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ORIGINAL PAPER

Sex-specific effects of glucose deprivation on cell-mediated immunity and reproduction in Siberian hamsters (*Phodopus sungorus*)

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Abstract In most species, sexes differ in levels of parasitism. These differences have traditionally been believed to be static, but a capacity for adjusting anti-parasite investments would allow sexes to allocate resources adaptively contingent on environmental conditions. During stressful periods, such as a food shortage, allocation decisions would be mandated in males and females, but the biasing of resources may differ depending on the value of various physiological alternatives to the fitness of each sex. To determine whether sexes sacrifice immune or reproductive capacity when stressed, male and female Siberian hamsters (*Phodopus sungorus*) were pharmacologically deprived of glucose. Glucose deprivation was expected to compromise immune activity (delayed-type hypersensitivity) more than reproductive capacity in males because male fitness is limited by reproductive opportunities. The opposite was predicted for females because of the greater value of surviving to breed in favorable conditions. Contrary to expectations, glucoprivation compromised immune activity in female, but not male, hamsters. Conversely, glucoprivation reduced male, but not female, reproductive organ masses. These results may reflect the adjustments made by wild hamsters during food shortages, or they may be influenced by the study design; neither sex was permitted to incur other behavioral and physiological costs, such as lactation and parental care. Regardless, our results indicate that sex differences in parasitism are likely to be plastic in many circumstances, but further work in free-living animals is critical to ascertain whether results of the present study are naturally representative.

Keywords 2-deoxyglucose · Cell-mediated · Delayed-type hypersensitivity · Immune · Rodent

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Introduction

Sex differences in parasitism and immunity are well-known (Rolff 2002; Zuk 1990), but whether these differences are fixed or labile remains understudied. In most species, female fitness is limited by egg production and parental care whereas male fitness is limited by mate attraction (Bateman 1948). The relative benefits to fitness of investments in immunity versus reproduction are contingent on the life history and reproductive strategy of particular species (Forbes 2007; Stoehr and Kokko 2006). Thus, sex differences in immunity and parasitism may not be fixed. The distinct priorities of each sex should lead to sex-specific promotion of different physiological processes depending on the context. When one physiological process is ongoing and resources are limited, individuals may be forced to sacrifice another physiological process. Which trait is sacrificed, however, is likely to be sex and environment specific. The costs and benefits of reproduction (Bronson 1985) and immunity (Martin et al. 2007) would often preclude sexes from investing in multiple functions simultaneously.

We tested the hypothesis that allocation decisions between immunity and reproduction are plastic by pharmacologically depriving virgin male and female Siberian hamsters of glucose, then examining the effects of glucose deprivation (i.e., glucoprivation) on one type of immune activity [T-cell dependent inflammation (i.e., delayed-type hypersensitivity, DTH)] and reproductive tissue masses. We predicted that glucoprivation would compromise DTH responses more than reproductive organ size in males because male fitness is usually limited by number of reproductive opportunities. The opposite was predicted for females: poor environmental conditions would preclude breeding, so glucoprivation should induce females to allocate resources so as to promote survival to the next breeding opportunity [e.g., immunity (Nelson and Demas 1996)].

We studied Siberian hamsters because they are seasonal breeders from demanding (arctic) environments (Wynne-Edwards 1998), which should have forced individuals to make adaptive allocation decisions among traits affecting fitness in previous generations. We conducted our study on captive, virgin animals, to minimize individual differences in behavior that may have confounded glucoprivation effects. In other words, we eliminated all reproductive investment opportunities available to hamsters except the opportunity to develop and maintain competent reproductive systems. A further asset to studying Siberian hamsters is that sex differences in immunity [females > males (Bilbo and Nelson 2003)] have been demonstrated previously. Further, the drug 2-deoxy-D-glucose (2DG), which we used in the present study, was used previously with this species to impair glucose uptake at the cellular level. The glucoprivation it induces (1) compromises cell-mediated (Demas et al. 1997) and humoral (Zysling and Demas 2007) immunity, (2) dampens hypothalamic-pituitary-gonadal (HPG) activity (Nagatani et al. 1996), and (3) induces anestrus (Schneider et al. 1993) in rodents.

