~2.5 g (Ancare, Bellmore, NY, USA) for the first 6 h of the dark phase (Baillie and Prendergast, 2008). The whole nestlet was weighed (to the nearest 0.1 g) before presentation, and the unshredded portion of the nestlet was weighed after presentation. When provided a nestlet, hamsters quickly start shredding the cotton to build a nest. Nest-building is an adaptive behavior to enhance energy conservation in low temperatures, however, hamsters readily build nests in room temperature (20–23 °C) (Puchalski et al., 1988). Presentation of nestlets continued daily through day 3 post-injection.

Leptin enzyme-linked immunosorbent assay (ELISA)

Circulating leptin levels were assayed via commercially prepared mouse leptin ELISA kits (Crystal Chem, Downers Grove, IL, USA). This kit has previously been used in another non-murine rodent (Johnson et al., 2004), and prior to testing samples, we validated the assay and determined the appropriate dilutions so that leptin concentrations lay within the detectable range of the ELISA. Samples were diluted 1:4 (non-leptin treated) or 1:8 (leptin treated) with sample diluent and run in duplicate. Intra-assay variabilities were 3.9% and 5.8% for the two plates.

Cortisol enzyme immunoassay (EIA)

We assessed circulating cortisol levels to determine if our photoperiod and leptin treatments affected the magnitude of LPS-induced HPA axis activation. Cortisol is the predominant glucocorticoid in Siberian hamsters, with concentrations ~100× that of corticosterone (Reburn and Wynne-Edwards, 2000). Serum cortisol concentrations were determined in multiple enzyme immunoassays (EIAs) from a commercially prepared kit (Cortisol EIA Kit; Enzo Life Sciences, Inc., Farmingdale, NY, USA). This assay was previously validated for use in Siberian hamsters (Demas et al., 2004) and is highly specific for cortisol; cross-reactivity with corticosterone is 27.7% and <4.0% for other steroid hormones. The sensitivity of the assay is 56.72 pg/ml. Samples were diluted to 1:80 with assay buffer and run in duplicate. Intra-assay variabilities were 6.7% and 7.4%.

Statistical analyses

All statistical tests were performed using JMP 10 (SAS Institute Inc., Cary, NC, USA), and a value of $P < 0.05$ was considered to be statistically significant. Residuals were checked for normality and homogeneity of variance, and those data that were non-normally distributed were transformed. Two LPS-treated animals were excluded from analyses (1 from SD-Leptin group; 1 from SD-Vehicle group) because they failed to exhibit any sickness symptoms. One LPS-treated animal

(from SD-Leptin group) was excluded from analyses because it displayed exaggerated LPS-induced sickness symptoms and was not showing any signs of recovery by the end of the experiment (as is typical for all LPS-treated SD animals). The final sample sizes were as follows: LD-Vehicle-Saline (n = 10), LD-Vehicle-LPS (n = 10), LD-Leptin-Saline (n = 10), LD-Leptin-LPS (n = 10), SD-Vehicle-Saline (n = 8), SD-Vehicle-LPS (n = 10), SD-Leptin-Saline (n = 8), and SD-Leptin-LPS (n = 9).

Non-normally distributed variables differed in their directions and degrees of skewness, and as such, each variable was transformed with the function that best fits the data to normality. Leptin concentrations and pre-injection saccharin solution intake were log transformed, pre-injection food intake was inverse transformed, post-injection percent change in saccharin solution intake was square root transformed, and post-injection percent change in nesting material shredded was square transformed. Finally, pre-injection percent nesting material shredded was not normally distributed and could not be transformed to meet the assumptions of normality, so a Kruskal–Wallis test was performed to determine if there were differences among groups for this measure.

Pre-injection baseline values were calculated for body mass, food intake, saccharin solution intake, and percent nesting material shredded by averaging the three daily measurements immediately prior to injections. To determine if there were effects of photoperiod or leptin treatment on pre-injection leptin levels, baseline body mass, baseline food intake, and baseline saccharin solution intake, two-way (photoperiod (2) × leptin treatment (2)) analyses of variance (ANOVAs) were performed. Pre-injection body mass was included as a covariate in the model for leptin levels to control for the effect of body mass on this dependent variable. Pre-injection body mass was initially included as a covariate in the model for pre-injection food intake but was removed from the model after it was determined to not be significant predictor ($P > 0.05$).

Changes in colonic temperature across groups and time were assessed via Linear Mixed Models (LMM) with leptin treatment, injection, time, and their interactions as fixed effects and animal ID as a random effect. Due to natural circadian variation in temperature between LD and SD hamsters, individual LMMs were run for each photoperiod. We used a LMM for this measure, as opposed to a repeated-measures ANOVA, because the thermometer failed during the temperature collection for six animals at the 4 h time-point, and the LMM allowed us to keep these animals in our analyses. Pairwise comparisons were conducted using *t*-tests between each LPS-injected group and their respective saline control at each time point.

Because photoperiod affected pre-injection body mass, food intake, and saccharin solution intake (see Results), post-injection changes in these measurements were expressed as percentages of each animal's baseline values for these variables. Although nest shredding was not affected by photoperiod, for continuity in presentation of results, this measurement was also expressed as a percentage of baseline. Differences in these repeated measures (i.e., percent change in food intake, percent change in body mass, percent change in saccharin solution intake, and percent change in nesting material shredded) were assessed via repeated-measures ANOVAs with photoperiod, leptin treatment, injection, and their interactions as between subjects variables and time and its interactions as the within-subjects variables. The within-subject comparisons for percent change in body mass, percent change in saccharin solution intake, and percent change in nesting material shredded violated the assumptions of sphericity and were Greenhouse–Geisser (GG)-corrected. Differences in serum cortisol concentrations among the groups were assessed with a three-way (photoperiod (2) × leptin treatment (2) × injection (2)) ANOVA. Post-hoc comparisons between pairwise means were conducted using Tukey's honestly significant difference (HSD) tests when the overall ANOVA was significant. Effect size estimates for ANOVAs and pairwise comparisons were calculated by eta squared (η^2) and Cohen's d, respectively.

