20 YEARS OF LEPTIN

Connecting leptin signaling to biological function

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Abstract

Hypothalamic leptin action promotes negative energy balance and modulates glucose homeostasis, as well as serving as a permissive signal to the neuroendocrine axes that control growth and reproduction. Since the initial discovery of leptin 20 years ago, we have learned a great deal about the molecular mechanisms of leptin action. An important aspect of this has been the dissection of the cellular mechanisms of leptin signaling, and how specific leptin signals influence physiology. Leptin acts via the long form of the leptin receptor LepRb. LepRb activation and subsequent tyrosine phosphorylation recruits and activates multiple signaling pathways, including STAT transcription factors, SHP2 and ERK signaling, the IRS-protein/PI3Kinase pathway, and SH2B1. Each of these pathways controls specific aspects of leptin action and physiology. Important inhibitory pathways mediated by suppressor of cytokine signaling proteins and protein tyrosine phosphatases also limit physiologic leptin action. This review summarizes the signaling pathways engaged by LepRb and their effects on energy balance, glucose homeostasis, and reproduction. Particular emphasis is given to the multiple mouse models that have been used to elucidate these functions *in vivo*.

Key Words
- leptin
- signal transduction
- obesity
- hypothalamus

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Introduction

Obesity and its many comorbidities present a significant challenge to public health in the USA. The health care costs associated with obesity totaled more than $147 billion annually. In addition to the economic burden, obesity results in premature death and disability from stroke, cardiovascular disease, and type 2 diabetes mellitus (http://www.cdc.gov/obesity/data/adult.html accessed 6/29/14). Furthermore, the obesity epidemic is no longer confined to the USA. Worldwide, more than 1.4 billion adults were overweight or obese in 2008 (Danaei *et al.* 2011). Clearly, the need for anti-obesity therapies is large and growing larger, yet no pharmacotherapies have been achieved more than minimal success in promoting long-term weight loss.

At its most basic level, body weight is determined by the amount of energy taken in relative to energy expenditure (Schwartz *et al.* 2000). If energy intake exceeds energy expenditure, excess energy accumulates in the form of triglycerides stored in adipose tissue, resulting in weight gain and obesity. However, the brain integrates signals of long-term energy stores with other physiologic inputs to modulate energy intake relative to energy expenditure. When adipose energy (fat) stores fall, hunger increases and energy expenditure decreases to defend body energy stores; conversely, the brain responds to nutritional surfeit by permitting increased energy expenditure and decreased feeding to maintain a constant body weight.

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One of the most important and widely studied players in the control of energy balance is the hormone leptin (Friedman & Halaas 1998, Elmquist *et al.* 2005). Leptin was discovered by Zhang *et al.* (1994). Defects in leptin production underlie the massive obesity observed in *ob/ob* mice. Leptin is produced in adipose tissue in proportion to triglyceride stores, and serves as a critical indicator of an organism's long-term energy status (Frederich *et al.* 1995*a*, Maffei *et al.* 1995). Leptin acts primarily in the brain, especially the hypothalamus, where its action is integrated with that of other adipokines, gastrokines, and other signals to coordinate energy homeostasis (Friedman & Halaas 1998, Bates & Myers 2003, Myers *et al.* 2009, Ring & Zeltser 2010). In addition to leptin-deficient *ob/ob* mice, rare human mutations resulting in leptin deficiency have also been identified; leptin-deficient mice and humans display hyperphagia, decreased energy expenditure, and early-onset obesity (Montague *et al.* 1997, Farooqi *et al.* 1999). Leptin receptor (LepRb)-deficient humans and *db/db* mice display a similar phenotype (Tartaglia *et al.* 1995, Chua *et al.* 1996). Numerous studies have elaborated the critical role of leptin in the modulation of energy balance: the lack of leptin, as in starvation or genetic leptin deficiency, increases hunger while promoting an energy-sparing program of neuroendocrine and autonomic changes, including decreased sympathetic nervous system tone, thyroid function, growth, and reproduction (Ahima *et al.* 1997). Leptin treatment largely reverses these changes (Farooqi *et al.* 1999, 2002). Decreased leptin also promotes a variety of other behavioral and physiologic changes to respond appropriately to low energy stores (Lu *et al.* 2006, Liu *et al.* 2010, 2011).

