

Reductions in total body fat decrease humoral immunity

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Mounting an immune response requires substantial energy, and it is well known that marked reductions in energy availability (e.g. starvation) can suppress immune function, thus increasing disease susceptibility and compromising survival. We tested the hypothesis that moderate reductions in energy availability impair humoral immunity. Specifically, we examined the effects of partial lipectomy (LIPx) on humoral immunity in two seasonally breeding rodent species, prairie voles (*Microtus ochrogaster*) and Siberian hamsters (*Phodopus sungorus*). Animals received bilateral surgical removal of epididymal white adipose tissue (EWATx), inguinal white adipose tissue (IWATx) or sham surgeries and were injected with the antigen keyhole limpet haemocyanin (KLH) either four or 12 weeks after surgery. In prairie voles, serum anti-KLH immunoglobulin G (IgG) did not differ significantly at four weeks. At 12 weeks, serum IgG was significantly reduced in IWATx, but not EWATx animals, compared with sham-operated animals. In Siberian hamsters, both IWATx and EWATx animals reduced serum IgG at four weeks. At 12 weeks, EWATx hamsters displayed a significant compensatory increase in IWAT pad mass compared with sham-operated hamsters, and serum IgG no longer differed from sham-operated animals. There was no significant increase in EWAT in IWATx hamsters compared with sham animals and IgG remained significantly reduced in IWATx hamsters. These results suggest that reductions in energy availability can impair humoral immunity.

Keywords: antibodies; adipose tissue; disease; energetics; immunity

1. INTRODUCTION

All organisms must maintain a balanced energy budget where energy intake is equal to or greater than energy output. For most mammals, maintenance of an energetic budget becomes challenging during times of increased energetic demands when food supplies are at their seasonal nadir (e.g. during winter). To cope with this energetic bottleneck, individuals of many mammalian species have evolved specific physiological adaptations to maintain energy homeostasis, including cessation of winter breeding and induction of torpor and/or hibernation (Bronson & Heideman 1994). Among these adaptations, both prairie voles (*Microtus ochrogaster*) and Siberian hamsters (*Phodopus sungorus*) undergo marked seasonal fluctuations in body mass, primarily in the form of body fat (Bartness & Wade 1985). Specifically, Siberian hamsters housed in short 'winter-like' photoperiods undergo a ca. 20–30% reduction in total body fat, presumably to reduce body size and thus energy demands; small bodies require less food than large bodies, and heat loss is reduced as body surface area is reduced. Interestingly, prairie voles, in contrast with Siberian hamsters, undergo marked *increases* in body fat in short compared with long days (Kriegsfeld & Nelson 1996). In addition to changes in body fat, voles and hamsters also display reduced humoral and cell-mediated immune function in short compared

with long days (Nelson *et al.* 1996; Yellon *et al.* 1999; Demas *et al.* 2001; Drazen *et al.* 2001).

The immune system, as all biological processes, requires substantial energy to maintain 'optimal' functioning. For example, mounting an antibody response to a specific non-replicating antigen results in a significant increase in oxygen consumption and metabolic heat production (Demas *et al.* 1997). More extreme immune challenges (e.g. sepsis) can increase metabolic rate by ca. 30–60% in humans (reviewed in Lochmiller & Deerenberg 2000). It is well-established that starvation impairs immunity and increases susceptibility to disease (Chandra 1996). Furthermore, it has recently been demonstrated that experimental induction of immunity under conditions of starvation significantly impairs subsequent survival (Moret & Schmid-Hempel 2000). It has been suggested that trade-offs among immune function and other energetically expensive processes exist such that individuals may tolerate relatively minor reductions in immunity at times when the energetic costs of mounting an immune response outweigh the benefits (Sheldon & Verhulst 1996; Nelson & Demas 1996; Lochmiller & Deerenberg 2000).

