Revised and resubmitted to *General and Comparative Endocrinology* on 20 September 2015

Glucose and insulin modulate sickness responses in male Siberian hamsters

Elizabeth D. Carlton* and Gregory E. Demas

Abstract

Mounting a sickness response is an energetically expensive task and requires precise balancing of energy allocation to ensure pathogen clearance while avoiding compromising energy reserves. Sickness intensity has previously been shown to be modulated by food restriction, body mass, and hormonal signals of energy reserves. In the current study, we tested the hypothesis that sickness intensity is modulated by glucose availability and an endocrine signal of glucose availability, insulin. We utilized male Siberian hamsters (*Phodopus sungorus*) and predicted that pharmacological induction of glucoprivation with 2-deoxy-D-glucose (2-DG), a non-metabolizable glucose analog that disrupts glycolysis, would attenuate energetically expensive sickness symptoms. Alternatively, we predicted that treatment of animals with insulin would enhance energetically expensive sickness symptoms, as insulin would act as a signal of increased glucose availability. Upon experimental treatment with lipopolysaccharide (LPS), we found that glucose deprivation resulted in increased sickness-induced hypothermia as compared to control- and insulin-treated animals; however, it did not have any effects on sickness-induced anorexia or body mass loss. Insulin treatment resulted in an unexpectedly exaggerated sickness response in animals of lesser body masses; however, in animals of greater body masses, insulin actually attenuated sickness-induced body mass loss and had no effects on hypothermia or anorexia. The effects of insulin on sickness severity may be modulated by sensitivity to sickness-induced hypoglycemia. Collectively, these results demonstrate that both glucose availability and signals of glucose availability can modulate the intensity of energetically expensive sickness symptoms, but their effects differ among different sickness symptoms and are sensitive to energetic context.

Keywords: 2-deoxy-D-glucose, ecoimmunology, infection-induced hypothermia, infection-induced anorexia, lipopolysaccharide

1. Introduction

Animals must obtain and utilize energy to fuel virtually every physiological and behavioral process required for their survival and reproductive success. In times of energy limitation, constraints may require energy to be shunted away from processes of a lesser priority for an organism's current needs and toward those processes most fundamental for survival (Sheldon and Verhulst, 1996). Mounting a sickness response to a pathogen is a process that requires precise balancing of energy allocation. Sickness is one of the first responses of the body to infection and is characterized by energetically expensive symptoms such as fever, anorexia, and body mass loss. While these responses may appear to be the result of infection-induced weakness, these symptoms have adaptive benefits (i.e., fever acts to inhibit growth of pathogens and elevated temperatures may enhance immunological efficiency; anorexia may lead to greater stringency in diet selection allowing an animal to alter its internal environment so that it is less favorable for pathogen growth (Hart, 1988; Kluger, 1986; Kyriazakis et al., 1998)) and reductions in their magnitude can negatively affect an animal's ability to clear its infection. However, if these symptoms are displayed too strongly, an animal may also succumb to death due to energy depletion (Adelman and Martin, 2009; Ashley and Wingfield, 2012; Hart, 1988; Moret and Schmid-Hempel, 2000).

Although sickness is an energetically expensive response, animals may also display sickness symptoms that act to counteract energy loss. For instance, some animals may display hypothermic responses, rather than fever, when they are energetically compromised, as decreasing body temperature can still provide an environment less favorable for pathogen growth than normal body temperature (Deen and Hutchison, 2001; Romanovsky and Szekely, 1998). Animals may also avoid hedonic behaviors or other energy consuming behaviors like nest-

building in thermoneutral environments so as to avoid expending energy on activities not required for survival (Aubert, 1999; Wen et al., 2007). Thus, making it through an infection requires precise coordination of the sickness response in regards to the energetic condition of the infected animal.

As such, sickness responses are not static, and animals are able to modulate sickness in response to their energy reserves. Previous work has shown that food restriction results in suppression of sickness symptoms (Bilbo and Nelson, 2002; MacDonald et al., 2014; MacDonald et al., 2011). Additionally, the intensity of the energetically costly symptoms of sickness are correlated with body mass in several species, such that animals with higher body masses show more intense sickness responses (Carlton and Demas, 2015; Owen-Ashley et al., 2008; Owen-Ashley et al., 2006; Pohl et al., 2014). Siberian hamsters (*Phodopus sungorus*) are a species that shows variation in sickness intensity that correlates with energetic state. For instance, hamsters show seasonal variation in both body mass and sickness intensity and exhibit the most intense sickness responses in the season in which they have the greatest body mass (Bilbo et al., 2002). We have previously manipulated body mass and an endocrine signal of fat stores (i.e., leptin) to determine their effects on sickness intensity variation in this species (Carlton and Demas, 2014; Carlton and Demas, 2015). These studies showed that hamsters modulate sickness symptoms in response to decreases in energy stores (i.e., attenuation of sickness-induced anorexia and body mass loss in hamsters that were food restricted to lose body mass; Carlton and Demas, 2015) and increases in circulating leptin levels (i.e., attenuation of sickness-induced hypothermia in hamsters provided exogenous leptin to simulate increased fat stores; Carlton and Demas, 2014).

In addition to signals of long-term energy stores, animals may also rely on signals of short-term energy availability (i.e., blood glucose levels) to modulate sickness. While an animal must avoid risking future survival by over-expending its excess energy stores, it may also be necessary for it to assess current environmental energy availability in order to avoid insufficient food resources during recovery. Glucose is the primary source of energy that is used by an animal, so blood glucose levels may provide the most immediate indicator of food availability. Furthermore, glucose is critical for fueling immune responses (Wolowczuk et al., 2008). Previous work has shown that reductions in glucose availability via treatment with 2-deoxy-D-glucose (2-DG), a non-metabolizable glucose analog that disrupts glycolysis and induces a state of glucoprivation (Horton et al., 1973), impairs antibody production, delayed-type hypersensitivity responses, splenocyte production, and leukocyte counts in Siberian hamsters, deer mice (*Peromyscus maniculatus*), and Lewis rats (Chou et al., 1996; Demas et al., 1997; Martin et al., 2008; Zysling and Demas, 2007). Although there is considerable evidence that reducing glucose availability modulates many aspects of the immune response, it remains unclear how glucose availability may affect sickness responses.

While we can manipulate actual glucose availability with 2-DG, we can manipulate signals of glucose availability via the pancreatic peptide hormone insulin. Insulin is secreted upon food consumption, and its release facilitates the storage of energy. In the short-term, increased insulin levels signal positive energy balance, and levels rapidly change in response to an organism's current energetic state (Benoit et al., 2004). Insulin receptors are expressed on activated lymphocytes, and administration of insulin to these activated lymphocytes increases cellular metabolism (Delmastro-Greenwood and Piganelli, 2013; Helderman, 1981). Insulin has been shown to modulate immune responses in Siberian hamsters, specifically enhancing

antibody production in the smaller short-day housed hamsters so that their antibody levels are comparable to those produced by the larger long-day housed hamsters (Garcia et al., 2010). These results suggest that insulin may act as a signal of current energy availability to allow coordination of energetically-appropriate immune responses.