Despite the initial heralding of leptin as a potential cure for human obesity, most obese humans exhibit high circulating leptin concentrations (Maffei *et al.* 1995). Serum leptin increases in proportion to body fat percentage; obese patients secrete leptin at levels appropriate for their increased adipose mass and display elevated leptin concentrations ('hyperleptinemia') relative to lean controls (Tobe *et al.* 1999). Clearly, however, these high circulating leptin levels do not suffice to restore body adiposity to lean levels, as might be predicted based on the sensitivity of organisms to decreases in leptin signaling. Whether this inability of leptin to suppress feeding in the face of obesity results from an intrinsic or acquired defect in leptin action, or rather simply reflects the inability of homeostatic controls to overcome hedonic feeding drives remains a matter of debate. This controversy serves to underscore the importance of developing a more complete understanding of leptin signaling, its cellular effects, target neural pathways, and integration with other determinants of energy homeostasis (Figs 1 and 2).

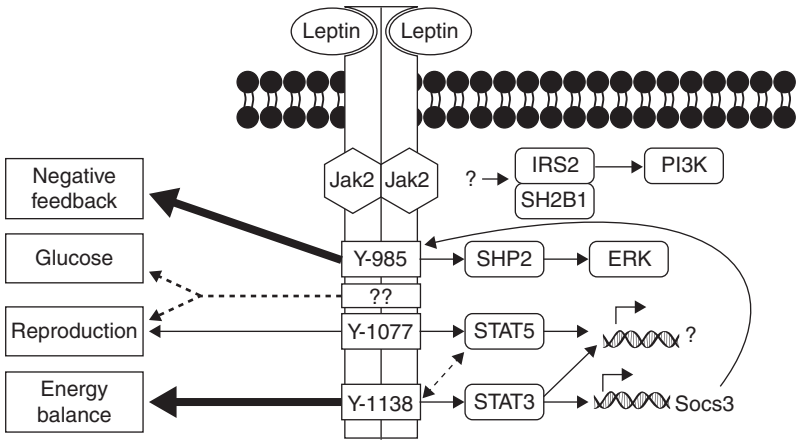

Figure 1
Leptin signaling and biological function. Leptin binds to LepRb, activating the associated JAK2 tyrosine kinase. Activated JAK2 phosphorylates the intracellular tail of LepRb on three tyrosine residues. Phosphorylated Tyr_{985} recruits SHP2, which participates in ERK signaling; Tyr_{985} also serves as a binding site for the negative feedback regulator, SOCS3. Phosphorylated Tyr_{1077} partially mediates leptin's control of reproduction; while STAT5 binds this site, STAT5 does not appear to participate in this effect of leptin. Phosphorylated Tyr_{1138} engages the STAT3 transcription factor. LepRb→STAT3 signaling represents the primary mechanism by which leptin regulates energy balance, although the target genes of STAT3 in LepRb neurons remain undiscovered. Leptin also recruits the IRS2→PI3K and SH2B1 pathways, although the mechanism of their recruitment to LepRb remains unclear. Some glucoregulatory and reproductive actions of LepRb appear to be mediated by unknown signals that function independently of LepRb tyrosine phosphorylation sites.

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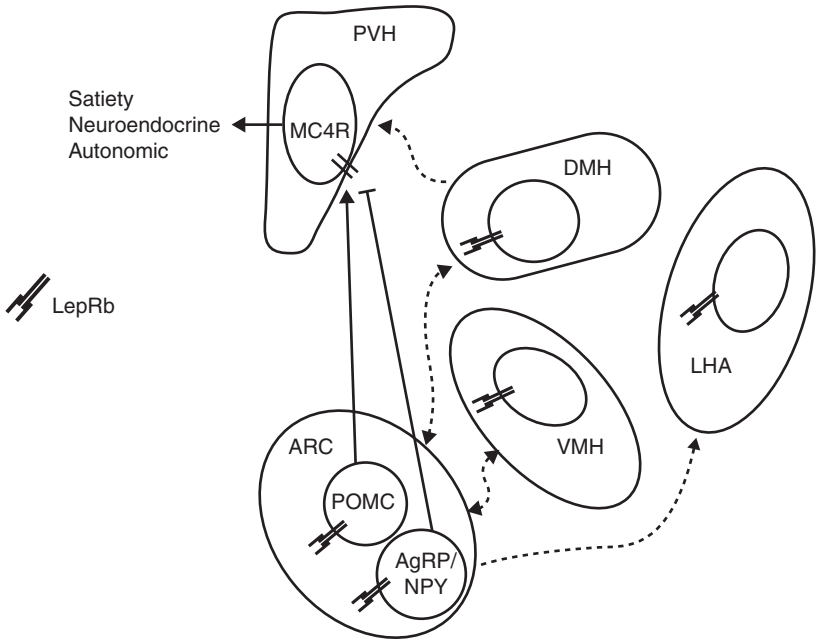