Despite the apparent link between energy availability and immunity, relatively little is known about changes in total body fat and immune function. On the one hand, a chronic positive imbalance between energy intake and expenditure leads to obesity and can impair immune function and increase disease susceptibility in both clinical populations and genetically obese animal models (Marti *et al.* 2001). On the other hand, marked reductions in energy availability without concomitant reductions in energy

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output can also lead to substantial suppression of immunity (Chandra 2002; Nova *et al.* 2002). Free fatty acids, one of the primary constituents of adipose tissue, provide a major fuel source for lymphocytes and may be used preferentially over glucose (Ardawi & Newsholme 1985). Furthermore, free fatty acids can either enhance or inhibit mitogen-induced proliferation of rodent and human lymphocytes *in vitro* (reviewed in Pond 1996). Alterations in free fatty acids can also affect responsiveness to infection and alter the severity of chronic infections (Erickson *et al.* 1992; Yaqoob *et al.* 1994). Immunological disorders (e.g. AIDS) trigger marked changes in whole-body lipid metabolism, suggesting an important role of adipose tissue in immunity (Pond 1996). More recently, the peptide hormone leptin (a member of the cytokine family), secreted primarily by adipose tissue, has been shown to enhance a variety of immunological parameters in rodents and humans (Lord *et al.* 1999; Faggioni *et al.* 2001) and leptin deficiency can increase susceptibility to infections (Faggioni *et al.* 2001). In addition, changes in serum leptin concentrations are positively correlated with measures of immune function in rodents (Drazen *et al.* 2000). Collectively, these findings suggest an important connection between body fat and the regulation of immunity.

A valuable model for studying the effects of energy availability on specific physiological responses is the surgical removal of adipose tissue (lipectomy, LIPx) (Dark *et al.* 1985; Mauer & Bartness 1994; Mauer *et al.* 2001). Partial LIPx reduces total body fat by reducing fat cell number, thus decreasing available energy stored as adipose tissue. Whether manipulations of this type result in permanent or relatively short-term decreases in total body fat, however, is a matter of debate. Interestingly, some studies in highly domesticated species (e.g. laboratory rats (*Rattus norvegicus*)) report a complete lack of fat restoration of the excised pad after LIPx (some compensation in the non-excised pad occurs (Faust *et al.* 1976)), whereas other studies suggest that rats are capable of partial compensation, but only following a prolonged period of time (e.g. six to eight months (Kral 1976)). Unlike highly domesticated rodent species, animals exhibiting naturally occurring seasonal changes in body fat (e.g. Syrian hamsters (*Mesocricetus auratus*) and ground squirrels (*Spermophilus lateralis*)) are generally capable of recovering seasonally appropriate levels of body fat after LIPx (Dark *et al.* 1985; Hamilton & Wade 1988; Mauer & Bartness 1994). For example, in Siberian hamsters (*P. sungorus*), surgical removal of the epididymal white adipose tissue (EWAT) surrounding the testes and epididymides results in a compensatory increase in the remaining intact fat pads (e.g. inguinal white adipose tissue (IWAT)), but only after 12 weeks (Mauer & Bartness 1994). IWAT LIPx, however, does not appear to trigger any subsequent compensatory increases in EWAT, regardless of the duration of recovery (Mauer & Bartness 1994). Thus, it has been suggested that compensatory increases in WAT after LIPx appear to be both species- and fat-pad-specific (Mauer & Bartness 1994).

In the present study we tested the hypothesis that decreases in energy stores (i.e. body fat) impair humoral immunity in two rodent species, prairie voles and Siberian hamsters. These species were chosen because they display robust and reliable seasonal fluctuations in both body fat

and humoral immunity (Nelson *et al.* 1996; Sinclair & Lochmiller 2000). Specifically, if reduced energy availability suppresses humoral immunity in these species, then experimental removal of body fat (partial LIPx) should reduce serum antibody levels. In addition, antibody levels should return to normal when seasonally appropriate levels of body fat are restored.

