

Molecular and anatomical determinants of central leptin resistance

Heike Münzberg & Martin G Myers, Jr

The increasing incidence of obesity in developed nations is an ever-growing challenge to health care, promoting diabetes and other diseases. The hormone leptin, which is derived from adipose tissue, regulates feeding and energy expenditure. Most forms of obesity are associated with diminished responsiveness to the appetite-suppressing effects of leptin. Here we review the mechanisms by which leptin activates intracellular signals, the roles of these signals in leptin action *in vivo*, and mechanisms that may attenuate leptin signaling, limiting its action in obese individuals. We highlight data regarding the expression of SOCS3 (a potential mediator of leptin resistance) in the arcuate nucleus of the hypothalamus.

Leptin is produced in proportion to fat stores; adequate leptin levels communicate the repletion of body energy stores to the CNS in order to suppress food intake and permit energy expenditure^{1–3}. Conversely, lack of leptin signaling owing to mutation of leptin (as in *ob/ob* mice) or the leptin receptor (as in *db/db* mice) in rodents and humans results in increased food intake in combination with a phenotype of reduced energy expenditure, reminiscent of the neuroendocrine starvation response^{1,2,4}. Leptin also regulates insulin sensitivity and glucose homeostasis by two mechanisms: first, by controlling energy balance and body fat (increased body adiposity leads to insulin resistance) and, second, through an adiposity-independent pathway mediated by the CNS control of hepatic glucose output^{5–8}. The fact that high circulating levels of leptin fail to promote weight loss in obesity have given rise to the notion of ‘leptin resistance’—that leptin action is limited in obese states.

The long form of the leptin receptor, LRB, is the form that mediates intracellular signaling and thus is crucial for leptin action^{9,10}. Many of the actions of leptin are attributable to effects in the CNS, particularly in the basomedial hypothalamus, including the arcuate, dorsomedial, ventromedial and premammillary nuclei^{2,11}. Within the arcuate nucleus, the best-characterized site of leptin action, LRB is found in at least two distinct populations of neurons. One population synthesizes neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the other synthesizes pro-opiomelanocortin (POMC)^{2,11,12}. In neurons expressing LRB and POMC, POMC is processed to produce α -melanocyte-stimulating hormone (α MSH), which signals anorexia (decreased appetite) by activating downstream melanocortin-3 and -4 receptors. LRB stimulates the synthesis of POMC and promotes the firing of these neurons^{11,14}. NPY is an orexigenic (appetite-stimulating) hormone, and AgRP is an inhibitor of melano-

cortin-3 and melanocortin-4 receptor signaling. Leptin acts through LRB to inhibit NPY/AgRP neurons and suppress expression of these neuropeptides^{11,14}. Thus, LRB signaling stimulates the elaboration of anorectic neuropeptides and suppresses the action of orexigenic peptides in the arcuate nucleus.

The finding that the arcuate-specific expression of LRB decreases appetite and decreases adiposity in rodents lacking LRB suggests that leptin is important in the arcuate nucleus for energy balance^{15,16}. A key component of the adiposity-independent regulation of glucose metabolism by leptin is also mediated by the arcuate nucleus¹⁶. It is clear, however, that leptin action in the arcuate nucleus represents only one component of its role in energy balance, as deletion of LRB from POMC neurons results in a relatively modest overweight phenotype, and the restoration of LRB expression specifically in the arcuate nucleus of *db/db* mice reverses only part of the hyperphagia and obesity of these animals^{16,17}.