Figure 2
Hypothalamic leptin action. Leptin acts on its receptor (LepRb) on neurons in a series of interconnected hypothalamic nuclei to regulate satiety, neuroendocrine function, and autonomic tone. In the arcuate nucleus, leptin controls the melanocortin system through its opposing actions on POMC and AgRP neurons. ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; LHA, lateral hypothalamic area; PVH, paraventricular hypothalamic nucleus; MC4R, melanocortin 4 receptor; POMC, pro-opiomelanocortin; AgRP, agouti-related peptide.

Leptin and the LepRb

Leptin is a 146 amino acid protein produced in white adipose tissue in proportion to triglyceride stores (Frederich *et al*. 1995*b*). Once secreted into the circulation, leptin travels to the brain, where it enters the CNS, presumably via the choroid plexus and circumventricular organs. In the brain, leptin acts by binding and activating the long form of LepRb, which is expressed primarily on specialized subsets of neurons in certain hypothalamic and brainstem nuclei (Tartaglia 1997, Elias *et al*. 2000, Scott *et al*. 2009, Patterson *et al*. 2011). Mutations that inactivate LepRb, as well as antagonists of LepRb activation, confirm that leptin binding to LepRb is required for its biological activity (Chen *et al*. 1996, Shpilman *et al*. 2011). While the *LEPR* gene encodes multiple isoforms (LepRa-f in rats), only LepRb contains the full intracellular domain necessary for the activation of critical second messenger pathways and normal leptin action (Chua *et al*. 1996, 1997, Lee *et al*. 1996, Tartaglia 1997). Many functions for the other ('short') forms of the receptor have been hypothesized, including actions as a serum-binding protein that functions in leptin stabilization or sequestration (Zastrow *et al*. 2003, Yang *et al*. 2004, Zhang & Scarpace 2009), or as a leptin transporter (Bjorbaek *et al*. 1998*a*, Kastin *et al*. 1999), but LepRb alone suffices for the control of energy balance, glucose homeostasis, and other leptin effects, and LepRb thus constitutes the focus of this review.

Peripheral actions of leptin

Multiple studies have attempted to assess the role of leptin in the periphery. Mice with ablated hepatic leptin signaling had normal body weight and blood glucose levels, but were protected from high-fat diet or age-induced insulin intolerance. Mice in which LepRb was deleted from the pancreas using a Pdx^{cre} or Rip^{cre} also showed improvements in glucose tolerance (Morioka *et al*. 2007, Huynh *et al*. 2010). However, interpretation of these results is confounded by hypothalamic CRE expression in both the *PDx* and *RIP* models (Schwartz *et al*. 2010, Wicksteed *et al*. 2010). LepRb expression has also been demonstrated in perivascular intestinal cells, although the function of LepRb in these cells has not been determined

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(Rajala *et al*. 2014). Studies examining the role of LepRb in the heart have been difficult to perform based on the negative effects of *cre* expression on cardiac function (Hall *et al*. 2011). One model revealed an additive role for cardiac-specific LepRb deletion in inducing cardiac failure, however, suggesting that LepRb may regulate the cardiovascular system through both central and peripheral mechanisms (Hall *et al*. 2012).

