REVIEW

Regulation of body mass and adiposity in the field vole, *Microtus agrestis*: a model of leptin resistance

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Abstract

Adult mammals are typically highly resistant to perturbations in their energy balance. In obese humans, however, this control appears to be lost. Apart from a few exceptional cases, this loss of control occurs despite appropriate levels of circulating leptin – suggesting that elevated adiposity may be a consequence of failure to respond to the leptin signal: leptin resistance. When cold-acclimated male field voles (*Microtus agrestis*) are transferred from short (SD, 8 h light) to long (LD, 16 h light) photoperiods, they increase dramatically in body mass and fatness for about 4 weeks. After this period, their mass stabilizes at a new plateau about 25% higher than animals maintained in SD. The increase in adiposity is not caused by significant increases in food intake, but reflects an increase in digestive efficiency. Measures of circulating leptin reveal that the increased adiposity is matched by increased circulating leptin. By infusing voles with exogenous leptin, we have demonstrated that SD voles are leptin sensitive (reducing both body mass and food

intake), whereas LD animals are leptin resistant. Voles may therefore be a useful model for understanding the process of leptin resistance. The change in leptin sensitivity in voles was not associated with changes in the levels of gene expression of the orexogenic or anorexogenic neuropeptides, such as neuropeptide Y, agouti-related peptide, POMC and cocaine- and amphetamine-regulated transcript, measured in the hypothalamic arcuate nucleus (ARC). During the phase that body mass was increasing, however, there was a transient increase in the ARC expression of suppressor of cytokine signalling-3 (SOCS3). These data suggest that the changes in the expression of SOCS3 in the ARC may be involved in leptin resistance. However, the mechanism by which these changes may be linked to alterations in digestive efficiency that underpin the changes in adiposity, or how the differences are signalled by changes in photoperiod, remains unclear.

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Introduction

There is a widespread belief that body mass and body fatness are regulated phenomena. This stems from observations in many mammal species that adult body mass and body fatness remain stable over protracted periods of time, in spite of changes in the availability and quality of food and levels of energy expenditure (Stallone & Stunkard 1991, Rosenbaum *et al.* 1997, Schwartz & Seeley 1997, Levin & Keesey 1998). The exact nature of the system regulating body mass and fatness, however, remains unclear, and a number of alternative models have been proposed that are all broadly consistent with the existing data (reviewed in Mercer & Speakman 2001, Speakman 2004). One model, originally proposed by Kennedy (1953) is that body fatness is regulated by a lipostatic system. In this system, body fat generates a signal that travels to the brain where it is compared with a target. Deviations of the adiposity signal away from the target, trigger

compensatory responses in energy intake and expenditure, which restore the defended level of body fatness (Schwartz *et al.* 2000). The discovery of the adipokine leptin (Zhang *et al.* 1994) and the signalling form of its receptor LRB (Tartaglia *et al.* 1995) provided a candidate molecular basis for the lipostatic control of adiposity. Subsequent characterization of the signalling system downstream from LRB, involving both orexogenic and anorexogenic neuropeptides located in the various nuclei of the hypothalamus, has greatly strengthened support for the lipostatic model of body fatness regulation (Schwartz *et al.* 2000, Berthoud 2002).

Obese humans do not appear to have the capability to regulate their weight and adiposity. Yet, apart from a few unusual cases (e.g. Montague *et al.* 1997), their adipose tissue seems to generate an appropriate leptin ‘adiposity’ signal (Considine *et al.* 1996). Treating humans that already have high levels of circulating leptin with recombinant leptin generates significant weight loss, but much less than might be

anticipated if the lipostatic signalling system were intact (Heymsfield *et al.* 1999). Focus of attention has therefore primarily been directed to understanding why the leptin signal from fat tissue does not generate an appropriate regulatory response – the so-called phenomenon of ‘leptin resistance’.

