Physiological Models of Leptin Resistance

A. Tups
Department of Animal Physiology, Faculty of Biology, Philipps University Marburg, Marburg, Germany.

The discovery of leptin revolutionised the field of obesity research since this hormone represents the long postulated ‘adiposity signal’ that provides the afferent signal required for the lipostatic theory (1), conveying information about the status of body fat stores to the brain. Despite the outstanding anorexigenic potential of leptin, catabolic effects are not always apparent. Since circulating leptin levels are proportionate to body fat stores, obesity in humans as well as in rodents is very often associated with increased leptin levels, but leptin does not suppress appetite effectively. This phenomenon, called leptin resistance, is often claimed as the key event for the onset of perturbed energy homeostasis (2, 3). Leptin resistance can either be a pathological state, for example in diet induced obesity, or it can be an adaptive response, to allow shifts in body weight set point, for example during pregnancy or in seasonal animals.

Insensitivity to leptin could be the result of changes at a number of levels in the signalling pathway from reduced access of the hormone to its receptor through to changes in receptor expression or changes in post-receptor signal transduction. Accumulating evidence suggests that accessibility of leptin to its target sites in the hypothalamus may be altered by a dose- and time dependent saturation in the intracellular domain of the leptin receptor. In seasonal animals, SOCS3, most importantly seems to act as a ‘molecular switch’ enabling a photoperiod-induced alteration in leptin signalling and subsequent adjustments in energy homeostasis to allow attainment of a new body weight set-point. These physiological models show that animals can exhibit leptin resistance as an adaptive response to meet new physiological or environmental challenges, promoting the survival of the species during times of increased metabolic demand. The molecular mechanisms mediating physiological and/or pathological leptin resistance, like during diet induced obesity, might be very similar involving hypothalamic SOCS3. Investigation of these models might further provide new insight into the dynamic complexity of energy homeostasis.

Key words: cytokines, neurepetides, melatonin, prolactin, receptors, membrane/nuclear.

doi: 10.1111/j.1365-2826.2009.01916.x
compared to the ventromedial hypothalamus (VMH), (8)], and leptin signalling through the janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway occurs more rapidly after peripheral injection of the hormone as compared to other hypothalamic sites (7). It still remains an unsolved question whether the ARC, like other hypothalamic nuclei, is protected by the blood brain barrier or whether some parts circumvent it. The latter point is supported by recent findings of Münzberg et al. (7). Thus, modulation of accessibility of leptin to its neuronal target sites represents one plausible mechanism of central regulation of leptin sensitivity. Alternatively, there could be a reduction in the availability of leptin receptors on the cell surface, although several studies suggest that this is unlikely (9–12). A large body of evidence suggests that leptin sensitivity is modulated by modification of intracellular signal transduction downstream of the leptin receptor, involving several distinct signal transduction pathways.

Central signalling pathways

Leptin conveys energy homeostasis encoding information to the central nervous system by acting directly on neurons in the hypothalamus. In this context a crucial event is binding of this hormone to its receptor (LepRb) that is expressed in several hypothalamic and extrahypothalamic brain regions, and in particular is abundantly expressed in the hypothalamic arcuate nucleus [ARC (13)]. Subsequent transduction of the peripheral signal is integrated into rapid neuronal responses by altering neuronal firing (14) as well as by affecting post-translational processing (e.g. phosphorylation events) of a set of signalling molecules (15, 16). Through these mechanisms leptin can exert rapid effects on e.g. glucose metabolism independent of its chronic alteration of gene transcription in orexigenic and anorexigenic downstream effectors.