Central actions of leptin

Within the brain, leptin acts on multiple populations of LepRb neurons – primarily in the hypothalamus and brainstem (Scott *et al*. 2009, Patterson *et al*. 2011). While leptin action in the nucleus of the solitary tract plays a role in the modulation of satiety, and ventral tegmental area LepRb contributes to the control of reward and aversion, hypothalamic LepRb appears to mediate the lion's share of leptin action on energy balance (Hommel *et al*. 2006, Hayes *et al*. 2010, Ring & Zeltser 2010). Within the hypothalamus, leptin acts on multiple populations of LepRb-expressing neurons, including those in the lateral hypothalamic area and the ventromedial, dorsomedial, ventral premammillary, and arcuate (ARC) nuclei (Scott *et al*. 2009, Patterson *et al*. 2011). Each of these sites contains multiple distinct types of LepRb cells, each of which contributes uniquely to leptin action. The most studied site of leptin action is the ARC, where leptin inhibits orexigenic agouti-related protein/neuropeptide Y-containing (AgRP/NPY) neurons and stimulates anorexigenic proopiomelanocortin (POMC)-containing neurons. POMC neurons produce anorexigenic neuropeptides, while AgRP is a potent antagonist of the melanocortin system and NPY mediates additional orexigenic signals (Schwartz *et al*. 2000).

LepRb signaling

LepRb is an IL6-type class I cytokine receptor, consisting of an extracellular leptin-binding domain, a single-pass membrane spanning domain, and an intracellular tail that contains binding domains for multiple signaling proteins (Tartaglia *et al*. 1995, Baumann *et al*. 1996). LepRb is present on the cell membrane as a mixture of monomers and dimers (Devos *et al*. 1997). Unlike many other cytokine receptors, ligand binding does not appear to activate LepRb by promoting receptor dimerization, but rather promotes a conformational change that results in the autophosphorylation and activation of JAK2, which is constitutively bound to Box1 and Box2 motifs in the membrane-proximal portion of LepRb (Banks *et al*. 2000, Kloek *et al*. 2002). Activated JAK2 phosphorylates LepRb on three tyrosine residues in mice: Tyr_{985}, Tyr_{1077}, and Tyr_{1138} (Banks *et al*. 2000, Gong *et al*. 2007). Each of these phosphorylated tyrosine (pY) residues represents a Src homology 2 (SH2)-binding motif that recruits specific SH2-containing effector proteins to the receptor to mediate subsequent signaling.

Leptin binding to LepRb results in the activation of several major signaling pathways. Importantly, phosphorylation of Tyr_{1138} results in the recruitment of STAT3 to LepRb, to permit its phosphorylation (pSTAT3) and activation by JAK2 (White *et al*. 1997, Banks *et al*. 2000). Activated pSTAT3 translocates to the nucleus, where it mediates changes in the expression of target genes, including suppressor of cytokine signaling 3 (*Socs3*) (which encodes a feedback inhibitor of LepRb signaling) (Bjorbaek *et al*. 1999). Phosphorylation of Tyr_{985} recruits protein tyrosine phosphatase 2 (SHP2; PTPN1) to LepRb, contributing to the activation of the ERK signaling pathway (Banks *et al*. 2000, Bjorbaek *et al*. 2001). Tyr_{985} also serves as the binding site for SOCS3 and thus plays a prominent role in the feedback inhibition of LepRb (Bjorbaek *et al*. 2000). Phosphorylated Tyr_{1077} promotes the recruitment and activation of STAT5; Tyr_{1138} may also contribute to STAT5 activation (Gong *et al*. 2007).

Another SH2 domain protein, SH2B1, also participates in LepRb signaling. In addition to increasing the amplitude of LepRb signaling via JAK2, SH2B1 may control specific downstream LepRb signals, including insulin receptor substrate (IRS)-proteins (Duan *et al*. 2004, Ren *et al*. 2005). IRS-proteins also participate in leptin action; they control the phosphatidylinositol 3-kinase (PI3K) pathway, and the subsequent regulation of Akt→FoxO1 and mTORC1 signaling (Niswender *et al*. 2001, Kim *et al*. 2006, Kitamura *et al*. 2006). The mechanism(s) whereby LepRb modulates this pathway remains obscure; some data suggest a potential role for poorly understood LepRb signaling that occurs independently of LepRb pY sites.

