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ORIGINAL PAPER

Metabolic stress suppresses humoral immune function in long-day, but not short-day, Siberian hamsters (*Phodopus sungorus*)

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Abstract Individuals of many species experience marked seasonal variation in environmental conditions and must adapt to potentially large fluctuations in energy availability and expenditure. Seasonal changes in immunity have likely evolved as an adaptive mechanism to cope with seasonal stressors. In addition, these changes may be constrained by seasonal fluctuations in energy availability. The goal of this study was to assess the role of energetic trade-offs associated with seasonal variation in immunity. In addition to body fat stores, metabolic fuels (e.g., glucose) may affect immune function in seasonally breeding rodents. In this study we experimentally reduced energy availability via injections of the metabolic inhibitor 2-deoxy-D-glucose (2-DG) in long- and short-day housed Siberian hamsters (*Phodopus sungorus*) and then examined antigen-specific antibody production. Metabolic stress decreased antibody response compared with control animals in long days. In contrast, no difference was observed between treatment groups in short days. These data suggest that reductions in energy availability suppress immunity and short days buffer organisms against glucoprivation-induced immunosuppression.

Keywords Photoperiod · Antibodies · Seasonal · Cortisol · Humoral immunity

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Abbreviations

2-DG	2-Deoxy-D-glucose
IgM	Immunoglobulin M
IgG	Immunoglobulin G
KLH	Keyhole limpet hemocyanin
PWAT	Parametrial white adipose tissue
IWAT	Inguinal white adipose tissue
RWAT	Retroperitoneal white adipose tissue
ELISA	Enzyme-linked immunosorbent assay
EIA	Enzyme immunoassay
PBS-T	Phosphate buffered saline with Tween-20
OD	Optical density
ANOVA	Analysis of variance
PVN	Paraventricular nucleus
AVP	Arginine vasopressin
CRH	Corticotropin-releasing hormone

Introduction

Individuals of most species are faced with marked seasonal fluctuations in environmental conditions (e.g., temperature, rainfall, food availability) and must adapt to large fluctuations in energy availability (Bronson 1989; Goldman and Nelson 1993; Bronson and Heideman 1994). Many non-tropical mammalian and avian species use photoperiodic (day length) information to phase energetically expensive activities (e.g., reproduction and immune function) to coincide with times of adequate resources (Nelson et al. 1990). The use of photoperiod to time life history events energetically prepares organisms for the upcoming season and is advantageous when factors are highly predictable from year to year (Goldman and Nelson 1993; Bronson and Heideman 1994).

Studies of seasonal changes in mammalian physiology have generally focused on reproductive function due to its high energetic costs and implications for fitness (Bronson 1989); however many other physiological processes display seasonal fluctuations. For example, many animals exhibit changes in metabolism, pelage, body mass, and thermoregulation (Heldmaier et al. 1989; Moffatt et al. 1993; Ruby and Zucker 1992). Furthermore, in numerous species seasonal fluctuations in disease and mortality exist (John 1994; Lochmiller et al. 1994; Demas and Nelson 1996; Dowell et al. 2003; Hosseini et al. 2004; Altizer et al. 2006). Although some of this can be explained by seasonal changes in pathogen prevalence, opportunistic infections are also likely to overwhelm defenses when allocation of limited energy to immunity is decreased (Lochmiller et al. 1994; Nelson and Demas 1996; Lochmiller and Deerenberg 2000; Mann et al. 2000).

Seasonally breeding rodents provide an ideal model with which to study the role of energetics in the regulation of immunity. Rodents exposed to short "winter-like" days under laboratory conditions display pronounced changes in immunity, including T-cell dependent humoral immunity, in vitro basal lymphocyte proliferation, circulating B- and T-lymphocyte numbers and wound healing (Yellon et al. 1999; Drazen et al. 2000; Prendergast et al. 2001, 2002; Bilbo et al. 2002; Demas et al. 2002; Kinsey et al. 2003).