Following binding to the LepRb, leptin’s anorexigenic action is mediated by three signal transduction pathways, each involving specific tyrosine residues on the receptor, and involving the receptor-associated JAK2 (17, 18). Upon binding, leptin activates JAK2 resulting in a subsequent phosphorylation of receptor tyrosine kinases. Through the so called JAK2-STAT3 pathway the key transcription factor, STAT3, is recruited to Tyr\textsuperscript{1138} and becomes transactivated ([phosphorylated]) (17, 19, 20]. A second pathway involves the extracellular-regulated kinase (ERK) signalling cascade whose activation is mainly critically dependent on the association of SH2-containing tyrosine phosphatase (SHP2) and the growth factor receptor binding protein (GRB2). This pathway is mediated via Tyr\textsuperscript{985} another tyrosine residue associated with the leptin receptor (17, 21, 22). However, 30% of transactivation of ERK is considered to be attributable directly upon phosphorylation by JAK2. The third tyrosine residue, Tyr\textsuperscript{1077}, of the leptin receptor may play a predominant role in recruiting another STAT molecule, STAT5, although Tyr\textsuperscript{1138} may also contribute to leptin-induced phosphorylation of STAT5 (23). A crucial enzyme that appears to facilitate crosstalk of hypothalamic leptin and insulin signalling is phosphatidylinositol 3-kinase (PI3K), which activates the key downstream target AKT [also known as protein kinase B; (17, 21)]. The specific molecular mechanism by which leptin activates this enzyme, however, is only poorly understood but it is plausible that JAK2 directly or one or several of the leptin receptor tyrosine residues are implicated in this process.

The central role of the inhibitory molecules SOCS3 and PTP1B

In assessing possible mechanisms for leptin resistance, there is particular interest in endogenous molecules that inhibit these leptin-responsive pathways. Here, the leptin-induced target gene, the suppressor of cytokine signalling 3 (SOCS3); and the protein tyrosine phosphatase 1B (PTP1B) may play crucial roles. Neuronal deficiency of SOCS3 in mice leads to enhanced leptin-induced phosphorylation of STAT3 in the hypothalamus, greater body weight loss and suppression of food intake. Furthermore, SOCS3-deficient mice exhibit resistance to diet-induced obesity (DIO) and retain insulin sensitivity (24, 25), suggesting that SOCS3 may underlie the leptin resistance that occurs in this state. Protein tyrosine phosphatase 1B deficiency in mice and diabetic rodents leads to enhanced central and peripheral insulin sensitivity (26–29). These animals maintain euglycemia (in the fed state) with one-half the level of insulin observed in wild-type littermates, and surprisingly are resistant to DIO. These observations suggest that these inhibitory signalling molecules play a crucial role in mediating changes in leptin and insulin sensitivity.

Physiological models of leptin resistance

Over the last couple of years the considerable therapeutic potential underlying treatment of leptin resistance led to an explosion of interest in unravelling the molecular identity of this phenomenon. Much of our knowledge about intracellular leptin signalling and the role of distinct components involved in this process has been derived from studies of genetically obese rodents or from models of imposed negative energy balance (30–33). These studies provided the basis for our current understanding of the neuroendocrine system underlying energy homeostasis. The aim of many of these studies has been limited to investigating leptin resistance as a pathological change. However, leptin resistance also occurs as an adaptive physiological response to allow plastic changes in homeostatic mechanisms enabling repeated and reversible alterations in the body weight set-point in certain situations. Although energy homeostasis is a fundamental mechanism regulating and optimising body functions that promote the survival of the animal during most periods of life, it is not always the most appropriate strategy. Sometimes, to meet the extreme pressures imposed by environmental changes, animals have developed anticipatory adaptations allowing a change in body weight set-point to a level above or below the ‘default value’ to cope with and survive this new challenge. For example, alterations in nutrient availability due to recurring changes in season that regularly expose animals to harsh winter conditions in cold or temperate latitudes require such adaptive responses in bodyweight homeostasis. An extreme facet of adaptation to deprived nutrient availability is reflected in daily torpor or hibernation, a physiological state in which energy expenditure is reduced while deprived nutrient availability is reflected in daily torpor or hibernation, a physiological state in which energy expenditure is reduced.
all Mammalian species including *Homo sapiens* is pregnancy and subsequent lactation. The enormous energetic cost of foetal growth, parturition and lactation demands significant changes of the mother’s body weight set-point above the “default line” resulting in increased appetite and food intake to prepare for this new challenge. In seasonal animals, pregnancy is even precisely timed in order that offspring are born into a favourable environment where food is plentiful.