Results

Pre-injection/post-leptin baseline measures

Leptin levels in both vehicle- and leptin-treated animals were related to the animal's pre-injection body mass ($F_{1,70} = 22.26$, $P < 0.001$, $\eta^2 = 0.116$). In addition, leptin levels were affected by photoperiod ($F_{1,70} = 5.67$, $P = 0.02$, $\eta^2 = 0.029$), leptin treatment ($F_{1,70} = 82.77$, $P < 0.001$, $\eta^2 = 0.430$) and the photoperiod × leptin interaction ($F_{1,70} = 12.01$, $P < 0.001$, $\eta^2 = 0.062$) (Table 1). Specifically, SD-Leptin hamsters showed leptin levels that were higher than the levels of SD-Vehicle hamsters ($P < 0.01$, d = 2.567) but statistically equivalent to LD-Vehicle levels ($P > 0.90$, d = 0.181).

Prior to injection, photoperiod affected baseline body mass ($F_{1,71} = 92.49$, $P < 0.001$, $\eta^2 = 0.548$), such that SD hamsters had significantly lower body masses than LD hamsters; however, neither leptin treatment ($F_{1,71} = 1.53$, $P = 0.220$, $\eta^2 = 0.009$) nor the photoperiod × leptin interaction ($F_{1,71} = 3.85$, $P = 0.0538$, $\eta^2 = 0.023$) had effects on pre-injection body mass (Table 1). Additionally, pre-injection baseline food intake was affected by both photoperiod ($F_{1,71} = 39.09$, $P < 0.001$, $\eta^2 = 0.320$) and leptin treatment ($F_{1,71} = 8.21$, $P = 0.006$, $\eta^2 = 0.067$) and a trend toward the photoperiod × leptin interaction ($F_{1,71} = 3.94$, $P = 0.051$, $\eta^2 = 0.0322$) (Table 1). Specifically, SD hamsters consumed less food per day than LD hamsters ($T = 6.25$, $P < 0.001$, d = −1.3471), and leptin-treated hamsters consumed less food per day than vehicle-treated hamsters ($T = 2.86$, $P = 0.003$, d = −0.490); however, the effect of leptin on food intake was largely driven by SD-Leptin hamsters exhibiting lower daily food consumption than SD-Vehicle hamsters.

Pre-injection baseline saccharin solution intake was affected by photoperiod ($F_{1,70} = 9.17$, $P = 0.003$, $\eta^2 = 0.116$) and the photoperiod × leptin interaction ($F_{1,70} = 5.38$, $P = 0.023$, $\eta^2 = 0.069$) but not leptin treatment alone ($F_{1,70} = 0.004$, $P = 0.953$, $\eta^2 = 0.0004$) (Table 1). Specifically, SD-Leptin hamsters consumed less saccharin solution than LD-Leptin hamsters. Pre-injection baseline nest shredding was not affected by our treatments, and percent nesting material shredded did not differ among groups ($H = 3.97$, $P = 0.265$) (Table 1).

Febrile/hypothermic responses

Within each photoperiod, colonic temperature was significantly affected by injection (LD: $F_{1,36} = 9.76$, $P = 0.004$; SD: $F_{1,31.11} = 7.667$, $P = 0.009$), time (LD: $F_{9,324} = 68.42$, $P < 0.001$; SD: $F_{9,\ 273.2} = 63.02$,

Table 1
Mean (±SEM) circulating leptin concentration, body mass, food intake, saccharin solution intake, and percent nesting material shredded prior to LPS and saline injections in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with vehicle- or leptin-filled osmotic mini-pumps. Baseline measurements were calculated by averaging the values collected for the measurements during the 3 days prior to LPS or saline injection. Groups with different letters indicate statistically significant differences between group means ($P < 0.05$); groups sharing the same letter are statistically equivalent.

	Vehicle	Leptin
Leptin concentration (ng/ml)		
LD	12.03 ± 1.08[a]	24.65 ± 2.54[c]
SD	4.06 ± 0.53[b]	12.90 ± 1.30[a]
Baseline body mass (g)		
LD	41.4 ± 1.2[a]	44.9 ± 1.0[a]
SD	33.2 ± 1.1[b]	32.4 ± 1.1[b]
Baseline food intake (g/day)		
LD	5.3 ± 0.2[a]	5.1 ± 0.1[a,b]
SD	4.5 ± 0.2[b]	3.9 ± 0.2[c]
Baseline saccharin solution intake (g/6 h)		
LD	2.1 ± 0.2[a,b]	2.5 ± 0.2[a]
SD	1.9 ± 0.2[a,b]	1.5 ± 0.2[b]
Baseline percent nesting material shredded		
LD	87.4 ± 4.5	97.2 ± 1.2
SD	92.0 ± 4.7	97.5 ± 1.8