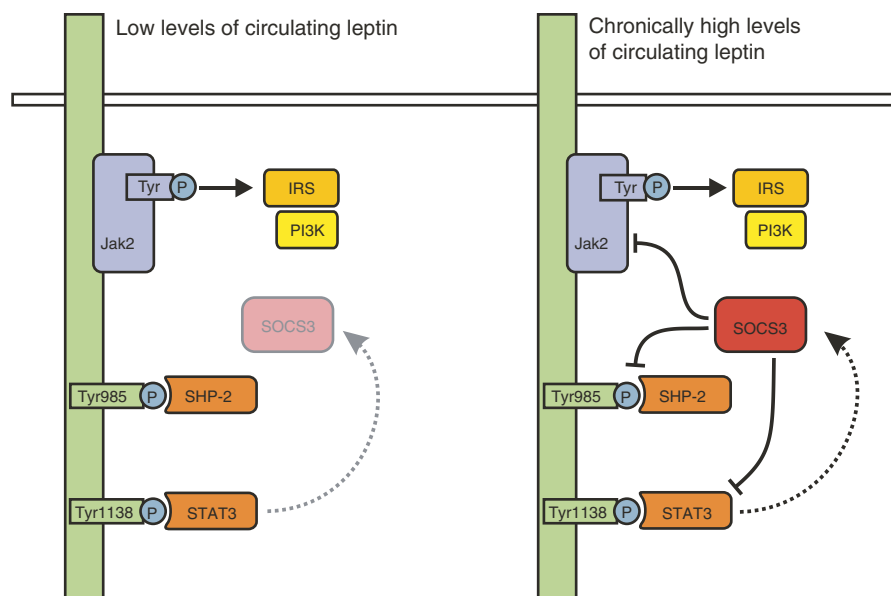
Leptin receptor signaling

Like other cytokine receptors, LRB does not have intrinsic enzymatic activity; instead, it signals by activating a noncovalently associated Jak family tyrosine kinase (Jak2) that autophosphorylates numerous tyrosine residues as well as phosphorylating two residues (Tyr985 and Tyr1138) on LRB during leptin stimulation^{10,18–20}. There are thus three distinct LRB signaling pathways (**Fig. 1**): those involving Tyr985 of LRB, those involving Tyr1138 of LRB and those originating directly from Jak2 tyrosine autophosphorylation sites³. The phosphorylation of Tyr985 recruits the tyrosine phosphatase SHP-2, which thereby mediates the activation of the canonical p21ras→ERK signaling pathway in cultured LRB-expressing cells. Although Tyr985 thus mediates most ERK stimulation during LRB signaling in cultured cells, part of the LRB-stimulated ERK activation is regulated independently of LRB phosphorylation, presumably via tyrosine phosphorylation sites on Jak2 (refs. 20,21). Tyr985 also attenuates LRB signal by binding the inhibitory SOCS3 molecule²².

Phosphorylation of Tyr1138 recruits the latent transcription factor STAT3 to the LRB-Jak2 complex, resulting in tyrosine phosphorylation and nuclear translocation of STAT3 so that it can regulate

Heike Münzberg and Martin G. Myers, Jr. are in the Division of Metabolism, Endocrinology and Diabetes, Department of Medicine and Department of Molecular and Integrative Physiology, University of Michigan Medical School, 1150 West Medical Center Drive, Ann Arbor, Michigan 48109, USA. Correspondence should be addressed to M.G.M. (mgmyers@umich.edu). Published online 26 April 2005; doi:10.1038/nn1454

Figure 1 LRB signaling and proposed role for SOCS3 in LRB signal attenuation. Leptin binding to LRB activates the LRB-associated Jak2 tyrosine kinase, leading to the autophosphorylation of tyrosine residues on Jak2 and the phosphorylation of Tyr985 and Tyr1138 on the intracellular tail of LRB. Although signaling by phosphorylation sites on Jak2 remains to be fully explored, Jak2 seems to activate the IRS-PI3K signaling pathway. Phosphorylation of Tyr1138 mediates the activation of the transcription factor STAT3. Among other targets, STAT3 induces the transcription (symbolized by the dashed arrows) of SOCS3 during LRB signaling. Tyr985 plays a dual role in LRB signaling, binding SHP-2 and also providing an important site of interaction for SOCS3 (SOCS3 also binds directly, albeit with lower affinity, to Jak2). SOCS3 binding to the LRB-Jak2 complex attenuates LRB-mediated signaling. We posit that the inhibitory feedback action of this LRB-SOCS3 pathway *in vivo* could explain the diminishing effectiveness of increasing leptin concentrations in obesity: at low concentrations of leptin, where baseline STAT3 activation is modest, induction of SOCS3 would also be modest, and incremental changes in leptin would be almost fully translated into changes in LRB signaling. At high levels of circulating leptin (as in obesity), the increased baseline STAT3 activation would result in increased expression of SOCS3, which would mitigate most of the expected increase in LRB signaling.



transcription^{19,20}. STAT3 activates the transcription of the neuropeptide POMC, the signaling inhibitor SOCS3 (refs. 20,23,24), and other genes. SOCS3 binds to Tyr985 of LRB to mediate inhibition of LRB→STAT3 signaling²²; SOCS3 also binds to a separate site on Jak2 itself^{25,26}. The relative importance of these two binding sites for SOCS3 remains an important area of investigation, as does the contribution of the Tyr1138→STAT3 pathway to the generation of SOCS3 in LRB-expressing tissues *in vivo*.

