Research paper

Effects of exogenous leptin on seasonal reproductive responses to interacting environmental cues in female Siberian hamsters

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A B S T R A C T

Animals living in temperate climates respond to environmental cues that signal current and future resource availability to ensure that energy resources are available to support reproduction. Siberian hamsters (Phodopus sungorus) undergo robust gonadal regression in short, winter-like photoperiods as well as in response to mild food restriction in intermediate photoperiods. The goal of the present study was to investigate whether leptin is a relevant metabolic signal in regulating gonadal regression in response to diminishing food availability. Adult female hamsters housed in short-day (winter-like) or intermediate (fall-like) photoperiods received either ad libitum access to food or mild food restriction (90% of ad libitum intake) and were treated with either leptin or a vehicle for five weeks in order to determine the ability of leptin to inhibit gonadal regression. At the end of five weeks, vehicle-treated hamsters showed physiological signs associated with ongoing gonadal regression, such as decreases in body mass and food intake, cessation of estrous cycling, and small decreases in reproductive tissue mass. Leptin did not modify changes in body mass, food intake, hormone concentration, or tissue mass, but showed a tendency to support estrous cycling, particularly in response to food restriction in the intermediate photoperiod treatment. Overall, leptin appears to play a minor role in coordinating reproductive responses to multiple environmental cues, at least in the early stages of gonadal regression.

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1. Introduction

Animals living in temperate climates experience extreme fluctuations in resource availability on a seasonal basis that influence survival and reproductive success. All components of reproductive function, from gametogenesis to parental care of offspring, are energetically expensive, particularly for females, and are typically not favored over survival if energy stores are insufficient (Bronson, 1985; Schneider, 2004). Many temperate zone species have thus evolved to restrict reproduction to times of year that are most favorable for survival of self and offspring (i.e., times of maximal energy availability) (Bronson, 1985; Bronson and Heideman, 1994; Goldman, 2001). In order to time reproduction appropriately, animals respond to cues signaling energy availability in the environment, as well as to internal metabolic signals. Seasonally breeding animals such as Siberian hamsters respond to multiple environmental factors in order to determine the appropriate time for reproduction. Siberian hamsters are reproductively active in long-day (LD), summer-like (>14 h of light per day) photoperiods and undergo robust gonadal regression accompanied by dramatic loss of body mass and decline in food intake in short-day (SD), winter-like (<12 h of light per day) photoperiods (Hoffmann, 1982; Bartness and Wade, 1985; Goldman, 2001). Photoperiod can be used to predict the future status of factors such as precipitation, presence of conspecifics, and perhaps most critical, food availability, and is therefore conceptualized as an “initial predictive” cue, which is used to coordinate broad physiological responses over time (gonadal regression) (Baker, 1938; Wingfield and Farner, 1980). Reproductive timing can then be fine-tuned in response to the immediate status of factors critical to offspring survival (e.g., food availability) acting as “supplementary” cues (Wingfield and Farner, 1980). To mimic the interactions of these two types of environmental cues, we employed a recently established laboratory
paradigm that is useful for exploring how animals respond to inter-acting signals of energy availability. Male Siberian hamsters housed in constant photoperiods of either greater than 14 h (summer) or less than 10 h (winter) of light per day in laboratory conditions show definitive physiological and behavioral states such as supplementary cues (e.g., food restriction) do not trigger reproductive responses (Paul et al., 2009a,b). However, if male hamsters develop within a constant, intermediate (13.5 h light) photoperiod, supplementary cues (food availability, social housing) can trigger the initiation of gonadal regression through neuroen-docrine mechanisms similar to those controlling the response to photoperiod (Paul et al., 2009a,b; Bailey et al., 2017).