$P < 0.001$), and the interaction between injection and time (LD: $F_{9,324} = 14.06$, $P < 0.001$; SD: $F_{9,273.2} = 22.21$, $P < 0.001$) (Fig. 1). In addition, the pattern of colonic temperature was influenced by the leptin × time interaction in LD animals only ($F_{9,324} = 3.06$, $P = 0.002$). Colonic temperature was not predicted by leptin treatment or any of the additional interaction terms in either photoperiods ($P > 0.05$ in all cases). LPS-injected LD-Vehicle, SD-Vehicle, and SD-Leptin hamsters had significantly higher T_c at 2 h after injections as compared to their respective saline-injected controls ($T > 3.06$, $P < 0.05$ in all cases, $d_{LD\text{-}Veh} = 1.374$, $d_{SD\text{-}Veh} = 2.408$, $d_{SD\text{-}Lep} = 1.837$). In the LPS-injected SD-Vehicle and SD-Leptin hamsters, the higher temperatures appear to be due to a transient hypothermic response in the saline-injected controls, rather than increases in temperature in LPS-treated animals. After these higher temperatures at 2 h post-LPS, all LPS-treated hamsters showed varying degrees of hypothermia. Specifically, LD-Vehicle, LD-Leptin, and SD-Leptin hamsters each showed hypothermia at two or three time points post-LPS treatment (LD-Vehicle: 6 h, 24 h; Fig. 1A; LD-Leptin: 6 h, 12 h, 24 h, Fig. 1B; SD-Leptin: 12 h, 16 h; Fig. 1B) as compared to their respective saline-injected controls ($T > 2.60$, $P < 0.05$ in all cases, LD-Veh: $d_{6h} = -2.634$, $d_{24h} = -1.644$; LD-Lep: $d_{6h} = -2.2637$, $d_{12h} = -1.814$, $d_{24h} = -1.170$; SD-Lep: $d_{12h} = -1.493$, $d_{16h} = -1.34$). In contrast, SD-Vehicle hamsters showed hypothermia at all measured time points from 6 h to 24 h post-LPS treatment as compared to their respective saline-injected controls ($T > 2.31$, $P < 0.05$ in all cases, $d < -1.048$; Fig. 1A).

Anorexia

Changes in food intake after injections were affected by photoperiod ($F_{1,67} = 20.15$, $P < 0.001$, $\eta^2 = 0.083$), injection ($F_{1,67} = 132.55$, $P < 0.001$, $\eta^2 = 0.548$), and the photoperiod × injection interaction ($F_{1,67} = 16.06$, $P < 0.001$, $\eta^2 = 0.066$), such that SD hamsters from both the vehicle- and leptin-treated groups showed decreased magnitudes of LPS-induced anorexia as compared to LD hamsters (Fig. 2). In addition, food intake changed across the course of the four days post injection (within subjects, $F_{3,65} = 122.78$, $P < 0.001$, $\eta^2 = 0.531$). Additional within subjects analyses revealed time × injection ($F_{3,65} = 50.51$, $P < 0.001$, $\eta^2 = 0.206$), time × photoperiod × injection ($F_{3,65} = 3.24$, $P = 0.028$, $\eta^2 = 0.011$), and time × photoperiod × leptin interactions ($F_{3,65} = 4.53$, $P = 0.006$, $\eta^2 = 0.017$). Specifically, LPS-treated LD-Vehicle hamsters showed decreased food intake on days 1, 2, 3, and 4 post-LPS treatment as compared to their respective saline treated controls ($P < 0.03$ for all comparisons, $d_{day1} = -4.931$, $d_{day2} = -3.587$, $d_{day3} = -1.989$, $d_{day4} = -1.799$), while LPS-treated SD-Vehicle hamsters showed decreased food intake on days 1 and 2 post-LPS as compared to controls ($P < 0.01$ for day 1 and 2 comparisons, $d_{day1} = -2.727$, $d_{day2} = -1.632$; $P > 0.90$ for day 3 and 4 comparisons, $d_{day3} = -0.356$, $d_{day4} = 0.498$; Fig. 2A). Alternatively, leptin-treated hamsters from both photoperiods showed attenuated LPS-induced anorexic responses as compared to vehicle-treated hamsters. Specifically, LPS-treated LD-Leptin hamsters only showed decreased food intake on days 1, 2, and 3 post-injection as compared to their respective saline-treated controls ($P < 0.01$ for day 1, 2, and 3 comparisons, $d_{day1} = -9.349$, $d_{day2} = -4.324$, $d_{day3} = -2.07$; $P > 0.08$ for day 4 comparison, $d_{day4} = -1.3149$), while LPS-treated SD-Leptin hamsters showed decreased food intake only on day 1 post-injection as compared to controls ($P < 0.05$ for day 1 comparison, $d_{day1} = -2.701$; $P > 0.80$ for day 2, 3, and 4 comparisons, $d_{day2} = -0.541$, $d_{day3} = -0.216$, $d_{day4} = 0.425$; Fig. 2B).

Body mass loss

Changes in body mass after injections were affected by injection ($F_{1,67} = 130.30$, $P < 0.001$, $\eta^2 = 0.631$) and the leptin × injection interaction ($F_{1,67} = 5.04$, $P = 0.0281$, $\eta^2 = 0.024$) (Fig. 3). Percent body mass

