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Short day lengths attenuate the symptoms of infection in Siberian hamsters

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Symptoms of infection, such as fever, anorexia and lethargy, are ubiquitous among vertebrates. Rather than nonspecific manifestations of illness, these responses are organized, adaptive strategies that are often critical to host survival. During times of energetic shortage such as winter, however, it may be detrimental for individuals to prolong energetically demanding symptoms such as fever. Individuals may adjust their immune responses prior to winter by using day length to anticipate energetically-demanding conditions. If the expression of sickness behaviours is constrained by energy availability, then cytokine production, fever, and anorexia should be attenuated in infected Siberian hamsters housed under simulated winter photoperiods. We housed hamsters in either long (14 L : 10 D) or short (10 L : 14 D) day lengths and assessed cytokines, anorexia and fever following injections of lipopolysaccharide (LPS). Short days attenuated the response to lipopolysaccharide, by decreasing the production of interleukin (IL)-6 and IL-1β, and diminishing the duration of fever and anorexia. Short-day exposure in hamsters also decreased the ingestion of dietary iron, a nutrient vital to bacterial replication. Taken together, short day lengths attenuated the symptoms of infection, presumably to optimize energy expenditure and survival outcome.

Keywords: fever; anorexia; seasonality; sickness behaviour; immune function; adaptation

1. INTRODUCTION

Exposure to pathogens or to inflammatory stimuli such as lipopolysaccharide (LPS) activates the immune system and initiates several physiological and behavioural responses that are collectively termed the acute phase response (Exton 1997). These responses are ubiquitous among vertebrates and include fever, iron-withholding, reductions in food and water intake, and reduced interest in sexual, parental and other social interactions (Hart 1988). Rather than being nonspecific manifestations of illness, these responses are initiated and maintained by the host via endogenous pro-inflammatory cytokines (e.g. interleukin (IL)-1), and are often critical to survival (Kluger & Rothenburg 1979; Wing & Young 1980; Dantzer *et al.* 1998*b*). For example, fever is universal among infected animals; most microbial organisms have adapted to grow best at or below the core body temperature of the host and reproduce poorly at elevated temperatures (Kluger *et al.* 1998). Mammals administered antipyrogenic substances such as flurbiprofen, a cyclooxygenase (COX) inhibitor, suffer increased mortality from infection (Van Miert *et al.* 1978); the prevention of behavioural fever in ectothermic animals, in which individuals move to warmer locations in the environment, is associated with increased recovery time and mortality (Kluger & Rothenburg 1979). In addition, infected animals often have decreased concentrations of blood iron (Hart 1988). Bacteria require iron to replicate (Wright *et al.* 1981), and during infection, iron is actively sequestered in the liver. The administration of iron supplements to mice and humans often increases the severity of infections (Van Miert *et al.* 1984; Hart 1988).

In addition to responses such as fever, behavioural responses to infection, collectively termed 'sickness behaviours', also appear to be highly organized, adaptive strategies for the host in overcoming infection more efficiently and effectively (Kent *et al.* 1992; Exton 1997; Langhans 2000). For example, behaviours such as decreased activity and increased sleep conserve energy and allow tissue repair during illness (Hart 1988). Immune function is energetically costly; the onset and maintenance of inflammation and fever, and the production of humoral immune factors all require substantial energy (Ardawi & Newsholme 1985; Demas *et al.* 1997; Moret & Schmid-Hempel 2000). Despite the high value of calories during illness, the anorexia of infection appears highly adaptive, and is hypothesized to reduce both energy spent foraging for food and water, as well as to optimize pathogen elimination by further reducing the concentrations of iron and other nutrients in the blood (Exton 1997; Langhans 2000). Mice that reduce food intake during infection recover more quickly than mice that maintain normal intake, and infected mice suffer increased mortality if force-fed (Wing & Young 1980). Food preferences also change following cytokine or LPS administration; voluntary intake of protein, which is more likely than fats or carbohydrates to contain iron, is selectively decreased in comparison with other macronutrients in rodents (Aubert *et al.* 1995).

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Despite their stereotyped onset, the physiological and behavioural adjustments associated with the course of infection may be motivated, modifiable responses (Aubert 1999); thus their onset or expression may be altered by intrinsic or extrinsic factors. For instance, lactating female rats are lethargic and reduce maternal nest building behaviour following injections of LPS during exposure to mild ambient temperatures in the laboratory. Importantly, the rats cease to exhibit this sickness behaviour when exposed to low temperatures that presumably endanger the lives of their dependent pups (Aubert *et al.* 1997). Thus, sick individuals retain the ability to respond to environmental stimuli during emergencies and, albeit with modified behavioural thresholds, reorganize their behavioural strategies during infection. Infected female animals engage in significantly fewer sexual interactions than non-infected females, presumably because pregnancy incurred during infection would probably be unsuccessful. The impact of infection on sexual behaviour in males is less pronounced than in females, because reproductive behaviour has fewer energetic costs in males (Avitsur & Yirmiya 1999). Thus, the energetic status or requirements of an individual may have a profound influence on the expression of sickness behaviour.