Environmental and metabolic signals that modulate reproductive function are integrated by the hypothalamo-pituitary-gonadal (HPG) endocrine axis. The hypothalamic gonadotropin releasing hormone (GnRH) neurons control secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, thereby leading to changes in gonadal function. HPG axis activity—specifically, the pulse frequency of GnRH neurons—controls reproductive function and is affected by both initial predictive and supplementary environmental cues as well as metabolic signals of energetic state, a notable example of which is leptin. Leptin is a peptide hormone that is released from white adipose cells, and is a critical signal in body mass homeostasis as well as an array of reproductive functions in both sexes such as the onset of puberty and maintenance of fertility (Cheung et al., 1997; Ahima and Flier, 2000; Fernandez-Fernandez et al., 2006; Quennell et al., 2009; Hausman et al., 2012; Landry et al., 2013; Chehab, 2014; De Bond and Smith, 2014; Pankov, 2015; Perez-Perez et al., 2015). Leptin-deficient animals exhibit infertility associated with a lack of GnRH and LH pulsatile release, delayed puberty, and impaired cycling and ovulation in females, all of which are alleviated through treatment with exogenous leptin (Barash et al., 1996; Chehab et al., 1996; Mouzinh et al., 1997; Schneider et al., 1998; Hill et al., 2008; Evans and Anderson, 2012; Luo et al., 2016).

Seasonally breeding animals, in addition to modifying reproductive function, exhibit seasonal changes in a host of physiological parameters, such as body mass, food intake, and immune function (reviewed in Bartness and Wade, 1985; Nelson et al., 2002). Concurrent with these changes, leptin expression and secretion fluctuates on a seasonal basis in response to photoperiod in several seasonally breeding species, such as hamsters, ground squirrels, and sheep (Klingenspor et al., 1996; Boyer et al., 1997; Marie et al., 2001; Bartness et al., 2002; Mercier et al., 2000; Rousseau et al., 2002). The potential role of leptin in regulating seasonal changes in metabolic and reproductive function is currently unclear, due to conflicting reports of the effects of exogenous leptin owing to differences in method of administration. For example, in Siberian hamsters, exogenous administration of leptin has photoperiod-specific effects on changes in body mass and immune function, but does not affect food intake or HPG axis activity within photoperiod treatments in the same way depending on administration (chronic/continuous vs. acute) (Atcha et al., 2000; Klingenspor et al., 2000; Drazen et al., 2001; Bartness et al., 2002; Carlton and Demas, 2014). Administration of leptin via daily injection triggered a decline in food intake in both LD- and SD-housed Siberian hamsters, but leptin administered continuously via mini-osmotic pump had no effect on food intake in similarly housed hamsters (Atcha et al., 2000; Klingenspor et al., 2000). Leptin appears to be an ideal candidate for an endocrine signal of energy availability relevant for seasonal reproductive function; however, chronic, continuous leptin administration is ineffective in stimulating reproductive physiology in SD photoperiod-housed, gonadally regressed male and female Siberian hamsters (Atcha et al., 2000).

The goal of the present experiment was to assess the role of leptin in signaling energetic state to the reproductive axis in environ-
h delivery rate. 42 days: Direc Corp., Cupertino, CA, USA). Pumps were filled with either 0.5 M Tris buffer or leptin at a concentration of 4.43 µg leptin/µL Tris according to assigned treatments. Murine leptin is effective as an adipose tissue endocrine signal in Siberian hamsters and the dose used in this experiment has been shown by several studies to modulate Siberian hamster seasonal responses (Atcha et al., 2000; Klingenspor et al., 2000; Drazen et al., 2001; Demas and Sakaria, 2005; French et al., 2009; Carlton and Demas, 2014).