The goals of the present study were to manipulate current glucose availability and a signal of glucose to determine their effects on sickness response intensity in male Siberian hamsters. If glucose availability is a limiting factor in the display of energetically expensive sickness symptoms, then we expected that animals experiencing 2-DG-induced glucoprivation would show weakened fever or enhanced hypothermia and attenuated anorexia and body mass loss in response to the bacterial mimetic lipopolysaccharide (LPS) as compared to LPS-treated control animals. Similarly, we expected animals receiving insulin as a signal of increased glucose availability would show enhanced LPS-induced fever or weakened hypothermia and enhanced anorexia and body mass loss as compared to LPS-treated control animals. We also measured behaviors that are modulated during sickness in this species, thermoregulatory nest building behavior and hedonic behavior. We predicted that animals in the glucose-deprived group would show lesser sickness-induced declines in nesting behavior in response to LPS than the other two groups, as nesting can provide energy saving benefits; however, we predicted that we would see no differences in the decreases in hedonic behavior among the groups, as this behavior is not largely energetically demanding in this context.

2. Material and methods

2.1. Animals and housing conditions

Adult (> 60 days of age) male Siberian hamsters ($n = 63$) were obtained from our breeding colony at Indiana University. All animals were initially group housed (2-5 per cage 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 x 17.5 x 13.0 cm) for one week prior to the start of the experiment. Animals were housed in long-day photoperiods for the entirety of the study. Food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and water were available *ad libitum* prior to and throughout the experiment. Temperature ($20 \pm 2^{\circ}\text{C}$) and humidity ($50 \pm 10\%$) were maintained at constant levels. All animal methods were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Indiana University.

2.2. Experimental methods

After one week of acclimation to individual housing, hamsters were quasi-randomly sorted (controlling for body mass, age, and genetic relatedness) into three groups: Control, 2-DG, or Insulin. Prior to receiving any experimental injections, daily measurements of body mass (to the nearest 0.1 g) and food consumption (to the nearest 0.1 g) were taken for five days. Food consumption was assessed by weighing the food pellets remaining in the hopper each day. Daily body mass and food consumption measurements continued through the entirety of the experiment.

After the five days of initial body mass and food intake measurements, hamsters started receiving daily injections. Animals in the Control group received one 0.2 ml intraperitoneal (i.p.)

injection of 0.9% sterile saline every other day and one 0.1 ml subcutaneous (s.c.) injection of 0.9% sterile saline every day until the end of the experiment. Animals in the 2-DG group received one 0.2 ml i.p. injection of 1250 mg/kg 2-DG (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.9% sterile saline every other day and one 0.1 ml s.c. injection of 0.9% sterile saline every day until the end of the experiment. This dose was chosen because it is greater than the 2-DG dose that affects least one immune measure (i.e., antibody production) in this species but well below the dose that induces torpor (Dark et al., 1994; Zysling and Demas, 2007). The Insulin group received one 0.2 ml i.p. injection of 0.9% sterile saline every other day and one 0.1 ml s.c. injection of the long-lasting form of insulin, protamine zinc insulin (PZI) (ProZinc, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA), dissolved in 0.9% sterile saline every day until the end of the experiment. The insulin dose was gradually increased over the course of 11 days to 20 U/kg, in order to avoid death-inducing severe hypoglycemia (Garcia et al., 2010). A dose of 20 U/kg was chosen because we wanted to modulate the signal of glucose availability without necessarily modulating actual blood glucose levels (Garcia et al., 2010). The PZI injections were administered according to the following dosing schedule: day 1: 0.625 U/kg; days 2-3: 1.25 U/kg; days 4-6: 5 U/kg; days 7-10: 10 U/kg; days 11-23: 20 U/kg. The 2-DG injections were provided on the odd-numbered experimental days.

On the 19th day of injections, a portion of the animals in each group were injected i.p. ~ 15 minutes before the onset of darkness (~1545 h) with 25 µg LPS (LPS from *Salmonella enterica* serotype typhimurium, Sigma-Aldrich, St. Louis, MO, USA; Carlton and Demas, 2014) suspended in 0.1 ml sterile 0.9% saline. Sickness responses were assessed throughout the four days following injections. The sample sizes were as follows: Control-Saline (n=10); Control-LPS (n=10); 2-DG-Saline (n=9); 2-DG-LPS (n=10); Insulin-Saline (n=11); Insulin-LPS (n=13).

2.3. *Sickness response measurements*

Following LPS injection, animals were monitored for signs of severe sickness, potentially indicating signs of sepsis, as outlined in our approved IACUC protocol. Animals that showed decreases in body temperature greater than 2°C below that of their group mean were removed from the study. These animals exhibited these temperature declines between 4 and 36 h post-injection. Due to a larger than expected number of animals showing these symptoms, we were able, post-hoc, to divide LPS-injected animals into two different groups: those that showed moderate sickness and remained in the study until the end ($< 2^{\circ}\text{C}$ decrease in colonic temperature) and those that showed severe sickness and were removed from the study ($> 2^{\circ}\text{C}$ decrease in colonic temperature).

2.3.1. *Fever, anorexia, and body mass*

On the day of LPS or saline 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 intake measurements continued until the end of the study.

2.3.2. *Hedonic 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 or saline injection, for the first 6 h of the dark phase (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.

2.3.3. Nest building behavior

To assess the effects of our treatments on thermoregulatory behavior, beginning five 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 nestlet was weighed (to the nearest 0.1 g) before presentation, and the unshredded portion 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.

2.4. Blood sampling and necropsies

Blood samples were drawn from each animal 4 h after the onset of darkness (2000 h) at two time points (two days before injection and on the day of injection) to assess circulating blood glucose and serum cortisol concentrations. 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, 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 five days after LPS injection and necropsies were performed. Testes, inguinal white adipose tissue (IWAT), epididymal WAT (EWAT), and retroperitoneal WAT (RWAT) were removed, cleaned of connective tissues, and weighed to the nearest 0.1 mg. A composite adipose tissue score was calculated by summing the individual WAT pad masses.

2.5. Blood glucose measurement

Blood glucose was measured from both blood samples. Immediately upon sampling, ~5 µl of whole blood was transferred onto the test strips of a blood glucose monitoring system (ReliOn, Micro Blood Glucose Monitoring System, Arkray USA, Inc., Minneapolis, MN, USA), and the readout was recorded. The meter was previously calibrated using an internal standard provided by the manufacturer.

