

REVIEW

Leptin re sensitisation: a reversion of leptin-resistant states

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Abstract

Leptin resistance refers to states in which leptin fails to promote its anticipated effects, frequently coexisting with hyperleptinaemia. Leptin resistance is closely associated with obesity and also observed in physiological situations such as pregnancy and in seasonal animals. Leptin re sensitisation refers to the reversion of leptin-resistant states and is associated with improvement in endocrine and metabolic disturbances commonly observed in obesity and a sustained decrease of plasma leptin levels, possibly below a critical threshold level. In obesity, leptin re sensitisation can be achieved with treatments that reduce body adiposity and leptinaemia, or with some pharmacological compounds, while physiological leptin resistance reverts spontaneously. The restoration of leptin sensitivity could be a useful strategy to treat obesity, maintain weight loss and/or reduce the recidivism rate for weight regain after dieting. This review provides an update and discussion about reversion of leptin-resistant states and modulation of the molecular mechanisms involved in each situation.

Key Words

- ▶ diet-induced obesity
- ▶ leptin resistance
- ▶ obesity
- ▶ leptin re sensitisation

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Introduction

Obesity results from an imbalance between energy intake and energy expenditure and represents a major threat to health across the globe. It is a chronic and multifactorial condition related to numerous diseases that often leads to premature disability and mortality. The discovery of the anorexigenic hormone leptin in 1994 (Zhang *et al.* 1994) opened a new field within the therapeutic strategies to combat obesity. Leptin, a product of the obesity (*ob*) gene, displays key anti-obesity actions as it inhibits food intake and induces energy expenditure (Pellemounter *et al.* 1995). This anti-obesity effect was demonstrated in individuals bearing congenital leptin

deficiencies (Farooqi *et al.* 1999) and raised expectations regarding its potential as a drug to reduce body weight in obese patients. However, leptin therapy turned out to be disappointing as a weight loss strategy since most obese individuals have hyperleptinaemia associated with loss of responsiveness to this hormone. Such lack of sensitivity to leptin inspired the notion of leptin resistance (Frederich *et al.* 1995). Leptin resistance has been described in several conditions (from obese individuals to pregnant females and seasonal animals) and encompasses numerous molecular and cellular mechanisms that have not yet been fully elucidated. The restoration of leptin sensitivity

could be a useful strategy to treat obesity, maintain weight loss and/or reduce the recidivism rate for weight regain after dieting (Chhabra *et al.* 2016). The idea of leptin resensitisation emerges as a new concept that refers to the reversion of leptin-resistant states, usually linked to reduction of body adiposity and leptinaemia. This review summarises the current knowledge about the reversion of leptin-resistant states.

General aspects of leptin and its receptor: molecular leptin signalling

Leptin is a 167-residue peptide hormone mainly produced by adipocytes and acts in the central nervous system to primarily coordinate the metabolic adaptations to fasting (Zhang *et al.* 1994, Margetic *et al.* 2002). Leptin is also expressed in other tissues, but their contribution to the total leptinaemia is negligible (Guilmeau *et al.* 2004, Odle *et al.* 2014). Except during prolonged fasting, leptinaemia correlates with the mass of adipose tissue (Frederich *et al.* 1995), thereby representing a key marker of energy storage. As discussed below, leptinaemia falls when energy stores decrease (e.g., starvation), increasing appetite and decreasing energy use. Conversely, leptinaemia increases with adequate energy stores.

Newly synthesised leptin is sorted into the secretory pathway and secreted at the same rate as it is synthesised; as a consequence, the stimulated secretion of leptin mainly depends on the transcription and translation rates of the *ob* gene (Coleman & Herrmann 1999, Cammisotto *et al.* 2006). Thus, leptinaemia shows little short-term variation and requires several hours (or even days) to modificate in response to changes in the nutritional status (Myers 2006). Leptin content in adipose tissue directly depends on the fat cell size and is regulated by the hormonal milieu: exposure to high insulinaemia and glucocorticoid levels increases leptin production (Lee *et al.* 2007). Besides, the sympathetic nervous system, via activation of beta-adrenergic receptors, inhibits leptin production (Ricci & Fried 1999).