2.4. Food restriction

For the five days prior to the start of experimental treatments, ad libitum food intake was measured to assess a baseline level of intake for each animal. Each hamster’s average daily intake across five days was used to calculate individual food-restriction rations, which were provided just prior to lights out each day (1500 h EST). FR animals in the ID photoperiod received 90% of their baseline intake (w/w, ±0.1 g) each day. FR animals in the SD photoperiod were given rations reflective of their AL counterparts, because food intake naturally decreases during reproductive regression in SD photoperiods. To accomplish this, SD-AL hamsters’ food intake was monitored daily and averaged as a group. Each SD-FR hamster received 90% of the mean SD-AL intake each day, unless an individual’s baseline intake was less than the SD-AL mean, in which case she received 90% of her original baseline intake in order to maintain the mild caloric restriction. In both photoperiods, upon providing FR rations, any remaining ration in the cage food hopper from the previous day was collected and weighed (±0.1 g) to gain a better approximation of actual intake, although it was not feasible to differentiate calorie consumption from hoarding or cheek pouch storage.

2.5. Estrous cycle monitoring

Estrous cycling of females was determined through cytological examination of vaginal epithelial cells for three 5-day periods in the experimental timeline (one set of five days prior to the start of the experiment [baseline], one set 2.5 weeks after transfer to photoperiod/beginning of food treatment [mid-experiment], and one during the last five days of the experiment [end-experiment]). A sample of vaginal epithelial cells was collected from each female each day at 1400 h EST by lavaging 30 µL of sterile 0.9% saline through the vaginal canal 3–5 times. The sample was placed on a glass microscope slide, allowed to dry, fixed in methanol, and stained with a 10% Giemsa solution (Sigma-Aldrich® Procedure No. GS-10). Cells were visualized using a light microscope and estrous cycle stages were identified according to the presence and proportions of nucleated and cornified epithelial cells, as well as leukocytes (Caligioni, 2009). Vaginal cytology is not as precise a method in this species as it is for other rodents (Dodge et al., 2002); thus, rather than analyzing an individual female’s specific cycle progression, we instead determined whether individual females were cycling (the population of cells progressed through clear estrous cycle stages during the 5-day evaluation period) or non-cycling (the population of cells was unchanged, usually in a late diestrus/early proestrus-like state, consisting of low numbers of irregularly-shaped parabalral cells).

2.6. Blood sampling and tissue collection

Three blood samples (one during the individual housing period, five days prior to the start of the experiment [baseline], one 2.5 weeks after transfer to photoperiod treatment/beginning of food treatment [mid-experiment] and one just before euthanasia and tissue collection on the final day [end-experiment]) were collected at 0900 h EST to verify the efficacy of leptin miniosmatic pumps and to monitor changes in LH concentrations in response to experimental treatments. Hamsters were lightly anesthetized with isoflurane and blood (~3.5% of the animal’s total blood volume) was drawn from the retro-orbital sinus and collected into microcentrifuge tubes. Samples were allowed to clot at room temperature for 1 h, clots were removed, and samples were centrifuged at 4 °C for 30 min at 2500 rpm. Serum was collected from the tubes and transferred to a −20 °C freezer for storage until performing LH and leptin assays.

On the last day of the experiment after the final blood sample was collected, hamsters were deeply anesthetized in isoflurane and necropsies were performed to collect ovaries, uterine horns, and parametrical white adipose tissue (PWAT), a fat pad that surrounds the ovary and likely provides metabolic support to reproductive functions (Jaubert et al., 1995; McInroy et al., 2000; Gui et al., 2006). Tissues were weighed to determine reproductive and energetic status as a result of experimental treatments.

2.7. Hormone measurement

Serum LH concentrations were determined via a radioimmunoassay (RIA) described previously (Legan et al., 2009) with slight modifications. The standard (rat LH, RP-3) and purified LH for iodination were obtained from Dr. A.F. Parlow at the National Hormone and Peptide Program, Torrance, CA, USA. Single 200 µl aliquots of each sample were diluted in 0.05 M phosphate-buffered saline (PBS) containing 0.1% gelatin (gel-PBS). The primary antibody was CSU 120 (provided by Dr. Terry Nett, Colorado State University, Fort Collins, CO, USA), diluted 1:10,000 in 1:100 normal rabbit serum (Millipore, St. Charles, MO). The tubes were incubated for 24 h at 22 °C after addition of 100 µL primary antibody, and after adding radiolabeled LH (~60,000 counts per minute/100 µL gel-PBS, iodinated by the iodogen method), and again following addition of the secondary antibody (anti-rabbit gamma globulin, diluted 1:50 in gel-PBS, Millipore, St. Charles, MO, USA). The LH results reported herein were obtained from four assays for which the mean sensitivity was 0.05 ng/ml, determined as two standard deviations below the maximum binding. Two replicates of each standard serum pools from male hamsters that inhibited binding on average to 71.4% and 83.7% were analyzed at the beginning, middle and end of each assay for determination of inter- and intra-assay coefficients of variation (CVs). The intra-assay CV was 13.4% and the mean intra-assay CV was 8.6%.