2.6. Cortisol enzyme immunoassay (EIA)

We assessed circulating cortisol levels to determine if our treatments influenced the magnitude of LPS-induced hypothalamic-pituitary-adrenal (HPA) axis activation. Glucocorticoids are released during sickness and are important for regulating its intensity (Sapolsky et al., 2000). Cortisol is the predominant glucocorticoid in Siberian hamsters, with concentrations ~100x 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 1:80 with assay buffer and run in duplicate. Intra-assay variabilities were 6.2%, 2.8%, and 3.1%.

2.7. Statistical analyses

All statistical tests were performed using JMP 10 (SAS Institute Inc., Cary, NC, USA). Residuals were checked for normality and homogeneity of variance, and those data that were non-normally distributed were transformed. Two animals were excluded from the final analyses: one from the LPS-treated 2-DG group which obtained an injury unrelated to the experimental procedures and one from the saline-treated Insulin group which exhibited sickness symptoms despite not receiving any LPS. The final sample sizes were as follows: Control-Saline (n=10); Control-LPS (n=10); 2-DG-Saline (n=9); 2-DG-LPS (n=9); Insulin-Saline (n=10); Insulin-LPS (n=13). Non-normally distributed variables differed in their degrees of skewness, so each variable was transformed with the function that best fit the data to normality. Baseline saccharin solution intake and percent change in saccharin solution intake were square root transformed, while cortisol concentrations were log transformed. Baseline percent nesting material shredded could not be transformed to meet the assumptions of normality, so a Kruskal-Wallis test was performed to determine if there were differences among the groups for this measure.

Pre-LPS 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 2-DG or insulin treatment on pre-injection baseline body mass, baseline food intake, baseline saccharin solution intake, and pre-injection blood glucose levels, one-way analyses of variance (ANOVA) on treatment (i.e., control, 2-DG, or insulin) were performed. Because expected values in some cells were less than

five, Fisher's Exact Test was performed to determine if treatment affected the frequencies of animals within each LPS-treated group that had to be removed from the study due to severe sickness. Comparisons between animals that exhibited moderate sickness and continued in the study and those that exhibited severe sickness and were removed from the study were performed with two-sided t-tests. Animals that showed severe sickness and were removed from the study were not included in subsequent between group comparisons of post-injection percent changes in body mass, food intake, saccharin solution intake, and nesting material shredded, post-injection blood glucose concentrations, cortisol concentrations, and tissue masses (Results section 3.3).

Post-LPS changes in body mass, food intake, saccharin solution intake, and percent nesting material shredded were expressed as percentages of each animal's baseline values. Repeated measures ANOVAs were performed on post-injection percent changes in body mass, food intake, saccharin solution intake, and nesting material shredded and colonic temperature, with treatment group and LPS as between-subjects factors and time as the within-subjects factor. Differences in post-injection glucose levels, cortisol concentrations, and tissue masses were assessed with two-way (treatment group (3) x LPS (2)) ANOVAs. Post-hoc comparisons were conducted using Tukey's honestly significant difference (HSD) tests when ANOVAs were significant.

3. Results

3.1. Pre-LPS baseline measures

Before receiving any 2-DG or insulin treatments, the groups did not differ in body mass (Mean = 49.5 g $\pm$ 0.8 SEM; $F_{2,58} = 0.88$, $P = 0.419$) or food intake (Mean = 4.8 g $\pm$ 0.7 SEM; $F_{2,58} = 0.25$, $P = 0.783$). After treatments, all three groups showed decreases in body mass ($T >$

4.54, $P < 0.001$ for all comparisons), and the Insulin group showed increased food intake ($T = 2.66$, $P = 0.014$) while the Control and 2-DG groups showed no change in food intake pre- to post-treatment ($T < 0.97$, $P > 0.347$). After treatments and before LPS or saline injection, baseline body mass ($F_{2,58} = 1.39$, $P = 0.256$), baseline saccharin solution intake ($F_{2,58} = 2.03$, $P = 0.140$), baseline percent nesting material shredded ($H = 4.07$, $P = 0.131$), and pre-injection blood glucose levels ($F_{2,58} = 1.82$, $P = 0.171$) did not differ among the Control, 2-DG and Insulin treatment groups (Table 1). Alternatively, pre-injection baseline food intake was affected by treatment group ($F_{1,58} = 3.19$, $P = 0.049$) (Table 1). Specifically, the Insulin group showed greater daily food intake than the Control group ($P = 0.041$). With the exception of baseline food intake in the 2-DG group (i.e., LPS-injected hamsters exhibited greater baseline food intake values than saline-injected hamsters; $T = 2.49$, $P = 0.025$), none of the baseline measures differed between LPS- and saline-injected hamsters within each group ($P > 0.085$ for all comparisons).

3.2. Animals displaying severe sickness

After LPS-injection, six of the 13 animals in the Insulin group exhibited severe sickness and were removed from the study. No LPS-treated animals from the Control or 2-DG groups were removed from the study. The frequencies of animal removal among the three LPS-treated groups were significantly different (Fishers Exact Test, $P = 0.005$). Within the LPS-injected Insulin group, those animals that exhibited severe sickness showed lower baseline body masses ($T = -5.29$, $P < 0.001$), higher pre-injection blood glucose levels ($T = 2.41$, $P = 0.035$), lower post-injection blood glucose levels ($T = -2.83$, $P = 0.018$), and higher post-injection cortisol concentrations ($T = 2.92$, $P = 0.014$) as compared to animals that exhibited moderate sickness (Table 2). There were no differences between the moderate and severe sickness animals in

baseline food intake, baseline saccharin solution intake, baseline percent nesting material shredded, or colonic temperature immediately prior to injection ($P > 0.063$ for all comparisons).

3.3. Sickness, physiological, and morphological measurements in animals showing moderate sickness responses

3.3.1. Colonic temperature

Post-injection colonic temperature was affected by LPS injection ($F_{1,49} = 16.08$, $P < 0.001$) but not by treatment group ($F_{2,49} = 0.65$, $P = 0.524$) or the LPS x treatment group interaction ($F_{2,49} = 0.24$, $P = 0.789$) (Figure 1). Temperature did change across time (within subjects, $F_{9,41} = 37.52$, $P < 0.001$) and with the time x LPS ($F_{9,41} = 9.14$, $P < 0.001$) and time x treatment group interaction ($F_{18,82} = 2.48$, $P = 0.003$). Specifically, all LPS-injected groups showed varying degrees of hypothermia post-injection. The LPS-injected 2-DG group exhibited temperatures lower than their respective saline-injected controls at the greatest number of timepoints post-injection (6, 10, 16, 20, and 24 h); whereas the LPS-treated Control and LPS-treated Insulin groups only showed lower temperatures than their saline-injected controls at two (6 and 16 h) and one (6 h) timepoints post-injection, respectively.