Reduced energy availability is an environmental variable that can act as a seasonal stressor and lead to suppressed immune function due to competition for resources among competing functions (Sheldon and Verhulst 1996; Demas 2004). Numerous empirical studies have demonstrated that energy reserves play an important role in regulating immune function in vertebrate species. Specifically, reduction of total body fat in long-day (LD) animals suppresses humoral immune function in both Siberian hamsters and prairie voles (*Microtus ochrogaster*) (Demas et al. 2003a). In addition to body fat, metabolic fuels such as glucose affect immune function in seasonally breeding rodents. Reduction in glucose availability inhibits splenic T-lymphocytes (Lysle et al. 1988) and mitogen-induced splenic proliferation in long-day housed deer mice (*Peromyscus maniculatus*) (Demas et al. 1997). Although energetics can affect immune function (Henken and Brandsma 1982; Demas et al. 1997; Demas et al. 2003a) few studies have examined the role of energetics in seasonal changes in immunity.

In theory, seasonal fluctuations in immune response and body mass have evolved as adaptive mechanisms to cope with seasonal stressors; furthermore, these changes may be constrained by seasonal fluctuations in energy availability. The goal of the present study was to assess the role of energetic trade-offs associated with seasonal variation in immune response. Specifically, we experimentally reduced energy availability in the form of glucose via the metabolic inhibitor 2-deoxy-D-glucose (2-DG) in long- and short-day housed Siberian hamsters (*Phodopus sungorus*). 2-DG is a non-metabolizable glucose analog, which causes a transient disruption of glycolysis, interfering with the cellular utilization of glucose and results in a state of glucoprivation (Horton et al. 1973). We then examined antigen-specific antibody production and cortisol levels.

We predicted that if animals are using photoperiod as a signal for forthcoming energy availability and consequently adjusting responses appropriately, then short-day animals will demonstrate a reduced antibody response as opposed to long-day animals. Additionally, if antibody production is indeed energetically costly as predicted, this response will be further attenuated in gluco-deprived individuals relative to control animals. Furthermore, the magnitude of the response may differ depending on photoperiodic condition. These results would suggest that variation in immune responsiveness may be constrained by seasonal fluctuations in energy availability and may also be a function of energy conserving strategies in response to these fluctuations.

Methods

Animals and housing

Adult female (>60 days of age) Siberian hamsters ($n = 60$) were obtained from our breeding colony and housed individually in polypropylene cages (40 × 20 × 20 cm). Temperature was held constant at 20 ± 2°C and relative humidity was maintained at 50 ± 10%. Food (Purina rat chow) and tap water were available ad libitum during the entire course of the experiment. Animals were weighed and pseudo-randomly (counterbalanced for body mass) assigned to one of two photoperiodic conditions: long-day (light:dark 16:8, $n = 20$) or short-day (light:dark 8:16, $n = 40$). A greater number of animals were housed in short days to control for reproductive non-responders (described below).

All animals were maintained within their respective photoperiod conditions for 8 weeks with ad libitum food and water. After 8 weeks animals were weighed

to the nearest 0.1 g daily for 2 days to establish a baseline (pre-2-DG) body mass. Food consumption was also assessed daily by weighing the food pellets remaining in the hopper and an average food intake was determined for each animal.

Experimental methods

Within each photoperiodic condition animals were further divided into one of two groups that received either control injections of saline or 2-DG. Following two days of baseline measurements, saline or 2-DG injections began while animals were still maintained in their photoperiodic conditions. 2-DG treated animals received a 0.1 ml injection of 2-DG at a concentration of 750 mg/kg dissolved in 0.9% sterile saline (Demas et al. 1997). This dose has been previously validated in rodent species and is significantly lower than doses used to induce torpor (2,500 mg/kg) (Dark et al. 1994). Control animals received a 0.1 ml injection of 0.9% sterile saline. Injections were administered every other day for 12 days, for a total of six injections. During this period animals were weighed to the nearest 0.1 g daily and food intake was monitored.

Following the first day of 2-DG or saline injection, all animals received a single subcutaneous injection of 100 μg of keyhole limpet hemocyanin (KLH) suspended in 0.1 ml sterile saline (day 0) in order to assess humoral immune function. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*). KLH generates a robust, non-replicating antigenic response in rodents, but does not make the animals sick (e.g., inflammation or fever) (Dixon et al. 1966).