LepRb signaling and physiology

LepRb→STAT3 signaling

Multiple LepRb signaling pathways coordinate the regulation of energy homeostasis. Of these, the Tyr_{1138}→pSTAT3 pathway plays an especially prominent role (Bates & Myers 2003). Mice containing a substitution mutation of LepRb Tyr_{1138} (which renders LepRb incapable of recruiting and activating STAT3; *s/s* mice) display

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hyperphagia and obesity approaching that of *db/db* animals (although linear growth, fertility, and glucose homeostasis are relatively protected in *s/s* relative to *db/db* mice) (Bates *et al*. 2003, 2004, 2005). Furthermore, brain-specific STAT3-knockout mice ($STAT3^{N-/-}$) exhibit severe obesity (Gao *et al*. 2004). Mice in which STAT3 was deleted specifically in LepRb neurons ($LepRb^{STAT3\text{-}KO}$) similarly develop hyperphagic obesity with some preservation of glucose homeostasis (Piper *et al*. 2007). These studies highlight the importance of LepRb Tyr_{1138}→STAT3 signaling for the regulation of body weight, but suggest some regulation of growth, reproduction, and glucose homeostasis by leptin independently of this pathway.

The role of STAT3 signaling in energy balance in discrete neural populations has been best characterized in the ARC. As might be expected, specific deletion of STAT3 from AgRP neurons results in moderate obesity, increased *Npy* expression, and decreased sensitivity to leptin (Gong *et al*. 2008). STAT3 deletion from POMC neurons also increases adiposity, but the effect is milder than that observed for the AgRP-specific knockout, suggesting a greater role for STAT3 in leptin action in AgRP neurons than in POMC cells (Xu *et al*. 2007). In contrast to STAT3 deletion studies, the interpretation of studies in which a mutant, transcriptionally active, form of STAT3 (STAT3-C) is expressed in ARC neurons is more complicated. While STAT3-C expression in AgRP neurons promotes leanness, STAT3-C expression in POMC neurons results in obesity (Mesaros *et al*. 2008, Ernst *et al*. 2009). *Agrp* expression is not altered in $Agrp^{STAT3\text{-}C}$ mice, consistent with the notion that *Agrp* expression is more sensitive to modulation by PI3K than by STAT3 (see below) (Mesaros *et al*. 2008). It is possible that the mild obesity resulting from STAT3-C action in POMC neurons results from altered transcriptional activity of this isoform relative to native STAT3, but STAT3-C also promotes *Socs3* expression, which could limit endogenous leptin action despite increased transcription mediated by STAT3-C. Interestingly, although the *Pomc* promoter contains known STAT3-binding sites (Munzberg *et al*. 2003) and *Pomc* expression is decreased in *s/s* mice and animals with neuronal STAT3 ablation (Bates *et al*. 2003, Gao *et al*. 2004), *Pomc* expression is decreased in $Pomc^{STAT3\text{-}C}$ animals (Ernst *et al*. 2009), suggesting that while *Socs3* represents a direct STAT3 target, the control of ARC *Pomc* expression may reflect the effects of additional and/or downstream LepRb signals, as well. Additionally, none of the phenotypes resulting from the modulation of LepRb→STAT3 signaling in POMC or AgRP neurons approach that of brain or hypothalamus-wide modulation, suggesting that LepRb→STAT3 signaling in other, non-ARC LepRb cells contributes to the control of energy balance during LepRb→STAT3 signaling.

Tyr_{985}-dependent signaling, SOCS3, and SHP2

In contrast to the obese phenotype that results from disruption of LepRb→STAT3 signaling, mice with a mutation in Tyr_{985} display a lean phenotype (which is especially pronounced in females). These mice also display decreased hypothalamic *Agrp* expression, increased pSTAT3, exaggerated sensitivity to exogenous leptin, and resistance to DIO (Bjornholm *et al*. 2007). These results are consistent with increased LepRb signaling due to decreased LepRb feedback inhibition via disruption of SOCS3 binding. Indeed, as for mice mutant for LepRb Tyr_{985}, disruption of *Socs3* in the brain decreases adiposity (more dramatically in female than in male mice) and increases the response to exogenous leptin (Mori *et al*. 2004).