3.3.2. Anorexia

Percent change in food intake after injection was affected by LPS injection ($F_{1,48} = 98.82$, $P < 0.001$) but not by treatment group ($F_{2,48} = 0.623$, $P = 0.541$) or the LPS x treatment group interaction ($F_{2,48} = 0.022$, $P = 0.978$) (Figure 2A). In addition, percent changes in food intake changed across time (within subjects, $F_{3,46} = 157.23$, $P < 0.001$) and with the time x LPS ($F_{3,46} =$

22.60, $P < 0.001$) and time x treatment group ($F_{3=6,92} = 3.87$, $P = 0.002$) interactions. Specifically, all LPS-injected groups showed greater percent decreases in food intake in comparison to their respective saline-injected controls at days 1, 2, and 3 post-injection ($P < 0.05$ for all comparisons). By day 4 post-injection, all LPS-injected groups no longer showed changes in food intake that differed from their respective saline-injected controls ($P > 0.09$ for all comparisons).

3.3.3. Body mass loss

Percent change in body mass loss after injection was affected by LPS injection ($F_{1,49} = 102.47$, $P < 0.001$), treatment group ($F_{2,49} = 7.95$, $P = 0.001$) and the LPS x treatment group interaction ($F_{2,49} = 3.71$, $P = 0.032$) (Figure 2B). In addition, percent change in body mass loss changed across time (within subjects, $F_{3,47} = 5.58$, $P = 0.002$) and with the time x LPS interaction (within subjects, $F_{3,47} = 22.74$, $P < 0.001$). Specifically, LPS-injected Insulin animals showed attenuated body mass loss as compared to LPS-injected Control and 2-DG animals. Whereas the LPS-injected Control and 2-DG groups showed greater percent decreases in body mass loss in comparison to their respective saline-injected groups at all four timepoints post-injection ($P < 0.001$ for all comparisons), the LPS-injected Insulin group only showed greater percent decreases in body mass loss in comparison to its saline-injected control group at days 2, 3, and 4 post-injection ($P = 0.068$ on day 1, $P < 0.001$ on other days). Furthermore, the LPS-injected Insulin group showed attenuated post-LPS body mass loss compared to the LPS-injected 2-DG group at day 2 post-injection and both the LPS-injected 2-DG and Control groups at day 3 post-injection ($P < 0.005$ for these comparisons).

3.3.4. *Saccharin solution intake*

The measure of hedonic behavior, percent change in saccharin intake after injection, was affected by LPS injection ($F_{1,49} = 12.38$, $P = 0.001$) but not by treatment group ($F_{2,49} = 0.50$, $P = 0.611$) or the LPS x treatment group interaction ($F_{2,49} = 0.48$, $P = 0.624$) (Figure 3A). Furthermore, saccharin solution intake changed with time (within subjects, $F_{3,47} = 14.17$, $P < 0.001$) and with the time x LPS interaction ($F_{3,47} = 4.75$, $P = 0.006$). Specifically, at the 0-6 h timepoint, animals in the LPS-injected 2-DG and Insulin groups showed greater percent decreases in saccharin solution intake as compared to their respective saline-injected controls ($P < 0.044$ for both comparisons), while there was no difference between the LPS-injected Control group and its respective saline-injected control ($P = 0.098$).

3.3.5. *Nesting material shredded*

Percent change in nesting material shredded after injection was affected by LPS injection ($F_{1,49} = 272.72$, $P < 0.001$) but not by treatment group ($F_{2,49} = 0.74$, $P < 0.481$) or the LPS x treatment group interaction ($F_{2,49} = 1.82$, $P < 0.173$) (Figure 3B). Percent nesting material shredded did change over time (within subjects, $F_{3,47} = 60.49$, $P < 0.001$) and with the time x LPS interaction (within subjects, $F_{3,47} = 88.39$, $P < 0.001$). Specifically, animals from all LPS-injected groups showed greater percent decreases in nesting material shredded than their respective saline-injected controls at the 0-6 h, 24-30 h, and 48-54 h timepoints ($P < 0.040$ for all comparisons); nest shredding returned to levels similar to saline-injected controls by the 72-78 h timepoint ($P > 0.998$ for all comparisons). In addition, animals in the LPS-injected 2-DG group showed a greater percent decrease in nesting material shredded in comparison with the LPS-injected Control group at the 48-54 h timepoint ($P = 0.016$).

3.3.6. Blood glucose

Post-injection blood glucose concentrations were affected by LPS injection ($F_{1,49} = 59.50$, $P < 0.001$) and treatment group ($F_{2,49} = 4.37$, $P = 0.018$) but not by the LPS x treatment group interaction ($F_{2,49} = 0.38$, $P = 0.688$) (Figure 4A). Specifically, all LPS-injected groups had lower blood glucose concentrations than their respective saline-injected controls ($P < 0.02$ for all comparisons), and insulin-treated animals, as a whole, had lower post-injection glucose levels as compared to animals not treated with insulin ($T = -2.77$, $P = 0.008$).

3.3.7. Serum cortisol

Post-injection serum cortisol concentrations were affected by LPS injection ($F_{1,49} = 55.33$, $P < 0.001$) but not by treatment group ($F_{2,49} = 0.82$, $P = 0.446$) or the interaction of LPS and treatment group ($F_{2,49} = 0.35$, $P = 0.706$) (Figure 4B). Specifically, all LPS-injected animals showed greater cortisol levels than their respective saline-injected controls ($P < 0.004$ for all comparisons).

3.3.8. Tissue masses

Paired testes mass was affected by treatment group ($F_{2,49} = 4.41$, $P = 0.017$) but not by LPS ($F_{1,49} = 0.42$, $P = 0.521$) or the interaction between LPS and treatment group ($F_{2,49} = 0.50$, $P = 0.608$). Specifically, as a whole, animals treated with 2-DG had lesser paired testes masses as compared to animals not treated 2-DG ($0.45 \text{ g} \pm 0.06 \text{ SE}$ vs. $0.63 \text{ g} \pm 0.03 \text{ SE}$, respectively) ($T = -2.57$, $P = 0.013$). Composite adipose tissue mass was not affected by any of the treatments or interactions ($F_{5,49} = 0.842$, $P = 0.526$).