Despite obvious discrepancies in the sensory and hormonal signals that drive fundamentally different events such as pregnancy or seasonal adaptations, remarkably, the alteration in energy homeostasis in both events may be mediated by a mutual neuroendocrine system. This involves the concerting key feature of inducible leptin resistance that contributes to a chronic change in energy homeostasis. As altered leptin sensitivity also underlies the pathological changes associated with obesity, it seems there might be much to learn by understanding these physiological adaptive responses. During the last 5 years it emerged that alterations in leptin sensitivity as occurring during seasonal body weight regulation and pregnancy are largely mediated by altered neuronal signalling events downstream of LepRb (35–40). Common features as well as a proposed model elucidating seasonal changes in leptin sensitivity will be discussed in this review.

**Small seasonal mammals represent a powerful tool to study leptin resistance**

Seasonal mammals such as the Siberian hamster (also known as the Djungarian hamster), *Phodopus sungorus* (39, 41, 42) and the field vole (*Microtus agrestis*) (35, 43) are powerful models for studies of leptin resistance. They exhibit a remarkable natural body weight cycle, accompanied by a biannual reversible switch in leptin sensitivity, mediated by the environmental cue photoperiod (44, 45). The neuroendocrine transducer of photoperiod information is the pineal hormone melatonin which acts on its receptor predominantly expressed in the pituitary pars tuberalis (PT) and the hypothalamic suprachiasmatic nucleus (SCN; [46]). Both species synchronise their physiology and behaviour to the seasonally programmed signal imposed by photoperiod, which is dramatically exemplified by an increase of body weight in a summer-like photoperiod and a reduction of body weight in a winter-like photoperiod. The changes induced by natural, and gradually changing photoperiod cues can be replicated in the laboratory by a simple square-wave switch from long day length (LD; 16 : 8 h light–dark) to short day length (SD; 8 : 16 h light–dark). Here, different functional *in vivo* studies investigating effects of exogenously applied leptin revealed a transition from leptin sensitivity in SD to leptin resistance in LD (38, 42, 47).

**The central role of SOCS3 in mediating seasonal changes in leptin sensitivity**

Findings by us and others provide substantial evidence for an involvement of central leptin signalling downstream of its receptor in mediating the biannual switch in leptin sensitivity. In this context it emerged very soon that SOCS3 may act as a central player. Both Siberian hamsters and the field vole seem to employ SOCS3 to sensitize the brain to a different reading of the leptin signal in opposite photoperiods. A switch of hamsters from one photoperiod to the other is associated with a rapid change in SOCS3 gene expression which clearly precedes the chronic body weight change induced by photoperiod (Fig. 1). In juvenile hamsters raised in LD, a rapid increase in arcuate SOCS3 gene expression occurs as early as 4 days after weaning. This rise can be completely prevented by transfer to SD on the day of weaning (39). Increased levels of SOCS3 mRNA in LD as compared to SD became significantly established 2 weeks after acclimation to the opposite photoperiods. These elevated levels were sustained during the whole course of photoperiod acclimation until the animals became photorefractory to SD induced by prolonged exposure. The regimen of inducing leptin sensitivity due to a reduction in SOCS3 gene expression by SD exposure is reversible. Animals that were transferred back to LD rapidly (also within 2 weeks) increased SOCS3...
mRNA to previous LD levels subsequently followed by an increase in body weight (40). Photoperiod-induced changes in SOCS3 that were caused by transferring hamsters from either LD to SD or SD to LD precede the subsequent alteration in body weight by about 3 weeks. Hamsters exhibit a phenomenon called photorefractoriness, a spontaneous body weight increase after prolonged (> 25 weeks) periods in SD. Around this time before animals fully returned to LD body weight, no difference in SOCS3 gene expression was detectable between LD and SD.