In addition to its role in feedback inhibition, Tyr_{985} may also coordinate energy homeostasis via SHP2/ERK signaling (Bjorbaek *et al*. 2000, 2001). As a tyrosine phosphatase, SHP2 was initially investigated as a potential negative regulator of leptin signaling. However, deletion of *Shp2* from the forebrain disrupts ERK signaling and promotes early-onset obesity (Zhang *et al*. 2004). Furthermore, deletion of *Shp2* from POMC neurons results in mild obesity and increased susceptibility to DIO (Banno *et al*. 2010). Similarly, female mice expressing a dominant active SHP2 mutant in the brain are resistant to DIO (He *et al*. 2012). Thus, these data are consistent with the notion that LepRb→SHP2 signaling is important for leptin action and the control of energy homeostasis, rather than SHP2-mediating feedback inhibition on LepRb. While SHP2 plays an essential role in the control of energy homeostasis, however, the promiscuity of SHP2 (which plays roles in many signaling pathways) renders it difficult to assess the specificity of SHP2 effects for LepRb signaling.

Tyr_{1077} and STAT5

LepRb→STAT5 signaling appears to have little impact on energy balance. While brain-wide STAT5 knockout mice develop late-onset obesity, this phenotype is quite mild (Lee *et al*. 2008). LepRb Tyr_{1077} mutants develop only mildly increased food intake and adiposity (Patterson *et al*. 2012). Furthermore, a recent study deleting STAT5 specifically in LepRb neurons has revealed no body weight phenotype; deleting both STAT3 and STAT5 that did not produce a more robust phenotype than deleting STAT3 alone (Singireddy *et al*. 2013). Also, Tyr_{1077} mutants enter

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puberty normally, but have a prolonged inter-estrus interval, suggesting mild subfertility in these animals. However, $LepR^{STAT5\text{-}KO}$ animals display normal estrus cycling and fertility. Altogether, these studies suggest that Tyr_{1077} plays a minor role in the control of feeding and reproductive functions, but that STAT5 may not be the binding partner that mediates this effect.

Other LepRb signals

Although the tyrosine phosphorylation of LepRb is essential for the majority of leptin's actions, mice in which Tyr_{985}, Tyr_{1077}, and Tyr_{1138} have all been replaced with phenylalanine ($LepRb^{3F}$) are less slightly less obese than *db/db* animals and display significant improvements in glucose homeostasis and fertility relative to *db/db* mice (Jiang *et al*. 2008). In contrast, mice express a LepRb truncation mutant ($LepRb^{\Delta 65}$) that retains JAK2 signaling and activity but lacks Tyr_{985}, Tyr_{1077}, and Tyr_{1138} phenocopy *db/db* animals and do not appear to be significantly protected from the obesity, diabetes and infertility that are hallmarks of impaired leptin signaling (Robertson *et al*. 2010). Thus, the improved phenotype seen in $LepRb^{3F}$ mice relative to *db/db* animals does not result from JAK2 signaling alone, as the $LepRb^{\Delta 65}$ model reveals that JAK2 signaling is not sufficient to mediate these improvements. The differing phenotypes between mice expressing $LepRb^{3F}$ and $LepRb^{\Delta 65}$ thus suggest the existence of non-canonical signaling pathway that may emanate from a distal site on LepRb, independently of LepRb pY sites. Further work will be required to identify this presumptive pathway.

While SH2B1 and IRS-protein/PI3K signaling contribute to leptin action, the mechanism(s) of their activation by LepRb remain somewhat unclear; no LepRb pY site has been definitively shown to mediate their recruitment. Thus, it is possible that one or both of these pathways constitute the presumptive LepRb pY-independent signaling pathway. Furthermore, these pathways may overlap, as SH2B1 recruits the IRS-protein/PI3K pathway during leptin signaling in cultured cells (Kim *et al*. 2000, Duan *et al*. 2004). However, the SH2B1 and IRS-protein/PI3K pathways contribute to energy balance *in vivo*. Sh2b1-null mice display severe early-onset obesity and hyperphagia (Ren *et al*. 2005). Furthermore, neuron-specific restoration of SH2B1 throughout the CNS rescues this phenotype, suggesting that CNS SH2B1 is crucial for the control of body weight (Morris *et al*. 2010). Unfortunately, the critical role of SH2B1 in insulin signaling (which is also significantly impacted by this deletion) as well as in signaling by other receptor tyrosine kinases renders it challenging to determine whether this phenotype results from only from the disruption of LepRb→SH2B1 signaling.