Many small mammals inhabiting temperate and arctic regions exhibit annual cycles in body mass and adiposity (e.g. Heldmaier & Steinlechner 1981, Stebbins 1984, Bartness & Wade 1985, Klingenspor *et al.* 1996, Bartness *et al.* 2002, Drazen *et al.* 2002). These seasonal changes are associated with corresponding alterations in circulating leptin. The seasonal change in body adiposity in animals, which are normally highly resistant to perturbations in their energy balance, provides a potentially useful model for exploring the phenomenon of leptin resistance. Since many of these annual changes require time to develop, they must be initiated in anticipation of the forthcoming season and many rodents rely on environmental cues such as increasing or decreasing day lengths to initiate the changes (e.g. Dark *et al.* 1983, Mrosovsky 1983, Bartness *et al.* 2002). The responses to changing photoperiod can be readily induced in the laboratory by acutely transferring animals between long- and short-day lengths. This amenability to manipulation makes such species attractive to study, and they may provide valuable insights into the mechanisms underlying regulation of body mass (Mercer & Speakman 2001, Bartness *et al.* 2002, Schuhler & Ebling 2006).

Although seasonal patterns of changes in body mass and fatness occur in a variety of mammalian species, they have been studied most extensively in the Siberian or Djungarian hamster (*Phodopus sungorus*; Steinlechner & Heldmaier 1982, Bartness & Goldman 1988, Ebling 1994, Klingenspor *et al.* 2000, Mercer *et al.* 2000, 2001), Syrian or golden hamster (*Mesocricetus auratus*; Campbell & Tabor 1983, Bartness & Wade 1985) and collared lemming (*Dicrostonyx groenlandicus*; Reynolds & Lavigne 1989, Nagy 1993, Nagy & Negus 1993, Nagy *et al.* 1994, Hunter & Nagy 2002). Some of these studies suggest that long-term physiological state is adjusted to a continuously altered set point that is regulated by seasonal changes in day length. The concept of the sliding body mass set point has been summarized in several previous reviews (e.g. Morgan & Mercer 2001, Bartness *et al.* 2002, Morgan *et al.* 2003, Schuhler & Ebling 2006). In the current review, we summarize our work on a novel seasonal model of leptin resistance – the field vole (*Microtus agrestis*; Król *et al.* 2005, 2006, 2007). The review is structured in three parts. In part one, we describe the general features of the photoperiod-induced changes in adiposity in the field vole, and measurements that reveal the energetic basis for the increase in fat mass. In the second part, we show that voles change their leptin sensitivity under different photoperiod regimes. Finally, we present some data on gene expression in the hypothalamic arcuate nucleus (ARC), a key site of both leptin signalling in the brain (Schwartz *et al.* 2000) and region-specific leptin resistance (Münzberg *et al.* 2004).

Photoperiod-induced adiposity in the field vole – energetics aspects

The pattern of change in body mass of cold-acclimated voles transferred from short-day (SD, 8 h light) to long-day (LD, 16 h light) photoperiod is illustrated in Fig. 1. During exposure to SD, body mass was stable and averaged 25.6 ± 3.3 g. However, on exposure to LD, body mass increased from a mean of 26.2 ± 3.7 g on day 0 to 36.3 ± 5.2 g on day 28. Following this period of dramatic increase, the voles regained stability over their mass which then remained stable at around 37.7 ± 5.4 g. This increase in body mass was associated with significant increases in the masses of all body components, including dry fat mass, dry lean mass and body water mass. At the organ level, voles killed during the plateau phase had significantly heavier dry masses of brown adipose tissue, subcutaneous, epididymal, perirenal and retroperitoneal fat depots (all $P < 0.001$).

Food intake of LD voles increased significantly throughout the experiment ($P < 0.001$, Fig. 2). Prior to LD exposure (days –14 to 0), food intake was constant and averaged 6.1 ± 0.8 g/day. Following LD exposure, voles increased their daily food intake from a mean of 6.3 ± 0.9 g on day 0 to 7.8 ± 1.2 g on day 28 (all comparisons between days 14–28 and days –14 to 0, $P < 0.05$). Over the next 4 weeks, food intake stabilized at a level of 8.1 ± 1.2 g/day. The trajectory of food intake (Fig. 2) for voles exposed to LD closely resembled the changes in body mass (Fig. 1). For SD voles, daily food intake averaged 6.2 ± 0.9 g on day –14 and 7.2 ± 1.1 g on day 56 (Fig. 2). To investigate the effect of photoperiod on food intake in relation to changes in body mass, we compared food intake of LD voles during the pre-exposure, increase and plateau phases, including body mass as a covariate in the analysis. Once body mass was included in the model, the effect of LD on food intake was not significant (photoperiod effect, $P = 0.95$). This suggests that LD voles responded to increases in body mass by increasing their food intake rather than the reverse.