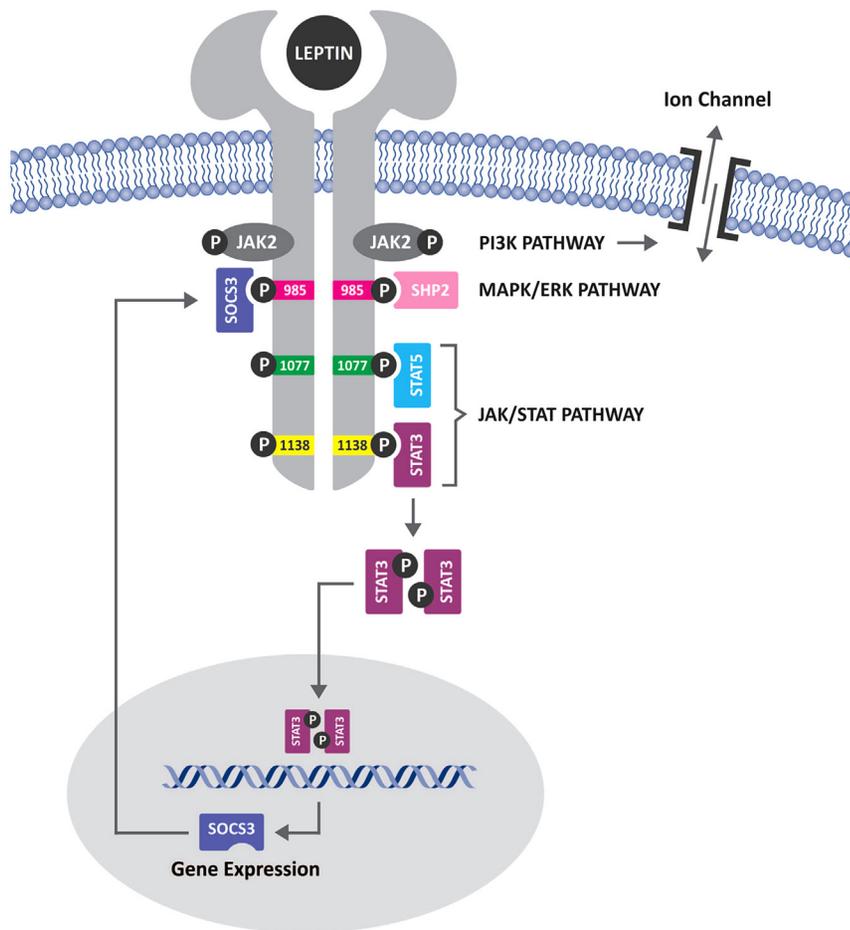
Leptin acts via its specific receptor, named LepR. Murine *Lepr* gene is alternatively spliced to give rise to six isoforms, LepRa-LepRf. LepR isoforms, except LepRe, differ in the length of the intracellular domain, and therefore, in their physiological role. LepRa isoform transports plasma leptin into the brain (Elmqvist *et al.* 1998, Hileman *et al.* 2002). Only LepRb contains all intracellular motifs required for full activation of the signalling pathways and, consequently, is essential for leptin action

(Tartaglia *et al.* 1995). LepR belongs to class 1 cytokine receptor family and lacks intrinsic enzymatic activity.

The binding of leptin to LepRb activates the tyrosine kinase janus kinase 2 (JAK2), which is associated with LepRb and phosphorylates the tyrosine residues Y985, Y1077 and Y1138 on LepRb (Fig. 1). These phosphorylated residues act as docking sites for signalling molecules that contain Src homology 2 (SH2)-domain and mediate the subsequent intracellular events (Banks *et al.* 2000). Phosphorylated Y1138 recruits the signal transducer and activator of transcription (STAT) 3, a latent transcription factor mediating major effects of LepRb (Bates *et al.* 2003, Gao *et al.* 2004). STAT3 is then phosphorylated by JAK2, generating pSTAT3 that undergoes dimerisation and translocates into the cell nucleus to act as an active transcription factor. Phosphorylated Y985 recruits a SH2-containing tyrosine phosphatase (SHP2), which activates mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK) signalling pathways (Banks *et al.* 2000). Phosphorylated Y985 also binds to the suppressor of cytokine signalling 3 (SOCS3), whose gene transcription depends on pSTAT3 and exerts an inhibitory effect on LepRb signalling (Bjorbaek *et al.* 1999). Phosphorylated Y1077 recruits STAT5, which mediates some effects of leptin on energy balance and reproduction as well as effects of prolactin (Tups 2009). Leptin-induced JAK2 activation also leads to the phosphatidylinositol 3-kinase (PI3K) activation, which is the major signalling pathway utilised by insulin and insulin-like growth factors (Hekerman *et al.* 2005). Notably, JAK2/STAT pathway mainly regulates gene expression while JAK2/PI3K pathway regulates not only gene expression but also fast cellular events by regulating ion channels (Donato *et al.* 2010, Gavello *et al.* 2016).

Biological effects of leptin

Leptin is a pleiotropic hormone that displays a variety of effects that seem to depend on its circulating level. Leptinaemia decreases under fasting, playing a critical role initiating the neuroendocrine response to starvation, including limiting procreation, decreasing thyroid thermogenesis and increasing secretion of stress steroids, which together are likely to have survival value during prolonged nutritional deprivation (Ahima *et al.* 1996). Leptin replacement blunts some of these fasting-induced adaptations, mainly concerning the gonadal, adrenal and thyroid axes (Ahima *et al.* 1996). Thus, studying the effects of systemic administration of leptin to fasted animals,

**Figure 1**

Leptin signalling. Binding of leptin to its receptor, LepRb, induces the activation of JAK2, which in turn phosphorylates the intracellular domain of LepRb, especially at tyrosines Y985, Y1077 and Y1138. Phosphorylated Y985 serves as a docking site for SHP2, activating MAPK/ERK signalling pathway. Phosphorylated Y1077 recruits STAT5. Phosphorylated Y1138 recruits STAT3, inducing its phosphorylation, dimerisation and nuclear translocation to activate the transcription of target molecules such as SOCS3. Induction of JAK2 can also stimulate PI3K, which regulates fast cellular events by regulating ion channels. LepRb also receives inhibitory signals from multiple negative feedback loops such as SOCS3 and PTP1B. ERK, extracellular signal-regulated kinase; JAK2, Janus kinase 2; LepRb, leptin receptor b; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; SHP2, Src homology 2-containing tyrosine phosphatase; SOCS3, suppressor of cytokine signalling 3; STAT3, signal transducer and activator of transcription 3; STAT5, signal transducer and activator of transcription 5. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0606>.

in doses that increase leptinaemia to levels similar to those found in fed animals, has helped to clarify some of the biologically relevant effects of leptin and supported the notion that leptinaemia in the lower concentration range is a key coordinator of the adaptation to negative energy balance conditions (Ahima *et al.* 1996). In contrast, the extent to which leptinaemia in the higher concentration range affects some physiological functions is still a matter of debate. Some groups suggest that hyperleptinaemia displays some actions in obesity that prevent further body weight gain (Myers *et al.* 2012, Ottaway *et al.* 2015), while others argue that the anti-obesity effect of hyperleptinaemia has not been clearly demonstrated (Ahima *et al.* 1996, Flier & Maratos-Flier 2017). In addition, some effects of leptin have been unmasked by administering pharmacological doses of leptin or by using non-physiological strategies of administration (e.g. chronic infusion, central infusions, infusions in specific brain areas); thus, the physiological implications of these observations are uncertain. Bearing these considerations in mind, some of the described biological effects of leptin are the following:

Leptin reduces food intake

Exogenous leptin, independently of the route of administration, reduces food intake in lean animals (Pellemounter *et al.* 1995, Halaas *et al.* 1997, Friedman & Halaas 1998, Ahima *et al.* 1999, Ravussin *et al.* 2014). Importantly, leptin infusion at doses that increase leptinaemia within physiological ranges leads to a transient reduction of food intake and weight loss (Halaas *et al.* 1997, Ahima *et al.* 1999, Ravussin *et al.* 2014).

The anorectic effect of leptin is mainly mediated by the hypothalamic arcuate nucleus (ARH) (Munzberg *et al.* 2004). In the ARH, leptin activates anorexigenic neurons that express proopiomelanocortin (POMC), which is cleaved into different neuropeptides, including α -melanocyte-stimulating hormone (α -MSH). α -MSH inhibits food intake via the melanocortin receptor 3 and 4 (MC3R/MC4R) (Coppari *et al.* 2005). In the ARH, leptin also inhibits orexigenic neurons that produce neuropeptide Y (NPY), agouti-related protein (AgRP) and gamma-aminobutyric acid (GABA) (Coppari *et al.* 2005). Leptin-responsive neurons outside the ARH could also

mediate the anorectic effects of the hormone. Particularly, the ventromedial nucleus (VMH) (Dhillon *et al.* 2006), the dorsomedial nucleus (DMH) and the paraventricular nucleus (PVH) of the hypothalamus are responsive to leptin and work as an interconnected circuit (Myers *et al.* 2009, Perello & Raingo 2013, Sutton *et al.* 2016). Other proposed targets of the anorexigenic effects of leptin include glutamatergic neurons of the median preoptic area (MPO) (Yu *et al.* 2016), dopamine neurons of the ventral tegmental area (VTA) (Fulton *et al.* 2006) and neurons of the nucleus of the solitary tract, which is an important relay of gastrointestinal sensory inputs (Garfield *et al.* 2012). In spite of these studies, the relative physiological relevance of these non-ARH areas mediating anorexigenic effects of leptin is still unclear. Regulation of food intake by leptin largely depends on pSTAT3, as point mutation of Y1138 or deletion of brain STAT3 lead to hyperphagic obesity (Bates *et al.* 2003). LepRb-induced PI3K signalling appears to be rather important for acute suppression of food intake (Xu *et al.* 2010).

Leptin increases energy expenditure and thermogenesis

A single intracerebroventricular (icv) injection or peripheral infusion of leptin slightly increase or have no effect on energy expenditure (Scarpace *et al.* 1997, Doring *et al.* 1998, Gautron & Elmquist 2011). Chronic administration of leptin to mimic the leptinaemia kinetics observed in obesity slightly decreases energy expenditure (Ravussin *et al.* 2014), and higher doses of leptin induce long-lasting effects that can completely deplete body fat stores in animals (Halaas *et al.* 1997, Montez *et al.* 2005). In addition, leptin deficiency causes a reduction in metabolic rate in *ob/ob* mice (Breslow *et al.* 1999). Thus, endogenous leptinaemia seems to be able to affect energy expenditure and thermogenesis under normal circumstances.

The effects of leptin on energy expenditure are mediated by both the autonomic nervous system and neuroendocrine hypothalamic–pituitary–thyroid (HPT) axis (Pandit *et al.* 2017). Leptin upregulates the activity of the sympathetic nervous system (Haynes *et al.* 1997) presumably via its action on multiple neuronal targets that include not only ARH POMC and NPY/AgRP/GABA neurons (Cowley *et al.* 2001, Harlan *et al.* 2011) but also MPO (Yu *et al.* 2016), VMH (Dhillon *et al.* 2006) and DMH (Enriori *et al.* 2011). Leptin activates the HPT axis via its direct action on thyrotropin-releasing hormone (TRH) neurons of the PVH (Perello *et al.* 2006) and also via an indirect action through ARH POMC and

NPY/AgRP/GABA neurons that provide potent stimulatory and inhibitory inputs, respectively, to PVH TRH neurons (Fekete *et al.* 2000).

Leptin increases heart rate and blood pressure

LepRb is expressed in brain regions and peripheral organs (e.g. heart, kidneys and adrenals) that are important in cardiovascular control and blood pressure regulation (Fruhbeck 2002). Central administration of leptin or direct injections in the DMH or the VMH increase both mean arterial pressure and/or heart rate in rodents (Dunbar *et al.* 1997, Casto *et al.* 1998, Marsh *et al.* 2003). However, the physiological significance of these observations is uncertain. In humans, chronic administration of leptin does not elevate blood pressure (Simonds *et al.* 2017).

Leptin decreases glycaemia

Leptin administration at a dose that does not affect body weight and food intake normalises blood glucose and insulin levels in otherwise hyperglycemic *ob/ob* mice (Pellemounter *et al.* 1995). Leptin decreases glycaemia by sensitising metabolically relevant tissues to insulin but also in an insulin-independent manner (Fujikawa & Coppari 2015). Leptin improves the glycaemic control via its effects at both the central and peripheral level, where leptin suppresses the production of glucagon and corticosterone, increases glucose uptake and inhibits hepatic glucose output (D'Souza *et al.* 2017). The central effects of leptin on glucose homeostasis strongly depend on the ARH (Coppari *et al.* 2005).