Comparing photoperiodic regulation of SOCS3 in hamsters and voles shows several parallels, but also important differences emerge. When voles are transferred from SD to LD it also took around 2 weeks (10–17 days) before a marked increase in arcuate SOCS3 gene expression could be observed. In contrast to hamsters, however, this effect only lasted up to 4 weeks (24–31 days) of exposure to the new photoperiod with a subsequent drop in SOCS3 expression after which it was not different from SD from day 38 onwards [Fig. 1 and (35)]. At this time the photoperiod-induced body weight differential is fully established and the trajectory of LD voles is maintained at a value 25% above the weight of SD voles. The conspicuous correlation between changes in arcuate SOCS3 and body weight in this species strongly implicates SOCS3 as being the molecular trigger for the photoperiod-induced alterations in energy homeostasis in this species. The relatively short (2–3 weeks) up-regulation of SOCS3 appears to induce chronic (through the LD season) leptin resistance increasing the body weight to the set-point imposed by the photoperiod. Distal effectors of leptin action mediating leptin resistance appear to be permanently altered. It is very unlikely that common neuropeptides such as neuropeptide NPY, cocaine and amphetamine regulated transcript (CART), agouti-related peptide (AGRP) and pro-opiomelanocortin (POMC) mediate these alterations since their gene expression was not regulated by photoperiod (35). Future studies are necessary to identify these as yet uncharacterised effectors. In hamsters, although SOCS3 seems to be a key component of the mechanisms mediating seasonal changes in leptin sensitivity its role appears to be slightly different. A permanent de-sensitisation of the leptin signal in LD seems to be important to keep body weight at the high level imposed by the long season since SOCS3 levels remain elevated through the entire LD season (Fig 1). Although hamsters and voles seem to have developed slightly different mechanisms, i.e. chronic as compared to transient upregulation of SOCS3 in adaptation to the LD season, they have in common that a central involvement of SOCS3 appears to be important for the seasonal alterations in energy homeostasis.

Our results imply that photoperiod exclusively accounts for the marked seasonal changes of this important leptin signalling inhibitor. By conducting a series of experiments in hamsters, we further excluded the possibility that the seasonal alterations in SOCS3 gene expression are secondarily mediated by changes in adiposity level, serum leptin, feeding status or gonadal alterations of the hamster. Together with our study conducted previously that revealed SOCS3 being induced by leptin only in SD but not in LD (39), these findings strongly imply this inhibitory molecule may be a critical modulator of seasonal body weight- and leptin sensitivity changes. Furthermore, they suggest a model by which a seasonally appropriate body weight may be rheostatically adjusted by dynamic modulation of leptin sensitivity via SOCS3.

These findings raise the important question: what is the molecular link between melatonin encoding photoperiod on the one hand and SOCS3 mediating body weight changes on the other hand? This missing link might be of endocrine nature and some evidence suggests that it might be the pleiotropic hormone prolactin. A long time ago prolactin was identified to change its circulating levels in the blood very rapidly after the hamster is exposed to a different photoperiod with elevated levels in LD (49–51). Reducing LD prolactin levels to SD by subcutaneous administration of the dopamine agonist bromoergocryptine altered fur colour and reduced body weight in the hamster. Opposite effects were observed by infusion of ovine prolactin in SD hamsters to mimic LD levels (49). Similarly, hypophysectomy, resulting in a massive drop of circulating prolactin levels in LD hamsters, resulted in similar effects to those observed after bromoergocryptine treatment. This phenomenon could be reversed by restoration of prolactin levels through daily subcutaneous injections (52). Further evidence for prolactin being regulated by melatonin is provided by the observation that infusion of melatonin into the suprachiasmatic nucleus (SCN) resulted in a drop of circulating prolactin levels (53). Prolactin can activate SOCS3 via the JAK2/STAT5b pathway of the prolactin receptor which is also expressed in the hypothalamic ARC (54–58). A study by Roy et al (60) revealed that prolactin- and leptin receptor are coexpressed in the hypothalamic paraventricular nucleus (PVN). Furthermore, in Chinese hamster ovary cells (CHO) stably coexpressing leptin- and prolactin receptor both leptin and prolactin induce SOCS3 and PTP1B protein to a similar extent. The mechanism by which melatonin alters seasonal changes in metabolism is not yet identified and a direct crosstalk of melatonin- and leptin signalling in hypothalamic populations of neurons expressing body weight regulatory neuropeptides is questionable. Among the hypothalamic nuclei expressing leptin receptors only the ventral premammillary nucleus (PMV) expresses also a significant amount of melatonin receptors (61). A hypothetical mechanism could involve elevated secretion of prolactin by the pituitary in LD, as a result of a reduced duration of melatonin secretion in this photoperiod. Enhanced binding of prolactin to its receptors in the hypothalamic ARC would lead to elevated levels in SOCS3 and then leptin resistance.