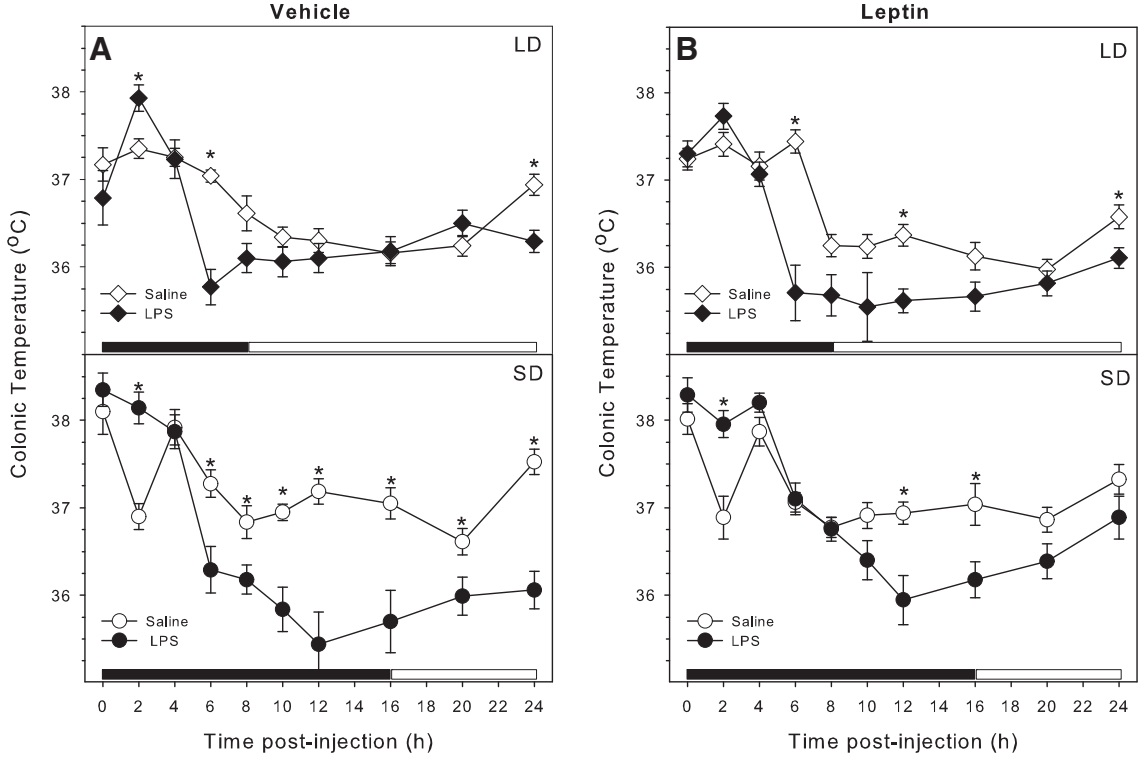

Fig. 1. Mean (±SEM) colonic temperature following LPS and saline treatments delivered at the 0 h time point in long-day (LD; top panels) and short-day (SD; bottom panels) housed male Siberian hamsters implanted with (A) vehicle-filled and (B) leptin-filled osmotic mini-pumps. Black and white bars at the bottom of the graphs indicate the active, dark (black) and inactive, light (white) phases of the light–dark cycle for each photoperiodic morph. Within each panel: $^*P < 0.05$ versus saline-treated group exposed to the same photoperiod and osmotic mini-pump treatment.

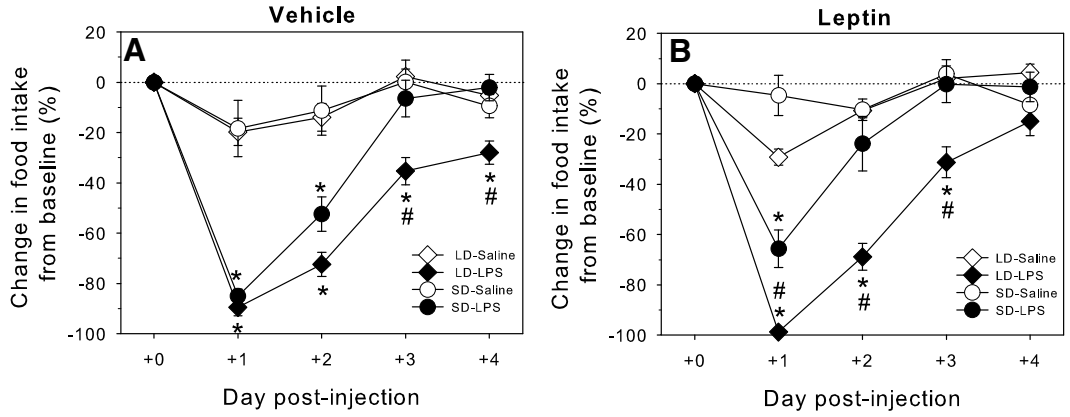

Fig. 2. Mean (±SEM) percent change in daily food intake from baseline following LPS and saline treatment delivered on day 0 in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with (A) vehicle-filled and (B) leptin-filled osmotic mini-pumps. Day +1 represents the time period from 0–24 h after LPS or saline injection, Day +2 represents the time period from 24–48 h after LPS or saline injection, and so forth. $^{*}P < 0.05$ versus saline-treated group exposed to the same photoperiod and osmotic mini-pump treatment. $^{\#}P < 0.05$ versus SD LPS-treated group exposed to the same osmotic mini-pump treatment.

loss changed across the course of the four days post injection (within subjects, $F_{2.03,136.31} = 30.75$, $P < 0.001$, G–G corrected, $\eta^2 = 0.190$). In addition, within subjects analyses revealed time × photoperiod ($F_{2.03,136.31} = 26.20$, $P < 0.001$, G–G corrected, $\eta^2 = 0.162$), time × injection ($F_{2.03,136.31} = 10.05$, $P < 0.001$, G–G corrected, $\eta^2 = 0.062$), and time × photoperiod × injection interactions ($F_{2.03,136.31} = 25.14$, $P < 0.001$, G-G corrected, $\eta^2 = 0.156$). Specifically, LPS-treated LD-Vehicle and SD-Vehicle hamsters showed body mass decreases that were greater than their respective saline-treated controls at all 4 days post-injection ($P < 0.03$ for all comparisons, LD: $d_{day1} = -2.686$, $d_{day2} = -4.087$, $d_{day3} = -3.596$, $d_{day4} = -3.409$; SD: $d_{day1} = -2.823$, $d_{day2} = -3.554$, $d_{day3} = -2.871$, $d_{day4} = -1.755$; Fig. 3A), although body mass appeared to be recovering toward baseline in SD-Vehicle hamsters by day 4. LPS-treated LD-Leptin hamsters only showed post-injection body mass decreases that were greater than their respective saline-treated controls at days 2, 3, and 4 post-injection ($P < 0.001$ for day 2, 3 and 4 comparisons, $d_{day2} = -1.421$, $d_{day3} = -1.721$, $d_{day4} = -1.702$; $P > 0.17$ for day 1 comparison, $d_{day1} = -1.176$; Fig. 3B). Alternatively, LPS-treated SD-Leptin hamsters showed post-injection body mass decreases that were greater than their respective saline-treated controls only at days 1, 2, and 3 post-injection ($P < 0.001$ for day 1, 2 and 3 comparisons, $d_{day1} = -2.898$, $d_{day2} = -2.917$, $d_{day3} = -2.907$), while the LPS-induced body mass loss did not persist to day 4 post-injection ($P > 0.57$, $d_{day4} = -1.108$; Fig. 3B).