Blood sampling and necropsies

On days 5 and 10 post KLH injection a blood sample was drawn from all animals via the retro-orbital sinus for later measurement of KLH-specific antibodies and serum cortisol concentrations. These sampling periods were chosen to capture peak immunoglobulin M (IgM; day 5) and IgG (day 10) levels. IgM is the first immunoglobulin class produced following an immune challenge and IgG is the predominant immunoglobulin class present in the blood during the course of the immune response (Demas et al. 1997; Drazen et al. 2000). Briefly, animals were lightly anesthetized with anhydrous diethyl ether (Sigma, St Louis, MO) and blood samples (~500 μl) were drawn from the retro-orbital sinus between 1,000 and 1,200 h EST. Blood samples were allowed to clot for 1 h, the clots were removed and the samples centrifuged at 4°C for 30 min at 2,500 rpm. Serum aliquots were aspirated and stored in sealable polypropylene microcentrifuge tubes at −80°C until assayed. Animals were euthanized and necropsies were performed at the completion of the study (day 11). Uterine horns and ovaries (reproductive mass), parametrial white adipose tissue (PWAT), inguinal WAT (IWAT), and retroperitoneal WAT (RWAT) were removed to determine the effects of energy deprivation on these tissues. All tissues were cleaned of connective tissues and weighed to the nearest 0.1 mg.

Following necropsies, as expected, a subset of short-day individuals did not reproductively respond to changes in photoperiod. This phenomenon has been previously documented in males of this species (Lynch et al. 1989; Gorman and Zucker 1997) and these individuals are referred to as reproductive "non-responders" (Puchalski and Lynch 1986). Based on our findings, short-day animals were further divided into reproductive responders ($n = 26$) and non-responders ($n = 14$). To accomplish this we first calculated relative reproductive mass by correcting reproductive mass for body mass (ratio of the combined uterine and ovarian masses divided by body mass) and found the 95% confidence interval for saline injected long-day animals. Any short-day animal falling below this lower bound was labeled a short-day responder (SD-R); anything above the lower bound was labeled a short-day non-responder (SD-NR).

Assessment of humoral immunity

To assess humoral immunity, serum antibody concentrations for KLH were assayed using an enzyme-linked immunosorbent assay (ELISA). For measurement of anti-KLH IgM and IgG concentrations (see Demas et al. 2003a) microtiter plates were coated with antigen by incubating overnight at 4°C with 0.5 mg/ml KLH in sodium bicarbonate buffer (pH 9.6). Plates were washed with phosphate buffered saline (PBS) (pH 7.4) containing 0.05% Tween 20 (PBS-T) at pH 7.4, then blocked with 5% non-fat dry milk in PBS-T overnight at 4°C to reduce non-specific binding, and washed again with PBS-T. Thawed serum samples were diluted 1:20 with PBS-T, and 150 μl of each serum dilution was added in duplicate to the wells of the antigen-coated plates. Positive control samples (pooled sera from hamsters previously determined to have high levels of anti-KLH antibody, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naïve hamsters, similarly diluted with PBS-T) were added in duplicate. Plates were sealed, incubated at 37°C for 3 h, and then washed with PBS-T. Secondary antibody

(alkaline phosphatase-conjugated-anti mouse IgG diluted 1:2,000 with PBS-T, Cappel, Durham, NC; alkaline phosphatase-conjugated-anti mouse IgM diluted 1:500 with PBS-T, Cappel, Durham, NC) was added to the wells, and the plates were sealed and incubated for 1 h at 37°C. Plates were then washed again with PBS-T and 150 µl of the enzyme substrate *p*-nitrophenyl phosphate (Sigma Chemical, St Louis, MO; 1 mg/ml in diethanolamine substrate buffer) was added to each well. Plates were protected from light during the enzyme-substrate reaction, which was terminated after 30 min by adding 50 µl of 1.5 M NaOH to each well. The optical density (OD) of each well was determined using a plate reader (Bio-Rad, Benchmark; Richmond, CA) equipped with a 405 nm wavelength filter, and the mean OD for each set of duplicate wells was calculated. To minimize inter- and intra-assay variability, the mean OD for each sample will be expressed as a ratio of its plate positive control OD for statistical analysis.

