Appetite regulation and seasonality: implications for obesity

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High circulating concentrations of leptin in obesity are associated with an apparent loss of its characteristic anorectic action within the hypothalamic region of the brain. Central insensitivity to leptin may therefore contribute to the aetiology of this disease, and an increased understanding of the underlying mechanisms will identify potential means of prevention and/or therapeutic targets. Seasonal animals such as sheep and Siberian hamsters (*Phodopus sungorus*) exhibit annual photoperiod-driven cycles of appetite and body weight. Increased food intake and weight gain in long days (summer) are associated with high circulating leptin, and decreased intake and weight loss in short days (winter) with low leptin. Critically, these cycles are associated with reversible changes in sensitivity to leptin. High sensitivity is seen in short days and relative insensitivity in long days, demonstrated both in sheep given leptin centrally via intracerebroventricular cannulas and in hamsters given leptin peripherally. In addition, primary hypothalamic appetite-regulating targets for leptin (i.e., neuropeptide Y, melanocortin and cocaine- and amphetamine-regulated transcript pathways) respond differently in these species to changes in circulating leptin and nutritional status induced by photoperiod as opposed to such changes induced by food restriction. Studies of seasonal animals will help to resolve causes of altered sensitivity to leptin and whether these changes reflect altered transport into the brain and/or altered signalling at the receptor or post-receptor level. Increased knowledge of the mechanism(s) and time-course for development and reversal of reduced central leptin sensitivity will provide new insights into the development and control of obesity.

Seasonal appetite cycles: Hypothalamic leptin sensitivity: Leptin signalling

The discovery approximately a decade ago of the anorectic adipose tissue hormone, leptin, provided a potent stimulus to obesity research, but it has yet to yield the originally anticipated breakthrough in effective treatment for prevention or cure of the disease (Zhang et al. 1994). One of the problems is that although leptin clearly has anorectic actions within the hypothalamus of all species studied to date (Ahima et al. 2000), obesity is accompanied by a state of apparent central leptin insensitivity or resistance. Thus, in man the obese individual has a high circulating concentration of leptin by virtue of the increased fat mass (for example, see Ruige et al. 1999), but this high leptin does not act to reduce appetite and body weight. Central resistance to leptin may contribute to the aetiology of obesity and an increased understanding of the underlying mechanisms will identify potential means of prevention and/or therapeutic targets.

Central leptin insensitivity may be caused by desensitisation at the receptor or post-receptor levels, by decreased transport of leptin across the blood–brain barrier, or indeed by a combination of these factors. However, the critical mechanism(s) remain unresolved despite considerable research effort using various animal models and *in vitro* systems. The present paper will discuss findings from seasonal animals that indicate their unique potential as models of reversible leptin insensitivity in which the mechanisms may be elucidated.

Species described as ‘seasonal’ are generally those in temperate latitudes that breed only at restricted times of the year; however, such species exhibit additional physiological adaptations to the environment. In particular, they show robust annual cycles of appetite and body weight that have now been well characterised in both sheep and Siberian hamsters (*Phodopus sungorus*). The environmental cue used to track the time of year is the day length or photoperiod, and the seasonal changes in the physiology of these species are reproducible experimentally in artificial photoperiod (Lincoln & Richardson, 1998; Mercer et al. 2001).

Abbreviations: AGRP, agouti-related peptide; ARC, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; DMN, dorsomedial nucleus; LD, long day; NPY, neuropeptide Y; OB-Rb, signalling form of the leptin receptor; POMC, proopiomelanocortin; SD, short day; SOCS, suppressors of cytokine signalling; STAT, signal transducers and activators of transcription.

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Despite the provision of unlimited food, both species exhibit increased appetite and body weight in long days (LD, spring–summer) and decreased appetite and body weight in response to short days (SD, autumn–winter). Much of the body-weight change is attributable to changes in adipose tissue mass, and the photoperiod-induced changes in nutritional status have predictable consequences for leptin secretion. Relatively lean sheep with low voluntary food intake in SD have lower plasma leptin concentrations than relatively fat sheep with high food intake in LD (Marie et al. 1996; Mercer et al. 2001). Thus, the seasonal pattern of circulating leptin concentrations reflects that of adiposity and matches the seasonal pattern of appetite drive. This situation seems paradoxical in view of the purported anorectic role of leptin but, critically, seasonal animals appear to adjust their sensitivity to the leptin signal accordingly. An anorectic response to high endogenous leptin in LD would counteract the photoperiodic drive to increase food intake and a reduction in sensitivity to leptin in LD would be required to maintain the seasonal appetite and body-weight cycles.

