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Metabolic stressors and signals differentially affect energy allocation between reproduction and immune function

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ABSTRACT

Most free-living animals have finite energy stores that they must allocate to different physiological and behavioral processes. In times of energetic stress, trade-offs in energy allocation among these processes may occur. The manifestation of trade-offs may depend on the source (e.g., glucose, lipids) and severity of energy limitation. In this study, we investigated energetic trade-offs between the reproductive and immune systems by experimentally limiting energy availability to female Siberian hamsters (*Phodopus sungorus*) with 2-deoxy-D-glucose, a compound that disrupts cellular utilization of glucose. We observed how glucoprivation at two levels of severity affected allocation to reproduction and immunity. Additionally, we treated a subset of these hamsters with leptin, an adipose hormone that provides a direct signal of available fat stores, in order to determine how increasing this signal of fat stores influences glucoprivation-induced trade-offs. We observed trade-offs between the reproductive and immune systems and that these trade-offs depended on the severity of energy limitation and exogenous leptin signaling. The majority of the animals experiencing mild glucoprivation entered anestrus, whereas leptin treatment restored estrous cycling in these animals. Surprisingly, virtually all animals experiencing more severe glucoprivation maintained normal estrous cycling throughout the experiment; however, exogenous leptin resulted in lower antibody production in this group. These data suggest that variation in these trade-offs may be mediated by shifts between glucose and fatty acid utilization. Collectively, the results of the present study highlight the context-dependent nature of these trade-offs, as trade-offs induced by the same metabolic stressor can manifest differently depending on its intensity.

1. Introduction

Animals are faced with the challenge of obtaining energetic resources. The energy available to most animals under natural conditions is finite and will depend on the quality and abundance of these energetic resources in the environment. Once an animal obtains energy, it is then faced with the challenge of balancing energy allocation among different physiological, biochemical, and behavioral processes (Ricklefs and Wikelski, 2002; Sheldon and Verhulst, 1996). The balancing of allocation toward diverse biological processes often results in energetic trade-offs among physiological systems. One commonly observed energetic trade-off occurs between the reproductive and immune systems (Demas et al., 2012; Fedorka, 2014), where increased investment into one system results in decreased investment to the other. Maintaining reproduction and activating and maintaining the immune system requires a substantial allocation of energy (Demas, 2004; Speakman, 2008). Therefore, the expression of constraints on these systems and trade-offs between them is often dependent on the animal's access to energetic resources in its environment. This dependence on the environment may explain why in some cases a reproduction-immune trade-off may be observed with relatively limited resources in the wild but is not observed in the laboratory where food is available ad lib (French and Moore, 2008; French et al., 2009b).

One possibility why the display of energetic trade-offs is often context-dependent is that the display of energetic limitations and trade-offs varies with the type of energy that is being used to fuel the physiological and behavioral processes. Energetic trade-offs may be manifested in different ways depending on an animal's access to both current food availability in the environment and stored energy availability in the form of body fat. For instance, food deprivation inhibits ovulation and estrous behavior in lean, but not fat, female Syrian hamsters (*Mesocricetus auratus*)

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(Schneider and Wade, 1989), suggesting that having large fat stores may be sufficient to overcome energetic deficits imposed by limited current energetic resources. Food restriction does not suppress antibody production in female Siberian hamsters (*Phodopus sungorus*) (Zysling et al., 2009); however, surgically removing body fat stores (i.e., lipectomy) from male prairie voles (*Microtus ochrogaster*) and male and female Siberian hamsters results in decreased antibody production (Demas et al., 2003; Demas and Sakaria, 2005). Collectively, these results provide support that different types of metabolic fuels (e.g., glucose from immediate food ingestion, free fatty acids from adipose tissue) may influence the expression of energetic trade-offs within and between these two systems.

One way to manipulate glucose availability is with treatment with 2-deoxy-D-glucose (2-DG). 2-DG is a non-metabolizable glucose analog, which causes a transient disruption of glycolysis by inhibiting glucose oxidation, resulting in a state of glucoprivation (Horton et al., 1973). Treating ad lib-fed female Syrian hamsters with high doses of 2-DG (1750 or 2000 mg/kg) induces anestrus, whereas a lower dose of 2-DG (750 mg/kg) induces anestrus only in hamsters that are food restricted (Schneider et al., 1993). Previous work in our lab has shown that treatment with 2-DG (750 mg/kg) results in reduced antibody production and reduced reproductive tissue mass in female Siberian hamsters (Zysling and Demas, 2007) and reduced splenocyte production in female deer mice (*Peromyscus maniculatus*) (Demas et al., 1997b). Thus, reducing glucose availability via 2-DG treatment suppresses energetic allocation to both reproduction and immunity.