Assessment of cortisol levels

Circulating glucocorticoids were measured as an index of metabolic-induced stress. In particular, cortisol was measured because it is the predominant glucocorticoid in Siberian hamsters, with concentrations ~100× that of corticosterone (Reburn and Wynne-Edwards 1999). Serum cortisol concentrations were determined in multiple enzyme immunoassays (EIA) from a commercially prepared kit (Correlate-EIA™, Assay Designs, Ann Arbor, MI). This assay was previously validated for use with Siberian hamsters (Demas et al. 2004). The antiserum used is highly specific for cortisol; cross-reactivity with corticosterone is 27.68 and <0.01% for other steroid hormones. The sensitivity of the assay is 56.72 pg/ml. Intra-assay variability was 2.24 and 3.35% for day 5 samples, and 1.41 and 2.95% for day 10 samples.

Statistical analyses

All statistical tests were performed using Minitab 14 (State College, PA). Data were checked for normality and homogeneity of variance and those data non-normally distributed were transformed where appropriate. Reproductive mass was corrected for body mass by taking the ratio of the combined uterine and ovarian masses divided by body mass (i.e., relative reproductive mass). The relative reproductive mass was not normally distributed and was square root transformed to best meet the normality and equality of variance assumptions of parametric tests. Cortisol concentrations at day 10 were also log-transformed to meet these assumptions. All differences except for repeated measures of cortisol were assessed via a two-way (photoperiod × treatment) ANOVA. Photoperiod was defined as long-day, short-day responder, or short-day non-responder. Treatment was defined as 2-DG or saline injection. Post hoc comparisons between pair-wise means were conducted using Tukey's honestly significant difference (HSD) tests when the overall ANOVAs were significant. Differences in cortisol levels between measurement time points were identified by use of a repeated-measures ANOVA with time as the within-subjects variable. For all statistical tests, the level of significance (α) was set at $P < 0.05$ and tests were two-tailed.

Results

Body mass

There was a significant effect of photoperiod ($F_{2,54} = 7.28$, $P = 0.002$; Fig. 1a) but not 2-DG treatment ($F_{1,54} = 0.002$, $P = 0.893$) on final body mass. There was no significant interaction between photoperiod and treatment on final body mass ($F_{2,54} = 1.32$, $P = 0.274$). Specifically, long-day animals were significantly heavier than short-day responders ($T = -3.790$, $P = 0.001$; Fig. 1a); short-day responders injected with 2-DG were significantly lighter than both long-day, 2-DG injected ($T = -3.434$, $P = 0.013$) and long-day, saline injected ($T = -3.071$, $P = 0.037$) animals.

There were no significant effects of photoperiod ($F_{2,54} = 0.79$, $P = 0.457$) or photoperiod × treatment ($F_{2,54} = 0.47$, $P = 0.676$) on the amount of weight change during the experiment. There was a trend towards treatment affecting body mass change ($F_{1,54} = 3.49$, $P = 0.067$) but it was not significant.

Food intake

There was a significant effect of photoperiod ($F_{2,54} = 4.08$, $P = 0.022$; Fig. 1b) but not 2-DG treatment ($F_{1,54} = 0.25$, $P = 0.616$) on food intake. There was no significant interaction between photoperiod and treatment on food intake ($F_{2,54} = 1.47$, $P = 0.239$). Specifically, short-day responders ate significantly less than long-day animals ($T = -2.680$, $P = 0.025$).

Relative reproductive mass and fat pads

Relative reproductive mass was significantly affected by photoperiod ($F_{2,54} = 22.61$, $P < 0.001$; Fig. 1c) but not 2-DG treatment ($F_{1,54} = 0.80$, $P = 0.375$). There was also a significant effect of photoperiod × treatment on relative