Winter is often an energetically demanding time during which animals are exposed to potentially harsh conditions such as low temperatures, coincident with reduced food and water availability and increased thermogenic demands. Siberian hamsters (*Phodopus sungorus*) cease breeding during the short days of winter. If exposed to short, winter-like day lengths in the laboratory, these hamsters undergo significant reductions of white adipose tissue and body mass (*ca*. 25%), despite mild temperatures and *ad libitum* access to food (Steinlechner & Heldmaier 1982). This adaptive strategy has presumably evolved because maintenance of a smaller body size throughout winter requires less food, and less heat loss occurs due to a reduced surface-to-volume ratio. Seasonal adaptations in behaviour and physiology improve the animals' ability to cope with the annual changes in environmental energy demands (Heideman & Bronson 1990; Nelson *et al.* 1998). Siberian hamsters may adjust their sickness responses prior to winter by using day length to anticipate energetically demanding conditions. If the expression of fever and anorexia are constrained by energy availability, then, similar to reproduction, these responses should be attenuated in short-day hamsters because they typically have fewer energetic reserves. The influence of 10 weeks of long- or short-day length exposure on the production of cytokines (i.e. IL-6 and IL-1β) and the expression of sickness behaviours (i.e. fever, anorexia) was examined in response to LPS. The hypothesis that infection-induced anorexia may serve as an iron-reducing mechanism was also tested by determining the influence of short-day exposure on the ingestive response to varying concentrations of iron in a food source following LPS.

2. METHODS

(a) *Animals*

Adult (four to six months of age) male Siberian hamsters (*P. sungorus*) from our breeding colony were used in this study. Animals were housed individually in colony rooms with a constant temperature and humidity of 21 ± 2 °C and 50 ± 5%, respectively, and *ad libitum* access to food and tap water. Hamsters were either housed in long-day (LD) conditions with a reverse 14 L : 10 D cycle (lights on at 24.00 EST), or in short-day (SD) conditions with a reverse 10 L : 14 D (lights on at 04.00 EST). All hamsters were maintained within their respective photoperiod conditions for 10 weeks prior to the start of the experiments. All studies were conducted after institutional animal care and use committee approval and were in compliance with all US federal animal welfare requirements.

(b) *Surgical procedures*

Fourteen LD and eight SD hamsters were implanted intraperitoneally (i.p.) with radio-telemetric transmitters (Mini-Mitter, Bend, OR) under sodium pentobarbital anesthesia and allowed a week's recovery. Cages were placed on TR-3000 receiver boards and connected to DP-24 DataPorts (Mini-Mitter) and a personal computer. Emitted temperature frequencies were collected in 10 min intervals (bins), and were converted to temperature values by interpolating from programmed calibration curves of individual transmitters.

(c) *Anorexia and fever assessment*

A subset of the implanted hamsters in both long ($n = 8$) and short days ($n = 8$) was then acclimatized to a sweetened condensed milk solution (Eagle brand; 1 : 1 with water) for a period of 6 h (14.00 to 20.00) each day for 5 days, beginning with the onset of the dark phase. On day 6, these animals received 0.1 cc i.p. injections of 25 μg LPS (Sigma) suspended in sterile saline at 13.00 EST. The remaining six LD implanted hamsters received injections of saline at this time as a control during fever monitoring. Milk intake was monitored in LPS-injected animals from 1–7 h post-injection, and again on days 7 and 8 for 6 h (14.00 to 20.00) each day. Total milk intake was recorded at the end of 6 h each day by subtracting end from start volume (±0.01 ml) using 10 ml drinking cups. *Ad libitum* food intake (food absent during the hours of milk presentation), body mass and body temperature were monitored daily.

(d) *Cytokine assessment*

Eighteen hours post-LPS injection (day 7) animals that had been used in the fever assessment ($n = 16$) were lightly anaesthetized with methoxyflurane vapours (Metofane, Schering-Plough), and blood samples (0.5 ml) were drawn from the retro-orbital sinus, allowed to clot for 1 h, the clot removed, and the samples centrifuged at 4 °C for 30 min at 3500 rpm. Handling was kept to less than 2 min. Serum aliquots were stored at −80 °C until assayed for IL-6 using an enzyme-linked immunosorbent assay (ELISA) (Biosource International).

A separate group of 10 LD and 10 SD hamsters that were previously unmanipulated were bled from the retro-orbital sinus (1 ml) at 13.00 EST. We measured IL-1β in culture supernatant because the incidence of IL-1 within the circulation tends to be very low (Rothwell 1997; Netea *et al.* 2000) and was undetectable in the serum in our samples. Lymphocytes were separated from whole blood by Ficoll gradient centrifugation as described elsewhere (Bilbo & Nelson 2001). Viable cells were adjusted to 1×10^6 cells ml^{-1} by dilution with culture medium, and 200 μl aliquots of each cell suspension were added to the wells of sterile round-bottom 96-well culture plates. LPS (2.5 μg; Sigma) was added directly to each cell suspension, and plates were incubated at 37 °C with 5% CO_2 for 48 h prior to supernatant extraction. Supernatant samples were frozen at −80 °C until

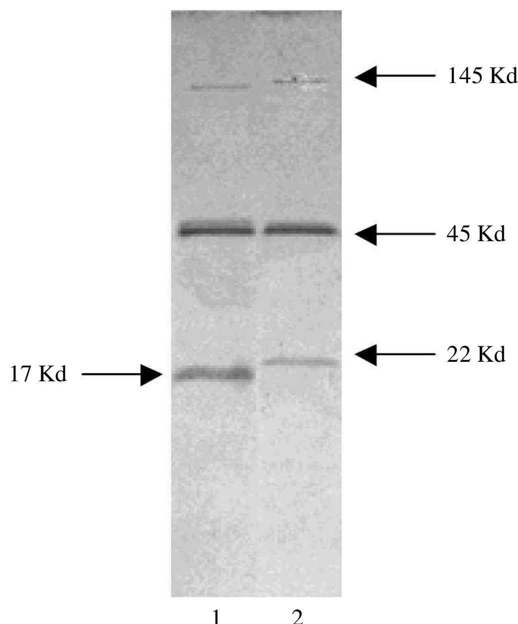

Figure 1. Immunoprecipitation of hamster plasma with antibodies against human IL resulted in three bands: two high molecular weight bands corresponding to the size of IgG (145 Kd) and protein A (45 Kd), respectively. The third band of *ca.*17 Kd (lane 1) corresponds to human IL-1β. The third band of *ca.* 22 Kd (lane 2) corresponds to human IL-6.