The dual role of LepRb associated Tyr\(^{985}\) in mediating seasonal leptin sensitivity

Since SOCS3 acts as a target gene of STAT3 in vitro and SOCS3 attenuates LepRb-mediated signalling in vitro and in vivo, we posit that the inhibitory feedback action of the LepRb-SOCS3 pathway may explain the different reading of the leptin signal in LD and SD. Therefore, studies were designed to scrutinise the molecular identity underlying inhibitory feedback of SOCS3 in the state of leptin resis-

\(^{1}\)Rheostasis: ‘condition or state in which homeostatic defenses are still present but over a span of time there is a change in the regulated level (48)’.
tance exhibited by hamsters acclimated to LD. Here of particular interest was the key question whether altered SOCS3 gene expression may be associated with impairment of the three distinct LepRb signalling pathways, JAK2-STAT3, ERK or PI3K. To answer this question we investigated whether post-translational modifications (phosphorylation) of key components within these pathways may be perturbed in leptin resistant LD hamsters. Consistent with the expected effect of elevated SOCS3, leptin-induced phosphorylation of the transcription factor STAT3 (critical for its activation) was dramatically reduced in LD compared to SD suggesting that the inhibitory feedback of increased SOCS3 in LD may be based on deactivation of this transcription factor (Fig. 2). In leptin sensitive SD animals the utilised time frame of 40 min post leptin injection was sufficient for leptin-induced phosphorylation of STAT3 which is followed by a subsequent rise in SOCS3 gene expression (our time course-study revealed that leptin needs 1h to induce SOCS3 gene transcription). Hence STAT3 mediated transcription of the SOCS3 gene in this photoperiod is plausible. However, in LD, when animals become insensitive to leptin, SOCS3 levels are elevated despite reduced leptin-induced STAT3 phosphorylation. This suggests that the increase in SOCS3 gene expression is mediated by a different signal than leptin. Future studies need to be designed to clarify whether this missing signal is prolactin.

Phosphorylation of other key components in the hypothalamus reflecting the activity of the ERK- and PI3K signalling pathways was not altered by leptin and either not affected by photoperiod (ERK) or counter-intuitively downregulated in SD (phospho-AKT, reflecting PI3K activity). These findings suggest that SOCS3 may not negatively feed back on activation of these alternative signalling pathways mediating leptin action. Interestingly, however, we detected a marked SD-induced increase of the signalling components SHP2 (Fig. 3) and GRB2 (unpublished data) which are crucial for LepRb associated Tyr985-mediated signalling that is required for activation of ERK. Since this distinct tyrosine residue may possess a dual role in LepRb signalling – binding SHP2 but also providing an important site for interaction with SOCS3 – we postulate a hypothetical negative feedback loop responsible for the precise adjustments in seasonal leptin sensitivity (Fig. 4): Leptin resistance revealed by LD-acclimated hamsters could be based on high expression of arcuate nucleus SOCS3 which may lead to competitive suppression of SHP2 binding to Tyr985 and association with GRB2. This may subsequently result in inhibition of JAK2 which might be

**Fig. 2.** Leptin stimulates hypothalamic nuclear phospho-STAT3 immunoreactivity (ir) in a photoperiod-dependent manner in adult male Siberian hamsters. Hamsters were held in long day length (LD) or transferred to short day length (SD) for 12 weeks. Coronal brain sections were subjected to immunohistochemistry using a phospho-specific-(Tyr705)-STAT3 antibody. Immunoreactive cells were counted and a rostro-caudal extension profile of phospho-STAT3 ir nuclei extending throughout the arcuate nucleus, ventromedial- and dorsomedial hypothalamus in LD and SD hamsters (n = 3-4 in each group) injected with recombinant mouse leptin or vehicle 40 min before death, was established rARC, rostral-; mARC, medial-; and cARC, caudal arcuate nucleus.