While it is clear from these studies that reducing glucose utilization can suppress reproductive and immune responses, the context-dependent nature of some of these results (e.g., lower doses of 2-DG suppress reproduction in only food restricted animals, not ad lib-fed animals) illustrates that glucose is not the only fuel source that animals can utilize to power reproduction and immunity. For instance, anestrus can be induced in ad lib-fed hamsters treated with a lower dose of 2-DG (750 mg/kg) if fatty acid utilization is simultaneously blocked (via methyl palmoxirate treatment), suggesting that changes in reproductive status may be driven by availability of all metabolic fuels, rather than just individual metabolic fuel types (Schneider and Wade, 1989). White adipose tissue (WAT), a primary energy source for organisms, provides storage for lipids that can be liberated to free fatty acids and glycerol when triggered by glucagon. WAT is not only a source of fuel, but it is also an endocrine organ that synthesizes and releases hormones (Ahima and Flier, 2000; Cinti, 2007). The peptide hormone leptin is one such adipose hormone, and circulating levels of leptin are directly proportional to the mass of adipose tissue in mammals (Maffei et al., 1995). Thus, high levels of leptin indicate adequate energy stores, whereas low circulating levels of leptin are consistent with an energy deficit. Furthermore, leptin not only influences lipid metabolism but it also increases glucose metabolism, glucose uptake, glucose turnover, and glucose oxidation (Kamohara et al., 1997).

While leptin was first characterized for its role in food intake and adiposity, there is now ample evidence that leptin plays a role in mediating both reproduction (Caprio et al., 2001; Schneider et al., 2012) and immunity (Carlton et al., 2012; La Cava and Matarese, 2004; Lord, 2002). Treatment with leptin restores estrous cycling in fasted female Syrian hamsters, however, when hamsters are fasted and treated with 2-DG, leptin does not restore estrous cycling. As 2-DG inhibits glucose oxidation, these results suggest that leptin influences energy allocation to reproduction via effects on metabolic fuel oxidation rather than through signaling of available adipose stores (Schneider et al., 1998). Additionally, leptin treatment counteracts the fasting-induced suppression of cell-mediated immunity in mice (Lord et al., 1998), and it attenuates the suppressive effects of surgical lipectomy on antibody production in male Siberian hamsters (Demas and Sakaria, 2005). Male Siberian hamsters treated with 2-DG show reductions in antibody production, and providing exogenous leptin alleviates this suppression of humoral immunity (Drazen, 2001) suggesting that the effects of leptin on humoral immunity are at least in part mediated through changes in signals of fat availability (Drazen, 2001; Drazen et al., 2001). Therefore, leptin may act differently in how it regulates energy allocation to reproduction versus immunity when glucose utilization is impaired.

The goal of the present study was to determine how leptin, as a neuroendocrine signal, affects energy allocation between the reproductive and immune systems in female Siberian hamsters experiencing glucoprivation. Specifically, we experimentally reduced glucose utilization with either a low (750 mg/kg) or high dose (1750 mg/kg) of 2-DG, and then supplemented animals with exogenous leptin. We assessed reproductive (i.e., estrous cycling, reproductive tissue mass) and immune (i.e., serum bacterial killing, antibody production) indices in response to our treatments. We predicted that mild glucoprivation (low 2-DG dose) would reduce reproductive tissue mass and antibody production and that leptin treatment should restore antibody production and may restore reproductive tissue mass. Additionally, we predicted that at more severe glucoprivation (high 2-DG dose), animals would be more energy limited and would show halted estrous cycling in addition to reduced reproductive tissue mass, antibody production, and bacterial killing ability. Because the high dose of 2-DG provides significant energy limitation, we expected that leptin supplementation would only provide a sufficiently large energetic signal to restore one system (e.g., the immune system because it is most important for survival) and this restoration might occur at a potential additional energetic cost to the reproductive system. Finally, in order to assess potential causation behind trade-offs, we assessed serum triglyceride and cortisol concentrations to determine the physiological mechanisms that may mediate energy allocation.

2. Materials and methods

2.1. Animals and housing

Adult female (>60 days of age) Siberian hamsters ($n = 58$) were obtained from our breeding colony at Indiana University. The progenitors of these animals were generously provided by Dr. Randy Nelson (Ohio State University) and Dr. Timothy Bartness (Georgia State University). In order to minimize the effects of inbreeding, our animals are outbred approximately every 10 generations. All animals were initially group housed (2–5 with same sex siblings on weaning at 17–18 days of age) in long-day photoperiods (light:dark, 16:8). Temperature (20 ± 2 °C) and humidity (50 ± 10%) were maintained at constant levels. For the experiment, animals were all housed in the same room where they were maintained on long days (16:8) and individually housed in polypropylene cages (27.8 × 17.5 × 13.0 cm). Food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and tap water were available ad lib during the entire course of the experiment. Animals used in this experiment came from 18 different litters across 8 different breeding pairs. All animal methods were reviewed and approved by the Institutional Animal Care and Use Committee at Indiana University Bloomington (protocol No. 10-038).

2.2. Experimental methods

For 8 days prior to experimental treatments, vaginal cell samples were obtained by vaginal lavage (with 0.9% sterile saline) from each animal between 0930 h and 1130 h EST to determine estrous cycle stage (Scotti et al., 2007). For 5 days prior to experimental