Leptin is a permissive factor for puberty and fertility

The administration of physiological amounts of leptin prevents the fasting-induced delay in ovulation (Ahima *et al.* 1996). Leptin signalling is required to enter puberty (de Luca *et al.* 2005). LepRb is not expressed in gonadotropin-releasing hormone neurons. Therefore, leptin indirectly controls the reproductive function via interneurons located at the ventral premammillary nucleus or through ARH POMC and NPY/AgRP/GABA neurons (Donato *et al.* 2011, Donato & Elias 2011).

Leptin resistance

Leptin resistance refers to the states in which leptin fails to promote its anticipated effects, frequently coexisting with

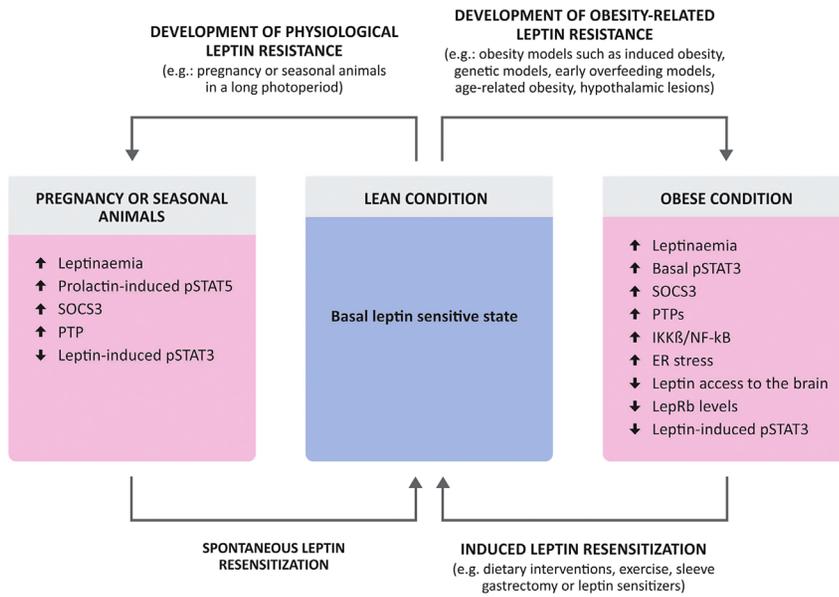


Figure 2

Schematic representation of the development of leptin resistance in physiological and obesity-related conditions, the reversion of this status (leptin re sensitisation) and the molecular mechanisms involved. ER, endoplasmic reticulum; IKKβ/NF-κB, IκB kinase-β/nuclear factor-κB; LepRb, leptin receptor b; PTPs, protein tyrosine phosphatases; SOCS3, suppressor of cytokine signalling 3; STAT, signal transducer and activator of transcription. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0606>.

marked hyperleptinaemia. A schematic representation of the development of leptin resistance can be seen in **Figs 2** and **3**. The assessment of leptin resistance encompasses diverse aspects. The standard biochemical marker for cellular LepRb action is leptin-induced STAT3 phosphorylation (Myers *et al.* 2010), and impairment of this induction is usually interpreted as an indication of leptin resistance. The measurement of the acute or chronic ability of exogenously-administered leptin to reduce body weight, adiposity and/or food intake is also used to estimate the sensitivity to leptin. Practically speaking, 'leptin resistance' is a broadly applied and context-dependent term with no universal, quantifiable and clinically useful definition (Myers *et al.* 2012).

Since a major physiological function of leptin is to signal energy deficiency, the implications of hyperleptinaemia and the concomitant notion of 'leptin resistance' become controversial. Besides, much of the evidence for leptin resistance relies on pharmacological studies that use non-physiological doses or routes of leptin administration. A variety of arguments suggest that a 'leptin resistance' underlies the development of obesity; however, it has been also considered that leptin action naturally faces a ceiling effect, beyond which it promotes little additional effect (Ahima *et al.* 1996, Myers *et al.* 2010, Rosenbaum & Leibel 2014, Flier & Maratos-Flier 2017). The concept of leptin resistance is also dependent on which of the biological effects of leptin are affected. The fact that in some forms

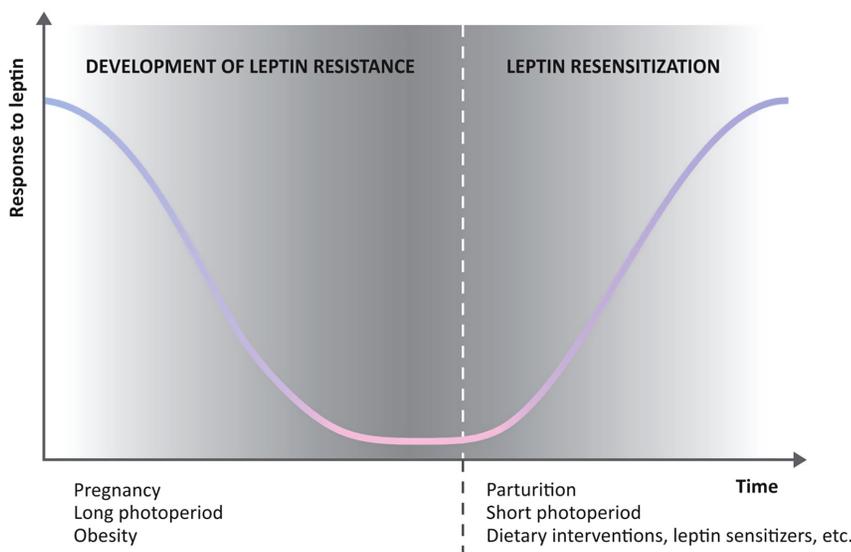


Figure 3

A graphical representation of the development of leptin resistance and subsequent leptin re sensitisation over time, in physiological and obesity-related conditions. Response to leptin represents any given leptin effect (e.g. pSTAT3, Socs3 expression or leptin-dependent suppression of food intake and body weight). Grey background colour intensity depicts plasma leptin levels. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0606>.

of obesity there may be resistance to the anorectic and weight-reducing actions of leptin but preservation of hypertension led to the concept of selective leptin resistance (Rahmouni *et al.* 2005, Mark 2013).