Methods

Animals

Hamsters were from our laboratory-bred colony at Ohio State University, which was initiated from wild individuals originally captured by K. Wynne-Edwards, Queen's University, Ontario. Hamsters were weaned 18 days after birth then they were housed singly in polycarbonate cages (28 × 17 × 12 cm), exposed to constant temperature (21 ± 4°C) and humidity (50 ± 10%) and given ad libitum access to food (Harlan Teklad 8640, Indianapolis, IN) and water until they reached sexual maturity (6 weeks later). To ensure hamsters were in breeding condition, photoperiod was maintained at 16L:8D during and before the experiment (lights-off at 1500 h EST). Thirteen males (saline = 6 and 2DG = 7) and eleven females (saline = 6 and 2DG = 5) were used in the experiment. All procedures were approved by the Ohio State ILACUC and comply with US regulations.

2DG administration

One day prior to DNFB (2,4-dinitro-1-fluorobenzene; Sigma, St. Louis, MO, USA) challenge and for the following 2 days, approximately half of the hamsters were injected (i.p.) twice daily with 1,500 mg kg^{-1} 2DG (Sigma D8375) in 100 µl 0.9% saline; all remaining individuals were injected with an identical volume of vehicle. This dose effectively alters glucose metabolism in rodents (Demas et al. 1997; Schneider et al. 1993) but does not induce torpor in Siberian hamsters (Dark et al. 1994). Injections occurred at ~0700 and ~1900 hours. Morning injections occurred concurrent with pinnae, body mass and food mass measurements; evening injections occurred in the room where animals were housed to minimize any stress associated with handling during the lights-off period. At both times of day, animals were randomly treated/measured to minimize anticipated handling by individuals and consequent stress.

Delayed type hypersensitivity (DTH)

DTH was induced by applying DNFB to the right pinna of each hamster after initial sensitization by application of DNFB to ~2 × 3 cm shaved area on the dorsum (Bilbo et al. 2002). A quantity of 25 µl DNFB [0.5% (wt/vol) in 4:1 acetone–olive oil vehicle] was applied to the shaved area for two consecutive days. Seven days later, baseline thickness of both left and right pinnae were quantified with a constant-loading dial micrometer (Long Island Indicator Service, Hauppauge, NY). Immediately thereafter, 20 µl DNFB [0.2% (wt/vol) in 4:1 acetone–olive oil] was applied to the dorsal surface of the right pinna while left pinnae were treated with vehicle. Left pinnae were treated with the vehicle to serve as a control to indicate whether any non-specific inflammation was induced. Pinnae thicknesses were measured every 24 h for the next 6 days following DNFB/vehicle treatment. Pinnae swellings were quantified by expressing daily thickness measures as a percentage of the baseline. Vehicle administration resulted in no significant swelling in the left pinnae. All DNFB treatments and pinnae measurements were performed between 0700 and 1000 hours on the same region of the pinna by the same person (Z. M. Weil). As DTH responses are sensitive to stressors and glucocorticoids (Bilbo et al. 2002), hamsters were brought into the testing room one at a time to minimize stress.

Statistical analysis

Data did not violate assumptions of parametric statistics, thus, repeated-measures ANOVA was used to assess effects of drug and sex and their interaction on DTH responses. Univariate ANOVA was used to identify effects of drug and sex and their interaction on reproductive, tissues, food consumption and body mass. For food consumption comparisons, body mass was used as a covariate in ANOVA models. Within sexes, *t*-tests were used to compare organ and body masses between treatment groups. Results were considered significant when $P < 0.05$.

Results

DTH

DNFB induced significant swelling in hamster pinnae ($F_{6,120} = 24.8$, $P < 0.001$). There was a significant interaction between treatment and sex, ($F_{6,120} = 2.31$, $P = 0.04$) so effects of drug on DTH were analyzed separately between sexes. In males, 2-DG treatment did not affect DTH ($F_{6,66} = 0.54$, $P = 0.78$; Fig. 1a), but in females, 2-DG dampened DTH ($F_{6,54} = 2.4$, $P = 0.04$; Fig. 1b). Females produced larger DTH responses than males ($F_{6,120} = 3.6$, $P = 0.003$), but when DTH responses were compared solely between 2-DG treated males and females, they did not differ between sexes ($F_{6,60} = 0.37$, $P = 0.90$).