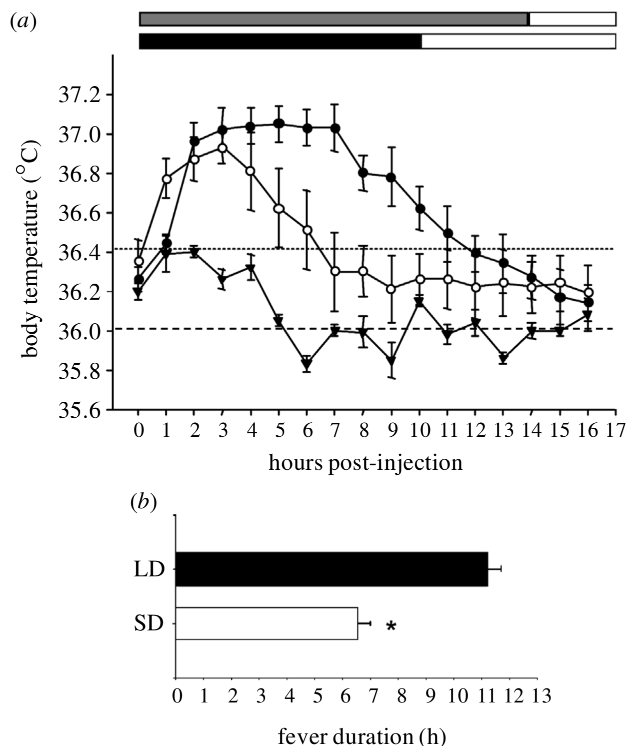

Figure 2. (*a*) Mean (± s.e.m.) body temperature from 0–16 h post-LPS injections in LD (black circles; $n = 8$) and SD (open circles; $n = 8$) hamsters, and after saline injections in control hamsters (triangles; $n = 6$). Black and grey bars above the graph indicate the active (dark) phase of the light–dark cycle in LD versus SD hamsters, respectively. Horizontal dashed and dotted lines represent mean baseline body temperature during the inactive (36 °C) versus active (36.4 °C) phases, respectively. (*b*) Mean (± s.e.m.) duration (h) of body temperatures higher than the active baseline in LD and SD hamsters following LPS injections. (Asterisk, significantly different from LD; $p < 0.05$.)

assayed for IL-1β using an ELISA (Biosource). The optical density (OD) of each well was determined for each assay with a microplate reader (Bio-Rad) equipped with a 450 nm wavelength filter. Mean duplicate OD values were used in subsequent analyses.

(e) *ELISA validation*

The ELISA method previously described uses antibodies against human IL-1β and IL-6, which appear to detect hamster IL-1β and IL-6 in a sensitive and reliable manner. To further verify these results, we performed immunoprecipitation and electrophoresis procedures. Plasma of hamsters ($n = 3$) injected with 25 mg kg^{-1} of LPS was harvested 3 h post-injection. 100 μg of plasma proteins were incubated with 2 μg of the appropriate Biosource capture and visualization antibodies against human IL-1β or IL-6. The resulting immune complexes were precipitated with 20 μl of protein A-argarose (sc-1002, Santa Cruz Biotechnology, Santa Cruz, CA). After washing away unprecipitated proteins, the precipitated proteins were run on a SDS-Page (12%) gel and stained by commassie blue and photographed. Figure 1 shows that immunoprecipitation of hamster plasma with antibodies against human IL resulted in three bands: two high molecular weight bands corresponding to the size of IgG (145 Kd) and protein A (45 Kd), respectively. The third band of *ca.* 17 Kd (lane 1) corresponds to human IL-1β (Dantzer *et al.* 1998*a*). Lane 2 shows antibodies against human IL-6 precipitated a hamster protein *ca.* 22 Kd, similar to the molecular weight of human IL-6 (Saper 1998). These results strongly suggest that the antibodies we used in our ELISA methods specifically recognized hamster IL-1β and IL-6.

(f) *Ingestion of dietary iron in milk*

One week after blood sampling, the 10 LD and 10 SD hamsters used for IL-1β assessment were acclimatized as described previously for 5 days to four solutions of sweetened condensed milk, to which finely ground crystalline ferrous sulfate ($FeSO_4$; Sigma) was added in concentrations of 0, 40, 400 or 4000 μg ml^{-1}. Zero and 40 μg ml^{-1} $FeSO_4$ correspond to minimal concentrations of iron based on normal dietary intake in rodents, whereas 400 and 4000 μg ml^{-1} are within the range of standard concentrations of daily dietary intake (Viteri *et al.* 1995). During acclimatization, hamsters were maintained on a low iron diet, and food was removed during the hours of milk presentation each day. On day 6, animals received 0.1 cc i.p. injections of 25 μg LPS suspended in sterile isotonic saline at 13.00 EST. A separate group of eight LD and eight SD hamsters that were previously unmanipulated were acclimatized to a zero (0 μg ml^{-1}) and high dose (4000 μg ml^{-1}) of $FeSO_4$-treated milk for 5 days as described above. On day 6, these animals received 0.1 cc i.p. injections of saline at 13.00. Milk intake was measured for a period of 6 h each day beginning with the onset of darkness (14.00), and body mass and food intake were measured daily for 3 days.