2. METHODS

(a) *Animals and housing conditions*

Fifty-four adult (older than 60 days of age) male prairie voles (*M. ochrogaster*) were used in experiment one. These animals were obtained from the breeding colony maintained at the Johns Hopkins University. (The progenitors of these animals were originally trapped near Champaign, IL (40.1° N latitude) and were generously provided by Dr C. Sue Carter, University of Illinois at Chicago.) All animals were housed individually in polypropylene cages (28 cm × 17 cm × 12 cm) in colony rooms with a 16 L : 8 D cycle (lights on at 0600 EST). Temperature was kept constant at 20 ± 2 °C and relative humidity was maintained at 50 ± 5%. Food (Prolab 2000) and tap water were available *ad libitum* throughout the experiment. All animals were treated in accordance with the Johns Hopkins University Institutional Animal Care and Use Committee.

Thirty-six adult (older than 60 days of age) male Siberian hamsters (*P. sungorus*) were used in experiment two. These animals were obtained from the breeding colony maintained at Georgia State University and were housed as described in experiment one. (The progenitors of these animals were originally trapped in 1990 and were generously provided by Dr Katherine Wynne-Edwards, Queens University.) Temperature and humidity were as described in experiment one. Food (Purina Rat Chow) and tap water were available *ad libitum* throughout the experiment. All animals were treated in accordance with the Georgia State University Institutional Animal Care and Use Committee.

(b) *Experimental procedures*

One-third of the animals in each experiment received bilateral LIPx of EWAT (EWATx), IWAT (IWATx), or sham surgeries. Surgery was performed under sodium pentobarbital anaesthesia (*ca.* 50 mg kg⁻¹). For EWATx, an abdominal incision was made on the ventral surface of the animal to allow access to both EWAT pads. The EWAT pads were isolated and carefully extirpated, with care taken to minimize disruption of the blood vessels supplying the testes. Half of the sham-operated animals received sham EWATx whereas the remaining animals received sham IWATx. Sham EWATx animals received a similar procedure except that EWAT pads were irrigated with 0.9% saline and returned to the body cavity. For IWATx, bilateral dorsal incisions were made and IWAT was removed by blunt dissection. Sham IWATx animals had IWAT dissected from the skin but not the underlying musculature. After surgeries, all animals were returned to the colony room for either four or 12 weeks. After four weeks, half of the animals from each experimental group received a single subcutaneous injection of 100 µg of the antigen keyhole limpet haemocyanin (KLH), to which all animals were previously naive, suspended in 0.1 ml sterile saline (day 0) and were then returned to the colony room. The remaining animals received injections of KLH after 12 weeks. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*). KLH was used because it gener-

ates a robust antigenic response in rodents, but does not make the animals sick (e.g. prolonged inflammation or fever). Blood was drawn from the retro-orbital sinus at two different sampling periods (days 5 and 10 post-immunization). These sampling periods were chosen to capture basal (day 5) and peak (day 10) immunoglobulin G (IgG) production (the predominant Ig class present in blood) during the course of the immune response to KLH (Demas *et al.* 1997; Drazen *et al.* 2000). On each sampling day, animals were brought into the surgery room individually, lightly anaesthetized with methoxyflurane vapours (Metofane; Pittman-Moore, Mundelein, IL), and blood samples (500 μ l) were drawn from the retro-orbital sinus between 1000 and 1200 EST. Samples were allowed to clot for 1 h, the clots were removed and the samples centrifuged (at 4 °C) for 30 min at 2500 rpm. Serum portions were aspirated and stored in sealable polypropylene microcentrifuge tubes at -80 °C until assayed for IgG. On the last day of sampling (day 10) animals were killed by cervical dislocation. EWAT pads were removed from IWATx and sham animals, IWAT pads were removed from EWATx and sham animals, retroperitoneal WAT (RWAT) and spleens were removed from all animals. All tissues were cleaned of connective tissue and weighed to the nearest 0.1 mg by laboratory assistants who were naive to the experimental hypotheses and treatment assignments.