Reproductive tissues

2DG treated males had smaller testes than vehicle injected hamsters ($t_{11} = 3.62$, $P = 0.004$; Fig. 1c). In females, 2DG did not significantly affect uterus ($t_9 = 1.1$, $P = 0.31$; Fig. 1d) or paired ovary ($t_9 = -0.16$, $P = 0.88$) masses. However, statistical power was low in both cases (uterus 31.9%; ovaries 6.8%) due to the small sample size. 2DG also did not affect body mass in males ($t_{11} = 0.3$, $P = 0.74$) or females ($t_9 = 0.52$, $P = 0.61$).

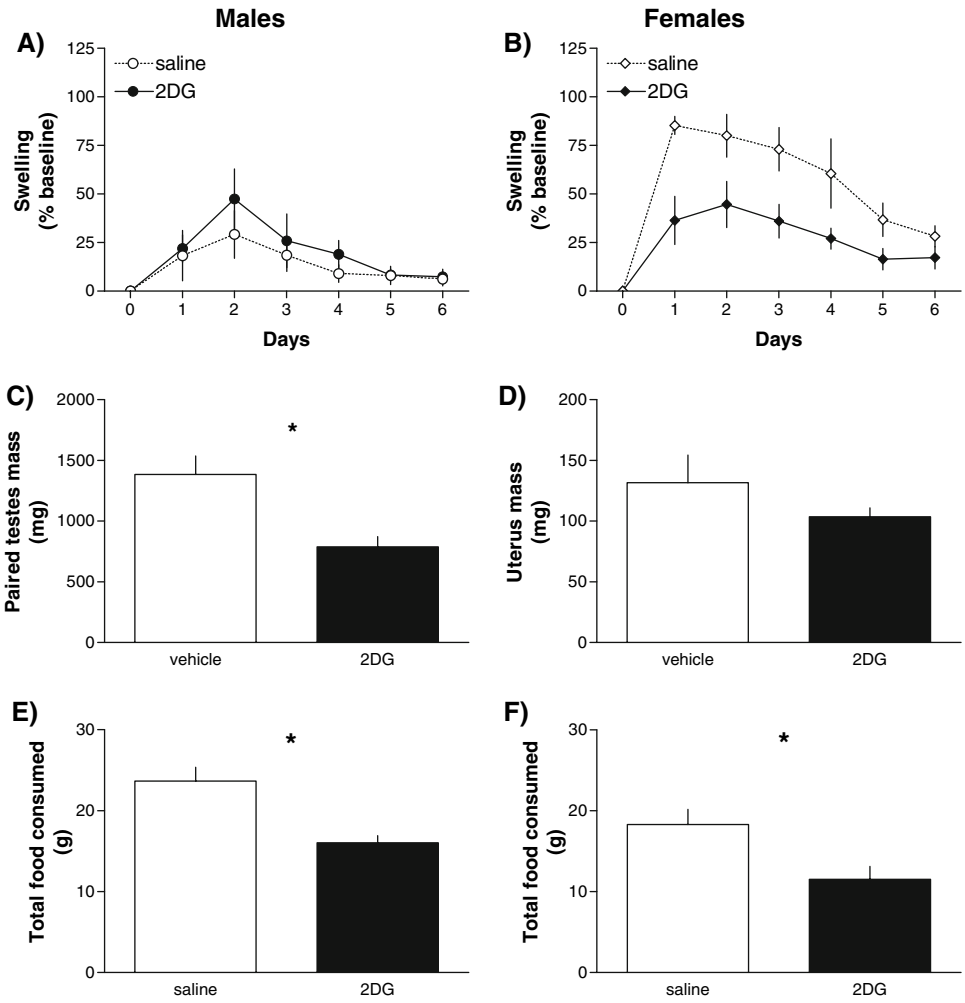

Fig. 1 Effects of saline (*open bars*) and 2-DG (*solid bars*) on delayed-type hypersensitivity (**a, b**), **c** testes and **d** uterine masses, and food intake (**e**, **f**) in male and female Siberian hamsters (*Phodopus sungorus*). Error bars are ± 1 Standard Error of the Mean (SEM) in **a** and **b**, + 1 SEM in **c–f**, and asterisks indicate $P < 0.05$