The roles for PI3K signaling in leptin action and the control of energy balance are also complicated. Leptin administration activates IRS-protein/PI3K signaling in the mediobasal hypothalamus, and ICV treatment with PI3K inhibitors inhibits leptin's anorexigenic effects (Niswender *et al*. 2001), along with the ability of exogenous leptin to suppress *Agrp* mRNA expression in fasted rats (Morrison *et al*. 2005). Furthermore, deletion of IRS2 specifically in LepRb neurons results in obesity (although it does not impact the ability of LepRb to stimulate pSTAT3) (Sadagurski *et al*. 2012). Both *in vitro* and *in vivo* studies have also implicated PI3K signaling in the acute actions of leptin. Leptin treatment induces the depolarization of POMC neurons in slice recordings; these effects are abrogated by pretreatment with PI3K inhibitors (Hill *et al*. 2008). This effect is also perturbed in mice lacking the PI3K regulatory subunits p85α and p85β in POMC neurons (Hill *et al*. 2008). While these mice do not display gross phenotypic abnormalities, leptin's ability to promote acute decreases in food intake is also disrupted. Studies in which the PI3K catalytic subunits p110α and p110β were deleted in AgRP or POMC neurons confirm these findings – mice lacking p110β in AgRP neurons are mildly lean, whereas mice lacking p110β in POMC neurons are more sensitive to DIO (Al-Qassab *et al*. 2009). It is unclear however, whether these results emanate from disrupted LepRb-PI3K signaling, or from alternations in IR-PI3K signaling, especially in light of data that suggests that leptin and insulin activate non-overlapping populations of POMC neurons (Williams *et al*. 2010). Together, these data suggest that leptin-induced PI3K signaling has a limited effect on energy balance. However, the importance of the LepRb-PI3K pathway for the glucoregulatory or reproductive functions of leptin is yet to be determined.

Negative regulation of leptin signaling

Multiple pathways and proteins inhibit LepRb. Given its role as an inhibitor of LepRb signaling, the mechanisms of action for SOCS3 have been a point of considerable interest. SOCS3 binds to LepRb Tyr_{985} and mediates negative feedback by directly inhibiting JAK2 activity and/or targeting the receptor-JAK2 complex for proteasomal degradation (Bjorbaek *et al*. 1998*b*, 1999, 2000). Neuron-wide deletion of *Socs3* using either nestin-cre ($Socs3^{N-/-}$) or synapsin-cre confers a significant resistance

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to diet-induced obesity (Mori *et al*. 2004). *Socs3*$^{N-/-}$ mice also display increased leptin sensitivity as measured by both leptin-induced food intake and STAT3 phosphorylation, as well as by increased PI3K activity. While *Socs3* has not been disrupted specifically in LepRb neurons, overexpression of *Socs3* in LepRb neurons (LepRb$^{Socs3\text{-}OE}$) yields an unexpected phenotype of slightly increased leanness (Reed *et al*. 2010). This may result from a compensatory increase in STAT3 at baseline and a corresponding increase in pSTAT3 levels after leptin treatment, although the mechanism for this is unclear and would seem to be a bit counter-intuitive. Clearly, however, the function of SOCS3 may not be as uniform or straightforward as initially thought.

Because high-fat diet induces *Socs3* expression in the ARC, ARC populations have been posited to be a major site of leptin resistance. As a result, the role of *Socs3* in arcuate POMC and AgRP neurons has been extensively studied. As with Socs3$^{N-/-}$ mice, POMC$^{Socs3\text{-}KO}$ mice are resistant to DIO, but display normal body weight on chow diet (Kievit *et al*. 2006). Interestingly, POMC$^{Socs3\text{-}KO}$ mice also have improved glucose homeostasis on a chow diet, suggesting that POMC neurons may be a critical site of LepRb/SOCS3 signaling in the control of peripheral blood glucose levels. Unlike LepR$^{Socs3\text{-}OE}$ mice, mice overexpressing *Socs3* in POMC neurons develop mild obesity on a chow diet, and acute leptin resistance (as assessed by leptin-induced inhibition of feeding) before any divergence in body weight (Reed *et al*. 2010). These animals also display a POMC neuron-restricted reduction in the pSTAT3 response to leptin, suggesting that potential compensatory mechanisms induced in the LepRb$^{Socs3\text{-}OE}$ model were not activated in this more restricted cell population. AgRP$^{Socs3\text{-}OE}$ mice also display early-onset leptin resistance, and slightly abnormal glucose homeostasis, but no alterations in body weight (Olofsson *et al*. 2013). Thus, while decreasing *Socs3* levels may prove protective against obesity, the modest body weight changes that occur with overexpression of *Socs3* suggest that increased *Socs3* levels may reflect hyperleptinemia and increased overall leptin signaling, rather than promoting obesity, *per se*.