Figure 1 Effect of exposure to long-day photoperiod (LD, 16 h light) on mean body mass of 12 male field voles measured between days –24 and 56 (day 0 is the day of exposure to LD). Data for 11 voles kept in short-day photoperiod (SD, 8 h light) are also shown. Error bars indicate 1 s.d.

Figure 2 Effect of exposure to long-day photoperiod (LD, 16 h light) on mean food intake of 12 male field voles measured between days -24 and 56 (day 0 is the day of exposure to LD). Data for 11 voles kept in short-day photoperiod (SD, 8 h light) are also shown. Error bars indicate 1 s.d.

The apparent digestive efficiency (calculated as the percentage of gross energy intake that was digested) averaged $74.2 \pm 5.5\%$ ($n=7$) in SD voles, $81.4 \pm 5.5\%$ ($n=8$) in voles during the increase phase and $80.3 \pm 4.1\%$ ($n=8$) during the plateau phase. These means were significantly different ($P=0.031$), with the digestive efficiency of LD voles during the increase and plateau phases being higher than in SD animals ($P<0.05$). Using the digestive efficiency data, we calculated metabolizable energy intake (MEI) for LD voles monitored between days -24 and 56 during the pre-exposure, increase and final plateau phases. After adjusting for differences in body mass, the effect of LD on MEI was significant (photoperiod effect, $P=0.006$), with MEI during the increase and plateau phases being significantly higher than during the pre-exposure phase ($P<0.05$; Fig. 3). The rate at which voles assimilated energy during the pre-exposure,

Figure 3 Metabolizable energy intake (MEI) as a function of body mass for 12 male field voles prior to exposure to long-day photoperiod (LD, 16 h light), during the increase phase (days 1–28 of LD exposure) and during the plateau phase (days 29–56 of LD exposure). Prior to exposure to LD, the animals were kept in short-day photoperiod (SD, 8 h light). After adjusting for differences in body mass, the effect of LD on MEI was significant. The relationships are described by $y=27.7+2.0x$ for pre-exposure, $y=26.0+2.4x$ for LD increase and $y=30.4+2.2x$ for LD plateau.

increase and plateau phases (adjusted to a common body mass of 32.7 g) averaged 95.3 , 103.7 and 102.9 kJ/day respectively.

Circulating leptin levels and leptin resistance

Levels of circulating leptin, in voles culled across the short- to long-day manipulation were positively correlated with dry fat mass ($r=0.84$, $P<0.001$; Fig. 4). The effects of photoperiod and day of exposure on the leptin levels were not significant if fat mass was included as an independent covariate. The voles were therefore generating an appropriate leptin signal for their changing adiposity as they progressed from SD to LD.

We implanted voles in both the SD conditions and during the increasing phase of exposure to LD (day 10 post-photoperiod switch) with mini-osmotic pumps, delivering either saline (PBS) or recombinant murine leptin for 7 days. The levels of circulating leptin averaged 5.8 ± 1.8 (LD-PBS), 21.2 ± 10.6 (LD-leptin), 4.7 ± 1.8 (SD-PBS) and 24.6 ± 11.5 (SD-leptin) ng/ml ($n=9$ for all means).

To evaluate the effect of photoperiod and leptin treatment on body mass, we calculated a base line body mass for each vole (mean body mass for days -4 to 0) and expressed all masses between days 2 and 17 post-photoperiod change as the difference from the base line (Fig. 5). In LD photoperiod, the day-to-day changes in body mass of leptin-infused voles over days 2–17 (prior to surgery and during the treatment) were not significantly different from those in PBS-infused controls. In contrast, the pattern of body mass changes in SD leptin-infused voles before and after surgery was significantly different from that of SD PBS-infused animals ($P<0.001$). This difference was related to days 16 and 17, when the body mass of leptin-infused voles became significantly lower than in PBS-infused controls. On average, the SD leptin-infused voles weighed 27.9 ± 4.4 g on day 10 and 25.5 ± 4.3 g on day 17.