Body mass and food intake

Male hamsters were significantly larger than females ($t_{22} = 3.1$, $P = 0.005$). The effects of 2DG on food intake were not different between the sexes (sex × drug interaction: $F_{1,20} = 0.14$, $P = 0.71$), but 2DG treated hamsters consumed less food than vehicle injected individuals ($F_{1,20} = 21.4$, $P < 0.001$; Fig. 1e, f), and females consumed less food than males ($F_{1,20} = 8.9$, $P = 0.007$). Body mass, independent of sex or drug treatment, had no additional effect on food consumption ($F_{1,23} = 1.6$, $P = 0.23$). In sum, 2DG had similarly decreased food consumption in males and females although females consumed less food than males.

Discussion

Contrary to our prediction, glucoprivation compromised immune activity in female, but not male, Siberian hamsters. Conversely, glucoprivation reduced reproductive tissue mass in males but not in females. These data mirror previous work in insects in which food restriction compromised immune activity in female, but not in male fruit flies [*Drosophila melanogaster* (McKean and Nunney 2005)] and crickets [*Teleogryllus oceanicus* (Zuk et al. 2004)]. Altogether, these three studies indicate that females do not generally have superior immune systems to males; sexes may simply have different investment priorities in survival versus reproduction that vary with the environmental context (Forbes 2007; Stoehr and Kokko 2006).

The specific findings in this study may be a consequence of high sustained levels of reproductive investment in males, which may have prevented them from sacrificing their immune defenses any further when on glucoprivation. In hamsters, DTH is at a nadir in males housed in long daylengths. This may be because of the large reproductive investments males make at this time of year, which impose trade-offs with other physiological processes including immunity (Martin et al. 2008). Indeed, females do not show as dramatic a decrease in immune responses in long versus short days; photoperiod manipulation dramatically alters DTH in males but much less so in females (Bilbo and Nelson 2003; Weil et al. 2006). Further, this study was conducted in captive animals when reproductive responsibilities in males and females were limited to gonad maintenance. Male gonads are large (relative to females) and can be catabolized and regenerated quickly (Furuta et al. 1994). Moreover, a ~50% decrease in testes mass, as observed in this study, would have diminished but not abolished reproductive capacity (Niklowitz et al. 1989). Thus, males may have used testes mass to survive glucoprivation whereas the relatively small size of the ovaries and uterus prevented nulliparous females from doing so. Had our study been conducted when females were engaged in energetically more expensive activities [e.g., carrying embryos or lactating (Speakman 2000)], depression of reproductive investments may have been marked (Martin et al. 2006) and subsequently immune function unaffected. Still, it is unclear whether the costs of maintaining a competent reproductive system produced the outcomes here. Indeed, no study to our knowledge has demonstrated directly that the costs of maintaining ovaries and uteri are lower than the costs of maintaining testes and other male reproductive structures. Greater magnitude of immune fluctuations in males in response to photoperiod changes may be due to other factors besides reproductive trade-offs.

A related alternative interpretation of our data is that males and females favor different types of immune defenses in different conditions. Stress sometimes induces redistribution of immune resources more so than it suppresses immunity (Dhabhar et al. 1995). Likewise, different immune defenses are favored in different contexts contingent on the cost of immune variants (Schmid-Hempel and Ebert 2003). In male Lewis rats (*Rattus norvegicus*), 2DG biases the immune system away from cell-mediated (Th1) defenses and towards humoral (Th2) ones (Chou et al. 1996). Perhaps depression of DTH in female hamsters represents immune redistribution to cheaper immune defenses in times of resource shortage; Th1 defenses are more expensive than other options in terms of use (Lee 2006).