Cellular and molecular mechanisms of leptin resistance

Brain accessibility

Leptin resistance may involve a limited accessibility of leptin to the brain. Leptin accesses the brain via a saturated transport system (Banks *et al.* 1996) and cerebrospinal fluid/serum leptin ratio has been shown to be decreased in obesity (Caro *et al.* 1996, Schwartz *et al.* 1996). Central administration of leptin to obese animals reduces food intake more potently than peripheral administration of the hormone, suggesting that leptin transport to the brain is impaired in obesity (Halaas *et al.* 1997, Van Heek *et al.* 1997). Interestingly, leptin transport across the blood–cerebrospinal fluid barrier (Kleinert *et al.* 2018), rather than across the blood–brain barrier (Harrison *et al.* 2018), seems to be impaired in obese animals. Notably, rats lacking functional LepRa display decreased transport of leptin to the brain and develop obesity (Kastin *et al.* 1999).

Hyperleptinaemia-induced downregulation of LepRb expression

Reduced hypothalamic LepRb expression is found in some models displaying hyperleptinaemia (Martin *et al.* 2000), and leptin resistance has been hypothesised to be secondary to the hyperleptinaemia found in obesity (Knight *et al.* 2010). In support of this notion, chronic hyperleptinaemia induced by transgene overexpression decreases hypothalamic LepRb levels and impairs leptin signalling (Wilsey *et al.* 2002). In addition, chronic high central leptin desensitises its physiological effects over time rendering lean rodents more susceptible to obesity (Scarpace *et al.* 2005).

Alteration of signalling cascade

Leptin resistance seems to involve intracellular proteins that impair LepR signalling. As stated above, SOCS3 blocks LepRb signalling. Since high hypothalamic SOCS3 expression is found in most hyperleptinaemic conditions, it is hypothesised that upregulation of SOCS3 in obesity impairs leptin-induced pSTAT3 (Bjorbaek *et al.* 1999). The key role of SOCS3 regulating leptin sensitivity has been confirmed by its either selective inactivation (Bjornholm *et al.* 2007, Pedroso *et al.* 2016) or

overexpression (Reed *et al.* 2010). Leptin action is also downregulated by protein tyrosine phosphatases (PTPs, such as PTP1B), which block leptin signalling through the dephosphorylation of LepRb, JAK2 or pSTAT3 and are increased in some models that display leptin resistance (St-Pierre & Tremblay 2012). Recently, matrix metalloproteinase-2 (Mmp-2) has been suggested as a key mediator of leptin resistance. Obesity promotes the hypothalamic Mmp-2 activity and its secretion from the astrocytes and AgRP neurons (Mazor *et al.* 2018). Mmp-2 cleaves the extracellular domain of LepRb and diminishes leptin action (Mazor *et al.* 2018).

Hypothalamic inflammation and oxidative stress

Leptin resistance has been linked to the hypothalamic inflammation, which involves the I κ B kinase- β /nuclear factor- κ B (IKK β /NF- κ B) signalling (de Git & Adan 2015). Activation of IKK β /NF- κ B pathways induces SOCS3 expression and production of proinflammatory cytokines, such as interleukin (IL) 1 and 6 and tumour necrosis factor- α (Zhang *et al.* 2008). Proinflammatory cytokines, in turn, increase SOCS3 and PTP1B, leading to leptin resistance (de Git & Adan 2015). Notably, constitutive activation of hypothalamic IKK β impairs leptin signalling and increases weight gain and food intake, while genetic or pharmacological inhibition of IKK β activity protects against obesity and improves leptin sensitivity in obese mice (Cakir & Nillni 2018a). Besides, it was recently shown that hypothalamic oxidative stress impairs the function of POMC neurons and suppresses leptin signalling in the hypothalamus, resulting in the development of systemic leptin resistance and obesity (Yagishita *et al.* 2017).

Endoplasmic reticulum (ER) stress

Impairment of leptin signalling has been linked to ER stress, which is caused by an excessive accumulation of unfolded proteins that activate the unfolded protein response (Cakir *et al.* 2013). This response promotes an improvement of the ER protein-folding capacity, the degradation of misfolded proteins and the reduction of the load of new proteins entering the ER to resolve protein-folding defects. Obesity-induced hypothalamic ER stress reduces the post-transcriptional processing of POMC and impairs the biosynthesis of α -MSH (Cakir *et al.* 2013). Notably, pharmacologically induced hypothalamic ER stress in lean animals increases SOCS3 and PTP1B and promotes leptin resistance (Cakir *et al.* 2013). Thus, obesity-related hypothalamic ER stress can promote central leptin resistance (Cakir & Nillni 2018b).

Table 1 Conditions displaying leptin resistance and their strategies of reversion.