**Fig. 3.** Leptin time course study depicting SHP2 mRNA in the arcuate nucleus (ARC) of juvenile female hamsters (8 weeks post weaning). Animals received an intraperitoneal injection of either leptin (LEP) or vehicle (VEH) and were sacrificed 15, 30, 60 or 120 min later (n = 3 in each group). Notably, SHP2 mRNA is markedly increased in SD-VEH compared to LD-VEH throughout the timecourse of injection. Leptin rapidly stimulates SHP2 binding to Tyr985 and association with GRB2. This may subsequently result in inhibition of JAK2 which might be

enhanced by the increased expression of PTP1B in LD (62), which in turn leads to reduced signalling via Tyr1138 followed by diminished STAT3 activation. In states of increased leptin sensitivity (SD-acclimated hamsters), however, low levels of arcuate nucleus SOCS3 expression would result in competitive inhibition of SOCS3 binding to Tyr985 and competitively displaces SHP2 which is associated with GRB2 (both factors are downregulated in LD). This may further reduce the already reduced phosphorylation of Tyr1138 resulting in attenuated activation of STAT3 In SD low levels of leptin (right panel) are associated with reduced PTP1B mRNA resulting in maximal phosphorylation of the two intrinsic tyrosine residues In this state elevated SHP2 and GRB2 may bind to Tyr985 and competitively displace SOCS3 SOCS3 which is already substantially decreased compared to LD fails to exhibit its inhibitory function. Conclusively, STAT3 phosphorylation is augmented leading to dimerisation and translocation to the nucleus where target gene transcription (e.g. SOCS3) becomes initiated. In LD a marked drop of circulating leptin induced by food restriction below the levels observed in SD is not associated with reduced SOCS3 mRNA suggesting that in this photoperiod high levels of SOCS3 are sustained by other mechanisms than the JAK-STAT pathway. This model suggests a crucial role for the dual function of Tyr985 in mediating seasonal changes in leptin sensitivity. Noteworthy, phosphorylation of STAT3 in LD is reduced to up to 50% of SD levels despite leptin levels are two to fourfold increased implying severe endogenous leptin resistance. (Implication of Tyr1077 in leptin signalling has not yet been satisfactorily resolved).

Fig. 4. Model proposing the molecular identity for the biannual switch in leptin sensitivity revealed by Phodopus sungorus. High circulating leptin levels in LD (left panel) are associated with increased gene expression of the inhibitory molecules SOCS3 and PTP1B. PTP1B deactivates JAK2 resulting in diminished phosphorylation of LepRb associated Tyr985 and Tyr1138. SOCS3 binds to Tyr985 and competitively displaces SHP2 which is associated with GRB2 (both factors are downregulated in LD). This may further reduce the already reduced phosphorylation of Tyr1138 resulting in attenuated activation of STAT3 In SD low levels of leptin (right panel) are associated with reduced PTP1B mRNA resulting in maximal phosphorylation of the two intrinsic tyrosine residues. In this state elevated SHP2 and GRB2 may bind to Tyr985 and competitively displace SOCS3 SOCS3 which is already substantially decreased compared to LD fails to exhibit its inhibitory function. Conclusively, STAT3 phosphorylation is augmented leading to dimerisation and translocation to the nucleus where target gene transcription (e.g. SOCS3) becomes initiated. In LD a marked drop of circulating leptin induced by food restriction below the levels observed in SD is not associated with reduced SOCS3 mRNA suggesting that in this photoperiod high levels of SOCS3 are sustained by other mechanisms than the JAK-STAT pathway. This model suggests a crucial role for the dual function of Tyr985 in mediating seasonal changes in leptin sensitivity. Noteworthy, phosphorylation of STAT3 in LD is reduced to up to 50% of SD levels despite leptin levels are two to fourfold increased implying severe endogenous leptin resistance. (Implication of Tyr1077 in leptin signalling has not yet been satisfactorily resolved).

Leptin resistance during pregnancy

Like seasonal leptin resistance, pregnancy is also a condition requiring predictive/anticipatory adaptation in bodyweight homeostasis to prepare for future metabolic demand. While both systems use changes in leptin responsiveness to achieve this goal, the specific mechanisms seem to be different.