Saccharin solution intake

Changes in saccharin solution intake after injections were affected by photoperiod ($F_{1,66} = 18.62$, $P < 0.001$, $\eta^2 = 0.184$), injection ($F_{1,66} = 5.41$, $P = 0.023$, $\eta^2 = 0.054$), and the photoperiod × injection interaction ($F_{1,67} = 4.47$, $P = 0.038$, $\eta^2 = 0.044$) (Fig. 4). Additionally, percent saccharin intake changed across time (within subjects, $F_{2.47,163.01} = 3.39$, $P = 0.027$, G–G corrected, $\eta^2 = 0.043$) and was affected by the time × photoperiod × leptin interaction ($F_{2.47,163.01} = 5.17$, $P = 0.004$, G–G corrected, $\eta^2 = 0.065$). As there was a lot of variance in this measure, post hoc analyses only revealed that, at the 48–54 h time point, LPS-treated LD-Vehicle hamsters showed a greater percent decrease in saccharin solution intake as compared to LPS-treated SD-Vehicle hamsters ($P < 0.01$, d = 1.562; Fig. 4A). However, LPS-treated hamsters (independent of photoperiod and leptin treatments) showed greater percent decreases in saccharin solution at the 0–6 h time point as compared to saline-treated hamsters ($T = 2.18$, $P < 0.04$, d = −0.532).

Nest building behavior

Percent changes in nesting material shredded were affected by injection ($F_{1,67} = 118.51$, $P < 0.001$, $\eta^2 = 0.626$), but not by photoperiod, leptin, or any of the interaction terms ($P > 0.05$ for all effects) (Fig. 5). In

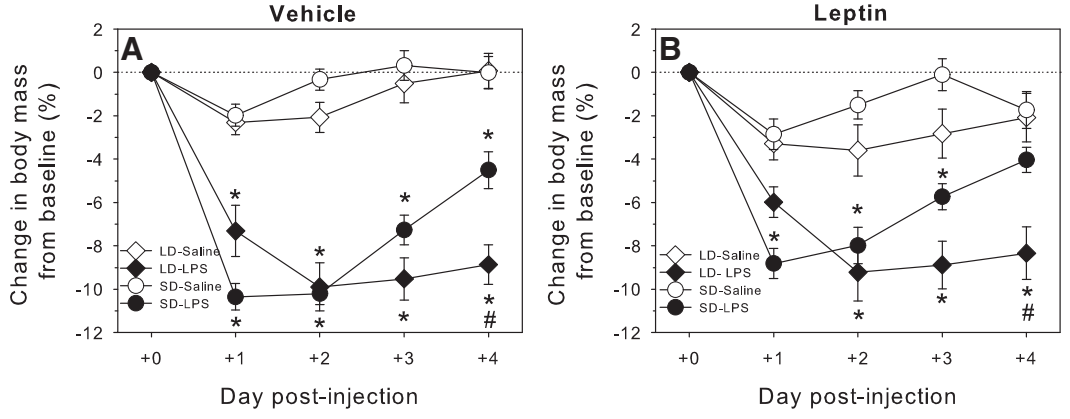

Fig. 3. Mean (±SEM) percent change in body mass from baseline following LPS and saline treatment delivered on day 0 in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with (A) vehicle-filled and (B) leptin-filled osmotic mini-pumps. Day +1 represents the time period from 0–24 h after LPS or saline injection, Day +2 represents the time period from 24–48 h after LPS or saline injection, and so forth. $^{*}P < 0.05$ versus saline-treated group exposed to the same photoperiod and osmotic mini-pump treatment. $^{\#}P < 0.05$ versus SD LPS-treated group exposed to the same osmotic mini-pump treatment.

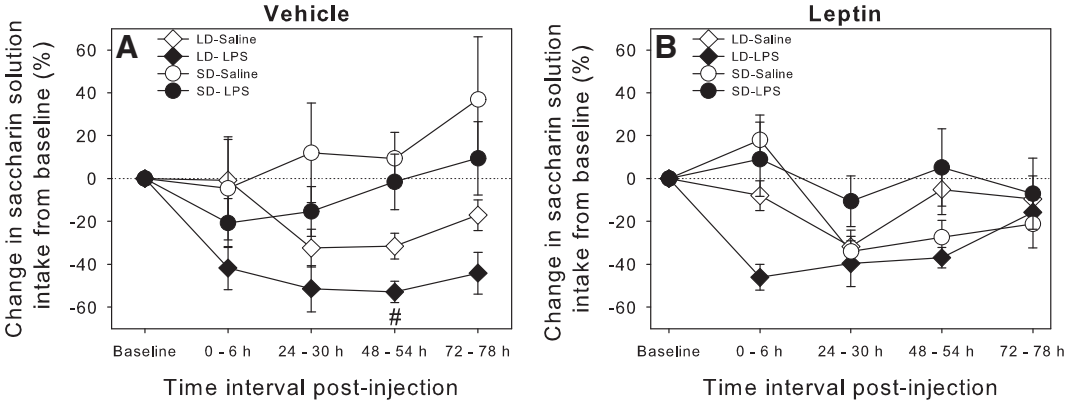

Fig. 4. Mean (±SEM) percent change in saccharin solution intake from baseline following LPS and saline treatment delivered at the 0 h time point in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with (A) vehicle-filled and (B) leptin-filled osmotic mini-pumps. $^{\#}P < 0.05$ versus SD LPS-treated group exposed to the same osmotic mini-pump treatment.