Serum leptin was determined via a commercially available enzyme-linked immunosorbent assay (ELISA) kit that has been validated for Siberian hamster and other non-murine rodent samples (Mouse Leptin ELISA Kit, Crystal Chem Inc., Downers Grove, IL, USA) (Johnson et al., 2004; Carlton and Demas, 2014). Serum samples were diluted for measurement on the absorbance standard curve and were run in duplicate; any set of duplicates with a CV > 10% was re-assayed. All results reported herein were obtained from four assays. The sensitivity of the assay was 0.2 ng/ml, determined as the lowest concentration of standard. Based on the leptin concentrations of two replicates of a male hamster serum pool that were analyzed in each assay and exhibited average absorbance of 0.298, the inter-assay CV was 16.4%. Based on multiple measurements of one of the provided standards per plate, the mean intra-assay CV was 4.9%.

2.8. Statistical analyses

Statistical tests were performed using JMP 12.0.1 (SAS Institute Inc., Cary, NC, USA) and VassarStats (Lowry, 2016). A value of p < 0.05 was considered to be statistically significant for all tests. Final sample sizes for each group were as follows: ID-AL-vehicle:
5; ID-AL-leptin: 7; ID-FR-vehicle: 8; ID-FR-leptin: 7; SD-AL-vehicle: 8; SD-AL-leptin: 7; SD-FR-vehicle: 7; SD-FR-leptin: 6. Data distributions were checked for homogeneity of variance and for normality of model residuals. Those distributions with unequal variances or producing non-normal residuals were transformed to best meet these assumptions for parametric tests. Leptin concentrations and percent change in food intake were square root-transformed and PWAT mass and LH concentrations were log-transformed. Uterine horn mass could not meet the assumption of homogeneity of variance, and was assessed using a non-parametric Wilcoxon rank sum test. One animal in the SD-ALvehicle treatment was excluded from food intake analysis because she was determined to be hoarding large amounts of food. Analysis of variance (ANOVA) models including the three treatments in a full factorial design were used to assess all measurements. Body mass was included as a covariate in analysis of leptin concentration and reproductive tissue masses. For measurements of body mass, food intake, leptin concentration, and LH concentration, a repeated measures ANOVA was used to detect treatment effects over time within and between subjects, with time as a within-subjects variable. Within-subjects comparisons for body mass and food intake violated assumptions of sphericity and were Greenhouse-Geiser (G-G) corrected. Comparisons of the eight group means were conducted using one-way ANOVAs with Tukey’s HSD post hoc tests. Paired t-tests were used to assess differences in leptin concentration between the baseline and final samples, and 2-tailed Student’s t-tests were used to assess differences in end-experiment leptin between vehicle- and leptin-treated groups. For analysis of estrous cycling, proportions of cycling females were compared among groups using χ² contingency tables at each time point. We assessed whether proportions of cycling and non-cycling females were different at the start vs. end of the experiment by constructing 3-way contingency tables for each treatment category (photoperiod, food, pump) with cycling status (cycling vs. non-cycling), time (start vs. end), and treatment (ID vs. SD, AL vs. FR, vehicle vs. leptin) as factors. Proportions of females changing in status from cycling to non-cycling were compared within each treatment category (e.g., photoperiod) between treatments (e.g., ID vs. SD), using 2-sample z-tests to compare proportions. To further demonstrate how these proportions changed over time, we assigned an “estrous cycling response score” from 1 to 4 to each animal to represent that animal’s change in cycling status from the beginning to the end of the experiment, such that a higher score signifies a change toward greater reproductive potential (1 = changed from cycling to not cycling; 2 = remained non-cycling across the experiment; 3 = remained cycling across the experiment; 4 = changed from non-cycling to cycling). Due to small sample size, it was not possible to conduct analyses of the effects of interactions between treatments on the proportions of cycling females.