Pregnancy in most mammals is associated with hyperleptinemia and leptin resistance. This phenomenon was most comprehensively described in rats (36, 37, 65–67). As mentioned above seasonal leptin resistance in small mammals as well as pregnancy-induced leptin resistance are related physiological states that are reversible and crucial for the survival of the species as a whole. Similar to seasonal leptin resistance the role of pregnancy-related hormones in mediating pregnancy-induced leptin resistance has not been fully understood.
Physiological Models of Leptin Resistance

Physiological Models of Leptin Resistance

**A** Physiological leptin resistance

Hamster (seasonal)

Vole (seasonal)

Rat (pregnant)

Mouse (DIO)

**B** Pathological leptin resistance

Leptin resistance might be induced by crosstalk with the inflammatory pathway IKKβ/NF-κB that induces SOCS3 in the hypothalamus in these states of leptin resistance consumed dietary fats enter the hypothalamus and may bind to the toll like 4 receptor inducing the inflammation pathway.

alternative mechanism involving protein kinase A (PKA) and SP1 (70). An important subject of future research might be a possible hypothalamic interaction of leptin and prolactin action via STAT5. Signal transducer and activator of transcription 5 is regarded as the main transcription factor for transducing the prolactin signal but its role in conveying the leptin signal is less clear. A potential crosstalk of prolactin and leptin in the hypothalamus might represent an important mechanism adjusting energy homeostasis to environmentally induced hormonal changes. In this context, STAT5 as a potential gatekeeper of leptin encoding information about energy stores on the one hand and prolactin encoding environmental adaptations on the other hand ought to be revisited. It is noteworthy; that hyperphagia induced by increased prolactin levels, despite the association with leptin resistance, might be directly linked through activation of the orexigenic neuropeptide Y (NPY). Arcuate nucleus mRNA levels of NPY were increased during the late course of pregnancy in the rat (day 14 and 21) at times when also prolactin levels are increased (71). Thus, a direct or indirect activation of NPY by prolactin in several nuclei involved in the control of food intake, specifically in the PVN, is plausible.

The above mentioned studies demonstrate, that the molecular mechanism characterising seasonal and pregnancy-induced leptin resistance reveals similarities. Particularly the JAK2-STAT3 pathway seems to play a predominant role. Differences in leptin signal transduction affecting different hypothalamic cell populations in pregnancy-induced leptin resistance compared to season-induced leptin resistance give new insights into the dynamic interaction of hypothalamic leptin target sites and their role in energy homeostasis. Seasonal leptin resistance in hamsters and voles and pregnancy-associated leptin resistance best studied in rats are fascinating anticipatory environmental adaptations which facilitate animals to adjust their metabolism on new demands they are facing during their lives. The study of these mechanisms may provide important insights into the pathogenesis underlying human obesity.

Comparison of physiological and diet induced leptin resistance

While leptin resistance during pregnancy and in seasonal animals is an adaptive response affecting virtually all animals in the particular metabolic state, at first glance leptin resistance during diet-induced obesity (DIO) appears fundamentally different. Although leptin resistance is a hallmark of DIO, not all animals fed a high fat diet become leptin resistant and obese. In rodents, the temporal development of diet induced leptin resistance is dependent on the species, and the strain and even in a DIO prone population not all individuals reveal the same susceptibility to develop leptin resistance. It is well established, for example, that Sprague Dawley rats divide into two populations after weaning, one resistant to DIO and the other particularly susceptible to develop leptin resistance on a high fat diet (72). Different mechanisms are thought to drive the susceptibility to develop leptin resistance on a high fat diet.