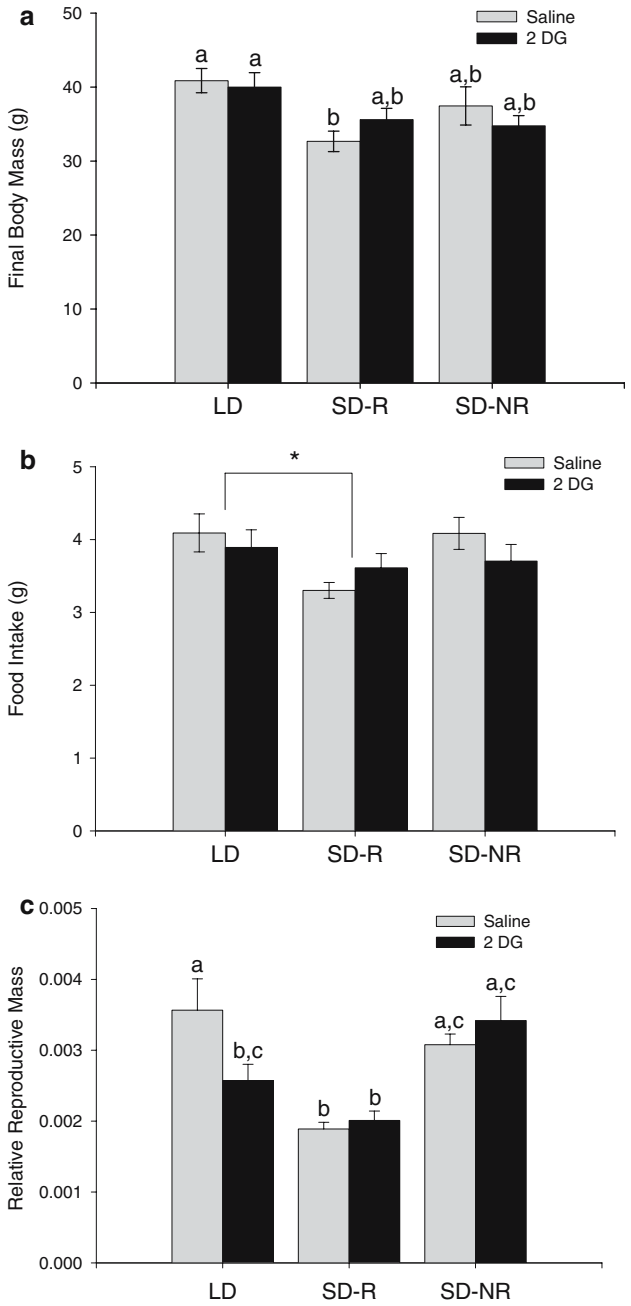

Fig. 1 Effects of 2-DG treatment on mean (±SEM) (**a**) final body mass, (**b**) food intake and (**c**) relative reproductive mass in long-day (*LD*), short-day reproductively responsive (*SD-R*), and short-day reproductively non-responsive (*SD-NR*) Siberian hamsters. In figure (**a**) and (**c**) *groups with different letters* indicate statistically significant differences between group means ($P < 0.05$); *group sharing the same letter* are statistically equivalent. Significant difference between pair-wise means in (**b**) are indicated by an *asterisk* if $P < 0.05$

reproductive mass ($F_{2,54} = 4.01$, $P = 0.024$). Short-day responders had smaller relative reproductive masses than both long-day ($T = -5.411$, $P < 0.001$; Fig. 1c) and short-day non-responder ($T = 5.871$, $P < 0.001$) animals. Furthermore, long-day 2-DG injected animals had smaller relative reproductive masses than long-day saline injected animals ($T = -2.88$, $P = 0.05$).

Differences in fat pad masses were also observed. Photoperiod significantly affected PWAT ($F_{2,54} = 3.99$, $P = 0.024$; Table 1), IWAT ($F_{2,54} = 6.52$, $P = 0.003$), total fat ($F_{2,54} = 6.59$, $P = 0.003$), but not RWAT ($F_{2,54} = 1.73$, $P = 0.186$). There were no significant effects of 2-DG treatment or photoperiod × treatment on any of the above variables ($P > 0.05$), except a trend towards photoperiod × treatment affecting RWAT mass ($F_{2,54} = 2.72$, $P = 0.075$). Most photoperiodic effects on body fat were due to long-day animals having significantly higher fat pad masses than short-day responders (PWAT $T = -2.793$, $P = 0.019$; IWAT $T = -3.458$, $P = 0.003$; total fat $T = -3.490$, $P = 0.003$; Table 1). Short-day responders also had significantly different fat pad masses than short-day non-responders in terms of IWAT ($T = -2.573$, $P = 0.033$) and total fat mass ($T = -2.553$, $P = 0.036$).