**Seasonal changes in leptin sensitivity**

Studies of exogenous leptin administration to Siberian hamsters and sheep support the hypothesis that there are seasonal changes in sensitivity to leptin. The first of these studies has shown that male hamsters decrease food intake and body weight in response to twice daily peripheral injections of leptin for 10 d, but the weight loss (fat depletion) is greater in SD than in LD (Klingenspor et al. 2000). Furthermore, in food-restricted hamsters in SD leptin injections blunt the increase in food intake and prevent the full recovery of body weight during ad libitum refeeding (Klingenspor et al. 2000). Subsequently, Atcha et al. (2000) have demonstrated that chronic leptin infusion (via subcutaneous osmotic minipumps) for 14 d causes body weight and fat loss in hamsters in SD but not in LD, with no effect on intake in either photoperiod. These effects have been seen in both males and ovariectomised females with oestadiol replacement, suggesting independence from gonadal steroid involvement (Atcha et al. 2000). Although the foregoing observations give a clear indication of seasonal changes in sensitivity to leptin, these changes may be attributable to peripheral and/or central effects. Specific seasonal changes in hypothalamic sensitivity to leptin in sheep have therefore been addressed (Miller et al. 2002).

In sheep (male castrates with steroid replacement) prepared with indwelling intracerebroventricular cannulas into the third ventricle at the centre of the hypothalamus, a single intracerebroventricular injection of ovine leptin in November acutely reduces food intake by 30% but the same treatment to the same animals in April fails to affect food intake (Miller et al. 2002). Thus, the hypothalamic appetite-regulating circuitry appears more sensitive to leptin in the SD of autumn than in the relatively LD of spring, and these observations have recently been repeated in artificial SD and LD photoperiods (CL Adam, DW Miller and PA Findlay, unpublished results). In addition, it is not only appetite but also the reproductive neuroendocrine hypothalamic pathway that responds to leptin (Adam et al. 2003), and this pathway also shows seasonal changes in sensitivity (Fig. 1; Miller et al. 2002). However, intriguingly, the changes in the (stimulatory) reproductive neuroendocrine response to leptin are the opposite of those in the (inhibitory) appetite response, being greatest in April (spring) compared with November (autumn). This finding suggests another level of complexity exists in leptin signalling, with these physiological axes utilising distinct hypothalamic neuronal pathways. Clarke et al. (2000) have also reported seasonal differences in sensitivity of the appetite axis to leptin in sheep. In apparent contrast to the findings mentioned earlier, their study with gonadectomised sheep has reported that intake is inhibited by intracerebroventricular leptin infusion (for 3 d) in the spring but not in the autumn. However, closer scrutiny reveals that the ‘spring’ treatment carried out in Australia by Clarke et al. (2000) was undertaken in January (Northern hemisphere equivalent) when day length would have been shorter than during their ‘autumn’ leptin treatment, which was conducted in late September. Thus, in both studies the appetite-depressing effect of leptin is greatest in shorter than longer natural day lengths, when the measured voluntary intake is at its lowest.

One of the postulated causes of leptin resistance in obesity is the chronic elevation of circulating leptin concentrations (Scarpace et al. 2002). Thus, it is pertinent to evaluate whether it is the photoperiod-induced changes in nutritional status and endogenous circulating leptin in seasonal species or photoperiod per se that gives rise to seasonally-altered leptin sensitivity. Chronic peripheral leptin infusion has been given to hamsters with low body weight, fat reserves and circulating leptin, brought about by either SD exposure or imposed food restriction in LD (Rousseau et al. 2002). This treatment causes body-weight and fat loss in SD but has no such effects in LD, suggesting that photoperiod rather than leptin is indeed the key regulator of leptin sensitivity in the seasonal model. Furthermore, preliminary data from sheep support this