4. Discussion

The results of this study demonstrate that both direct manipulations of glucose availability, as well as signals of glucose availability, modulate the intensity of some of the energetically expensive symptoms of sickness. Specifically, reductions in glucose availability in the 2-DG group resulted in a greater hypothermic response as compared to the other two groups, potentially as an energy saving mechanism. Alternatively, contrary to our predictions, animals experiencing reduced glucose availability showed a more pronounced sickness-induced decrease in nesting behavior as compared to control animals. Furthermore, among the animals that showed moderate sickness, there were no treatment effects on sickness-induced anorexia and contrary to our predictions, insulin-treated animals showed an attenuation of sickness-induced body mass loss as compared to the other two groups.

In addition to these effects on specific sickness symptoms, we also saw that a portion of the animals that were provided signals of increased glucose availability via insulin treatment displayed severe sickness after LPS injection. Although all insulin-treated animals showed hypoglycemia after LPS injection, the insulin-treated animals that exhibited severe sickness showed lower glucose levels post-injection than animals exhibiting moderate sickness. This relationship between post-LPS glucose levels and severe sickness is consistent with prior work in rats that showed that severe endotoxemia is associated with greater hypoglycemia (Lang et al., 1985). Although it seems that the drop in blood glucose may be predictive of which animals showed severe sickness, it is still not clear why the animals that exhibited severe sickness were also the animals with the lowest body masses in the insulin-treated group. One possible explanation is that these smaller individuals had increased sensitivity to sickness-induced hypoglycemia due to differences in LPS dosing. Even though the insulin doses were corrected

for body mass, all animals received a standard dose of LPS (25 μ g). Therefore, smaller animals received a higher dose of LPS relative to their body masses. As all LPS-injected groups were matched for body mass prior to injection, it is not likely that this severe sickness response was only due to dose differences among smaller and larger animals, as none of the smaller animals from the Control and 2-DG groups showed severe sickness. Therefore, it appears that insulin treatment may have increased the sensitivity of animals to LPS treatment.

Insulin treatment resulted in increased food intake in these animals relative to controls. However, this insulin-induced increase in food intake was largely driven by hamsters in the insulin-treated, moderate sickness group, as the insulin-treated hamsters displaying severe sickness showed baseline food intake values that were comparable to the baseline food intake values of the control and 2-DG-treated groups. This lack of increase in food intake in the insulin-treated, severe sickness animals could have contributed to this enhanced sickness response. Because sickness is such an energetically-costly process, it is important to have glucose readily accessible to utilize for energy during this response. However, insulin does the opposite of providing readily accessible energy, and instead, promotes the storage of glucose of later use (Woods et al., 2006). If an animal shows increased food intake during insulin treatment, the effects of insulin on lowering blood glucose may be somewhat alleviated (e.g., what we would expect in the insulin-treated, moderate sickness group), but if an animal does not increase food intake during insulin treatment, they may initiate their sickness response while in a hypoglycemic state (e.g., what we would expect in the insulin-treated, severe sickness group). Yet, our results do not suggest that initiating the sickness response in a hypoglycemic state is the cause of this severe sickness, as the severe sickness group actually showed greater pre-LPS blood glucose levels in comparison to the moderate sickness group. Thus, it appears that the

animals that experienced severe sickness actually had more readily accessible glucose as compared to those in the moderate sickness group.

While blood glucose levels prior to LPS injection do not seem a likely mechanism facilitating the differences in sickness severity between the two insulin-treated groups, it is possible that blood glucose levels after injection may be critical mediators of this variation in sickness severity. Interleukin (IL)-1, a pro-inflammatory cytokine released in response to LPS and infection, affects glucose homeostasis during infection and induces hypoglycemia, as it modulates glucose regulation via the brain and stimulates increases in glucose utilization by peripheral tissues (del Rey and Besedovsky, 1989; del Rey et al., 2006; Vogel et al., 1991). While animals in the current study exhibited hypoglycemic responses to LPS treatment, it is also common for human and non-human animals to exhibit hyperglycemic responses to infection (McGuinness, 2005). In cases of sickness-induced hyperglycemia, insulin treatment improves survival in the critically ill, and this is likely mediated through a normalization of glucose levels via decreases in pro- and increases in anti-inflammatory cytokine release (Jeschke et al., 2004; van den Berghe et al., 2001). In the current study, all LPS-treated animals showed hypoglycemia, so administering insulin during experimental infection likely was the cause of the potentiated hypoglycemic response in the insulin-treated animals as compared to the other two groups. Thus, the potential enhanced hypoglycemic effects of the greater LPS dose in these smaller animals coupled with the hypoglycemic effects of insulin treatment may explain why the smaller animals in the insulin-treated group showed the most severe sickness responses of all groups.

Whereas insulin treatment facilitated an enhanced sickness response in the smaller individuals in that group, the larger individuals that exhibited moderate sickness and continued in the study actually showed attenuation of an energetically expensive sickness symptom. The

insulin-treated animals that remained in the study showed lesser percent decreases in body mass after LPS injection as compared to the Control and 2-DG groups. This attenuation of body mass loss in the insulin-treated group is not a product of differences in sickness-induced anorexia, as all three LPS-treated groups showed similar patterns and intensities of anorexia post-LPS. Furthermore, this attenuation is surprising because the animals that displayed moderate sickness in the LPS-treated Insulin group had an average body mass that was 6-8 grams greater than animals in the LPS-treated Control and 2-DG groups. In Siberian hamsters and white-crowned sparrows (*Zonotrichia leucophrys*), pre-sickness body mass is negatively correlated with percent decrease in body mass loss after LPS-injection, such that larger individuals show greater percent decreases in body mass after injection, even when all animals are given the same quantity of LPS (Carlton and Demas, 2015; Owen-Ashley et al., 2008; Owen-Ashley et al., 2006). Based upon these previous results, it was expected that the LPS-injected Insulin group would show even greater percent decreases in body mass loss than we initially predicted (i.e., when we expected that all LPS-treated groups would share similar average body masses). Although we did not see insulin attenuate sickness responses in the smaller individuals in the group, these heavier animals that continued until the end of the study could have experienced some of the anti-inflammatory properties of insulin (Jeschke et al., 2004). The LPS-injected Insulin group showed attenuated body mass loss compared to the other groups at days 2 and 3 post-LPS. At this point in the study, if animals were no longer hypoglycemic, insulin-treatment could have induced anti-inflammatory mechanisms rather than exacerbating hypoglycemia as it did in the few hours after LPS injection.