(c) Assessment of humoral immunity

To assess humoral immunity, serum anti-KLH IgG concentrations were assayed by using an enzyme-linked immunosorbent assay. Microtiter plates were coated with antigen by incubating overnight at 4 °C with 0.5 mg ml⁻¹ KLH in sodium bicarbonate buffer (pH 9.6), 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 nonspecific binding, and washed again with PBS-T. Thawed serum samples were diluted 1 : 20 with PBS-T, and 150 μ l of each serum dilution was added in duplicate to the wells of the antigen-coated plates. Positive control samples (pooled sera from hamsters or voles previously determined to have high levels of anti-KLH antibody, similarly diluted with PBS-T) and negative control samples (pooled sera from KLH-naive hamsters or voles, similarly diluted with PBS-T) were also added in duplicate to each plate; plates were sealed, incubated at 37 °C for 3 h, then washed with PBS-T. Secondary antibody (alkaline phosphatase-conjugated-anti-mouse IgG diluted 1 : 2000 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 washed again with PBS-T and 150 μ l of the enzyme substrate p-nitrophenyl phosphate (Sigma Chemical, St Louis, MO (1 mg ml⁻¹ in diethanolamine substrate buffer)) was added to each well. Plates were protected from light during the enzyme-substrate reaction, which was terminated after 20 min by adding 50 μ l 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 intra-assay variability, the mean OD for each sample was expressed as a percentage of its plate positive control OD for statistical analyses.

(d) Statistical analyses

Because sham EWATx and IWATx animals did not differ in any physiological parameter measured, the data from these

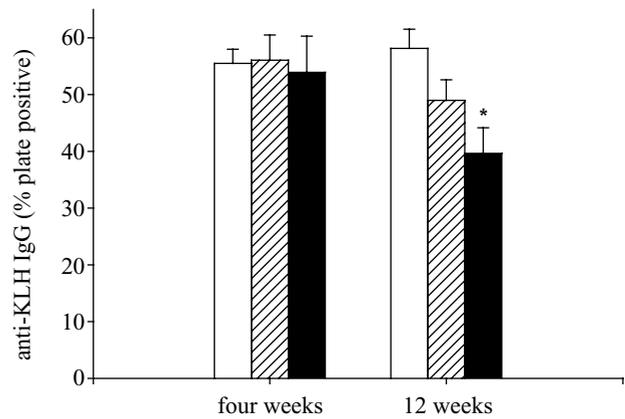


Figure 1. Mean (\pm s.e.m.) serum anti-KLH IgG levels in prairie voles receiving EWATx (hatched bars), IWATx (filled bars), or sham surgeries (open bars). Significant differences between pairwise means are indicated by an asterisk if $p < 0.05$.

groups were combined into a single sham group for all statistical analyses. Body masses were analysed by using a two-way mixed model analysis of variance (ANOVA). Tissue mass and IgG data were analysed using separate two-way between-groups ANOVAs (Sigma Stat, Jandel Scientific, San Rafael, CA). Differences between groups within each time period were assessed by using individual one-way ANOVAs. *Post hoc* comparisons between pairwise means were conducted using Tukey-HSD tests when the overall ANOVAs were significant. In all cases, differences between group means were considered statistically significant if $p < 0.05$.

3. RESULTS

(a) Experiment one

As expected, serum anti-KLH IgG was basal at day 5 post-immunization and did not differ among the experimental groups (data not shown). At four weeks, serum anti-KLH IgG at day 10 did not differ among any of the experimental groups ($p > 0.05$). At 12 weeks, however, serum IgG was significantly reduced in IWATx voles compared with EWATx or sham-operated animals ($F_{2,19} = 6.84$; $p < 0.05$); there was no significant difference between EWATx and sham-operated animals ($p > 0.05$; figure 1). There was no significant difference in IWAT pad mass between EWATx and sham-operated animals ($p > 0.05$) or in EWAT pad mass between IWATx and sham-operated animals ($p > 0.05$) at either four or 12 weeks ($p > 0.05$; figure 2*a,b*). There were no significant differences among body or RWAT masses among any of the groups at either four or 12 weeks ($p > 0.05$; figure 2*c*; table 1).