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**Fig. 1.** Contrasting seasonal responses to leptin shown by the reproductive neuroendocrine axis, in terms of lutetising hormone (LH) secretion (a) and the appetite axis (b). Adequately-nourished sheep were given a single intracerebroventricular injection of leptin (1) in the autumn (○, ■) or in the spring (○, □). Appetite was measured as the voluntary food intake during the 2 h immediately post injection. Values are means with their standard errors represented by vertical bars. Mean value was significantly different from that for the control group: *P*<0·05. (From Miller et al. 2002.)
interpretation (CL Adam, DW Miller and PA Findlay, unpublished results). The appetite-suppressive effect of a single intracerebroventricular injection of leptin is already increased in sheep at 4–5 weeks following an abrupt switch from artificial LD to SD, before any discernible changes in voluntary food intake, fat reserves and presumably circulating leptin. Thus, there is physiological evidence to indicate direct photoperiodic adjustment of central sensitivity to leptin.

Leptin transport into the brain

Central resistance to circulating leptin may in part be caused by a reduced ability of the blood–brain barrier to transport leptin into the brain. This mechanism is supported by the observation that obese subjects have a markedly reduced cerebrospinal fluid:blood concentration for leptin (Schwartz et al. 1996), and it is widely assumed that the transport mechanism is saturated in obesity. If this assumption is valid, which comes first? Does an impaired blood–brain barrier leptin transport mechanism cause leptin-resistant obesity or does it result from the chronic elevation in circulating leptin? In a murine model of age-related obesity transport rates of intravenously-administered radiolabelled leptin into the brain decrease with age, in parallel with, but not preceding, their increased adiposity (Banks & Farrell, 2003). Furthermore, blood–brain barrier transport rates in these obese mice are increased by weight loss induced by either fasting or leptin administration. This finding indicates that the impaired transport is reversible, and although it develops alongside the developing obesity, it is unlikely to be caused by the increase in leptin itself (Banks & Farrell, 2003). Seasonal changes in leptin transport into the brain have yet to be determined in sheep and hamsters. Although photoperiod may alter hypothalamic sensitivity to peripheral leptin in this way, it is unlikely to be the only mechanism, since seasonally-altered responses to leptin delivered directly into the hypothalamus have been observed (Miller et al. 2002). Thus, investigation of seasonal changes within the hypothalamus of leptin receptor and neuronal targets for leptin may throw some light on the mechanism(s).

Seasonal changes in hypothalamic gene expression

In common with non-seasonal rodents, in situ hybridisation studies have shown that the signalling form of the leptin receptor (OB-Rb) is expressed in the hypothalamus of Siberian hamsters and sheep largely within the arcuate nucleus (ARC) and the ventromedial nucleus, but also in the dorsomedial nucleus (DMN), paraventricular nucleus and lateral hypothalamic area (Williams et al. 1999; Mercer et al. 2000). Leptin actions on appetite and energy balance are transduced primarily by neuronal populations within the ARC that express OB-Rb, notably neuropeptides co-expressing neuropeptide Y (NPY) and agouti-related peptide (AGRP) and neuropeptides co-expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART; Ahima et al. 2000). These target neuropeptides constitute opposing orexigenic and anorexigenic pathways respectively, with extensive projections between the ARC and paraventricular nucleus, a hypothalamic region known to be pivotal to appetite regulation. Thus, a high leptin signal suppresses NPY/AGRP and activates POMC/CART pathways whereas a reduction in leptin signal activates NPY/AGRP and suppresses POMC/CART, the latter being an important facet of the compensatory survival response to starvation (Ahima et al. 2000). In addition, the classic receptor response to the fall in leptin caused by food deprivation or restriction is a marked up-regulation of OB-Rb in the ARC (Ahima et al. 2000). The intriguing issue in seasonal species is how the expression of leptin receptor and primary target neuropeptides varies with season and seasonally-altered concentrations of circulating leptin.