3. Results

3.1. Body mass and food intake

There were no significant treatment effects or differences between groups in baseline body mass (F7,47 = 0.69, p = 0.68). Measurements of body mass each week were unaffected by treatments, with the exception of a significant effect of food treatment in week 5 such that FR animals exhibited lower mass at this stage (F1,47 = 4.92, p = 0.032) (Fig. 1a). Repeated measures analysis showed no between-individual treatment effects (F2,46 = 1.00, p = 0.44), but within individuals, hamsters lost mass according to time × photoperiod (G-G corrected, F1,56.92.91 = 9.34, p < 0.001) and time × food (F1,58.92.91 = 11.59, p < 0.001), with SD and FR ani-

mals declining more in mass than ID and AL animals, respectively. Total percent change in mass was significantly different among groups (F7,47 = 5.74, p < 0.001), and was significantly affected by photoperiod (F1,47 = 16.44, p < 0.001) and food (F1,47 = 19.011, p < 0.001) treatments, with SD and FR animals losing a greater total percentage of mass than ID and AL animals (Fig. 1c).

There were no significant treatment effects or differences between groups in baseline food intake (F2,46 = 2.07, p = 0.066). Photoperiod and food treatments affected food intake throughout the experiment, beginning after 1 week of treatments and continuing through the final week (week 1: photoperiod: F1,46 = 7.08, p = 0.011; food: F1,46 = 66.31, p < 0.001. Similar results in weeks 2–5) (Fig. 1b). Similarly, total percent change in food intake was significantly affected by photoperiod (F1,46 = 31.63, p < 0.001) and food (F1,46 = 22.10, p < 0.001), but not pump (F1,46 = 0.20, p = 0.89), treatments (Fig. 1d). Repeated measures analysis revealed a significant effect of food treatment on food intake between individuals (F1,46 = 32.95, p < 0.001) and effects of time × photoperiod (G-G corrected, F3.47,159.54 = 11.87, p < 0.001) and time × food (F3.47,159.54 = 24.18, p < 0.001) within individuals, such that intake over time differed between AL and FR individuals and decreased within individuals in time in SD and FR treatments.

3.2. Serum leptin

Serum leptin did not differ significantly among groups in the baseline measurement (F7,47 = 0.39, p = 0.91) and was significantly correlated with body mass at each measurement point (baseline: F1,53 = 49.15, p < 0.001, R² = 0.48; mid-experiment: F1,53 = 11.81, p = 0.001, R² = 0.18; end-experiment: F1,53 = 33.48, p < 0.001, R² = 0.39). Leptin pump treatment successfully enhanced serum leptin levels; in both mid- and end-experiment blood samples, only a significant effect of pump treatment was observed (mid-experiment: F1,46 = 90.00, p < 0.001; end-experiment: F1,46 = 29.15, p < 0.001) (Fig. 2). Similarly, in repeated measures analysis, only pump treatment produced differences between individuals over time (F1,47 = 47.59, p < 0.001), and serum leptin changed within individuals over time according to time × pump (F2,46 = 29.85, p < 0.001). However, leptin levels in pump-treated animals declined between the mid-experiment and end-experiment time points. Individual Student’s t-tests between vehicle- and leptin-treated hamsters in the end-experiment sample showed leptin-treated hamsters having significantly higher leptin concentrations than their vehicle-treated counterparts in all photoperiod/food combinations except for SD-FR (ID-AL: t(9) = 3.24, p = 0.010; ID-FR: t(12) = 2.23, p = 0.046; SD-AL: t(13) = 4.84, p < 0.001; SD-FR: t(8) = 1.74, p = 0.12), but more conservative Tukey’s HSD post hoc comparisons among groups indicated that the SD-AL-leptin group was the only group with statistically significantly higher leptin levels than its vehicle-treated counterpart at the end of the experiment (F4,46 = 13.20, p < 0.0001) (Fig. 2). All vehicle-treated groups declined in leptin concentration from the baseline to end-experiment samples (ID-AL-veh: t(4) = –3.26, p = 0.031; ID-FR-veh: t(7) = –4.63, p = 0.002; SD-AL-veh: t(7) = –4.42, p = 0.003; SD-FR-veh: t(6) = –9.49, p < 0.001), whereas end-experiment lep-