(b) Experiment two

As in experiment one, anti-KLH IgG was basal in day 5 serum samples and did not differ among experimental groups (data not shown). At four weeks, both EWATx and IWATx hamsters had significantly reduced anti-KLH IgG at day 10 compared with sham-operated animals ($F_{2,15} = 13.05$; $p < 0.05$; figure 3). There was no significant difference in IWAT pad mass between EWATx and sham-operated animals ($p > 0.05$) or in EWAT pad mass

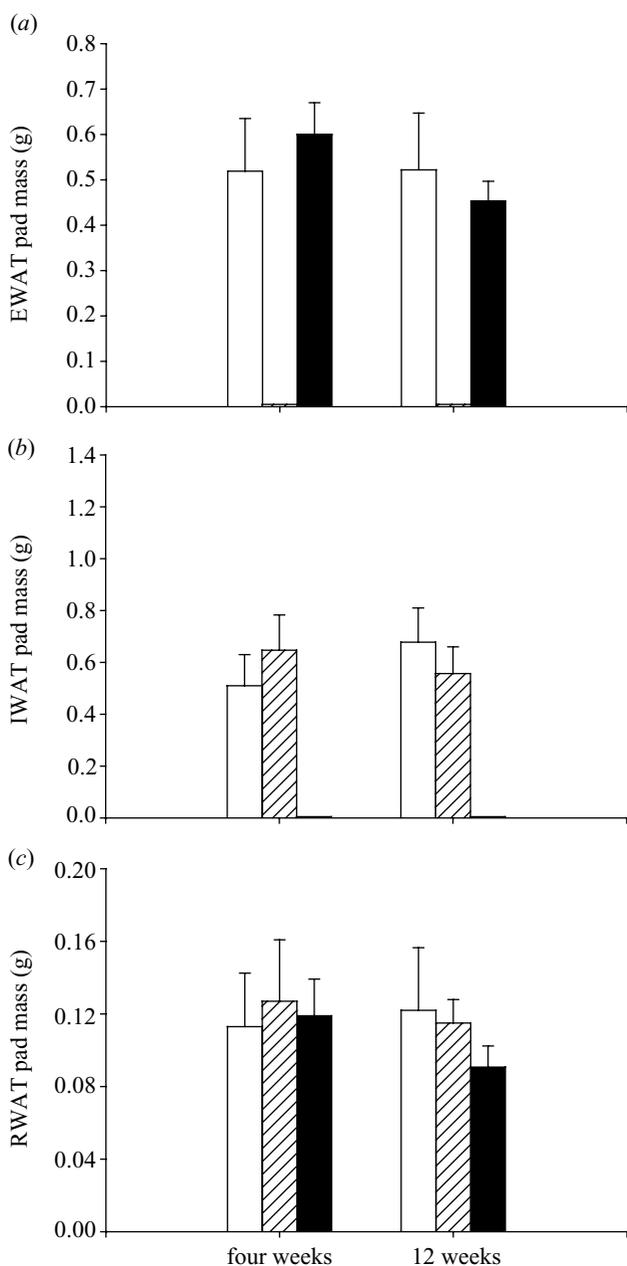


Figure 2. Mean (\pm s.e.m.) fat pad masses of (a) EWAT, (b) IWAT and (c) RWAT in prairie voles receiving bilateral EWATx (hatched bars), IWATx (filled bars), or sham surgeries (open bars). Statistical conventions are as described in figure 1.

between IWATx and sham-operated animals ($p > 0.05$; figure 4b). At 12 weeks, IWATx hamsters had significantly reduced serum anti-KLH IgG compared with either EWATx or sham-operated hamsters ($F_{2,15} = 16.57$; $p < 0.05$; figure 3). EWATx and sham-operated animals did not differ in serum anti-KLH IgG ($p > 0.05$). EWATx hamsters displayed a significant compensatory increase in IWAT pad mass compared with sham-operated hamsters ($F_{1,10} = 9.90$; $p < 0.05$; figure 4b). EWAT pad mass of IWATx hamsters, however, did not differ from sham-operated hamsters ($p > 0.05$; figure 4a). There were no significant differences between body or RWAT pad masses among any of the groups at either four or 12 weeks ($p > 0.05$; figure 4c; table 2).