addition, percent nesting material shredded changed over time (within subjects, $F_{2.7,183.4} = 46.63$, $P < 0.001$, G–G corrected, $\eta^2 = 0.270$) and changed according to the time × injection interaction ($F_{2.7,183.4} = 52.32$, $P < 0.001$, G–G corrected, $\eta^2 = 0.303$). LPS-treated LD-Vehicle, SD-Vehicle, and SD-Leptin hamsters showed greater decreases in nesting material shredded as compared to saline-injected controls at the 0–6 h and 24–30 h time points ($P < 0.02$ for all comparisons, LD-Veh: $d_{0-6\ h} = -1.383$, $d_{24-30\ h} = -4.933$; SD-Veh: $d_{0-6\ h} = -1.684$, $d_{24-30\ h} = -4.306$; SD-Lep: $d_{0-6\ h} = -2.830$, $d_{24-30\ h} = -4.416$; Figs. 5A and B). Nest building behavior in these groups had recovered to levels equivalent to saline-injected controls by the 48–54 h time point. LPS-treated LD-Leptin hamsters showed greater percent decreases in nesting material shredded as compared to saline-injected controls at the 24–30 h and the 48–54 h time points ($P < 0.02$, $d_{24-30\ h} = -23.403$, $d_{48-54\ h} = -1.721$; Fig. 5B), and nest building behavior had recovered in this group by the 72–78 hour time point.

Serum cortisol

Post-injection circulating cortisol levels were affected by photoperiod ($F_{1,67} = 19.55$, $P < 0.001$, $\eta^2 = 0.148$) and injection ($F_{1,67} = 41.73$, $P < 0.001$, $\eta^2 = 0.317$) (Fig. 6). Specifically, SD hamsters had higher levels of circulating cortisol than LD hamsters ($T = 4.42$, $P < 0.01$, $d = 0.880$), and LPS-treated hamsters had higher cortisol levels than saline-treated hamsters ($T = 6.46$, $P < 0.01$, $d = 1.377$).

Discussion

Our findings demonstrate that leptin acts as a neuroendocrine regulator of sickness. Specifically, leptin mediated some but not all symptoms of seasonal sickness response variation in Siberian hamsters. In this experiment, we experimentally elevated the circulating leptin levels of short-day hamsters so that they were comparable to the leptin levels of long-day hamsters and then assessed various components of their sickness responses to LPS. Although animals showed mainly hypothermic responses to LPS, short-day leptin-treated hamsters showed patterns of LPS-induced hypothermia that were greatly attenuated as compared to short-day vehicle-treated hamsters. The attenuated pattern of LPS-induced hypothermia in short-day leptin-treated hamsters was more similar to the hypothermic responses exhibited by long-day vehicle- and long-day leptin-treated hamsters. Conversely, while we predicted that short-day leptin-treated hamsters would show longer durations and greater magnitudes of LPS-induced anorexia and body mass loss as compared to short-day vehicle-treated hamsters, we actually found that short-day leptin-treated hamsters had the shortest durations and lowest magnitudes of LPS-induced anorexia and body mass loss of all four groups. Finally, our measures of anhedonic behavior (i.e., saccharin solution intake), thermoregulatory behavior (i.e., nesting material shredding), and circulating cortisol were affected by LPS injection. LPS injection resulted in decreased saccharin solution intake and nest material shredding and increased circulating

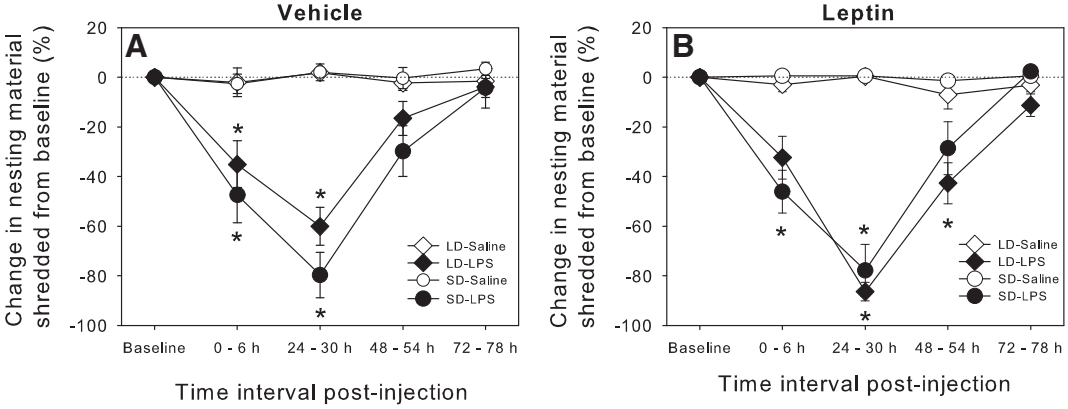

Fig. 5. Mean (±SEM) percent change in nesting material shredded from baseline following LPS and saline treatment delivered at the 0 h time point in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with (A) vehicle-filled and (B) leptin-filled osmotic mini-pumps. $^{*}P < 0.05$ versus saline-treated group exposed to the same photoperiod and osmotic mini-pump treatment.