Animals experiencing glucose deprivation showed the most pronounced hypothermic responses of the three LPS-treated groups. Hypothermic responses to LPS treatment or infection,

rather than febrile responses, are hypothesized to be advantageous for pathogen clearance when energy availability is low or in cases where animals are experiencing more severe inflammatory responses (Deen and Hutchison, 2001; Romanovsky and Szekely, 1998). In the context of this study, the hypothermic response exhibited by all LPS-treated groups is more likely a response to greater inflammation rather than energy availability, as animals in the Control and Insulin groups were experiencing no sources of energy limitation. Alternatively, the 2-DG group was experiencing energy limitation, and the increased hypothermic response shown by this group could have been a mechanism of energy conservation. In a previous study, we showed that hamsters that were experimentally provided with a hormonal signal of increased energy stores (i.e., leptin) showed lesser LPS-induced hypothermic responses as compared to animals receiving a vehicle control (Carlton and Demas, 2014). The results of the current study further support the conclusion that the magnitude of LPS-induced hypothermia can be modulated by energetic state and signals of energetic state, as hypothermia was enhanced when animals were experimentally deprived of glucose.

While 2-DG disrupts glycolysis and induces glucoprivation, it is often used in experimental contexts as a “metabolic stressor,” as its effects on glucose use are often accompanied by activation of the HPA axis and increased circulating glucocorticoid levels (Demas et al., 1997; Weidenfeld et al., 1994). In the current study, there were no effects of 2-DG on cortisol levels in both the saline- and LPS-injected animals. This lack of increased cortisol in response to 2-DG has been observed in two other studies in this species, so it was not necessarily unexpected (Carlton et al., 2014; Zysling and Demas, 2007). Glucocorticoids are critical for the regulation of inflammatory responses and sickness symptoms (Sapolsky et al., 2000). Specifically, lack of glucocorticoid regulation of the inflammatory response during infection can

augment the amplitude and duration of fever, enhance behavioral depression, and in some cases, increase susceptibility to death; however, glucocorticoid replacement can ameliorate these effects (Coelho et al., 1992; Goujon et al., 1995a; Morrow et al., 1993; Ruzek et al., 1999). Glucocorticoids are also involved in the regulation of sickness-induced hypothermia (Goujon et al., 1995b); however, since there were no treatment effects on either saline- or LPS-injected cortisol levels, this suggests that the increased hypothermic response in the LPS-injected 2-DG group was mediated by changes in energy availability rather than glucocorticoids produced as a product of metabolic stress.

One reason why we may not have seen attenuation of sickness-induced anorexia, body mass loss, or percent decreases in nesting material shredded in the LPS-injected 2-DG group was that animals in this group were shifting energy away from other physiological systems in order to allocate energy to the immune system. 2-DG-treated animals had lower paired testes masses than the animals in the Control and Insulin groups, which suggests that animals in this group were shifting energy away from reproductive maintenance. Previous work has found that when experiencing glucose deprivation while mounting a delayed-type hypersensitivity (DTH) immune response, male Siberian hamsters show reduced testes mass but no reductions in the magnitude of their DTH response as compared to animals not experiencing glucose deprivation (Martin et al., 2008). In females, the reverse is found, as glucose-deprived animals show no reductions in reproductive tissue masses but show decreases in DTH response as compared to controls (Martin et al., 2008). Thus, in the current study, if energy had not been shifted away from reproductive maintenance in 2-DG-treated animals, then we may have seen attenuation of these additional energetically-expensive sickness symptoms in this group.

In conclusion, our data show that sickness symptoms in Siberian hamsters can be modulated by glucose deprivation and by signals of increased glucose availability. Insulin treatment appears to have both enhancing and suppressive effects on sickness in this species, as it facilitated severe sickness responses in smaller individuals but attenuated sickness-induced body mass loss in the larger individuals that displayed moderate sickness. Glucose deprivation resulted in increased sickness-induced hypothermia, but its lack of effect on the other energetically expensive sickness symptoms suggests that energy may have been reallocated from another physiological system (i.e., the reproductive system) to maintain the full expression of sickness symptoms in this group. Collectively, these results suggest that glucose availability can modulate sickness symptoms but that its effects depend on factors such as infection severity and energetic trade-offs among multiple physiological systems.

Acknowledgements

We thank Chryssa Athans, Nikki Rendon, Allison Bailey, and Kristyn Sylvia for help with experimental procedures. Thanks to the Indiana University animal care staff for excellent animal care. This work was supported by a National Science Foundation (NSF) Graduate Research Fellowship, a National Institutes of Health T32 training grant (HD049336), and a NSF Doctoral Dissertation Improvement Grant (IOS-1310749) to EDC and NSF IOS-0919911 to GED.

References

- Adelman, J.S., Martin, L.B., 2009. Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integr Comp Biol* 49, 202-214.
- Ashley, N.T., Wingfield, J.C., 2012. Sickness Behavior in Vertebrates: Allostasis, Life-History Modulation, and Hormonal Regulation, in: Demas, G.E., Nelson, R.J. (Eds.), *Ecoimmunology*. Oxford University Press, New York, pp. 45-91.
- Aubert, A., 1999. Sickness and behaviour in animals: a motivational perspective. *Neuroscience and biobehavioral reviews* 23, 1029-1036.
- Baillie, S.R., Prendergast, B.J., 2008. Photoperiodic regulation of behavioral responses to bacterial and viral mimetics: a test of the winter immunoenhancement hypothesis. *Journal of biological rhythms* 23, 81-90.
- Benoit, S.C., Clegg, D.J., Seeley, R.J., Woods, S.C., 2004. Insulin and leptin as adiposity signals. *Recent Prog Horm Res* 59, 267-285.
- Bilbo, S.D., Drazen, D.L., Quan, N., He, L., Nelson, R.J., 2002. Short day lengths attenuate the symptoms of infection in Siberian hamsters. *Proceedings. Biological sciences / The Royal Society* 269, 447-454.
- Bilbo, S.D., Nelson, R.J., 2002. Melatonin regulates energy balance and attenuates fever in Siberian hamsters. *Endocrinology* 143, 2527-2533.
- Carlton, E.D., Cooper, C.L., Demas, G.E., 2014. Metabolic stressors and signals differentially affect energy allocation between reproduction and immune function. *Gen Comp Endocr* 208, 21-29.
- Carlton, E.D., Demas, G.E., 2014. Leptin mediates seasonal variation in some but not all symptoms of sickness in Siberian hamsters. *Horm Behav* 66, 802-811.
- Carlton, E.D., Demas, G.E., 2015. Body mass affects seasonal variation in sickness intensity in a seasonally breeding rodent. *J Exp Biol* 218, 1667-1676.
- Chou, S.H., Kojic, L.D., Messingham, K.N., Cunnick, J.E., 1996. Characterization of the effect of 2-deoxy-D-glucose(2-DG) on the immune system. *Brain, behavior, and immunity* 10, 399-416.
- Coelho, M.M., Souza, G.E.P., Pela, I.R., 1992. Endotoxin-Induced Fever Is Modulated by Endogenous Glucocorticoids in Rats. *American Journal of Physiology* 263, R423-R427.
- Dark, J., Miller, D.R., Zucker, I., 1994. Reduced glucose availability induces torpor in Siberian hamsters. *The American journal of physiology* 267, R496-501.
- Deen, C.M., Hutchison, V.H., 2001. Effects of lipopolysaccharide and acclimation temperature on induced behavioral fever in juvenile Iguana iguana. *J Therm Biol* 26, 55-63.