(g) *Reproductive measures*

At the conclusion of all procedures, all LPS-injected hamsters used in fever, anorexia, cytokine and Fe^{2+} milk intake assessments for each photoperiod group ($n = 18$, each photoperiodic group) were killed via rapid cervical dislocation. Paired testes,

Table 1. Mean (± s.e.m.) body mass (g), reproductive organ and fat pad masses (mg) in LD and SD hamsters.

	long day ($n = 18$)	short day ($n = 18$)
body mass	35.6 ± 0.9	30.0 ± 1.0*
paired testes	572 ± 65.5	60.0 ± 7.0*
paired epididymides	223 ± 24.0	40.0 ± 4.0*
paired seminal vesicles	81.0 ± 15.0	18.0 ± 5.0*
epididymal white adipose tissue (EWAT)	74.7 ± 4.1	18.9 ± 3.1*
brown adipose tissue (BAT)	23.8 ± 3.0	15.3 ± 1.0*

* $p < 0.05$.

epididymides, seminal vesicles, epididymal white adipose tissue (EWAT), and brown adipose tissue (BAT) were removed, cleaned of connective tissue and fat, and weighed by laboratory assistants unaware of the experimental conditions of the animals. The average paired testes mass for LD animals was determined, and all SD animals lying ⩾ 2 standard deviations below this mean were considered reproductively responsive to short days.

(h) *Data analysis and statistics*

Baseline body temperatures for the active and inactive phases of the light cycle were determined for each animal using mean values for the 5 days prior to LPS. Temperatures were analysed for the 18 h following LPS injections across all time points using a repeated measures analysis of variance (ANOVA) (photoperiod × time) in order to assess the overall amplitude of fever. We defined fever as temperatures significantly ($p < 0.05$) higher than the active phase baseline for each 15 min interval using two-tailed *t*-tests. The total duration of temperatures above the baseline were compared between groups using a one way ANOVA. Latency to onset of fever was compared using a one way ANOVA. The circadian increase in body temperature (*ca.* 0.4 °C) that occurs 1–2 h prior to lights out was also analysed between groups using a one way ANOVA to assess whether LD and SD animals may increase body temperature according to different circadian timers. Anorexia was assessed as a percent of baseline food or milk intake for the day of LPS injections and each day following, and compared between groups using one way ANOVAs. Fe^{2+} intake was assessed following injections of LPS as percent of baseline intake of each $FeSO_4$ milk concentration and analysed using a repeated-measures ANOVA. Each dose was analysed individually to compare pre- and post-LPS intake using one way ANOVAs. Post-hoc differences between all means were analysed using Tukey's honestly significant difference test. All tests were considered statistically significant if $p < 0.05$.

3. RESULTS

(a) *Reproductive measures*

SD hamsters ($n = 18$) had significantly lower body, reproductive organ, and fat pad masses than LD hamsters ($n = 18$) after 10 weeks of photoperiod manipulation ($p < 0.001$ for all; table 1).

(b) *Fever*

LPS significantly increased body temperature in both LD and SD hamsters ($n = 8$, each photoperiodic group). Saline injections did not cause fever ($n = 6$; figure 2*a*). Neither fever amplitude nor latency to onset differed significantly as a function of day length. However, duration of fever (h), defined as temperatures significantly higher than active baseline, was markedly attenuated in SD (6.5 ± 0.74 h) compared with LD hamsters (11.1 ± 1.22 h) ($p < 0.01$; figure 2*b*). Neither baseline body temperatures nor the circadian increase in body temperature prior to lights off differed between LD and SD hamsters (data not shown).

(c) *Anorexia and weight loss*

Hamsters significantly decreased their intake of sweetened condensed milk (figure 3*a*) and laboratory food (figure 3*b*; $p < 0.001$ in both cases) following LPS injections. On days 7 and 8, SD hamsters ($n = 8$) consumed significantly more milk and food (47% more calories) than did LD hamsters ($n = 8$) ($p < 0.005$ in both cases). SD hamsters resumed normal food intake within 24 h of infection, and normal milk intake within 48 h. By contrast, LD animals did not approach the normal milk and food intake throughout the 48 h period. Consequently, LD hamsters lost more weight (46% more) in the days following LPS injections than SD hamsters ($p < 0.005$; figure 3*c*). Saline injections did not significantly influence milk intake (either Fe^{2+} dose), food intake, or body mass (data not shown).

(d) *Cytokine concentrations*

Serum IL-6 was decreased in SD compared to LD animals ($n = 8$, each group) following induction of fever (18 h post-LPS; $p < 0.05$; figure 4*a*). LPS-stimulated production of IL-1β was attenuated in the culture supernatant from unmanipulated SD compared to LD hamsters ($n = 10$, each group; $p < 0.001$; figure 4*b*).

(e) *Fe^{2+} milk intake*

Baseline intake of four $FeSO_4$ milk solutions did not differ between LD and SD hamsters ($n = 10$, each group) during the acclimatization period (figure 5*a*). As expected, all hamsters reduced their milk intake following LPS injection by reducing all four concentrations of $FeSO_4$-treated milk compared with intake during the acclimatization period ($p < 0.01$ for all; figure 5*b*). However, SD hamsters significantly reduced consumption of only the two highest concentrations of milk (400 and 4000 μg ml^{-1} $FeSO_4$) compared with 0 and 40 μg ml^{-1} doses, and LD hamsters ($p < 0.001$ for both). LD hamsters exhibited no distinction among doses and reduced intake of all four equally.