Jak2 tyrosine phosphorylation during LRB stimulation mediates some signals independently of tyrosine phosphorylation sites on LRB (for example, some of the ERK activation)²⁰. The individual tyrosine phosphorylation sites on Jak2 are beginning to be enumerated^{27–31}, but many more remain to be identified; the binding partners and signals mediated by many sites are not known, limiting our understanding of the mechanisms that mediate Jak2-dependent signals. Although signaling by the Jak2-LRB complex by means of the IRS proteins (a class of intracellular tyrosine kinase substrates) is not fully understood, activated Jak2 tyrosine phosphorylates IRS proteins, which subsequently recruit the phosphatidylinositol 3'-kinase (PI3K) to activate downstream PI3K signals^{3,32,33}.

LRB signaling in the regulation of physiology

Many intracellular signaling pathways function in the regulation of energy balance. In mice, deletion of one of the four IRS proteins, IRS-2, results in obesity³⁴. Although this approach says little about the specificity of the resulting phenotype to leptin action, the finding that the pharmacological inhibition of PI3K blocks the suppression of feeding by leptin, but not by melanocortin agonists, suggests that IRS-2→PI3K signaling contributes to the regulation of energy balance by leptin³⁵. Conditional deletion of SHP-2 or STAT3 in forebrain neurons results in overfeeding and obesity^{36,37}. It is not clear from this knockout approach whether the resulting obesity actually stems from perturbations in leptin signaling *per se* or from less specific consequences of the widespread removal of these promiscuous signaling molecules. However, the role of LRB→STAT3 signaling in

energy balance has been clearly established by the study of rodents with a homologous replacement of LRB by a receptor mutant for Tyr1138, which is the site of STAT3 binding^{24,38}. The LRB Tyr1138→STAT3 signal, although dispensable for some neuroendocrine functions of leptin, is required for the normal regulation of POMC and AgRP expression in the hypothalamus and thus for the control of feeding and energy expenditure by leptin. Notably, whereas the LRB Tyr1138→STAT3 pathway is thus central to the adiposity-dependent regulation of insulin sensitivity, this signaling pathway is dispensable for the adiposity-independent regulation of glucose homeostasis by leptin³⁹. Thus, another signal, such as the IRS-protein→PI3K pathway, must mediate the adiposity-independent regulation of glucose homeostasis by leptin.

Leptin resistance and obesity

Obesity is a growing health problem in the western world and is a major risk factor for type 2 diabetes and cardiovascular disease⁴⁰. After the discovery of leptin, it was initially hoped that exogenous leptin therapy might induce satiety and weight loss in obese humans^{1,2,9}. Indeed, leptin administration reduces appetite and promotes weight loss in obese, genetically leptin-deficient humans and rodents^{4,41}, and it ameliorates the hyperphagia and endocrine abnormalities associated with very low body-fat content caused by a number of lipodystrophic and eating disorders^{42–44}. Unfortunately, the scale of the weight loss achieved with leptin therapy in most obese humans has been modest⁴⁵. Indeed, most obese individuals have elevated circulating levels of leptin as a consequence of their large fat mass, but they do not adequately respond to these increased leptin levels with reduced food intake⁴⁶. This under-responsiveness to endogenous and exogenous leptin in most forms of obesity has given rise to the idea that obesity is associated with or even caused by a state of relative leptin resistance similar to the insulin resistance of type 2 diabetes^{47,48}. Although leptin has a profound effect on the transcriptional regulation of the neuropeptides POMC, AgRP and NPY, there is little consensus regarding the potential dysregulation of specific neuropeptides in diet-induced obesity.