631 del Rey, A., Besedovsky, H., 1989. Antidiabetic effects of interleukin 1. *Proc Natl Acad Sci U S*
632 *A* 86, 5943-5947.

633 del Rey, A., Roggero, E., Randolph, A., Mahuad, C., McCann, S., Rettori, V., Besedovsky, H.O.,
634 2006. IL-1 resets glucose homeostasis at central levels. *Proc Natl Acad Sci U S A* 103, 16039-
635 16044.

636 Delmastro-Greenwood, M.M., Piganelli, J.D., 2013. Changing the energy of an immune
637 response. *American journal of clinical and experimental immunology* 2, 30-54.

638 Demas, G.E., DeVries, A.C., Nelson, R.J., 1997. Effects of photoperiod and 2-deoxy-D-glucose-
639 induced metabolic stress on immune function in female deer mice. *The American journal of*
640 *physiology* 272, R1762-1767.

641 Demas, G.E., Johnson, C., Polacek, K.M., 2004. Social interactions differentially affect
642 reproductive and immune responses of Siberian hamsters. *Physiology & behavior* 83, 73-79.

643 Garcia, N.W., Greives, T.J., Zysling, D.A., French, S.S., Chester, E.M., Demas, G.E., 2010.
644 Exogenous insulin enhances humoral immune responses in short-day, but not long-day,
645 Siberian hamsters (*Phodopus sungorus*). *Proceedings. Biological sciences / The Royal Society*
646 277, 2211-2218.

647 Goujon, E., Parnet, P., Aubert, A., Goodall, G., Dantzer, R., 1995a. Corticosterone regulates
648 behavioral effects of lipopolysaccharide and interleukin-1 beta in mice. *The American journal of*
649 *physiology* 269, R154-159.

650 Goujon, E., Parnet, P., Cremona, S., Dantzer, R., 1995b. Endogenous glucocorticoids down
651 regulate central effects of interleukin-1 beta on body temperature and behaviour in mice. *Brain*
652 *research* 702, 173-180.

653 Hart, B.L., 1988. Biological basis of the behavior of sick animals. *Neuroscience and*
654 *biobehavioral reviews* 12, 123-137.

655 Helderman, J.H., 1981. Role of insulin in the intermediary metabolism of the activated thymic-
656 derived lymphocyte. *The Journal of clinical investigation* 67, 1636-1642.

657 Horton, R.W., Meldrum, B.S., Bachelard, H.S., 1973. Enzymic and cerebral metabolic effects of
658 2-deoxy-D-glucose. *Journal of neurochemistry* 21, 507-520.

659 Jeschke, M.G., Klein, D., Bolder, U., Einspanier, R., 2004. Insulin attenuates the systemic
660 inflammatory response in endotoxemic rats. *Endocrinology* 145, 4084-4093.

661 Kluger, M.J., 1986. Is Fever Beneficial. *Yale J Biol Med* 59, 89-95.

662 Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998. Towards a functional explanation for the
663 occurrence of anorexia during parasitic infections. *Anim Behav* 56, 265-274.

664 Lang, C.H., Bagby, G.J., Spitzer, J.J., 1985. Glucose kinetics and body temperature after lethal
665 and nonlethal doses of endotoxin. *The American journal of physiology* 248, R471-478.

666 MacDonald, L., Hazi, A., Paolini, A.G., Kent, S., 2014. Calorie restriction dose-dependently
667 abates lipopolysaccharide-induced fever, sickness behavior, and circulating interleukin-6 while
668 increasing corticosterone. *Brain, behavior, and immunity* 40, 18-26.

669 MacDonald, L., Radler, M., Paolini, A.G., Kent, S., 2011. Calorie restriction attenuates LPS-
670 induced sickness behavior and shifts hypothalamic signaling pathways to an anti-inflammatory
671 bias. *American journal of physiology. Regulatory, integrative and comparative physiology* 301,
672 R172-184.

673 Martin, L.B., Weil, Z.M., Bowers, S.L., Nelson, R.J., 2008. Sex-specific effects of glucose
674 deprivation on cell-mediated immunity and reproduction in Siberian hamsters (*Phodopus*
675 *sungorus*). *Journal of comparative physiology. B, Biochemical, systemic, and environmental*
676 *physiology* 178, 623-628.

677 McGuinness, O.P., 2005. Defective glucose homeostasis during infection. *Annual review of*
678 *nutrition* 25, 9-35.

679 Moret, Y., Schmid-Hempel, P., 2000. Survival for immunity: the price of immune system
680 activation for bumblebee workers. *Science* 290, 1166-1168.

681 Morrow, L.E., McClellan, J.L., Conn, C.A., Kluger, M.J., 1993. Glucocorticoids alter fever and
682 IL-6 responses to psychological stress and to lipopolysaccharide. *The American journal of*
683 *physiology* 264, R1010-1016.

684 Owen-Ashley, N.T., Hasselquist, D., Raberg, L., Wingfield, J.C., 2008. Latitudinal variation of
685 immune defense and sickness behavior in the white-crowned sparrow (*Zonotrichia leucophrys*).
686 *Brain Behavior and Immunity* 22, 614-625.

687 Owen-Ashley, N.T., Turner, M., Hahn, T.P., Wingfield, J.C., 2006. Hormonal, behavioral, and
688 thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living white-
689 crowned sparrows (*Zonotrichia leucophrys gambelii*). *Horm Behav* 49, 15-29.

690 Pohl, J., Sheppard, M., Luheshi, G.N., Woodside, B., 2014. Diet-induced weight gain produces a
691 graded increase in behavioral responses to an acute immune challenge. *Brain, behavior, and*
692 *immunity* 35, 43-50.

693 Puchalski, W., Bulova, S.J., Lynch, C.B., Lynch, G.R., 1988. Photoperiod, temperature and
694 melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiology & behavior*
695 42, 173-182.

696 Reburn, C.J., Wynne-Edwards, K.E., 2000. Cortisol and prolactin concentrations during repeated
697 blood sample collection from freely moving, mouse-sized mammals (*Phodopus spp.*).
698 *Comparative medicine* 50, 184-198.

699 Romanovsky, A.A., Szekely, M., 1998. Fever and hypothermia: two adaptive thermoregulatory
700 responses to systemic inflammation. *Med Hypotheses* 50, 219-226.

701 Ruzek, M.C., Pearce, B.D., Miller, A.H., Biron, C.A., 1999. Endogenous glucocorticoids protect
702 against cytokine-mediated lethality during viral infection. *Journal of immunology* 162, 3527-
703 3533.