tin in leptin-treated animals did not differ from baseline (ID-AL-lep:

1 = 1.82, p = 0.12; ID-FR-lep: t(6) = –0.16, p = 0.88; SD-AL-lep:

1 = –0.55, p = 0.60; SD-FR-lep: t(5) = –1.18, p = 0.29).

3.3. Estrous cycling

Comparing all groups at each time point, the proportion of females cycling did not differ significantly at the baseline time point (χ²(7) = 12.29, p = 0.092) or the mid-experiment time point (χ²(7) = 8.73, p = 0.27). However, statistically significant differences among groups did occur at the end-experiment time point.
At the end of the experiment, ID-AL (leptin and vehicle groups), ID-FR-leptin, and SD-AL-leptin groups contained cycling females (Fig. 3a). Three-way contingency table analyses comparing the proportion of animals cycling at the beginning and end of the experiment were performed for each treatment category (photoperiod, food, pump) separately. For each treatment category, the 3-way interaction between the treatment, start vs. end, and cycling vs. non-cycling was significant (photoperiod: $\chi^2(4) = 28.68, p < 0.001$; food: $\chi^2(4) = 25.18, p < 0.001$; pump: $\chi^2(4) = 23.14, p < 0.001$). Thus, changes in the proportions of cycling vs. non-cycling females were in part a function of treatment. Estrous cycling response scores were higher in AL and leptin-treated groups compared to their counterparts (Fig. 3b). As our hypotheses addressed the likelihood of females within each group to undergo gonadal regression, i.e., lose reproductive potential through cessation of cycling, we directly compared the proportion of animals that stopped cycling during the course of the experiment within each treatment category. Photoperiod (ID proportion: 0.22, SD proportion: 0.61, $z(2) = 2.9, p = 0.004$) and food (AL proportion: 0.57, FR proportion: 0.26, $z(2) = 2.3, p = 0.019$) treatments showed significant differences in proportions of females that stopped cycling, but comparing pump treatments revealed no significant difference in the proportion of females to stop cycling between vehicle and leptin treatments (vehicle proportion: 0.54, leptin proportion: 0.30, $z(2) = 1.8, p = 0.072$).
3.4. Reproductive tissue mass and serum LH

Uterine horn mass did not differ significantly among groups (χ²(7) = 11.53, p = 0.12, Fig. 4a). A visualization of uterine horn mass in cycling and non-cycling females (end-experiment cycling measure) indicates that within groups, cycling females exhibited larger uterine horns (Fig. 4b). PWAT mass was affected by photoperiod treatment such that SD animals had lower PWAT mass when larger uterine horns (Fig. 4b). PWAT mass was affected by photoperiod (F1,46 = 5.25, p = 0.027) between individuals (Fig. 5a). While LH changed over time within individuals, this was not affected by treatment, as there were no interactions between time and treatments (time effect: F7,46 = 10.26, p < 0.001). There were no significant differences among groups in the total change in LH (F7,46 = 1.17, p = 0.34) (Fig. 5b).