Condition	Experimental model	Main features (key references)	Tested strategies to reverse leptin-resistant state (key references)
Obesity	DIO	Most DIO models display hyperleptinaemia, higher basal pSTAT3, diminished leptin-induced pSTAT3, increased SOCS3 and ER stress (Munzberg <i>et al.</i> 2004, Enriori <i>et al.</i> 2007, Cakir <i>et al.</i> 2013, Ottaway <i>et al.</i> 2015). Features of DIO models depend on experimental factors such as species, diet composition and duration of the manipulation	<ul style="list-style-type: none"> Fasting (Pedroso <i>et al.</i> 2016, Caron <i>et al.</i> 2018) Energy restriction (Morabito <i>et al.</i> 2017) Switch to low fat diets (Enriori <i>et al.</i> 2007, Morabito <i>et al.</i> 2017) Fructose removal from the diet (Shapiro <i>et al.</i> 2008) Sleeve gastrectomy (Stefater <i>et al.</i> 2010) Exercise (Shapiro <i>et al.</i> 2011, Kang <i>et al.</i> 2013)
	Monogenic mutations	Several genetic models are useful tools to study leptin resistance. Most of them display one or more features of diminished leptin sensitivity (Bates <i>et al.</i> 2003, Gao <i>et al.</i> 2004, Elmquist <i>et al.</i> 2005, Myers <i>et al.</i> 2010, Liao <i>et al.</i> 2012, Lutz & Woods 2012)	<ul style="list-style-type: none"> Normal body weight is restored in obese <i>pomc</i>-knockout mice if POMC is re-expressed after a reduction in leptinaemia (Bumaschny <i>et al.</i> 2012, Chhabra <i>et al.</i> 2016)
	Obesity-prone rats	Obesity-prone rats are early leptin-resistant and display diminished leptin-induced pSTAT3 and reduced LepRb expression without hyperleptinaemia prior to the development of obesity (Levin <i>et al.</i> 1997, 2003, Irani <i>et al.</i> 2007, Bouret <i>et al.</i> 2008)	<ul style="list-style-type: none"> Rearing in large litters (Patterson <i>et al.</i> 2010). Postweaning exercise (Patterson <i>et al.</i> 2009).
	Early overfeeding	Reduction of the litter size leads to decreased pSTAT3 and increased SOCS3 (Plagemann <i>et al.</i> 2012)	<ul style="list-style-type: none"> Partial reversion in adulthood (Sominsky <i>et al.</i> 2017).
	ARH ablation	Monosodium glutamate-treated rodents display increased adiposity, hyperleptinaemia and resistance to the anorexigenic effects of leptin (Perello <i>et al.</i> 2003)	<ul style="list-style-type: none"> Total reversion by prolonged energy restriction and partial reversion by short-term fasting (Perello <i>et al.</i> 2009). Transient resensitisation by adrenal enucleation (Perello <i>et al.</i> 2003).
	Age-related obesity	Ageing is associated with body weight gain, central leptin resistance, upregulation of hypothalamic SOCS3, reduced leptin-induced pSTAT3 and diminished LepR expression (Scarpace & Tumer 2001).	<ul style="list-style-type: none"> Food restriction (Moyse <i>et al.</i> 2012).
Pregnancy	Pregnant rodents	Increase in food intake and adiposity are associated to hyperleptinaemia and leptin resistance compromising the VMH. Prolactin and placental lactogens induce impaired leptin-induced pSTAT3 and pSTAT5, increased expression of SOCS3, reduced LepRb expression and impairment of leptin transport (Ladyman & Grattan 2005, Tups 2009, Ladyman <i>et al.</i> 2010, Trujillo <i>et al.</i> 2011).	<ul style="list-style-type: none"> Total reversion by parturition (Ladyman & Grattan 2005, Tups 2009, Ladyman <i>et al.</i> 2010, Trujillo <i>et al.</i> 2011).
Seasonal animals	Siberian hamsters and field voles	Seasonal animals show hyperleptinaemia and high food intake during long day photoperiod (summer) with impairment of leptin-induced pSTAT3, prolactin-induced increase of pSTAT5 and increased SOCS3 and PTP1B (Anderson <i>et al.</i> 2006, Tups <i>et al.</i> 2004, Tups 2009).	<ul style="list-style-type: none"> Total reversion by switch to short day photoperiod (Tups <i>et al.</i> 2004, Tups 2009).

DIO, diet-induced obesity; ER, endoplasmic reticulum; LepRb, leptin receptor b; POMC, proopiomelanocortin; PTP1B, protein tyrosine phosphatase 1 B; SOCS3, suppressor of cytokine signalling 3; STAT3, signal transducer and activator of transcription 3; STAT5, signal transducer and activator of transcription 5; VMH, ventromedial nucleus.

Conditions displaying leptin resistance

Obese animals

Several rodent models of obesity are used to investigate the molecular basis of leptin resistance (Myers *et al.* 2010). Such models include diet-induced obesity (DIO),

genetic models, obesity-prone models, early overfeeding, age-related obesity and animals with hypothalamic lesions (Table 1).

DIO is likely the most frequent animal model used to investigate leptin resistance because it shares many characteristics with the common form of human obesity,

including an attenuated response to the anorexigenic effect of leptin (Van Heek *et al.* 1997, Myers *et al.* 2010). DIO models are obtained by feeding animals with highly palatable and hypercaloric diets for different periods of time. Notably, obesity *per se* in DIO models is not sufficient for the development of leptin resistance, and the presence of hyperleptinaemia is also required (Knight *et al.* 2010). An evidence for this notion comes from *ob/ob* mice implanted with osmotic pumps to bring their leptin levels to those observed in lean wild-type mice: this DIO normoleptinaemic *ob/ob* model remains leptin sensitive despite developing obesity comparable to a DIO wild-type model (Knight *et al.* 2010). Notably, only 8 days of high-dose leptin treatment are necessary to induce leptin resistance in wild-type mice (Montez *et al.* 2005). Besides, mice chronically overexpressing central leptin show leptin resistance and increased susceptibility to high-fat (HF) diet (Scarpace *et al.* 2005).