Immune measures

There were significant effects of 2-DG treatment ($F_{2,54} = 12.43$, $P = 0.001$; Fig. 2a), but not photoperiod

Table 1 Effects of 2-DG treatment on mean (±SEM) parametrial white adipose tissue (PWAT), inguinal WAT (PWAT), retroperitoneal WAT (WAT), total body fat (composite of WAT), and serum day 5 and 10 cortisol concentrations in long-day (LD), short-day reproductively responsive (SD-R), and short-day reproductively non-responsive (SD-NR) Siberian hamsters

	Control	2-DG
PWAT		
LD	0.405 ± 0.051^{a}	0.277 ± 0.032^{a}
SD-R	$0.161 \pm 0.064^{a,b}$	0.244 ± 0.038^{a}
SD-NR	0.260 ± 0.074^{a}	0.223 ± 0.041^{a}
IWAT		
LD	2.078 ± 0.249^{c}	1.654 ± 0.223^{c}
SD-R	$0.990 \pm 0.196^{c,d}$	1.276 ± 0.186^{c}
SD-NR	1.320 ± 0.318^{c}	1.128 ± 0.069^{c}
RWAT		
LD	0.225 ± 0.018	0.158 ± 0.021
SD-R	0.121 ± 0.028	0.170 ± 0.032
SD-NR	0.171 ± 0.039	0.113 ± 0.016
Total body fat		
LD	2.708 ± 0.282^{e}	2.089 ± 0.262^{e}
SD-R	$1.217 \pm 0.276^{e,f}$	1.690 ± 0.216^{e}
SD-NR	1.751 ± 0.422^{e}	1.465 ± 0.084^{e}
Day 5 cortisol		
LD	84.991 ± 6.561	92.757 ± 8.749
SD-R	96.799 ± 7.786	88.473 ± 5.611
SD-NR	91.549 ± 6.018	96.169 ± 10.901
Day 10 cortisol		
LD	94.998 ± 4.813	98.142 ± 5.941
SD-R	87.537 ± 4.230	86.145 ± 7.097
SD-NR	89.151 ± 8.281	87.246 ± 5.992

Groups with different letters indicate statistically significant differences between group means ($P < 0.05$); group sharing the same letter are statistically equivalent

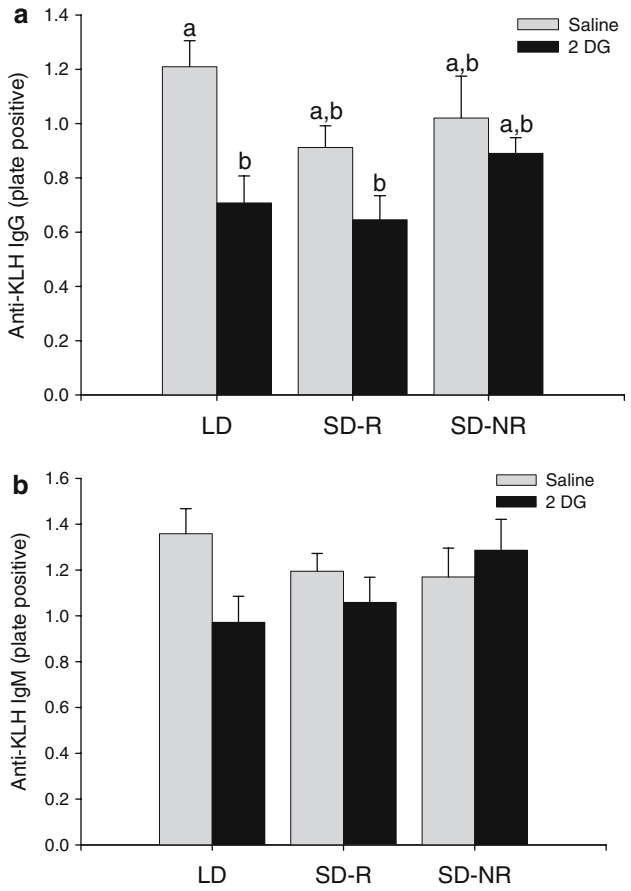

Fig. 2 Effect of 2-DG treatment on mean (±SEM) (**a**) serum anti-KLH immunoglobulin G (IgG) and (**b**) serum anti-KLH immunoglobulin M (IgM) in long-day (*LD*), short-day reproductively responsive (*SD-R*), and short-day reproductively non-responsive (*SD-NR*) Siberian hamsters. Groups with different letters indicate statistically significant differences between group means ($P < 0.05$); group sharing the same letter are statistically equivalent