Protein tyrosine phosphatases (PTPases) also modulate the amplitude and duration of LepRb signaling. Protein tyrosine phosphatase 1B (PTP1B) has been the most extensively studied of these, but other PTPs such as TCPTP and RPTPe also play critical roles in both leptin and insulin signaling (see review by Tsou & Bence (2013)). PTP1B is a promiscuous phosphatase that attenuates signaling by the receptor for insulin as well as other receptors, in addition to LepRb. *In vitro*, PTP1B dose-dependently suppresses the leptin-stimulated phosphorylation of Jak2 and pSTAT3 (Zabolotny *et al*. 2002). *In vivo*, whole-body PTP1B knockout (PTP1B^{TKO}) results in a lean phenotype, resistance to DIO, and increased sensitivity to exogenous leptin, consistent with the interpretation that PTP1B is a negative regulator of LepRb signaling (Klaman *et al*. 2000). Interpretation of the PTP1B^{TKO} model is complicated by the promiscuity of PTP1B and its broad pattern of expression, however, provoking more focused studies of the sites and mechanisms of its action. Pan-neuronal deletion of PTP1B also induces a lean phenotype, whereas liver or muscle-specific deletion has no effect, and adipose-specific deletion actually causes weight gain (perhaps due to enhanced adipose insulin signaling) (Bence *et al*. 2006). LepRb neuron-specific PTP1B deletion (LepRb$^{PTP1B\text{-}KO}$) results in a leaner phenotype than that observed in the PTP1B^{TKO} mice, suggesting that this model may have unmasked an even more important role for PTP1B in LepRb neurons that may have been opposed by other tissue (e.g., adipose) effects in the PTP1B^{TKO} model (Tsou *et al*. 2012). The specificity of PTP1B action on LepRb for the development of the lean phenotype is supported by the similar phenotypes of hypothalamic LepRb knockout and LepRb/PTP1B double-knockout mice, suggesting the LepRb dependence of the lean phenotype of PTP1B-null animals (Tsou *et al*. 2014). Interestingly, heterozygous LepRb$^{PTP1B+/-}$ mice display as strong a phenotype as LepRb$^{PTP1B\text{-}KO}$, underscoring the importance of appropriate levels of phosphatase action in the control of LepRb signaling (Tsou *et al*. 2012).

Future directions: leptin signaling and gene transcription

Despite the early identification of LepRb→STAT3 signaling as the primary mechanism for leptin's control of energy balance, LepRb→STAT3 target genes remain poorly defined. Currently, the list of genes known to be regulated by leptin *in vivo* is short: *Socs3*, *Pomc*, *Cart* (*Cartpt*), *Agrp*, and *Npy*. LepRb→STAT3 signaling is required for appropriate *Socs3*, *Pomc*, and *Agrp* gene expression, although (as noted above) *Pomc* and *Agrp* may represent indirect targets of STAT3 and/or may be partly controlled by other pathways; PI3K appears to play a role in the control of *Agrp* and *Npy* expression. Furthermore, of these five genes, only *Socs3* is thought to be induced in multiple LepRb populations; *Pomc*, *Agrp*, *Npy*, and *Cart* expression are restricted to circumscribed populations and do not contribute to leptin action in

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the majority of LepRb neurons. This dearth of information about LepRb→STAT3 target genes can largely be attributed to the challenge of specifically isolating LepRb neurons from the hypothalamic milieu; LepRb neurons comprise approximately <5% of all hypothalamic neurons, making it challenging to identify cell-autonomous changes in gene transcription for any subset of neurons. Clearly, more work will be necessary to identify the hypothalamic gene targets of LepRb and STAT3 signaling. These transcripts will be responsible for much of leptin action and may represent potential targets for therapy, in addition to shedding light on the mechanisms of leptin action.

Declaration of interest

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