Mean food intake at the beginning of the experiment (day -4) was 6.4 ± 0.8 (LD-leptin), 6.4 ± 0.9 (LD-PBS),

Figure 4 Serum leptin concentration plotted against the dry fat mass for male field voles exposed to either short-day photoperiod (SD, 8 h light, $n=38$) or long-day photoperiod (LD, 16 h light, $n=52$). The fitted line represents reduced major axis regressions for the pooled data ($n=90$).

Figure 5 Mean body mass difference from the base line for leptin- and PBS-infused male field voles exposed to long- (LD, 16 h light) and short-day (SD, 8 h light) photoperiod ($n=9$ for each group). Day 0 is the day of exposure to LD. Mini-osmotic pumps were implanted on day 10, after recording body mass. The base line was calculated for each vole as a mean body mass prior to exposure to LD (days -4 to 0). Arrows indicate period of leptin/PBS infusion. Error bars represent 1 s.d.

6.8 ± 0.9 (SD-leptin) and 6.3 ± 0.8 (SD-PBS) g/day (Fig. 6). Prior to leptin treatment (days 2–10), the LD and SD voles did not differ in their food intake. This is consistent with our previous results, which indicated that the food intakes of LD and SD voles were not significantly different until day 18 of exposure to LD (above and Król *et al.* 2005). All voles responded to the implantation of mini-osmotic pumps by decreasing their food intake on day 12 compared with day 10. In LD and SD voles infused with PBS, this decrease averaged 0.9 ± 0.8 and 0.9 ± 1.0 g/day respectively. Between days 14 and 17, the food intake of LD and SD voles infused with PBS returned to the level observed prior to surgery. In LD photoperiod, changes in food intake of leptin-infused voles over days 2–17 were similar to the changes observed in PBS-infused controls. In the SD photoperiod, however, the response of leptin-infused voles was significantly different to PBS-infused animals ($P=0.041$). Specifically, SD leptin- and PBS-infused voles did not differ in food intake between days 2 and 12 ($P>0.05$), but on days 14–17, the voles infused with leptin ate significantly less food than those infused with PBS (day 14, $P=0.022$; day 16, $P=0.016$; day 17, $P=0.030$). Over the 7-day leptin treatment (days 10–17), the difference

Figure 6 Mean food intake difference from the base line for leptin- and PBS-infused male field voles exposed to long- (LD, 16 h light) and short-day (SD, 8 h light) photoperiod ($n=9$ for each group). Day 0 is the day of exposure to LD. Mini-osmotic pumps were implanted on day 10, after recording food intake. The base line was calculated for each vole as a mean food intake prior to exposure to LD (days -4 to 0). Arrows indicate period of leptin/PBS infusion. Error bars represent 1 s.d.

between mean absolute food intakes of SD PBS- and leptin-infused voles was 2.5 g.

Neuroendocrine correlates of leptin sensitivity and resistance

We examined hypothalamic *ARC* gene expression of suppressor of cytokine signalling-3 (SOCS3) and four leptin-responsive neuropeptides that are important to energy homeostasis – neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). mRNA levels were quantified by *in situ* hybridization in 20 μ m coronal sections using techniques described previously (Simmons *et al.* 1989, Mercer *et al.* 1995, 1997). Antisense riboprobes complementary to fragments of hamster SOCS3 (Tups *et al.* 2004), rat NPY (Mercer *et al.* 1995), hamster AgRP and POMC (Mercer *et al.* 2000) and rat CART (Adam *et al.* 2000) were transcribed from cloned cDNA templates. Coronal hypothalamic sections of vole brains were cut on a cryostat and collected throughout the extent of the ARC onto a set

of eight slides, with six or seven sections mounted on each slide. Autoradiographic images were quantified using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD, USA). Image analysis was performed on four to five sections for SOCS3, NPY, AgRP and POMC, and on three to four sections for CART. Final results were expressed as the mean value of the three highest readings (SOCS3, NPY, AgRP and POMC) or as the mean value of the two highest readings (CART).