4. DISCUSSION

Physiological and behavioural defences against pathogenic infection may assist individuals in regaining strength

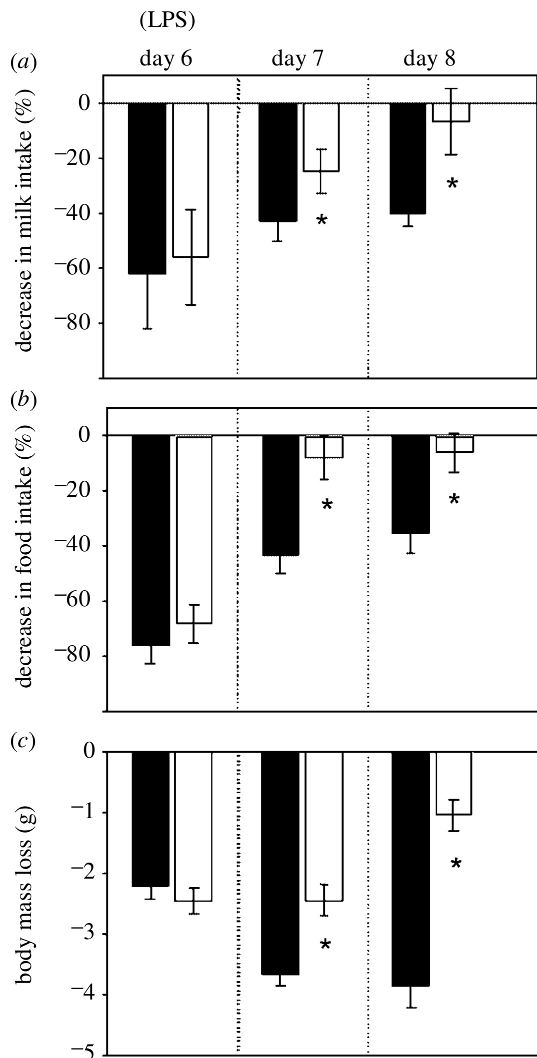

Figure 3. (*a*) Mean (± s.e.m.) decrease in sweetened condensed milk intake as a percent of baseline on the day of LPS injections and days 7 and 8 of testing in LD (black bars; $n = 8$) and SD (white bars; $n = 8$) hamsters. (*b*) Mean (± s.e.m.) decrease in food intake as a percent of baseline on the day of LPS injections and days 7 and 8 of testing in LD and SD hamsters. (*c*) Mean (± s.e.m.) loss of body mass (g) on the day of LPS injections and days 7 and 8 of testing in LD and SD hamsters. (Asterisk, significantly different from LD; $p < 0.05$.)

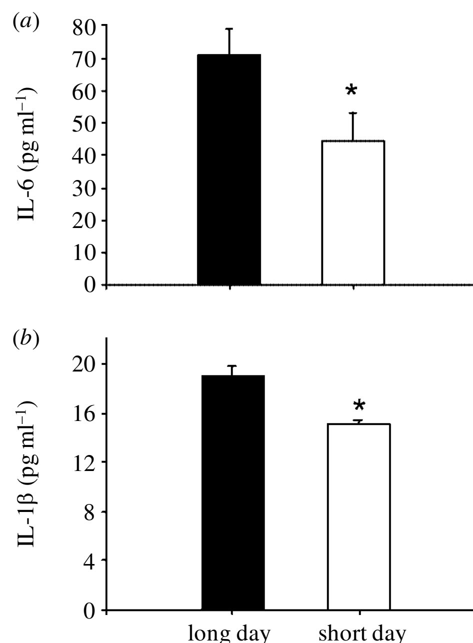

Figure 4. (*a*) Mean (± s.e.m.) serum IL-6 concentrations in LD (black bars; $n = 8$) and SD (white bars; $n = 8$) hamsters 18 h post-LPS injections. (*b*) Mean (± s.e.m.) IL-1β concentrations in LPS-stimulated culture supernatant from LD (black bars; $n = 10$) and SD (white bars; $n = 10$) hamsters. (Asterisk, significantly different from LD; $p < 0.05$.)

and overcoming infection (Hart 1988; Kent *et al.* 1992). However, responses such as fever and anorexia may be dependent on seasonal fluctuations in energy availability. Siberian hamsters housed in winter-like, short day lengths initiate significant reductions in body mass and reproductive function in preparation for winter (Steinlechner & Heldmaier 1982). SD hamsters reduced fever duration following injections of LPS and lost less body mass following LPS compared with LD animals. SD hamsters resumed normal feeding within 24 h of infection, whereas LD animals did not approach their normal milk and food intake throughout the 48 h post-LPS period. Importantly, the ability to initiate an immune response was unaltered in SD; neither fever amplitude nor latency to onset differed as a function of day length. Conversely, reductions in body mass and fat deposits, and thus energy stores, were correlated with reduced febrile and anorexic responses. Coincident with these behaviours, concentrations of serum IL-6 and culture supernatant IL-1β were lower in short days than in long days.

Our behavioural results are consistent with data in rats, in which obese animals exhibit greater anorexia after IL-1β infusions than lean rats (Plata-Salaman *et al.* 1997), and rats that are food restricted to 6 and 12% of normal body weight do not exhibit anorexia in response to inflammation as severely as *ad libitum* fed animals (Lennie 1998). Similarly, high body mass in iguanas (*Iguana iguana*) is correlated with choosing warmer temperatures in the environment during infection, and thus inducing higher fever, compared with those animals of low body mass (Deen & Hutchison 2001). The low body mass set point of SD Siberian hamsters appears to be strictly defended, because extended fever and anorexia may be counterproductive rather than beneficial in energetically compromised animals. For example, chronic anorexia in humans is a common clinical manifestation that can lead to cachexia and a longer recovery time following infection (Plata-Salaman *et al.* 1997).