Table 1. Mean (\pm s.e.m.) body masses of sham-operated prairie voles and voles receiving EWATx or IWATx 4 or 12 weeks after surgical manipulations.

	initial body mass (g)	terminal body mass (g)
week four		
sham	41.09 \pm 1.77	40.98 \pm 2.60
EWATx	43.48 \pm 2.32	41.51 \pm 2.38
IWATx	44.55 \pm 2.88	44.90 \pm 2.49
week 12		
sham	43.68 \pm 2.95	43.03 \pm 3.37
EWATx	39.70 \pm 1.12	40.26 \pm 1.09
IWATx	45.09 \pm 4.19	40.04 \pm 1.65

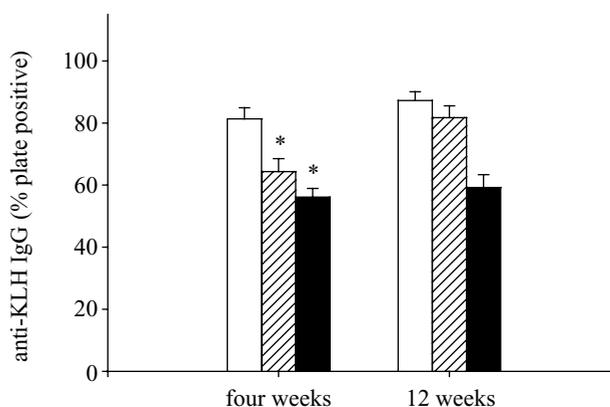


Figure 3. Mean (\pm s.e.m.) serum anti-KLH IgG levels in Siberian hamsters receiving EWATx (hatched bars), IWATx (filled bars), or sham surgeries (open bars). Statistical conventions are as described in figure 1.

4. DISCUSSION

The results of the present study demonstrate that reductions in total body fat reduce humoral immunity in both prairie voles and Siberian hamsters. Specifically, IWATx reduced humoral immunity in both species, whereas EWATx reduced immunity in Siberian hamsters, but not prairie voles. Consistent with previous findings, Siberian hamsters experienced a compensatory increase in IWAT after EWATx at 12 weeks and immune function returned to pre-LIPx levels. By contrast, prairie voles did not experience any compensation after either EWATx or IWATx and thus humoral immunity was reduced in IWATx hamsters after 12 weeks compared with sham-operated animals. Collectively, these results suggest that reductions in total body fat can reduce humoral immunity in both prairie voles and Siberian hamsters and these changes in immune function parallel changes in total body fat in these species.

In common with previous reports suggesting decreases in energy availability can impair immunity (reviewed in Lochmiller & Deerenberg 2000), the results of the present study support the notion of a trade-off between energy availability and immune function to the extent that immunity is compromised at times when long-term energy stores are reduced. Surgical removal of EWAT and IWAT in the present study typically results in a lipid deficit of ca. 5% and 10% in EWATx and IWATx animals, respectively