In comparisons of hypothalamic ARC gene expression between Siberian hamsters kept in SD and LD consistent findings (Adam et al. 2000; Mercer et al. 2000, 2001) are decreased OB-Rb and POMC and increased CART in SD, whereas there are no consistent differences in NPY and AGRP between photoperiods. Such a profile is unexpected for animals with a low leptin signal in which (from food-restriction or food-deprivation experiments in non-seasonal rodents, see p. 415) increased gene expression for OB-Rb, NPY and AGRP and decreased expression for CART and POMC would be predicted. However, only POMC changes in the predicted direction, and OB-Rb and CART gene expression change in the opposite direction to that predicted. The lack of consistent change in NPY and AGRP mRNA suggests that hamsters remain in energy balance despite appetite reduction and weight loss when these are induced by SD rather than by imposed food restriction. This scenario reflects the ‘anticipatory’ nature of appetite and body-weight change in the seasonal animal (Adam & Mercer, 2001). However, hamsters clearly retain the ability in either photoperiod to respond as predicted to food restriction, which elicits the classic compensatory responses as opposed to anticipatory changes (Mercer et al. 2001). Likewise in food-restricted or fasted sheep, OB-Rb and the orexigenic NPY and AGRP genes are up regulated in the ARC, and CART and POMC genes are down regulated, as predicted with the decline in leptin (Adam et al. 2002; Archer et al. 2002). However, when circulating leptin is reduced in sheep as a result of SD exposure (Marie et al. 2001), none of these changes are seen. Instead, there is no change in NPY gene expression and up regulated CART gene expression (as in SD hamsters) but no change in OB-Rb mRNA (unlike SD hamsters) and increased POMC mRNA levels (opposite to SD hamsters; Archer et al. 1999; CL Adam, ZA Archer and PA Findlay, unpublished results). In agreement with these findings, another group (Clarke et al. 2003) has reported increased POMC gene expression in the ARC of sheep kept in SD as opposed to LD. However, they also report increased OB-Rb and NPY gene expression in ARC in SD, in apparent contrast to the data described earlier. The discrepancy may be explained by the lack of a SD-induced decrease in adiposity and circulating leptin in the study of Clarke et al. (2003).

It appears, first, that seasonal animals undergoing SD weight loss remain in energy balance. The hypothalamus does not perceive the animal to be in negative energy balance, unlike the scenario when weight loss is involuntary.
and compensatory orexic pathways are activated. Second, the leptin receptor is not up regulated in SD, suggesting that it is somehow primed to expect a low leptin signal at this time. Third, the changes in POMC gene expression cannot be attributable to changes in circulating leptin or photoperiod since gene expression is increased in LD in hamsters but in SD in sheep (Mercer et al. 2000; Clarke et al. 2003). A possible explanation lies in the opposite reproductive responses to photoperiod shown by the two species, coupled with the stimulatory effect of gonadal steroids on POMC expression in the ARC (Cheung & Hammer, 1995). This explanation is supported by the lack of photoperiod-induced change in POMC gene expression in castrated sheep with constant-release steroid implants (Archer et al. 1999). However, it is not supported by the SD-induced reduction in POMC mRNA levels seen in ovariectomised hamsters with constant-release steroid replacement (Rousseau et al. 2002). Altogether, it seems unlikely that changes in POMC drive seasonal changes in appetite or leptin sensitivity. Fourth, although the SD-induced up-regulation of CART gene expression in both sheep and hamster ARC could be driving the seasonal anorexia (Mercer et al. 2003), it is open to speculation at this stage as to whether CART also sensitises ARC neurones to leptin feedback.

Furthermore, a comparison of hypothalamic neuropeptide responses to peripheral leptin infusion reveals no difference between the photoperiods in Siberian hamsters, with similar decreases in NPY gene expression and no other changes observed (Rousseau et al. 2002). Similarly, responses to a reduced leptin signal brought about by food restriction show no evidence for altered sensitivity of the anorectic neuropeptides POMC and CART between SD and LD, although the orexigenic neuropeptides NPY and AGRP are more markedly elevated in SD than LD (Mercer et al. 2001). Although this finding suggests that increased sensitivity to inappropriately low leptin in SD may be mediated in NPY/AGRP neurones, it does not readily explain the reduced sensitivity to inappropriately-high leptin in LD.