The hypothesis that infection-induced anorexia may serve as an iron-reducing mechanism was also examined. The administration of iron supplements to humans often increases the severity of infections (Hoen 1999). Conversely, treatment with iron-chelating compounds is associated with improved recovery from bacterial infections, including malaria, tuberculosis and *Mycobacterium avium*, a common complication of HIV infection and AIDS (Hershko *et al.* 1992; Cabantchik *et al.* 1999; Gomes *et al.* 1999). When presented with four different concentrations of iron-supplemented milk, SD hamsters selectively reduced consumption of sweetened milk solutions

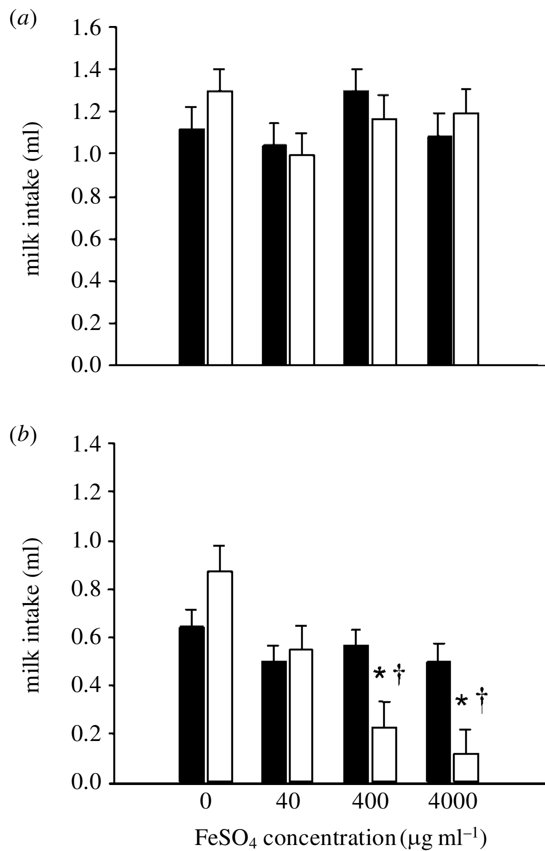

Figure 5. Mean (± s.e.m.) intake of four different concentrations of $FeSO_4$-treated milk in LD (black bars; $n = 10$) and SD (white bars; $n = 10$) hamsters across a 5 day acclimatization period (*a*), and following injections of LPS on day 6 (*b*). (Asterisk, significantly different from LD; Dagger, significantly different from 0 and 40 μg ml^{-1} doses within the SD group; $p < 0.05$.)

containing moderate to high concentrations of iron following injections of LPS, whereas LD hamsters exhibited no distinction among doses. Thus, SD animals ingested more food following infection than LD hamsters, because they probably could not afford to lose critical body mass, but SD hamsters decreased intake selectively when micronutrients essential for the growth of pathogenic microorganisms were contained in the food (i.e. Fe^{++}). It is unclear from these data whether anorexia may serve as an iron-reducing mechanism, *per se*, as LD hamsters did not distinguish among doses. LPS administration in rats reduces the threshold for detection of quinine in sucrose water and accelerates a change from appetitive to aversive reactions to quinine, compared with uninfected controls (Aubert 1999). The present data suggest that day length (and thus energy status) may mediate the response threshold to micronutrients in food during infection in hamsters, thus preventing energetically compromised animals from absorbing harmful compounds that might otherwise be tolerated in normal weight animals.

The immune system has been considered a diffuse sensory organ and regulator of metabolism that works throughout the body to achieve and maintain homeostasis (Husband 1995). Recent evidence implicates the involvement of cytokines in metabolic, as well as pathological processes (Husband 1995; Inui 2000). For instance, IL-1 is released downstream of leptin, the protein product of the *ob* gene and an important regulator of energy balance; IL-1 in turn plays a role in food intake and metabolism (Luheshi *et al.* 1999). We suggest that the decreased production of cytokines in SD hamsters may reflect an adaptive reorganization (i.e. attenuation) of sickness behaviours in preparation for the harsh conditions of winter. Thus, Siberian hamsters may use day length information to orchestrate the response to infection prior to the onset of challenging environmental conditions. During infection, the release of pro-inflammatory cytokines (i.e. IL-1β and IL-6) is strongly correlated with the expression of fever and sickness behaviours in a dose-dependent manner in rats and mice (Dantzer *et al.* 1998*b*; Dunn & Swiergiel 1998). IL-6 concentrations were lower in serum from SD than LD hamsters 18 h post-LPS, despite the fact that fever was no longer present in either group of animals. Similarly, the increased concentrations of IL-1β observed in LD compared with SD supernatant reflect the production of IL-1β from baseline conditions. These results may reflect lower concentrations of cytokine-producing cells in short days in this species. Alternatively, immune reactions in SD hamsters may be more efficient than in LD animals; truncated fever and anorexia may reflect elimination of inflammatory elements more quickly, and thus a faster return to baseline. These hypotheses remain to be fully investigated.

The pineal hormone, melatonin, encodes day length (photoperiod) information, and appears to be the primary hormone mediating seasonal changes in reproduction and physiology in mammals (Bartness *et al.* 1993). Melatonin is secreted only at night, and the duration of its release is proportional to the length of the dark phase (Illnerova *et al.* 1984); consequently, SD hamsters experience longer nightly durations of melatonin than LD hamsters. The role of melatonin as an immunomodulator is well established, and its influence on immune function has been elucidated for a variety of species (Maestroni 1993; Nelson & Blom 1994; Liebmann *et al.* 1997; Nelson & Demas 1997; Demas & Nelson 1998; Nelson & Drazen 1999; Conti *et al.* 2000; Drazen *et al.* 2000, 2001). Melatonin attenuates the LPS-induced production of cytokines and prostaglandins in rat serum (Nava *et al.* 1997), and reduces body temperature in humans (Deacon & Arendt 1995), as well as fever following LPS treatment in rats (Nava *et al.* 1997). Other sickness behaviours, including hyperalgesia and adipsia, are also attenuated following melatonin treatment (Nava *et al.* 1997; Raghavendra *et al.* 2000). In seasonally breeding animals, centrally acting melatonin is crucial for reproductive involution (Bartness *et al.* 1993), and its role as part of an integrative system to coordinate reproductive, immune and other physiological processes makes it a probable candidate for the seasonal mediation of sickness behaviours.