One way males and females may redistribute (or suppress) immune activity in response to glucoprivation is via glucocorticoids. 2DG elevates corticosterone in mice (Demas et al. 1997; Dreau et al. 1997), and this hormone affects the immune and reproductive systems of most vertebrates (Sapolsky et al. 2000). Moreover, female mice are reproductively (Nagatani et al. 1996) and immunologically (Dreau et al. 1997) more sensitive to 2DG. Other hormones, including estrogens or hypothalamic peptides (Dreau et al. 1998; Nagatani et al. 1996), may be more important in Siberian hamsters. DTH is enhanced by mild stressors in this species (Bilbo and Nelson 2003), not suppressed after 2DG treatment as occurred here. Still, glucocorticoid effects on immunity in hamsters are greater in females than males (Bilbo and Nelson 2003), which may be relevant in the present study given that our experimental protocol required multiple handlings of individuals. Additionally, as changes in fat constitute large proportion of seasonal changes in body mass (Geiser and Heldmaier 1995) and have significant effects on immune parameters in this species (Demas et al. 2003), the effects of 2DG on immunity via its effects on fat abundance warrant study.

Although the lack of effect of 2DG on reproductive tissue in females may be partly a consequence of low statistical power, the conservative interpretation of our data is

that 2DG effects were sex specific; reproductively competent, nulliparous females sacrificed one component of immunity in response to glucose shortage whereas virgin males sacrificed testes mass. Future studies should replicate this work with larger sample sizes and identify the neuroendocrine mechanisms producing the outcomes of this study. It would be especially informative to determine whether the effects of 2DG on testes were local (and thus may have arisen via changes in negative feedback of testosterone on the hypothalamus) or central (leading to decreased LH tone and subsequent gonadal regression). Also, it would be intriguing to determine whether morphological effects of 2DG were coupled to behavioral effects. In other words, does 2DG suppress the male but not female proceptive and receptive sex behavior as it suppressed male but not female reproductive tissue mass? Inclusion of gonadectomized and hormone-replacement treatment groups could shed light on this question, and also determine what role estrous cyclicity in female hamsters may have had on this study. Lastly, comparisons of changes in immune versus reproductive activities between sexes during other demanding life stages could clarify why these particular responses to glucoprivation occurred, and whether sex differences in immunity are even more complex than indicated in the present study. 2DG treatment induces both glucoprivation and anorexia in some species, but only glucoprivation (and sometimes increased food intake) in others. Additional studies of the specific effects of glucoprivation versus overall decreased food and nutrient intake would thus be useful to illuminate the particular directional changes in immunity between the sexes in this study. Ultimately, such integrative work will add to the basic and biomedical understanding of sex differences in infection and autoimmunity.

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References

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368

Bilbo SD, Nelson RJ (2003) Sex differences in photoperiodic and stress-induced enhancement of immune function in Siberian hamsters. Brain Behav Immun 17:462–472

Bilbo SD, Dhabhar FS, Viswanathan K, Saul A, Yellon SM, Nelson RJ (2002) Short day lengths augment stress-induced leukocyte trafficking and stress-induced enhancement of skin immune function. Proc Natl Acad Sci USA 99:4067–4072

Bronson FH (1985) Mammalian reproduction—an ecological perspective. Biol Reprod 32:1–26

Chou SH, Kojic LD, Messingham KN, Cunnick JE (1996) Characterization of the effect of 2-deoxy-D-glucose (2-DG) on the immune system. Brain Behav Immun 10:399–416

Dark J, Miller DR, Zucker I (1994) Reduced glucose availability induces torpor in Siberian hamsters. Am J Physiol 267:R496–R501

Demas GE, DeVries AC, Nelson RJ (1997) Effects of photoperiod and 2-deoxy-D-glucose-induced metabolic stress on immune function in female deer mice. Am J Physiol Regul Integr Comp Physiol 41:R1762–R1767

Demas GE, Drazen DL, Nelson RJ (2003) Reductions in total body fat decrease humoral immunity. Proc R Soc Lond B Biol Sci 270:905–911

Dhabhar FS, Miller AH, McEwen BS, Spencer RL (1995) Effects of stress on immune cell distribution—dynamics and hormonal mechanisms. J Immunol 154:5511–5527