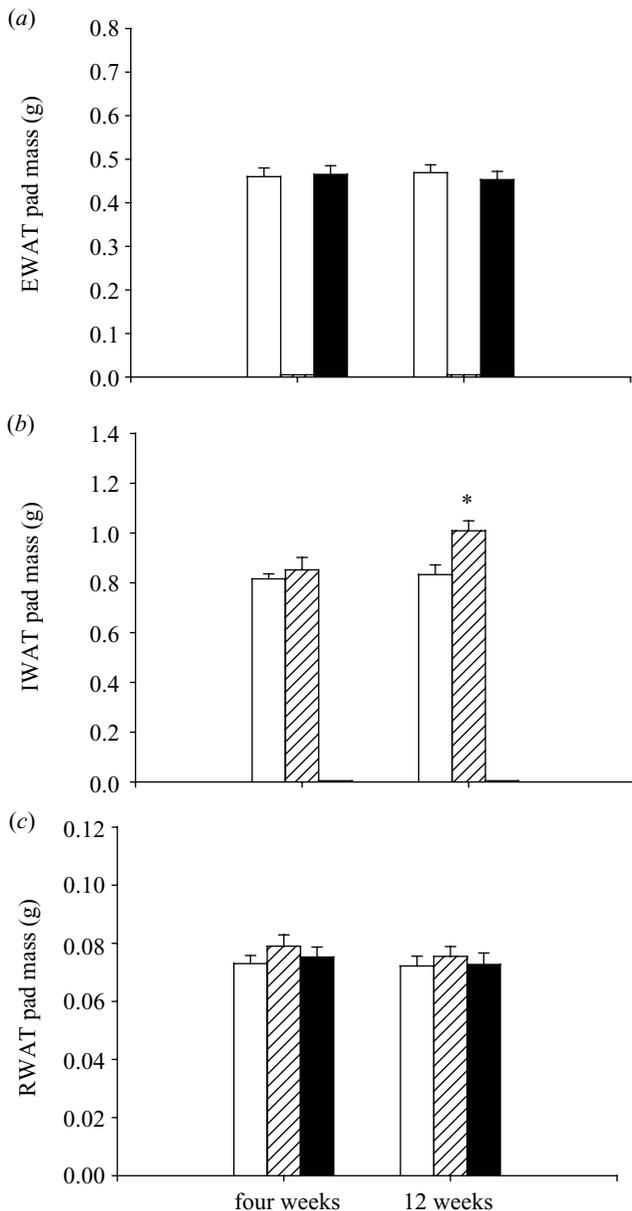


Figure 4. Mean (\pm s.e.m.) fat pad masses of (a) EWAT, (b) IWAT and (c) RWAT in Siberian hamsters receiving EWATx (hatched bars), IWATx (filled bars), or sham surgeries (open bars). Statistical conventions are as described in figure 1.

(Mauer & Bartness 1994). Because these relatively small amounts are not necessarily a substantial proportion of the total body fat of prairie voles or Siberian hamsters, it may be argued that partial surgical LIPx is not a biologically meaningful manipulation of total body fat (cf. Mauer & Bartness 1994). However, given that these modest reductions in body fat elicited marked reductions in antibody production and triggered compensatory increases in the remaining fat pads (at least in Siberian hamsters), we would argue that these rodents are clearly responsive to these manipulations and, thus, partial LIPx provides a meaningful, biologically relevant model with which to examine the interactions between total body fat and immunity. As a general rule, the greater the LIPx-induced deficit, the greater the compensatory fat pad mass increase is (Mauer & Bartness 1997). Although greater

Table 2. Mean (\pm s.e.m.) body masses of sham-operated Siberian hamsters and hamsters receiving EWATx or IWATx four or 12 weeks after surgical manipulations.

	initial body mass (g)	terminal body mass (g)
week four		
sham	41.27 \pm 1.23	43.47 \pm 1.20
EWATx	41.04 \pm 1.41	43.58 \pm 1.37
IWATx	42.06 \pm 1.26	43.31 \pm 1.02
week 12		
sham	40.66 \pm 1.24	43.51 \pm 0.97
EWATx	41.13 \pm 1.49	43.12 \pm 1.17
IWATx	40.85 \pm 0.94	43.48 \pm 0.68

immunological impairments may occur with additional lipid deficits, these manipulations would require more invasive surgeries (e.g. removal of more internally located fat pads) which could possibly compromise survival of the animals.