The distribution of SOCS3, NPY, AgRP, POMC and CART mRNAs in the field vole hypothalamus was consistent with other rodent species (Mercer *et al.* 2000, Peacock *et al.* 2004, Tups *et al.* 2004). Although the probe for SOCS3 hybridized to the ARC, ventromedial and dorsomedial nuclei, expression outside the ARC was highly variable and generally not strong enough to allow quantification by image analysis. Apart from SOCS3, the ARC was also the main expression site for NPY, AgRP and POMC mRNAs. CART mRNA was widespread throughout the hypothalamus and also strongly expressed in the ARC.

Hypothalamic ARC gene expression of SOCS3, NPY, AgRP, POMC and CART was highly correlated with dry fat mass. The correlation was positive for SOCS3 ($r=0.62$, $P<0.001$) and catabolic neuropeptides POMC ($r=0.47$, $P<0.001$) and CART ($r=0.52$, $P<0.001$), but negative for anabolic neuropeptides NPY ($r=-0.59$, $P<0.001$) and AgRP ($r=-0.56$, $P<0.001$). There was a significant effect of photoperiod on the hypothalamic ARC gene expression of SOCS3 (dry fat mass, $P<0.001$; photoperiod, $P<0.001$; day of exposure, $P=0.105$; interaction photoperiod $\times$ day, $P=0.005$; Fig. 7). As indicated by the significant interaction between photoperiod and day of exposure, temporal changes in SOCS3 mRNA in LD voles differed from those in SD voles. At the early stage of exposure (day 3), there was no significant difference in SOCS3 expression between LD and SD voles ($P>0.05$). The levels of SOCS3 mRNA in LD voles then increased to 82.4% above the SD levels on days 10–17 ($P=0.001$) and to 69.0% above the SD levels on days 24–31 ($P=0.012$). However, this increase was not maintained, and on days 38–52 and 59–73, SOCS3 expression in LD voles returned to the same level as in the SD controls ($P>0.05$). In contrast to SOCS3, expression of AgRP, NPY, POMC and CART was unaffected by photoperiod or day of exposure.

Discussion

Seasonal body mass changes in field voles are characterized by a clear delineation between two levels where animals regulate

Figure 7 Hypothalamic ARC gene expression of SOCS3, NPY, AgRP, POMC and CART in male field voles exposed to either short- (SD, 8 h light) or long-day (LD, 16 h light) photoperiod for 3, 10–17, 24–31, 38–52 and 59–73 days ($n=90$ in total). Data are least squares means adjusted to a common dry fat mass of 2.0309 g ($n=90$). P values indicate significant differences between the means, as assessed by Tukey *post hoc* comparisons. Error bars represent 1 s.d.

their body mass and body fatness, and by the rapid rate at which they switch between these levels (Król *et al.* 2005; Fig. 1). During this transition in adiposity, serum leptin concentrations increase as a direct function of dry fat mass. The highly significant positive correlation between leptin levels and dry fat mass (Fig. 4) is consistent with the similar pattern observed in many other species (e.g. Frederich *et al.* 1995, Considine *et al.* 1996, Wolden-Hanson *et al.* 1999) as well as the potential role of leptin as an adipostatic signal (e.g. Friedman & Halaas 1998, Woods *et al.* 2000). Therefore, the absence of a response to the increasing levels of adiposity was not because the voles modulated the peripheral production of leptin. This observation corresponds with similar patterns observed in other seasonal mammals (e.g. Drazen *et al.* 2000, Concannon *et al.* 2001, Johnson *et al.* 2004, Li & Wang 2005).