Dreau D, Morton DS, Foster M, Swiggett JP, Sonnenfeld G (1997) Immune alterations in male and female mice after 2-deoxy-D-glucose administration. Physiol Behav 62:1325–1331

Dreau D, Morton DS, Foster M, Fowler N, Sonnenfeld G (1998) Effects of 2-deoxy-D-glucose administration on immune parameters in mice. Immunopharmacology 39:201–213

Forbes MR (2007) On sex differences in optimal immunity. Trends Ecol Evol 22:111–113

Furuta I, Porkkaheiskanen T, Scarbrough K, Tapanainen J, Turek FW, Hsueh AJW (1994) Photoperiod regulates testis cell apoptosis in Djungarian hamsters. Biol Reprod 51:1315–1321

Geiser F, Heldmaier G (1995) The impact of dietary fats, photoperiod, temperature and season on morphological variables, torpor patterns, and brown adipose-tissue fatty-acid composition of hamsters, *Phodopus sungorus*. J Comp Physiol B 165:406–415

Lee KA (2006) Linking immune defenses and life history at the levels of the individual and species. Integr Comp Biol 46:1000–1015

Martin LB, Han P, Kwong J, Hau M (2006) Cutaneous immune activity varies with physiological state in female house sparrows (*Passer domesticus*). Physiol Biochem Zool 79:775–783

Martin LB, Navara KJ, Weil ZM, Nelson RJ (2007) Immunological memory is compromised by food restriction in deer mice, *Peromyscus maniculatus*. Am J Physiol Regul Integr Comp Physiol 292:R316–R320

Martin LB, Weil ZM, Nelson RJ (2008) Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philos Trans R Soc Lond B Biol Sci 363:321–339

McKean KA, Nunney L (2005) Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. Evolution 59:1510–1517

Nagatani S, Bucholtz DC, Murahashi K, Estacio MAC, Tsukamura H, Foster DL, Maeda KI (1996) Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. Endocrinology 137:1166–1170

Nelson RJ, Demas GE (1996) Seasonal changes in immune function. Q Rev Biol 71:511–548

Niklowitz P, Khan S, Bergmann M, Hoffmann K, Nieschlag E (1989) Differential effects of follicle stimulating hormone and luteinizing hormone on Leydig cell function and restoration of spermatogenesis in hypophysectomized and photoinhibited Djungarian hamsters (*Phodopus sungorus*). Biol Reprod 41:871–880

Rolff J (2002) Bateman's principle and immunity. Proc R Soc Lond B Biol Sci 269:867–872

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89

Schmid-Hempel P, Ebert D (2003) On the evolutionary ecology of specific immune defence. Trends Ecol Evol 18:27–32

Schneider JE, Friedenson DG, Hall AJ, Wade GN (1993) Glucoprivation induces anestrus and lipoprivation may induce hibernation in Syrian hamsters. Am J Physiol 264:R573–R577

Speakman JR (2000) The cost of living: field metabolic rates of small mammals. Adv Ecol Res 30:177–297

Stoehr AM, Kokko H (2006) Sexual dimorphism in immunocompetence: what does life-history theory predict? Behav Ecol 17:751–756

Weil ZM, Pyter LM, Martin LB, Nelson RJ (2006) Perinatal photoperiod organizes adult immune responses in Siberian hamsters (*Phodopus sungorus*). Am J Physiol Regul Integr Comp Physiol 290:R1714–R1719

Wynne-Edwards KE (1998) Evolution of parental care in *Phodopus*: conflict between adaptations for survival and adaptations for rapid reproduction. Am Zool 38:238–250

Zuk M (1990) Reproductive strategies and disease susceptibility—an evolutionary viewpoint. Parasitol Today 6:231–233

Zuk M, Simmons LW, Rotenberry JT, Stoehr AM (2004) Sex differences in immunity in two species of field crickets. Can J Zool 82:627–634

Zysling DA, Demas GE (2007) Metabolic stress suppresses humoral immune function in long day, but not short day, Siberian hamsters (*Phodopus sungorus*). J Comp Physiol B 177:339–347