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Glucose and insulin modulate sickness responses in male Siberian hamsters

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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. 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.

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, the 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

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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, 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, 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, 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 × 17.5 × 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 ± 2 °C) and humidity (50 ± 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

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