Molecular changes linked to hypothalamic leptin resistance in DIO models (Fig. 2) include higher basal pSTAT3 (Cakir *et al.* 2013), diminished leptin-induced pSTAT3 (Munzberg *et al.* 2004), increased SOCS3 (Enriori *et al.* 2007, Ottaway *et al.* 2015) and ER stress (Cakir *et al.* 2013). Hypothalamic inflammation has also been detected as early as 24 h after HF diet and prior to any weight gain (Thaler *et al.* 2012). Interestingly, hypothalamic expression of PTPs is increased in obese mice and their selective ablation improves leptin sensitivity and partially prevents obesity (St-Pierre & Tremblay 2012).

Importantly, specific features of DIO models strongly depend on factors such as animal model (strains of mice or rats), diet composition and duration of the manipulation, among other factors. Commercially available diets used to induce DIO include HF or HF/high sugar content. Although it is difficult to discern the effects of each macronutrient, it is known that saturated fats strongly impair leptin signalling in the hypothalamus (de Git & Adan 2015) and that high fructose intake also leads to leptin resistance (Shapiro *et al.* 2008). The hypercaloric diet period also impacts on the phenotype and the molecular features detected. In mice, the development of DIO as a consequence of HF diet has been divided into three stages: (a) an early stage in which mice gain weight and remain sensitive to leptin; (b) a further stage in which mice have increased leptin production and still retain central sensitivity to leptin and (c) a later stage in which central leptin sensitivity is decreased (Lin *et al.* 2000). However, it has been reported that reduced leptin sensitivity can be detected as early as after 6 days on HF diet, when the increase in body weight and circulating leptin is first

detectable (Munzberg *et al.* 2004). Another study showed that 24 h of HF diet led to impaired leptin signalling in the ARH, suggesting that a reduction of leptin sensitivity in the ARH precedes the increase in body weight induced by HF diet (Rizwan *et al.* 2017).

It is interesting to note that DIO mice are considerably less obese than *ob/ob* mice and maintain their reproductive function, energy expenditure, sympathetic outflow and other leptin-regulated processes, in contrast to *ob/ob*- or LepR-deficient (*db/db*) mice (Rahmouni *et al.* 2005, Myers *et al.* 2010). Also, the administration of a LepRb antagonist to DIO mice induces similar effects on energy balance as seen in lean mice (Ottaway *et al.* 2015). These observations suggest that leptin resistance in DIO models selectively affects the anorectic effect of the hormone, while endogenous hyperleptinaemia is able to exert several effects biologically relevant in DIO animals (Myers *et al.* 2010). In line with this possibility, it has been shown that the degree of leptin resistance in DIO models differs among brain areas. Thus, leptin fails to decrease food intake or increase pSTAT3 in the ARH of DIO mice while its action in other hypothalamic regions is retained (Munzberg *et al.* 2004). The action of endogenous leptin in the DMH has been proposed to increase blood pressure in DIO mice (Simonds *et al.* 2014). Similarly, DIO rats are resistant to the anorectic effect of leptin, although endogenous hyperleptinaemia acts in the PVH TRH neurons upregulating the HPT axis and increasing thermogenesis (Perello *et al.* 2010). In addition, oxytocin-producing neurons of the PVH remain sensitive to leptin and mediate leptin effects on body weight in DIO rats (Perello & Raingo 2013).

Animal models of obesity concerning monogenic mutations in the leptin pathway have also been used to study leptin resistance (Bates *et al.* 2003, Gao *et al.* 2004). These models include *db/db* mice and their counterpart, the Zucker rats. Additionally, POMC-deficient mice, MC4R-deficient mice and Otsuka Long Evans Tokushima Fatty rats, which lack functional receptors for cholecystokinin, display diminished leptin sensitivity (Elmqvist *et al.* 2005, Myers *et al.* 2010, Lutz & Woods 2012). Interestingly, mice with truncations of the brain-derived neurotrophic factor gene are obese, hyperphagic, hyperleptinaemic and resistant to the anorectic action of leptin, but display normal leptin-induced pSTAT3 in the hypothalamus suggesting that some biochemical markers of LepR signalling do not necessarily reflect the physiological response to leptin (Liao *et al.* 2012).

A different model is the obesity-prone rat, a polygenic substrain developed by selectively breeding outbred

Sprague–Dawley rats highly prone to develop DIO (Levin *et al.* 1997). Obesity-prone rats are early (possibly inborn) leptin resistant. Prior to the development of obesity, they show diminished hypothalamic leptin-induced pSTAT3 (Bouret *et al.* 2008) and reduced LepRb expression (Levin *et al.* 2003) without hyperleptinaemia (Irani *et al.* 2007).

Another obesity model displaying leptin resistance is early overfeeding by reduction of the litter size, a manoeuvre that increases the availability of breast milk in the postnatal period and leads to decreased pSTAT3 and increased SOCS3 in the hypothalamus (Plagemann *et al.* 2012).

Some animal models of obesity are generated by selective hypothalamic lesions, such as ARH-ablated animals, which are obtained using postnatal administration of monosodium glutamate to rodents (Perello *et al.* 2003). ARH-ablated animals display increased adiposity, hyperleptinaemia and resistance to the anorexigenic effects of leptin presumably due to the absence of one of the key hypothalamic targets of leptin (Perello *et al.* 2003).

Age-related obesity is also associated with body weight gain, central leptin resistance, upregulation of hypothalamic SOCS3, reduced leptin-induced pSTAT3 and diminished LepR expression (Scarpace & Tumer 2001).