4. Discussion

We tested the hypothesis that leptin is a relevant metabolic cue for seasonal reproductive function, specifically in an environmental context in which future resource availability is uncertain. We exposed female Siberian hamsters to combinations of photoperiodic and food availability cues, and predicted that leptin administration would affect their reproductive responses to declining food availability in an intermediate photoperiod, but not to SD photoperiod. Our leptin treatment effectively increased circulating leptin above baseline levels for at least half of the experiment, but unexpectedly decreased to baseline levels on average by the end of the experiment, while vehicle-treated animals showed a decline in leptin levels below baseline. We did not observe effects of leptin treatment on seasonal changes in body mass and food intake that have previously been observed in this species (Atcha et al., 2000; Klingenspor et al., 2000). There was a trend for leptin to support estrous cycling in females exposed to inhibitory environmental cues, but it did not attain significance. Leptin treatment did not modify other reproductive parameters such as reproductive tissue mass or serum LH. Thus, our hypothesis that leptin would modify reproductive responses to interacting environmental cues relevant for seasonal reproduction—specifically that it would prevent the cessation of estrous cycles and atrophy of reproductive tissues—was not fully supported.

Our leptin treatment may not have increased leptin levels long enough to produce clear effects, as circulating leptin concentrations in leptin-treated animals dramatically declined between the mid-experiment and end-experiment time points. This most likely indicates degradation of the exogenous protein, as the pumps should maintain functionality for six full weeks and we evaluated leptin five weeks post-implantation. In our previous studies, there was no degradation of leptin administered in the same manner for two weeks (Drazen et al., 2001; Demas and Sakaria, 2005; French et al., 2009; Carlton and Demas, 2014). However, this is the first attempt we know of to administer leptin continuously for five weeks, and no data are available regarding the stability of recombinant murine leptin at hamster body temperature. It is possible that this decline affected the ability of exogenous leptin to modulate physiological responses to the environmental treatments, but there is also evidence to suggest it may have maintained some effectiveness through the end of the experiment. All vehicletreated groups showed declines in circulating leptin in the final sample compared to baseline, whereas leptin-treated animals maintained their baseline leptin concentration. When compared directly, all photoperiod/food combinations except SD-FR showed leptin-treated animals having significantly higher leptin concentrations than vehicle-treated animals. However, because the final leptin concentrations in leptin-treated groups were not significantly different than those of the vehicle-treated groups when comparing all groups using Tukey’s HSD post hoc comparisons, with the exception of the SD-AL groups, it cannot be determined from these results whether leptin treatment was effective for the entire experiment duration.

Contrary to previous reports, our leptin treatment did not affect typical seasonal changes in body mass, food intake, or HPG axis function. Our goal was to examine whether providing continuous exogenous leptin, mimicking a continuous secretion of leptin from adipose tissue (but lacking the variability in leptin secretion accompanying food intake), would affect reproductive responses
to certain environmental conditions in real time. Thus, we chose to administer leptin at the start of exposure to environmental treatments and continuing for five weeks. Gonadal regression and its associated physiological changes take eight to ten weeks to reach completion, and previous studies examining differences in the effects of leptin treatment between photoperiods have observed Siberian hamsters that were fully regressed, i.e., leptin treatment was started after at least eight weeks of SD photoperiod exposure (Atcha et al., 2000; Klingenspor et al., 2000). Examining physiological characteristics essentially halfway through regression may therefore resulted in less obvious environmental effects. Because of this, we also saw very few effects of our treatments on reproductive tissue masses, with only a significant effect of photoperiod on PWAT mass. This PWAT finding, combined with photoperiod and food treatment effects on repeated measures analysis of body mass, food intake, and serum LH, confirm that regression was proceeding, but the end result is less dramatic than if we were able to track the entire regression process. Furthermore, this experiment examined females that developed within an intermediate photoperiod rather than a long-day (LD) photoperiod, in which previous comparisons to SD photoperiod hamsters have been performed (Atcha et al., 2000; Klingenspor et al., 2000). Male hamsters that develop in an intermediate photoperiod are predisposed to be responsive to supplementary cues in ways that LD males are not (Paul et al., 2009a,b); assuming that females are similar, it is possible that ID females are not similar enough to LD females to act as an equivalent control group to compare effects of exogenous leptin.