Our results suggest that the expression of sickness behaviours, while stereotyped, may be organized according to the seasonal and energetic requirements of the individual. Many animals, including humans, are not reproductively responsive to changes in day length; however, occurrence and susceptibility to many diseases occur on a seasonal basis (Nelson *et al.* 2002), and the extent to which day length influences the severity or duration of illness remains unspecified. An appreciation of the mech-

anisms underlying the interaction of environment and the onset and attenuation of sickness behaviours could have important and novel clinical applications.

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REFERENCES

Ardawi, M. S. & Newsholme, E. A. 1985 Metabolism in lymphocytes and its importance in the immune response. *Essays Biochem.* **21**, 1–44.

Aubert, A. 1999 Sickness and behaviour in animals: a motivational perspective. *Neurosci. Biobehav. Rev.* **23**, 1029–1036.

Aubert, A., Goodall, G. & Dantzer, R. 1995 Compared effects of cold ambient temperature and cytokines on macronutrient intake in rats. *Physiol. Behav.* **57**, 869–873.

Aubert, A., Goodall, G., Dantzer, R. & Gheusi, G. 1997 Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. *Brain Behav. Immun.* **11**, 107–118.

Avitsur, R. & Yirmiya, R. 1999 The immunobiology of sexual behavior: gender differences in the suppression of sexual activity during illness. *Pharmacol. Biochem. Behav.* **64**, 787–796.

Bartness, T. J., Powers, J. B., Hastings, M. H., Bittman, E. L. & Goldman, B. D. 1993 The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J. Pineal Res.* **15**, 161–190.

Bilbo, S. D. & Nelson, R. J. 2001 Sex steroid hormones enhance immune function in male and female Siberian hamsters. *Am. J. Physiol.* **280**, R207–R213.

Cabantchik, Z. I., Moody-Haupt, S. & Gordeuk, V. R. 1999 Iron chelators as anti-infectives; malaria as a paradigm. *FEMS Immunol. Med. Microbiol.* **26**, 289–298.

Conti, A. Maestroni, G. J. M. McCann, S. M. Sternberg, E. M. Lipton, J. M. & Smith, C. C. (eds) 2000 *Neuroimmunomodulation. Perspectives at the new millenium*, vol. 917, Annals of the New York Academy of Sciences.

Dantzer, R., Bluthe, R. M., Gheusi, G., Cremona, S., Laye, S., Parnet, P. & Kelley, K. W. 1998*a* Molecular basis of sickness behavior. *Ann. NY Acad. Sci.* **856**, 132–138.

Dantzer, R., Bluthe, R. M., Laye, S., Bret-Dibat, J. L., Parnet, P. & Kelley, K. W. 1998*b* Cytokines and sickness behavior. *Ann. NY Acad. Sci.* **840**, 586–590.

Deacon, S. & Arendt, J. 1995 Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. *Brain Res.* **688**, 77–85.

Deen, C. M. & Hutchison, V. H. 2001 Effects of lipopolysaccharide and acclimation temperature on induced behavioral fever in juvenile *Iguana iguana*. *J. Thermal Biol.* **26**, 55–63.

Demas, G. E. & Nelson, R. J. 1998 Exogenous melatonin enhances cell-mediated, but not humoral, immune function in adult male deer mice (*Peromyscus maniculatus*). *J. Biol. Rhythms* **13**, 245–252.

Demas, G. E., Chefer, V., Talan, M. I. & Nelson, R. J. 1997 Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.* **273**, R1631–R1637.

Drazen, D. L., Klein, S. L., Yellon, S. M. & Nelson, R. J. 2000 *In vitro* melatonin treatment enhances splenocyte proliferation in prairie voles. *J. Pineal Res.* **28**, 34–40.

Drazen, D. L., Bilu, D., Bilbo, S. D. & Nelson, R. J. 2001 *In vitro* melatonin enhancement of splenocyte proliferation is attenuated in mice by luzindole, a melatonin receptor antagonist. *Am. J. Physiol.* **280**, 1476–1482.

Dunn, A. J. & Swiergiel, A. H. 1998 The role of cytokines in infection-related behavior. *Ann. NY Acad. Sci.* **840**, 577–585.

Exton, M. S. 1997 Infection-induced anorexia: active host defence strategy. *Appetite* **29**, 369–383.

Gomes, M. S., Dom, G., Pedrosa, J., Boelaert, J. R. & Appelberg, R. 1999 Effects of iron deprivation on *Mycobacterium avium* growth. *Tuberculosis Lung Dis.* **79**, 321–328.

Hart, B. 1988 Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* **12**, 123–137.

Heideman, P. D. & Bronson, F. H. 1990 Photoperiod, melatonin secretion, and sexual maturation in a tropical rodent. *Biol. Reprod.* **43**, 745–750.

Hershko, C., Gordeuk, V. R., Thuma, P. E., Theanacho, E. N., Spira, D. T., Hider, R. C., Peto, T. E. & Brittenham, G. M. 1992 The antimalarial effect of iron chelators: studies in animal models and in humans with mild falciparum malaria. *J. Inorg. Biochem.* **47**, 267–277.