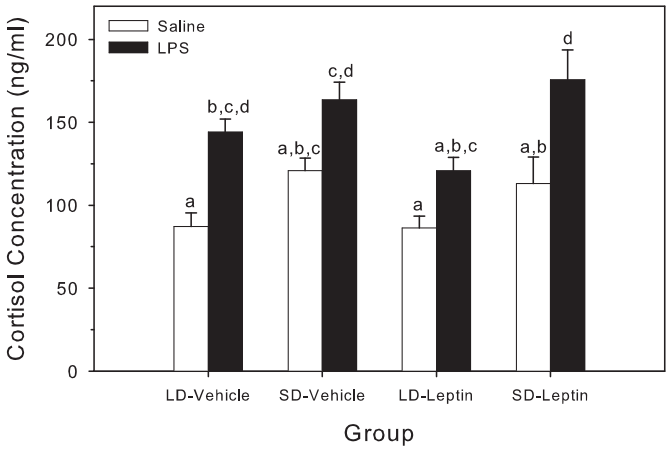

Fig. 6. Mean (±SEM) circulating serum cortisol taken 4 h following LPS and saline treatments in long-day (LD) and short-day (SD) housed male Siberian hamsters implanted with vehicle-filled or leptin-filled osmotic mini-pumps. Groups with different letters indicate statistically significant differences between group means ($P < 0.05$); groups sharing the same letter are statistically equivalent.

cortisol levels, however, none of the LPS-induced decreases and increases in these measures were modulated by leptin treatment.

Collectively, these results suggest that leptin acts as a mediator of seasonally appropriate fever/hypothermic responses. Although we did not initially predict that animals would show hypothermic responses to LPS, hypothermic responses to LPS are not uncommon and have been observed in several species of birds and mammals in experimental settings where a hypothermic response was not predicted (Burness et al., 2010; Martin et al., 2008a; Owen-Ashley et al., 2008). Hypothermic responses may be beneficial as they can help improve survival chances during severe systemic inflammation and sepsis (Romanovsky and Szekely, 1998). Additionally, hypothermia may be an adaptive strategy used for fighting infection when energy levels are low, as fever can only be beneficial if its heightened energy demands can be supported (Romanovsky and Szekely, 1998). In support of this hypothesis, green iguanas (*Iguana iguana*) can show both febrile and hypothermic responses to LPS, and the type of response that a lizard displays is dependent on its body mass (Deen and Hutchison, 2001). Heavier lizards choose to behaviorally thermoregulate to higher body temperatures (i.e., fever), while smaller lizards choose to behaviorally thermoregulate to lower body temperatures (i.e., hypothermia) after LPS-injection (Deen and Hutchison, 2001). Further support for this hypothesis comes from studies showing that negative energy balance, induced via fasting, results in attenuated fever responses to LPS in rats and hamsters (Bilbo and Nelson, 2002; Inoue and Luheshi, 2010; Inoue et al., 2008). There is also evidence that circulating leptin levels play a role in fever and hypothermic responses to LPS (Steiner and Romanovsky, 2007), as neutralizing leptin via a leptin anti-serum depresses or abolishes fever in rats treated with LPS (Harden et al., 2006; Sachot et al., 2004). Leptin receptor-deficient rats (Koletsky *f/f*) show increased durations of LPS-induced hypothermia as compared to rats with functional receptors (Koletsky *F/?*) (Steiner et al., 2004); however, these fever- and hypothermia-modulating effects of leptin are not always seen in all experimental systems or at all LPS doses (Inoue and Luheshi, 2010; Ivanov and Romanovsky, 2002). Therefore, it is possible that the short-day vehicle-treated hamsters showed the greatest magnitude of LPS-induced hypothermia because they had the lowest energy stores and leptin levels and were engaging in energy conservation. Additionally, increased circulating leptin levels in short-day leptin-treated hamsters may have acted as a signal of increased energy stores in these animals and mediated the attenuated hypothermic response.

We did not expect that long-day hamsters would show LPS-induced hypothermia, as they were not energetically challenged and because other studies in this species have shown that both long-day and short-day hamsters exhibit febrile responses to LPS (Bilbo et al., 2002; Wen et al., 2007). Although we used the same dose of LPS in our study as was used in the previous studies, this difference in our results from those of the other studies may be due to differences in the species and serotypes of the LPS used in these studies. In the current study, we used LPS from *S. enterica*, whereas LPS from *Escherichia coli* was used in other studies. Different serotypes of *E. coli* can produce different patterns of LPS-induced fever and hypothermia (Akarsu and Mamuk, 2007); therefore, it seems likely that LPS from different species could also elicit different fever and hypothermia patterns. We have previously used LPS from *S. enterica* in our lab and found that it induced hypothermia, rather than fever in hamsters (French et al., 2013). These species differences in LPS may also explain why we did not see photoperiodic differences in LPS-induced decreases in nest building behavior as previous studies have reported (Prendergast et al., 2008; Wen et al., 2007). For instance, huddling in a nest would likely prevent radiative heat loss and reduce hypothermia, so animals exhibiting sickness-induced hypothermia may want to avoid nesting during illness. As such, if sickness-induced hypothermia is adaptive in this experimental context, then we would not expect to see short-day attenuation of LPS-induced decreases in nest building behavior. However, as we still saw seasonal variation in sickness responses between the long-day and short-day groups in several of our measures (i.e., temperature, anorexia, body mass loss, cortisol levels), these LPS species differences should not influence our ability to make meaningful comparisons to previous studies.