While it seems, however, that physiological leptin resistance is fundamentally different to something like diet induced leptin resistance, the central underlying mechanisms of both states might be very similar. Central signalling events downstream of the leptin receptor are impaired in DIO in a manner similar to that in physiological models of leptin resistance (31). Ventromedial hypothalamic neurons are less sensitive to leptin excitation (73) and even a permanent disruption of hypothalamic neuronal projections was reported (74), suggesting that neural changes which are induced by DIO are not entirely reversible. This is in contrast to leptin resistance in seasonal animals and during pregnancy from which at least the first is entirely reversible. Although our knowledge about central changes during DIO has ever grown at a very high rate, two central questions about the cause of this metabolic state remain unanswered: (i) Does leptin resistance lead to obesity? If this is the case and (ii) How can diet affect leptin resistance? As discussed above, in seasonal animals, changes in leptin signalling events significantly precede the subsequent body weight alterations, although the causal connection remains to be established. Hence, it seems possible that the increase in body weight during DIO might also be mediated by leptin resistance. Although causative studies of leptin resistance are still missing it was demonstrated that dietary prone rats revealed an impaired leptin-induced STAT3 phosphorylation in the hypothalamus prior to the development of obesity. This was in contrast to the dietary resistant rats which had unaltered STAT3 phosphorylation in response to leptin (75). This study although not causative indicates that both in seasonal- and DIO-mediated leptin resistance, this phenomenon precedes the subsequent body weight increase.

Hypothalamic inflammation as a possible cause of leptin resistance in DIO

In contrast to physiological models of leptin resistance, mediated by photoperiod and/or hormonal induction of SOCS3, it is less clear what might cause leptin resistance in DIO. Very recent data support the hypothesis that hypothalamic inflammation induced by dietary fats might be a cause of developing leptin resistance (76, 77). It has been known for a long time that pro-inflammatory cytokines such as interleukin 6, tumor necrosis factor α (TNFα) and possibly interleukin 18 interfere with leptin signalling pathways in the hypothalamus. For example, all three cytokines induce the phosphorylation and transcriptional activation of STAT3. The idea, however, that hypothalamic inflammation might be the cause for developing leptin and also insulin resistance emerged very recently. A study performed by Zhang et al. (77) revealed that overnutrition activates the inflammatory pathway IKKβ/NF-κB in the hypothalamus, which in turn blunts hypothalamic leptin- and insulin signalling. Moraes et al. (78) demonstrated that dietary fats, independently of caloric intake, induce apoptosis of hypothalamic neurons involving toll like receptor 4, whose exact function in these processes remains to be clarified. A third study performed by Posey et al. (76) showed that hypothalamic infusion of palmitate induced hypothalamic inflammation and insulin resistance. Furthermore, central IKKβ inhibition reduced food intake and was associated with increased insulin sensitivity in rats on high fat diet. Intriguingly, IKKβ/NF-κB signalling promotes SOCS3 expression in vitro and in vivo (77). Medial basal hypothalamic overexpression of IKKβ by lentiviral delivery significantly elevated SOCS3 mRNA and protein.
Taken together these findings reveal that also in DIO upregulation of SOCS3 might be the molecular means of increased body weight induced by high fat feeding. Intriguingly, the central role of SOCS3 as a potential molecular mediator of leptin resistance appears to be preserved whether the cause of leptin resistance is either physiological (in seasonal animals or during pregnancy) or patho-physiological during DIO. The process leading to increased hypothalamic SOCS3 expression, however, seems different and dependent on the origin of leptin resistance. In physiological states when leptin resistance is intended and necessary for the survival it might be driven by the hormone prolactin whereas in patho-physiological states, in which leptin resistance is the consequence of malnutrition, hypothalamic inflammatory processes might be the cause (Fig. 5).

Concluding remarks

During the last few years, significant advances in understanding the molecular basis of the phenomenon of leptin resistance were achieved. Despite the obvious advantages of utilizing novel transgenic animals, to improve our understanding of the pathogenesis of leptin resistance in physiological states, in which leptin resistance is the consequence of malnutrition, hypothalamic inflammatory processes might be the cause (Fig. 5).

Acknowledgements

I am grateful to Professor Dave Grattan and Professor Julian Mercer for their review of this manuscript. The author was funded by the Boehringer Ingelheim Fonds, the Health Research Council of New Zealand and a young investigator programme of the German Ministry of Research and Education.

References


rat during late pregnancy and lactation. Endocrinology 2006; 147: 4996–5005.
75 Levin BE, Dunn-Meynell AA, Banks WA. Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. Am J Physiol Regul Integr Comp Physiol 2004; 286: R143–R150.