The mechanisms underlying leptin resistance remain a matter of debate, but the two hypotheses that have received the most attention are failure of circulating leptin to reach its targets in the brain and inhibition of the intracellular LRB signaling cascade. With respect to the former hypothesis, leptin may gain access to the brain by a number of mechanisms, including a specific transport mechanism functioning across the blood-brain barrier, diffusion from the circumventricular organs (such as the median eminence), and direct access from the blood to neuroendocrine neurons that project to the circulation. Leptin is clearly transported across the blood-brain barrier by a saturable transport system⁴⁹, the activity of which seems to be decreased in rodents with diet-induced obesity (a classical model of obesity and leptin resistance in which rodents are made obese by high-fat feeding)⁵⁰. However, the extent to which leptin transport across the blood-brain barrier contributes to leptin action is not clear, especially in the arcuate nucleus, which is close to the median eminence and the portal circulation and may not be fully protected by the blood-brain barrier^{51,52}.

Regardless of whether defective leptin transport contributes to leptin resistance, it is clear that the ability of leptin to activate hypothalamic signaling is decreased in diet-induced obesity^{47,48,53}. Indeed, numerous studies provide support for potential roles for two inhibitory molecules, SOCS3 and the protein tyrosine phosphatase PTP1B (refs. 22,54–58), in the regulation of LRB signaling *in vitro* and *in vivo*. Overexpression of PTP1B in cultured cells attenuates leptin signaling, and leptin signaling is increased in fibroblasts lacking PTP1B (refs. 54,55). In intact animals, the increased insulin sensitivity in PTP1B-null mice is at least in part attributable to the leanness of these animals, suggesting that increased leptin sensitivity may underlie the metabolic phenotype of these animals^{54,55}.

Similarly, exogenous SOCS3 expression blocks LRB signaling in cultured cells, whereas LRB signaling is enhanced by RNAi-mediated knockdown of SOCS3 and by mutation of Tyr985 (the SOCS3 binding site on LRB). In contrast, overexpression, blockade or knockdown of SHP-2 in cultured cells minimally alters the amplitude of LRB signals (with the exception of SHP-2-dependent ERK activation)^{21,22,56}. The leanness and leptin sensitivity of mice haploinsufficient for SOCS3 and mice lacking SOCS3 in the CNS^{57,58} suggest that SOCS3 limits LRB action *in vivo*.

Clearly, PTP1B and SOCS3 each act to decrease LRB signal strength *in vivo*. Although hypothalamic PTP1B levels are not known to be altered in obesity, SOCS3 expression is increased in several rodent models of leptin-resistant obesity, consistent with a potential role for SOCS3 in leptin resistance^{53,56}. The stimulation of SOCS3 expression by leptin in cultured cells and in the hypothalamus suggests that high levels of leptin may induce SOCS3 expression and thus mediate the attenuation of LRB signaling in obesity^{12,20,22,53,56}. Thus, we postulate that the action of this LRB→SOCS3 pathway *in vivo* could explain the diminishing effectiveness of increasing leptin concentrations in obesity (Fig. 1). When leptin concentrations are low and thus baseline STAT3 activation is modest, SOCS3 expression is low, and incremental changes in leptin would be almost fully translated into increased LRB signaling. When circulating leptin levels are high (as in obesity), the increased baseline STAT3 activation would result in increased expression of SOCS3, mitigating much of the effect of increased leptin binding to LRB. In line with this model, data from cultured cells suggest that chronic high-level LRB activation induces its own feedback inhibition, effectively limiting the efficacy of high concentrations of leptin during chronic exposure^{20,22,26,56}. This feedback inhibition of LRB signaling depends both on the STAT3 pathway (which stimulates the expression of SOCS3 as well as the expression of some mediators of leptin action) and on

the expression of SOCS3 itself²⁶. It is possible that other signals may increase expression of SOCS3 and other mediators of leptin resistance *in vivo*, however. These other potential inducers of SOCS3 expression include inflammatory mediators and cytokines such as IL-6 and TNF α , fatty acids and other lipids, and activators of counter-regulatory signals, such as corticosteroids.