($F_{1,54} = 2.33$, $P = 0.107$), or photoperiod × treatment ($F_{2,54} = 1.53$, $P = 0.226$) on IgG levels. When short-day non-responders are excluded from the analyses, there is a nearly significant effect of photoperiod ($F_{1,42} = 3.84$, $P = 0.057$). Saline injected animals had higher IgG responses that 2-DG injected animals ($T = -3.525$, $P < 0.001$; Fig. 2a). In addition, long-day saline injected animals had higher IgG responses than both long-day 2-DG injected ($T = -3.540$, $P = 0.01$) and short-day responder 2-DG injected ($T = -4.298$, $P = 0.001$) animals. There were no significant differences in IgG levels between short-day responder 2-DG injected and short-day responder saline injected animals ($T = -2.136$, $P = 0.285$) or short-day non-responder 2-DG injected and short-day non-responder saline injected animals ($T = -0.761$, $P = 0.973$). There were no significant effects of photoperiod ($F_{2,54} = 0.37$, $P = 0.690$; Fig. 2b), treatment ($F_{1,54} = 2.07$, $P = 0.156$), or photoperiod × treatment ($F_{2,54} = 2.12$, $P = 0.129$) on IgM levels.

Serum cortisol

There were no significant effects of photoperiod (day 5 $F_{2,53} = 0.22$, $P = 0.802$, Table 1; day 10 $F_{2,53} = 1.97$, $P = 0.150$; Table 1), treatment (day 5 $F_{1,53} = 0.05$, $P = 0.831$; day 10 $F_{1,53} = 0.01$, $P = 0.907$) or photoperiod × treatment (day 5 $F_{2,53} = 0.73$, $P = 0.486$; day 10 $F_{2,53} = 0.15$, $P = 0.864$) on plasma cortisol levels at either time point.

Discussion

We asked if metabolic stress would suppress humoral immune function and whether these responses would differ depending on photoperiodic condition. Consistent with previous findings (Yellon et al. 1999), there appeared to be a photoperiodic effect on immune function with long-day animals tending to have a higher concentration of IgG antibodies compared with short-day reproductive responders. In addition, short-day responders had significantly lower body mass and fat pad masses than long-day animals. 2-DG-induced glucoprivation led to a reduction in both IgG antibody production and relative reproductive mass in long-day animals. In short-day responders and short-day non-responders however, there was a buffering of immune suppression following 2-DG administration. One possibility is that this response was due to a "floor effect" in which the decreased immune function in response to short days could not be depressed further by 2-DG. This is unlikely for several reasons. First, it has been demonstrated that other types of stressors can push immune function lower than normal short-day values (Demas et al. 2003b). Secondly, short-day induced buffering against 2-DG induced immunosuppression has been observed in deer mice, a species that shows short day immunoenhancement (Demas et al. 1997). Lastly, a similar result was found in a previous pilot study in our lab (Zysling et al. unpublished), suggesting that variation in immune responsiveness across seasons may be a function of energy conserving strategies in response to seasonal energetic fluctuations or stressors.

The physiological mechanisms regulating this buffering effect are currently unknown. One possible explanation is the differential secretion pattern and/or response to the pineal hormone melatonin. Melatonin is an indoleamine hormone that is rhythmically synthesized and secreted almost exclusively by the pineal gland at the onset of darkness and is suppressed during daylight hours (Cassone 1990). Thus, the duration of melatonin secretion codes for day length (i.e., a short- or long-day) (Goldman and Elliot 1988; Bartness et al. 1993) and mediates many physiological adaptations in short days

including reproductive regression, decreased body mass, and fat storage (Bartness and Goldman 1989).