We observed a general lack of change in circulating LH concentrations. Gonadal regression is associated with a downregulation of HPG axis function (Goldman, 2001). In this study, LH showed effects of photoperiod and food treatments over time between individuals, but showed little absolute change within groups and was not different among groups in overall change. This is likely due to the short observation period as well as the fact that basal LH levels are very low (near assay sensitivity) and pulsatile, so differences could not be detected based on only three samples collected weeks apart from each other. We expected leptin treatment to stimulate circulating LH levels because of its stimulatory effects on the HPG axis (Chehab, 2014), but because we did not observe detectable declines in LH in response to environmental cues, it is not surprising that we could not detect any modifications of these changes by leptin. Leptin effects on HPG axis function and LH levels within a seasonal context are generally poorly understood. A study comparing LD and SD Siberian hamsters found no effect of continuous leptin treatment on pituitary LH levels in either sex (Atcha et al., 2000). In contrast, a study on male LD and SD Syrian hamsters, another species of seasonally breeding
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Hamster, found that an acute dose of leptin reduced LH levels in serum in both photoperiods (Boggio et al., 2013). If leptin does play a role in regulating seasonal reproduction, it is unclear whether this role involves mediating HPG axis function.

The possibility that leptin treatment was effective in preserving estrous cycling in animals undergoing regression is intriguing, but difficult to confirm. Because of the limitations of our sample size, there was no feasible way of interpreting interactions between treatments when dealing with results in proportion form. Generally, treatment combinations did affect estrous cycling, as the proportion of females cycling within each group differed significantly among groups at the end of the experiment, but not at the beginning or middle. There were also significant interactions between the proportions of cycling and non-cycling females at the start and end of the experiment within each treatment (photoperiod, food, pump); thus, each treatment alone contributed to the change in the proportion of cycling females. Looking specifically at females that stopped cycling within each treatment category, proportions differed across photoperiod and food treatments individually, but not according to pump treatment. This would indeed be better tested in a setting in which treatment interactions could be examined; we predicted that leptin treatment would modify responses to the environmental treatments rather than produce responses of its own, which our results suggest may have occurred. For example, it is perhaps notable that at the end of the experiment, when many groups failed to contain any cycling females, that the groups with remaining cycling females were the two ID-AL control groups, and the leptin-treated ID-FR and SD-AL groups (this is also reflected in our innovated “estrous cycling response score”). In the case of the SD-AL cycling female, it is likely that she was a SD photoperiod “non-responder”: a subset of the Siberian hamster population fails to respond to the SD photoperiod and maintains a reproductively active phenotype year-round in wild populations and under laboratory conditions (Prendergast et al., 2001), although after only five weeks in photoperiod, we were unable to determine this definitively. Interestingly, if we do classify the cycling SD-AL female as a non-responder, we are left with only one non-control group containing cycling females: the ID-FR-leptin group. This would support our hypothesis that leptin is effective in signaling for energy allocation toward reproduction when a hamster is presented with an inhibitory supplementary cue, but not an initial predictive cue, and may be promising for future investigation of the mechanisms by which hamsters respond to different types of environmental cues.

Overall, our findings demonstrate that leptin, when administered continuously during the beginning of seasonal gonadal regression in female Siberian hamsters, has little modifying effect on physiological responses to photoperiod and food-related environmental cues. To definitively determine if leptin plays a role in supporting reproductive functions, such as estrous cycling, further investigation is required. Future study of the mechanisms by which leptin supports and regulates seasonal reproductive function will continue to elucidate how animals successfully interpret environmental cues to maximize survival and reproductive success.

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References