From the foregoing discussion, no unequivocal mechanism emerges for seasonally-altered hypothalamic sensitivity to leptin. The increased or unchanged leptin receptor expression in LD intuitively cannot explain the decreased sensitivity at this time, and although it seems that NPY/AGRP neurones may be less sensitive to low leptin in LD than SD, the photoperiod-induced changes in hypothalamic neuropeptides also fail to provide an adequate explanation. It is pertinent, therefore, to examine post-receptor leptin signal transduction and how this process may be seasonally altered.

**Seasonal changes in post-receptor leptin signal transduction**

The effects of leptin are mediated at the cellular level by activation of diverse intracellular signalling pathways (for recent review, see Sweeney, 2002). In particular, the Janus kinase/signal transducers and activators of transcription (STAT) signalling pathway plays a critical positive role, with Janus family tyrosine kinases utilised by the intracellular domain of the leptin receptor to phosphorylate STAT proteins, specifically phosphorylation of STAT3, which then facilitates transcription of the target genes. The intracellular effects of leptin are also negatively regulated by suppressors of cytokine signalling (SOCS) proteins, notably SOCS-3, which inhibit Janus kinase/STAT activity. Thus, leptin activates SOCS3, which then reduces intracellular signalling by inhibiting Janus kinase/STAT. It is axiomatic that alterations in the activities of these intracellular signalling pathways could potentially underlie observed changes in leptin responsiveness, and these alterations in signalling activity have been investigated in rodent models of obesity with leptin resistance. For example, leptin-induced phosphorylated STAT3 levels, as well as the leptin-induced decrease in NPY mRNA levels, are diminished in the hypothalamus of rats with age-related obesity (Scarpone & Tumer, 2001). In addition, induction by leptin of phosphorylation of STAT3 has been demonstrated in POMC neurones of the rat hypothalamus, indicating that STAT3 mediates leptin stimulation of POMC gene transcription (Munzberg et al. 2003). On the other hand, expression of SOCS-3 mRNA in the hypothalamus is increased in leptin-resistant murine obesity (Bjorbaek et al. 1998) and in aged rats that have become obese (Peralta et al. 2002). In the latter case food restriction partially reverses the age-related increase in SOCS-3 mRNA, suggesting that the developing obesity and hyperleptinaemia in part causes the increase in SOCS-3 rather than vice versa (Peralta et al. 2002). In rats the increase in SOCS-3 mRNA in the ARC following acute leptin treatment occurs concomitantly with increases in POMC mRNA and decreases in NPY mRNA levels in the ARC (Proulx et al. 2002).

An important question in the present context is how these leptin signal transduction pathways vary with the reversible changes in sensitivity to leptin exhibited in seasonal species. Preliminary work (A Tups, C Ellis and JG Mercer, unpublished results) has demonstrated, by in situ hybridisation, the co-localisation of SOCS-3 mRNA with POMC and NPY mRNA in the Siberian hamster ARC and up-regulation of ARC SOCS-3 gene expression in LD compared with SD. Furthermore, SOCS-3 mRNA in the ARC is acutely stimulated by peripheral leptin injection only in SD and not in LD. These findings support the notion that reduced SOCS-3 activity contributes to the increased sensitivity to leptin in SD and, conversely, that increased SOCS-3 activity contributes to the relative leptin insensitivity seen in LD. Given that SOCS-3 is clearly modulated by leptin, it is important to establish whether these differences in its expression are caused by photoperiod per se or by photoperiod-induced differences in adiposity and circulating leptin. Preliminary findings (A Tups, CL Adam and JG Mercer, unpublished results) support the former interpretation. Hamsters weight-matched to SD counterparts by food restriction in LD do not have similarly low amounts of SOCS-3 mRNA; instead the SOCS-3 gene expression remains high, matching that of fat ad libitum-fed hamsters in LD. It is therefore tempting to speculate that the changing photoperiod primes ARC sensitivity to circulating leptin by adjusting the activity of SOCS-3 within neurones of this hypothalamic nucleus.
Sites of melatonin–leptin interaction