Although the precise mechanisms mediating changes in immune function in response to changes in total body fat are not known and were not examined in the present study, one possibility is that reduced immunity in LIPx animals may be due to decreases in circulating leptin. Leptin is a peptide hormone produced almost exclusively by adipose tissue and is released in direct proportion to body fat; thus circulating leptin concentrations are a relatively accurate reflection of total body fat (Klingenspor *et al.* 2000; Woods & Seeley 2000). A recent study from our laboratory has provided support for this hypothesis. Specifically, Siberian hamsters housed in short days for 10 weeks displayed significant reductions in body fat, as well as reduced humoral immunity. Administration of exogenous leptin restored short-day reductions in immunity to levels comparable with long day animals; leptin, however, had no effect on immunity in long-day animals (Drazen *et al.* 2001). The present results suggest that short-day decreases in humoral immunity in Siberian hamsters are due, at least in part, to reductions in body fat. The extent to which modest reductions in total body fat (e.g. 5–10% in the present study) reduce circulating leptin concentrations, however, is not known. Thus, whether LIPx-induced decreases in immunity are due to decreased leptin or rather, occur via a leptin-independent mechanism requires further research. For example, mice with genetic alterations in leptin (e.g. ob/ob and db/db mice) are able to compensate following LIPx despite the lack of functional leptin or leptin receptors, respectively, suggesting that the regulation of total body fat is independent of leptin, at least in these mice (Harris *et al.* 2002).

It is interesting to note that, although LIPx reduced anti-KLH antibodies in prairie voles and Siberian hamsters, there were noteworthy differences in physiological responses between these species. For example, prairie voles experienced reduced humoral immunity, but only after prolonged reductions in body fat (e.g. 12 weeks). By contrast, Siberian hamsters displayed reduced immunity after only four weeks. Although it is not known why these species differ in their responsiveness to LIPx, this difference may be due to differences in hormonal 'feedback'. For example, immunological responsiveness to lev-

els of total body fat may be mediated by a hormonal feedback signal (such as leptin suggested above). As with most endocrine signals, there can be a substantial time-lag between removal of the source of the hormone (e.g. LIPx) and a concomitant decrease in the signal (e.g. serum leptin concentrations). It is possible that prairie voles and Siberian hamsters differ in the time-course for this hormonal feedback. Alternatively, the immune system of Siberian hamsters may simply be more responsive to decreases in body fat compared with prairie voles. Recall that Siberian hamsters and prairie voles differ in their physiological responsiveness to changing day lengths with Siberian hamsters undergoing decreases and prairie voles displaying increases in body fat in short days. This difference may underlie the differences in metabolic responses to LIPx between the two species. Although this hypothesis is possible, it is unlikely given that Syrian hamsters, a species that also increases body mass in short days, is capable of compensatory increases in body fat following LIPx (Hamilton & Wade 1988).

Another important difference between prairie voles and Siberian hamsters is that Siberian hamsters, unlike prairie voles, demonstrated compensatory increases in the remaining fat pads after EWATx. These findings are consistent with previous findings in hamsters (Mauer & Bartness 1994). Prairie voles, by contrast, failed to display compensatory increases in WAT in response to LIPx, consistent with previous reports in rats (Faust *et al.* 1976). It is unclear why prairie voles did not display increased WAT pad mass typical of Siberian hamsters. One possible reason is that prairie voles are capable of compensatory increases in WAT after LIPx, but that 12 weeks was an insufficient amount of time for such compensation to occur. Alternatively, consistent with the delayed immune responsiveness to LIPx in voles discussed above, prairie voles may be generally less responsive to changes in total body fat.

The results of the present study suggest that modest reductions in energy availability in the form of reduced body fat can reduce humoral immune function, and the restoration of seasonally appropriate levels of body fat ameliorate these reductions in immunity. Given that changes in immunity can have marked consequences for disease resistance, these findings support the idea that reduced energy availability can lead to increased disease susceptibility in a variety of mammalian species. If the present results generalize to humans, then modest decreases in total body fat may also lead to reduced immunity and possibly increased disease (see Nelson *et al.* 2002). Future studies are needed to address this important issue.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.