Exogenous administration of melatonin is generally immunoenhancing (Caroleo et al. 1992; Maestroni 1993; Demas and Nelson 1998; Drazen et al. 2001). It is therefore possible that melatonin may be modulating differences in immune effects between long- and short-day hamsters by attenuating the effects of glucoprivation on immunity in short days, a time of increased duration of secretion. One potential difficulty with this explanation is that, although both short-day groups (i.e., responders and non-responders) display a buffering effect, short-day non-responders generally display a melatonin signal duration in short days that is not different than the normal long day pattern (Margraff et al. 1991). Furthermore, short-day non-responders are reproductively responsive to exogenous short-day-like melatonin, therefore post-pineal mechanisms function normally (Puchalski and Lynch 1988). One interesting, and previously unexplored, explanation may lie in the peak rather than the duration of melatonin secretion. In Siberian hamsters, the peak amplitude of melatonin is twice as high under short days as long days (Hoffman et al. 1985; Ribelayga et al. 2000). Differences in immune function may be driven by peak amplitude or a combination of peak amplitude and duration of secretion. To our knowledge, it is currently unknown whether short-day non-responders display a peak melatonin concentration like that of short-day responders or long-day animals. In order to test this possibility, melatonin rhythms in short-day non-responders, specifically the peak amplitude, need to be determined.

Another possibility is that the buffering effect is mediated by photoperiod independent of melatonin secretion. Pre-pineal mechanisms are likely candidates as we would expect these circuits to be operating similarly in short-day responders and non-responders. For example, the paraventricular nucleus (PVN), which is part of the photoperiodic circuit, responds to physiological stressors by increasing the synthesis of hormones involved in modulating aspects of immune function and the release of glucocorticoids, such as arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) (Yates et al. 1971; Motawei et al. 1999). The PVN plays a critical role in the neuroendocrine response to hypoglycemia (Evans et al. 2001). Furthermore, glucocorticoids (e.g., cortisol) have also been demonstrated to have immunomodulatory effects (Dhabhar 2000) and, although we did not detect an increase in cortisol with 2-DG injection, 2-DG induced glucoprivation is a known stimulus for secretion of glucocorticoids (Weidenfeld et al. 1994; Demas et al. 1997). The lack off an effect of 2-DG on cortisol in the present study was unexpected, especially given previous reports as well as the immunosuppressive effects in the present study. It is possible that we did not sample close enough to the injection time point to capture the cortisol peak. Furthermore, there may be effects farther upstream in the system (e.g., levels of CRH or changes in receptor densities) that were not assessed in the current study.

Although gonadal steroid hormones, such as testosterone and estradiol have been demonstrated to modulate many aspects of immunity in male and female Siberian hamsters (Bilbo and Nelson 2003) we found that short-day non-responders maintain reproductive mass and presumably, elevated steroid hormone concentrations. It is therefore likely that the buffering effect observed in both short-day responders and non-responders is independent of steroid hormones. Interestingly, we found a decrease in relative reproductive mass in long-day animals; this effect was not seen in short-day non-responders. These results suggest that there may be energetic constraints and/or trade-offs between reproduction and immunity that are photoperiod dependent.

Finally, we found a decrease in both body and fat pad mass in short-day responders compared with long-day animals. Short-day Siberian hamsters undergo a reduction in body mass primarily due to reductions in body fat (Bartness and Goldman 1989). This decrease in body mass is presumed to decrease absolute energy requirements and foraging time, thus limiting exposure to unfavorable climatic conditions (Iverson and Turner 1974; Dark and Zucker 1983; Bartness and Wade 1985). In our study there was no effect of 2-DG treatment on body mass, fat pad mass or food intake in any group and the buffering of immune changes were seen in both short-day responders and non-responders. Short-day responders lose body/fat pad mass whereas non-responders maintain their mass so it is unlikely that these factors alone regulate this effect. In examining the strength of the response in both groups, however, it appears that the buffering effect is more pronounced in short-day non-responders. Short-day non-responders may be better able to maintain immune function in the face of an energetic challenge due to an increased availability of resources, suggesting that factors such as fat pad mass may play an important role in fine tuning immune responses.

In summary, our data indicate that, although immune function is compromised during metabolic stress in long days, short day lengths appear to buffer organisms against this effect. These results are likely due to differential responsiveness of long- and short-day hamsters to metabolic stress; however, the mechanisms regulating these differences are currently unknown. Regardless of the precise mechanisms, changes in immunity have likely evolved as an adaptive mechanism to cope with seasonal stressors such as

decreased food availability and lowered temperatures. These changes are likely constrained and modulated by seasonal fluctuations in energy availability; future studies will address the specific physiological mechanisms underlying these responses. Collectively, these data provide support for the ideas that energetic trade-offs play a role in mediating seasonal variation in immune function.

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