The foregoing discussion indicates that photoperiod can clearly affect central sensitivity to leptin. The photoperiod signal to the brain is mediated humorally by melatonin (Morgan et al. 1994). Receptors for melatonin have been localised in the hypothalamus of seasonal species in the suprachiasmatic nucleus, DMN and anterior hypothalamic area, but they are not present in the ARC (Morgan et al. 1994; C Ellis, JG Mercer and PJ Morgan, unpublished results). This finding rules out a simple interface of melatonin signalling with leptin signalling within primary targets in the ARC. Co-localisation of both receptors elsewhere has not been reported but this explanation cannot be ruled out since, for example, both receptors have independently been localised in the DMN. A disadvantage at present is that the phenotype is unknown of neurones expressing melatonin receptors in the suprachiasmatic nucleus and DMN and of those expressing leptin receptors in the DMN, ventromedial nucleus and paraventricular nucleus. However, the phenotype of leptin receptor-expressing neurones in the ARC is known, i.e. NPY/AGRP and POMC/CART neurones. Neuronal projections are known to exist from the suprachiasmatic nucleus, DMN and the ARC to the paraventricular nucleus, a critical regulatory centre for appetite control (Morgan & Mercer, 2001) and through which melatonin and leptin feedback may thereby be coordinated (Fig. 2).

Conclusions and implications for obesity

The evidence suggests that insensitivity or resistance to leptin can occur within its primary targets in the ARC, possibly mediated by desensitisation of intracellular signal transduction. This process may contribute to the leptin resistance of obesity, with or without additional impairment of leptin transport into the brain. It remains equivocal as to what extent it is the hyperleptinaemia of increasing adiposity itself that causes changes in sensitivity and whether sensitivity (and an anorectic response) can be restored. Although correcting the obesity with an enforced drastic reduction in food intake and adiposity can restore sensitivity, there would be therapeutic potential in being able to induce an increase in leptin sensitivity that could then drive physiological weight loss. In seasonal animals there is a physiological tool (photoperiod), as opposed to imposed food restriction, with which leptin sensitivity may be adjusted reversibly, providing a previously unexploited model in which to elucidate the mechanism(s).

In the context of the present symposium, it is clear from studies of various animal models that appetite control and obesity may be programmed in utero and that programmed offspring exhibit leptin resistance both peripherally and centrally (Breier et al. 2001). It remains to be determined to what extent the development of central leptin resistance postnatally is attributable to fetal programming of the neuroendocrine hypothalamus, but clearly an important step in such an investigation is to determine the basic molecular mechanism(s) whereby sensitivity to leptin is adjusted. Once an increased understanding has emerged from studies in adult animal models, such as those discussed here involving seasonal species, targeted approaches to the phenomenon in fetal and neonatal life may be devised. The ontogeny of the hypothalamic neuronal pathways involved in leptin feedback and appetite regulation has yet to be fully elucidated. By in situ hybridisation in the ovine fetus (110 d; term 147 d), adult-like localisation of gene expression for the primary orexigenic and anorexigenic leptin-sensitive hypothalamic pathways have been found; i.e. OB-Rb, NPY, AGRP, POMC and CART mRNA are expressed in the ARC (G Rouzaud, PA Findlay, SM Rhind and CL Adam, unpublished results). How these pathways may be affected, short- or long-term, by nutritional status in utero remains unresolved, although there is already evidence that maternal undernutrition can increase levels of NPY mRNA in the fetal hypothalamus of both sheep (Warnes et al. 1998) and rats (Huizinga et al. 2001). In addition, offspring of diabetic rats show persistent hyperphagia and overweight, associated with persistently increased hypothalamic expression of NPY (Plagemann et al. 1999). Conversely, offspring of low-protein malnourished rats have reduced body weight associated with reduced NPY expression in the ARC (Plagemann et al. 2000).

Thus, there are good indications that the wiring of the neuroendocrine hypothalamus is sensitive in the fetus to nutritional feedback. It is postulated that detecting the extent of these changes and their persistence postnatally will contribute to the understanding of the putative programming of central leptin resistance and obesity. It could be argued that the hypothalamus of seasonal species is programmed biannually to become leptin resistant in LD and leptin sensitive in SD. Elucidating this reversible mechanism at the cellular and/or molecular level will provide insights into the causes of leptin insensitivity and
obesity and, importantly, may provide novel therapeutic approaches.

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References


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