One of our initial predictions was that if leptin mediated seasonal changes in sickness responses, then we expected that providing short-day hamsters with supplemental leptin would increase the magnitude and duration of LPS-induced anorexia and body mass loss. Rather, we found that leptin treatment attenuated these measures in short-day hamsters. Leptin was first characterized for its role in regulating food intake and body mass, with experimentally increased leptin levels resulting in decreases in food intake and body mass (Friedman and Halaas, 1998). Furthermore, circulating leptin levels are increased in response to LPS injection (Sarraf et al., 1997). Because LPS increases circulating leptin levels, and increased leptin levels decrease food intake and body mass, it seems that leptin could be a likely candidate for regulating infection-induced anorexia and body mass loss; however, the evidence supporting this relationship is mixed (Carlton et al., 2012). Our results suggest that leptin modulates LPS-induced anorexia and body mass loss because these measures are attenuated in both the long-day and short-day leptin treated groups; however, as it does not regulate these responses in our predicted manner, it is likely not regulating these responses in this seasonal context.

Another possible explanation for the unexpected attenuation of LPS-induced anorexia and body mass loss in the short-day leptin-treated group is that anorexia and body mass loss were attenuated in this group to counteract the effects of the weakened hypothermic response. If reducing hypothermia resulted in greater energy expenditure, then these animals might have had to preserve energy by reducing anorexia in order to avoid reaching a level of survival-risking negative energy balance (Ashley and Wingfield, 2012; Owen-Ashley and Wingfield, 2007). If reductions in LPS-induced anorexia in short-day leptin-treated animals are the result of an energy conservation mechanism, then it is possible that seasonal changes in infection-induced fever/hypothermia are regulated by circulating leptin levels while changes in infection-induced anorexia are regulated by some other energetic mechanism. There are several other orexigenic and anorexigenic hormones (e.g., ghrelin, neuropeptide Y, alpha-melanocyte-stimulating hormone, cholecystokinin, corticotropin-releasing hormone) that have been experimentally linked to infection-induced anorexia (Carlton et al., 2012). Therefore, it is likely that leptin may be just one among many hormones that act to coordinate seasonally appropriate sickness.

Further support of this idea that different symptoms of seasonal sickness responses are regulated by different hormonal and physiological mechanisms comes from previous studies that have examined the roles of other seasonally variable traits in sickness response modulation (Adelman and Martin, 2009). For example, removing the endogenous melatonin signal that organizes the seasonal response in Siberian hamsters (i.e., the short-day morph is induced when animals secrete melatonin for long durations; the long-day morph is induced when animals secrete melatonin for short durations) via pinealectomy causes hamsters housed in short days to display long-day-like patterns of LPS-induced anorexia, body mass loss, and reductions in nesting material use (Wen et al., 2007). Providing short-day pinealectomized hamsters with peripheral melatonin implants restores short-day-typical responses for these three LPS-induced measures (Freeman et al., 2007). Surprisingly, short-day pinealectomized animals still show short-day-typical fever responses (Wen et al., 2007), suggesting that this response is mediated by some factor other than melatonin. Long-day hamsters that are castrated show shortened durations of LPS-induced anorexia and decreased magnitudes of LPS-induced body mass loss as compared to uncastrated long-day controls; however, castration results in no attenuations in LPS-induced decreases in nesting material use (Prendergast et al., 2008).

While these studies show that manipulating different seasonally variable traits (e.g., photoperiodic rhythms, melatonin, testosterone) affects some of the same and some different components of sickness responses, manipulating these traits also have effects on the animal's body mass. Pinealectomy results in short-day hamsters displaying long-day-like body masses, while providing peripheral melatonin to these pinealectomized animals once again renders their body masses short-day-like (Freeman et al., 2007; Wen et al., 2007). Additionally, castrating long-day hamsters results in body mass decreases (Prendergast et al., 2008). Even though our study suggests that leptin does not influence all components of seasonal sickness response variation, we cannot eliminate the hypothesis that energetic state is a primary driver of the seasonal differences in any of these symptoms of sickness.

Because we did not manipulate actual body mass in this study (and leptin is only one among many signals of energy) and prior studies have not controlled for changes in body mass while manipulating other seasonally-changing variables, there could be other energetic hormones modulating these responses. Additional support for the need to continue to test the hypothesis that energetic state drives seasonal variation in sickness responses comes from observations that these correlations between sickness responses and body mass extended beyond seasonal breeders. For instance, in rats, ranging from lean to obese, there are positive correlations between body mass and the length of time it takes for an animal to recover from LPS-induced sickness (i.e., heavier animals take longer to recover) and the number of sickness symptoms that an animal displays (i.e., heavier animals display more sickness symptoms) (Pohl et al., 2014). Exploring the connections between other energetic hormones and seasonal variation in sickness responses will hopefully allow us to narrow in on the mechanisms promoting these correlations between body mass and sickness response intensity in seasonally breeding animals, as well as non-seasonal model organisms. We may also gain a better understanding of the mechanisms driving these relationships between energy stores and sickness responses by investigating the role of cytokines as intermediate factors between energy and sickness. Leptin (and other seasonal and energetic factors) is likely driving seasonal differences in sickness response intensity through effects on cytokine release and sensitivity (Dantzer, 2004; Zimmerman et al., 2014). Previous studies have shown photoperiodic differences in cytokine release (Bilbo et al., 2002), gene expression (Pyter et al., 2005), and sensitivity (Wen and Prendergast, 2007), so the effects of seasonal leptin variation on these factors should be a target of future investigations.

In conclusion, our data show that leptin does play a role in regulating seasonal variation in sickness response symptoms. Although leptin may not influence all the components of sickness responses in Siberian hamsters, leptin supplementation clearly attenuated the hypothermic response to LPS in short-day hamsters. This leptin-induced suppression of hypothermia in short-day hamsters may require animals to conserve energy by reducing their displays of LPS-induced anorexia. Our results, and the results of previous studies, do not exclude the hypothesis that seasonal variation in sickness responses is a product of seasonal changes in energetic stores. Instead, our results suggest that individual symptoms in the sickness responses of these seasonally breeding animals may be modulated by various energetic and non-energetic hormones, providing opportunities for future investigations.

Acknowledgments

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