704 Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress
705 responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*
706 21, 55-89.

707 Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-
708 offs in evolutionary ecology. *Trends in ecology & evolution* 11, 317-321.

709 van den Berghe, G., Wouters, P., Weekers, F., Verwaest, C., Bruyninckx, F., Schetz, M.,
710 Vlasselaers, D., Ferdinande, P., Lauwers, P., Bouillon, R., 2001. Intensive insulin therapy in
711 critically ill patients. *The New England journal of medicine* 345, 1359-1367.

712 Vogel, S.N., Henricson, B.E., Neta, R., 1991. Roles of interleukin-1 and tumor necrosis factor in
713 lipopolysaccharide-induced hypoglycemia. *Infection and immunity* 59, 2494-2498.

714 Weidenfeld, J., Corcos, A.P., Wohlman, A., Feldman, S., 1994. Characterization of the 2-
715 Deoxyglucose Effect on the Adrenocortical Axis. *Endocrinology* 134, 1924-1931.

716 Wen, J.C., Dhabhar, F.S., Prendergast, B.J., 2007. Pineal-dependent and -independent effects of
717 photoperiod on immune function in Siberian hamsters (*Phodopus sungorus*). *Horm Behav* 51,
718 31-39.

719 Wolowczuk, I., Verwaerde, C., Viltart, O., Delanoye, A., Delacre, M., Pot, B., Grangette, C.,
720 2008. Feeding our immune system: Impact on metabolism. *Clin Dev Immunol*, 1-19.

721 Woods, S.C., Lutz, T.A., Geary, N., Langhans, W., 2006. Pancreatic signals controlling food
722 intake; insulin, glucagon and amylin. *Philosophical transactions of the Royal Society of London.*
723 *Series B, Biological sciences* 361, 1219-1235.

724 Zysling, D.A., Demas, G.E., 2007. Metabolic stress suppresses humoral immune function in
725 long-day, but not short-day, Siberian hamsters (*Phodopus sungorus*). *Journal of Comparative*
726 *Physiology B-Biochemical Systemic and Environmental Physiology* 177, 339-347.

727
728
729
730
731
732
733
734
735
736

Figure Captions

Figure 1. Mean ($\pm$ SEM) colonic temperature following LPS (black icons) or saline (white icons) injection delivered at the 0 h timepoint in control- (●), 2-DG- (▲), or insulin- (■) treated male Siberian hamsters. Black and white bars at the top of the graph indicates the active, dark (black) and inactive, light (white) phases of the light-dark cycle. * $P < 0.05$ versus saline-injected group given the same treatment.

Figure 2. Mean ($\pm$ SEM) percent changes in (A) food intake and (B) body mass from baseline following LPS (black icons) or saline (white icons) injection delivered on day 0 in control- (●), 2-DG- (▲), or insulin- (■) treated male Siberian hamsters. 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. Within each panel: * $P < 0.05$ versus saline-injected group given the same treatment. # $P < 0.05$ versus LPS-injected Insulin group. ^ $P < 0.05$ versus other saline-injected groups.

Figure 3. Mean ($\pm$ SEM) percent changes in (A) saccharin solution intake and (B) percent nesting material shredded from baseline following LPS (black icons) or saline (white icons) injection delivered at the 0 h timepoint in control- (●), 2-DG- (▲), or insulin- (■) treated male Siberian hamsters. * $P < 0.05$ versus saline-injected group given the same treatment. # $P < 0.05$ versus LPS-injected Control group.

Figure 4. Mean ($\pm$ SEM) (A) blood glucose and (B) circulating serum cortisol concentrations taken 4 h following LPS or saline injection in control-, 2-DG-, or insulin-treated male Siberian hamsters. Within each panel, groups with different letters indicate statistically significant differences between group means ($P < 0.05$); groups sharing the same letter are statistically equivalent.

Table 1

Table 1. Mean ($\pm$ SEM) baseline body mass, food intake, saccharin solution intake, percent nesting material shredded, and blood glucose levels prior to LPS or saline injection in control, 2-DG, and insulin-treated male Siberian hamsters. Baseline measurements were calculated by averaging the values collected for the measurements during the three 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 (or no letters) are statistically equivalent.

	Control	2-DG	Insulin
Baseline Body Mass (g)	43.9 $\pm$ 1.4	45.7 $\pm$ 1.2	47.0 $\pm$ 1.3
Baseline Food Intake (g/day)	4.7 $\pm$ 0.1 ^a	4.9 $\pm$ 0.2 ^{a,b}	5.2 $\pm$ 0.1 ^b
Baseline Saccharin Solution Intake (g / 6 h)	1.6 $\pm$ 0.2	1.8 $\pm$ 0.2	2.2 $\pm$ 0.2
Baseline Percent Nesting Material Shredded	93.7 $\pm$ 2.7	91.6 $\pm$ 4.7	83.6 $\pm$ 4.3
Pre-injection blood glucose concentration (mg/dl)	107 $\pm$ 4	98 $\pm$ 5	96 $\pm$ 4

Table 2. Mean ($\pm$ SEM) baseline body mass, food intake, saccharin solution intake, percent nesting material shredded and colonic temperature and blood glucose levels prior to LPS injection and blood glucose and serum cortisol levels 4 h after LPS injection in insulin-treated male Siberian hamsters that exhibited moderate or severe sickness symptoms. Hamsters were classified as exhibiting moderate sickness and kept in the study if they showed a decrease in colonic temperature $< 2^{\circ}\text{C}$ from the group mean after LPS injection and as severe sickness and removed from the study if they showed a decrease in colonic temperature $> 2^{\circ}\text{C}$ from the group mean after LPS injection. $*P < 0.05$ and $**P < 0.001$ for differences between the two groups.

Measure	Moderate	Severe
Baseline Body Mass (g)	52.5 ± 1.2	$40.1 \pm 2.0^{**}$
Baseline Food Intake (g/day)	5.6 ± 0.1	4.8 ± 0.3
Baseline Saccharin Solution Intake (g/6h)	2.9 ± 0.5	2.1 ± 0.5
Baseline Percent Nesting Material Shredded	88.1 ± 5.8	70.7 ± 12.3
Pre-injection Colonic Temperature ($^{\circ}\text{C}$)	37.1 ± 0.2	36.7 ± 0.2
Pre-injection Blood Glucose (mg/dl)	85 ± 8	$112 \pm 8.0^{*}$
Post-injection Blood Glucose (mg/dl)	51 ± 5	$31 \pm 5^{*}$
Post-injection Cortisol (ng/ml)	134.6 ± 12.9	$189.3 \pm 13.6^{*}$

Figure 1

Figure 2

Figure 3

Figure 4