The anatomical distribution of leptin resistance

Leptin-stimulated STAT3 activation is greatly attenuated in the hypothalamus of rodents with diet-induced obesity⁴⁸. The arcuate nucleus can show a specific defect in leptin-stimulated STAT3 phosphorylation (detected by immunohistochemistry) in the context of continued leptin sensitivity of other hypothalamic and extrahypothalamic sites⁵³. Most studies have assayed LRB→STAT3 signaling to examine leptin signaling in leptin-resistant states (STAT3 activation is the most robust test for LRB signaling *in vivo*). The attenuation of leptin-induced STAT3 phosphorylation in diet-induced obese rodents, however, presumably reflects the diminution of all leptin-stimulated signals, although this has not been formally demonstrated. Arcuate nucleus-specific resistance correlates with greater expression of SOCS3 in the arcuate nucleus than in other hypothalamic nuclei (such as the ventromedial and dorsomedial hypothalamic nuclei), suggesting a role for SOCS3 expression in the arcuate-specific development of leptin resistance. Arcuate-restricted leptin resistance and SOCS3 expression are also seen in Siberian hamsters adapted to short photoperiods⁵⁹. SOCS3 inhibits not only leptin signaling to STAT3, but also the activation of all known leptin signaling pathways, including the IRS-protein→PI3K pathway^{36,60–62}.

These data cannot exclude the possibilities that important mediators of leptin resistance act on signaling pathways other than STAT3 and that these putative mediators of leptin resistance act in other hypothalamic nuclei to contribute to the leptin resistance of diet-induced obese rodents. Some data suggest roles for sites other than the arcuate nucleus in the regulation of body weight by leptin^{16,17}. Because diet-induced obesity results in relatively small increases in daily food intake that are manifest by their cumulative effect over time, however, modest changes in leptin action in the arcuate nucleus could presumably mediate these minor alterations of energy balance in the absence of other major sites of leptin resistance.

Numerous data suggest that the arcuate nucleus integrates signals from hormones other than leptin (such as insulin and ghrelin) and from metabolic fuels (such as glucose and lipids)⁶³. The crucial role of the arcuate nucleus in glucose homeostasis renders the hypothesis of arcuate nucleus-specific leptin resistance even more tantalizing: impairment of the regulation of these neurons should theoretically lead not only to obesity, but also to impaired glucose homeostasis. Thus, arcuate nucleus leptin resistance could potentially link obesity to the predisposition to diabetes with which it goes hand-in-hand.

The means by which the arcuate nucleus could be specifically subject to leptin resistance is less clear. One possible explanation is its increased accessibility to circulating factors: the close apposition of the arcuate nucleus to the median eminence and the direct contact of secretory neurons in the arcuate nucleus with the circulation may render this region more sensitive to circulating mediators of leptin resistance^{51,52}. Indeed, the arcuate nucleus of immature rodents is specifically sensitive to the neurodegenerative effects of peripherally applied monosodium glutamate, suggesting that the arcuate nucleus is accessible to circulating molecules, at least in juveniles⁶⁴. Similarly, young animals are more sensitive to the development of diet-induced obesity than are mature animals. Thus, it is theoretically possible that greater exposure of the arcuate nucleus to circulating mediators of leptin resistance

(for instance, leptin or other cytokines), as compared with the relative protection afforded the rest of the brain by the blood-brain barrier, could mediate arcuate nucleus-specific induction of SOCS3 and might therefore trigger the development of leptin resistance.

Summary

Since the discovery of leptin in 1994, our understanding of the homeostatic mechanisms that regulate food intake and energy expenditure have increased logarithmically. It has also become clear, however, that these homeostatic mechanisms do not adequately diminish feeding and promote weight loss in obesity. Although we do not fully understand the mechanisms that limit the body's ability to respond appropriately to obesity, this is an important problem that is receiving a great deal of attention. It is clear not only that leptin fails to adequately limit feeding and mediate weight loss, but also that hypothalamic leptin signaling is suppressed in an anatomically distinct manner in most obese states. Several explanations have been proposed to underlie the limitation of leptin action in the face of high circulating leptin levels of obesity (known as leptin resistance). One reasonable mechanism derives from elevated SOCS3 expression in leptin-responsive neurons of the arcuate nucleus. Going forward, it will be important to understand the diverse signaling pathways activated by LRB and to dissect the identity and function of the myriad uncharacterized populations of leptin-responsive neurons in the CNS. In addition to rigorously examining the intracellular mechanisms by which leptin action may be attenuated in obese states, understanding neuroanatomical determinants of leptin resistance will be crucial to defining the mechanisms underlying the development and maintenance of obesity, in order to develop more effective therapies for this increasingly important public health problem.

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The authors declare that they have no competing financial interests.

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Web address

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