Hoen, B. 1999 Iron and infection: clinical experience. *Am. J. Kidney Dis.* **34**(Suppl. 2), S30–S34.

Husband, A. J. 1995 The immune system and integrated homeostasis. *Immunol. Cell Biol.* **73**, 377–382.

Illnerova, H., Hoffmann, K. & Vanecek, J. 1984 Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster *Phodopus sungorus*. *Neuroendocrinology* **38**, 226–231.

Inui, A. 2000 Transgenic approach to the study of body weight regulation. *Pharmacol. Rev.* **52**, 35–61.

Kent, S., Bluthe, R. M., Kelley, K. W. & Dantzer, R. 1992 Sickness behavior as a new target for drug development. *Trends Pharmacol. Sci.* **13**, 24–28.

Kluger, M. J. & Rothenburg, B. A. 1979 Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science* **203**, 374–376.

Kluger, M. J., Kozak, W., Conn, C. A., Leon, L. R. & Soszynski, D. 1998 Role of fever in disease. *Ann. NY Acad. Sci.* **856**, 224–233.

Langhans, W. 2000 Anorexia of infection: current prospects. *Nutrition* **16**, 996–1005.

Lennie, T. A. 1998 Relationship of body energy status to inflammation-induced anorexia and weight loss. *Physiol. Behav.* **64**, 475–481.

Liebmann, P. M., Wolfler, A., Felsner, P., Hofer, D. & Schauenstein, K. 1997 Melatonin and the immune system. *Int. Arch. Allergy Immunol.* **112**, 203–211.

Luheshi, G. N., Gardner, J. D., Rushforth, D. A., Loudon, A. S. & Rothwell, N. J. 1999 Leptin actions on food intake and body temperature are mediated by IL-1. *Proc. Natl Acad. Sci. USA* **96**, 7047–7052.

Maestroni, G. J. 1993 The immunoneuroendocrine role of melatonin. *J. Pineal Res.* **14**, 1–10.

Moret, Y. & Schmid-Hempel, P. 2000 Survival for immunity: the price of immune system activation for bumble-bee workers. *Science* **290**, 1166–1168.

Nava, F., Calapai, G., Facciola, G., Cuzzocrea, S., Giuliani, G., De Sarro, A. & Caputi, A. P. 1997 Melatonin effects on inhibition of thirst and fever induced by lipopolysaccharide in rats. *Eur. J. Pharmacol.* **331**, 267–274.

Nelson, R. J. & Blom, J. M. 1994 Photoperiodic effects on tumor development and immune function. *J. Biol. Rhythms* **9**, 233–249.

Nelson, R. J. & Demas, G. E. 1997 Role of melatonin in mediating seasonal energetic and immunologic adaptations. *Brain Res. Bull.* **44**, 423–430.

Nelson, R. J. & Drazen, D. L. 1999 Melatonin mediates seasonal adjustments in immune function. *Reprod. Nutr. Dev.* **39**, 383–398.

Nelson, R., Demas, G. & Klein, S. 1998 Photoperiodic mediation of seasonal breeding and immune function in rodents: a multi-factorial approach. *Am. Zool.* **38**, 226–237.

Nelson, R. J., Demas, G. E., Klein, S. L. & Kriegsfeld, L. J. 2002 *Seasonal patterns of stress, immune function, and disease.* New York: Cambridge University Press.

Netea, M. G., Kullberg, B. J. & Van der Meer, J. W. 2000 Circulating cytokines as mediators of fever. *Clin. Infect. Dis.* **31**(Suppl. 5), S178–S184.

Plata-Salaman, C. R., Vasselli, J. R. & Sonti, G. 1997 Differential responsiveness of obese (fa/fa) and lean (Fa/Fa) Zucker rats to cytokine-induced anorexia. *Obes. Res.* **5**, 36–42.

Raghavendra, V., Agrewala, J. N. & Kulkarni, S. K. 2000 Melatonin reversal of lipopolysaccharides-induced thermal and behavioral hyperalgesia in mice. *Eur. J. Pharmacol.* **395**, 15–21.

Rothwell, N. J. 1997 Neuroimmune interactions: the role of cytokines. *Br. J. Pharmacol.* **121**, 841–847.

Saper, C. B. 1998 Neurobiological basis of fever. *Ann. NY Acad. Sci.* **856**, 90–94.

Steinlechner, S. & Heldmaier, G. 1982 Role of photoperiod and melatonin in seasonal acclimatization of the Djungarian hamster, *Phodopus sungorus*. *Int. J. Biometeorol.* **26**, 329–337.

Van Miert, A. S., van Duin, C. T. H., Busser, F. J. M., Perie, N., Ingh, T. S. G. A. M. & Neys-Backers, M. H. H. D. 1978 The effect of flurbiprofen, a potent non-steroidal anti-inflammatory agent, upon *Trypansosoma vivax* infection in goats. *J. Vet. Pharmacol. Ther.* **1**, 69–76.

Van Miert, A. S., van Duin, C. T. H., Schotman, A. J. & Franssen, F. F. 1984 Clinical, haematological and blood biochemical changes in goats after experimental infection with tick-borne fever. *Vet. Parasitol.* **16**, 225–233.

Viteri, F. E., Xunian, L., Tolomei, K. & Martin, A. 1995 True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. *J. Nutr.* **125**, 82–91.

Wing, E. J. & Young, J. B. 1980 Acute starvation protects mice against *Listeria monocytogenes*. *Infect. Immun.* **28**, 771–776.

Wright, A. C., Simpson, L. M. & Oliver, J. D. 1981 Role of iron in the pathogenesis of *Vibrio vulnificus* infections. *Infect. Immun.* **34**, 503–507.

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