Peripheral infusion with murine leptin for 7 days through mini-osmotic pumps, allowed us to demonstrate that SD voles are sensitive to both weight-reducing and appetite-reducing effects of leptin (Król *et al.* 2006). Conversely, LD voles infused with leptin between days 10 and 17 of exposure showed no significant response, indicating a state of leptin resistance. The changes in responsiveness to leptin treatment induced by photoperiod were similar to those observed in Siberian hamsters (Atcha *et al.* 2000, Klingenspor *et al.* 2000, Rousseau *et al.* 2002). A novel aspect of our study is the demonstration that the development of leptin resistance in voles starts as early as 10–17 days, following transfer from SD to LD. In contrast, comparable studies of Siberian hamsters focused on the changes in leptin sensitivity that occurred at least 8 weeks after the photoperiod switch (Atcha *et al.* 2000, Klingenspor *et al.* 2000, Rousseau *et al.* 2002). In voles, the period between days 10 and 17 of exposure to LD corresponded with the highest increase in hypothalamic ARC gene expression of SOCS3, an inhibitor of intracellular leptin signalling, which rose to 82.4% above the SD levels (Fig. 7). The simultaneous occurrence of a diminished responsiveness to leptin along with hypothalamic induction of SOCS3 and rapid body mass increase in LD voles, clearly suggests that their resistance to leptin may involve SOCS3-mediated inhibition of the leptin signal.

The importance of SOCS3 in mediating seasonal changes in leptin sensitivity may be more generally applicable. Specifically, transfer of male Siberian hamsters from LD to SD conditions was associated with rapid down-regulation of hypothalamic SOCS3 mRNA followed by a decrease in body mass (Tups *et al.* 2004), whereas transfer back from SD to LD induced an increase in SOCS3 expression that preceded any changes in body mass by at least 2 weeks (Tups *et al.* 2006). Furthermore, neural cell-specific SOCS3 conditional knockout mice have greater responsiveness to the weight- and appetite-reducing effects of exogenous leptin than their wild-type littermates (Mori *et al.* 2004). Enhanced leptin sensitivity and attenuation of diet-induced obesity are also observed in mice with heterozygous SOCS3 deficiency (Howard *et al.* 2004). Finally, leptin-resistant lethal yellow (A^y/a) mice (Bjørbaek *et al.* 1998), diet-induced obese C57BL/6J mice (Münzberg *et al.* 2004) and age-induced

obese F344×BN (Scarpace *et al.* 2002) and Wistar (Peralta *et al.* 2002) rats have all been shown to have excessive hypothalamic expression of SOCS3, indicating its importance in aetiologically different forms of obesity.

There is also growing evidence that SOCS3 may modulate central leptin action by changing transcriptional activity of leptin-responsive genes (Mori *et al.* 2004, Higuchi *et al.* 2005, Münzberg & Myers 2005). However, in spite of substantial increases in SOCS3 gene expression in the ARC of voles exposed to LD, we found no significant changes in the hypothalamic expression of mRNA for *NPY*, *AgRP*, *POMC* and *CART* genes. Changes in *NPY*, *AgRP*, *POMC* and *CART* were also not detected in our previous studies, where bank voles (*Clethrionomys glareolus*) were exposed to SD or LD for 12 weeks (Peacock *et al.* 2004). However, these changes in the first-order neuropeptides believed to control food intake were perhaps not surprising because we have also shown that exposure to LD did not significantly affect food intake, once differences in body mass were accounted for (Peacock *et al.* 2004, Król *et al.* 2005). Rather, the increase in mass and adiposity was mediated primarily by an increase in digestive efficiency. The nature of the potential link between leptin signalling in the ARC, its inhibition by SOCS3, and digestive efficiency remains unclear. However, possible links between central leptin action and digestive efficiency have also been inferred in arctic ground squirrels *Spermophilus parryii* (Boyer *et al.* 1997) and Siberian hamsters (Klingenspor *et al.* 2000). This is a potentially important finding because differences in digestive efficiency between individuals as a contributing cause of obesity in humans are almost universally ignored.

Conclusions

Our data on field voles suggest that changes in expression of SOCS3 in the ARC may contribute to the phenomenon of photoperiod-induced changes in leptin sensitivity, and perhaps underpin leptin resistance in other situations such as human obesity. Moreover, our results imply that the transfer of animals from SD to LD alters the defended level of body mass and adiposity. The seasonal changes in the body mass set point, the modulation of the leptin signal by SOCS3 and the important role of changes in digestive efficiency in field voles provide an interesting model system that may allow us to study mechanisms contributing to the development of obesity in humans.

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