Pregnancy

Gestation is associated to hyperleptinaemia and leptin resistance (Table 1). Gestational leptin resistance is characterised by impaired leptin-induced pSTAT3 in the hypothalamus and likely plays a role in the adaptations observed during pregnancy, including increase in food intake and adiposity (Ladyman *et al.* 2010). Some evidence indicates that prolactin and placental lactogens are involved in gestational leptin resistance (Ladyman *et al.* 2010). Prolactin levels are elevated during the first half of pregnancy, while placental lactogens progressively increase during late gestation (Augustine & Grattan 2008). Many hypothalamic LepR-expressing neurons are directly responsive to prolactin, which increases hypothalamic expression of SOCS3 via activation of pSTAT5 and abolishes the anorexigenic effects of leptin (Trujillo *et al.* 2011). Notably, ablation of *Socs3* gene in LepRb-expressing cells of female mice does not affect fertility but increases leptin sensitivity during gestation and mitigates pregnancy-induced metabolic changes (Zampieri *et al.* 2015). Gestational leptin resistance could also include reduced hypothalamic expression of LepRb mRNA (Ladyman & Grattan 2005) and impairment of leptin transport into the brain due to the sustained hyperprolactinemia

(Trujillo *et al.* 2011). Unlike DIO models, impairment of leptin sensitivity during pregnancy seems to compromise the VMH (Ladyman & Grattan 2005, Tups 2009). However, SOCS3 ablation in steroidogenic factor-1 neurons of the VMH only causes modest metabolic effects during pregnancy (Ramos-Lobo *et al.* 2017).

Small seasonal animals

Seasonal mammals, such as Siberian hamsters and field voles, exhibit a natural body weight cycle, accompanied by a biannual reversible switch in leptin sensitivity (Table 1). Such seasonal leptin resistance is observed during long day photoperiod (summer), when the animals show hyperleptinaemia and high food intake (Tups *et al.* 2004). At a molecular level, seasonal leptin resistance is characterised by an impairment of leptin-induced pSTAT3 in the ARH. This phenomenon seems to be also mediated by a prolactin-induced increase of pSTAT5, which in turn increases SOCS3 and PTP1B and further blunts leptin's central action (Anderson *et al.* 2006, Tups 2009). Therefore, hypothalamic SOCS3 is employed to sensitise the brain to a different reading of leptin signal in opposite photoperiods (Tups *et al.* 2004, Tups 2009).

Leptin resensitisation

Leptin resensitisation refers to the reversion of leptin-resistant states (Fig. 3). Normalisation of circulating leptin levels after chronic hyperleptinaemia has been shown to affect the sensitivity to the hormone (Montez *et al.* 2005). In obese animals, leptin resistance can be reversed with treatments that reduce body adiposity and leptinaemia, while physiological leptin resistance reverts spontaneously when pregnant animals give birth or when seasonal animals are exposed to a short-day photoperiod. The reversion of leptin-resistant states is achieved through the modulation of diverse molecular mechanisms depending on the case (Fig. 2 and Table 1).

Reduction of circulating leptin after a chronic exogenously induced hyperleptinaemia

Since hyperleptinaemia is required for the development of leptin resistance, normalisation of leptin levels after chronic hyperleptinaemia should improve leptin sensitivity. To the best of our knowledge, only two studies investigated the effects of the withdrawal of leptin after its pharmacological administration. One study showed that mice treated with leptin for 8 days decrease their food

intake in the first 4 days and then normal food intake is recovered (Montez *et al.* 2005). After leptin withdrawal, food intake transiently increases the first 3 days, while body weight returns to initial values. Thus, mice seem to develop a transient leptin-resistant state after few days of hyperleptinaemia that quickly reverts when the treatment is over. Notably, food intake increments and weight gain can be partially suppressed depending on the magnitude of the leptinaemia reduction, showing that leptin sensitivity is recovered at least partially in the first few days (Montez *et al.* 2005). A more recent study also showed that the initial weight gain after leptin withdrawal is not sustained in time and that mice slightly gain weight and adiposity after ~4 months of leptin withdrawal (Ravussin *et al.* 2014). Strikingly, leptinaemia remains elevated in this period (Ravussin *et al.* 2014). These findings suggest that after chronic exogenously induced hyperleptinaemia, the reversion of leptin resistance and the reduction of circulating leptin are not immediate. Unfortunately, none of these studies was designed to investigate leptin resistance *per se*, nor the molecular mechanisms involved in the adaptations from hyperleptinaemia to normoleptinaemia.

Reversion of leptin resistance in obesity models

Since DIO leads to leptin resistance, it is expected that treatments that reduce body adiposity and hyperleptinaemia should improve leptin sensitivity. Dietary interventions, including fasting, energy restriction and the switch from HF to low-fat diets, induce leptin re sensitisation. One day fasting robustly decreases leptinaemia and partially restores leptin responsiveness in obese mice, and fasting-induced hyperphagia and weight regain involve changes in SOCS3 expression (Pedroso *et al.* 2016). Fasting-induced decrease in leptinaemia is partly regulated by sympathetic outflow to the white adipose tissue regulating leptin biosynthesis and secretion (Caron *et al.* 2018). Energy restriction also reduces leptinaemia and restores leptin-induced pSTAT3 response in several brain regions including VMH (Morabito *et al.* 2017). Additionally, diet switch from HFD to regular chow reverses leptin resistance in some DIO models. In particular, decreasing the dietary fat content in mice fed with HFD for 20 weeks reverses obesity and hypothalamic leptin sensitivity in the ARH after 7 weeks of diet switch (Enriori *et al.* 2007). Another study showed that 12 weeks of low-fat diet after 30 weeks of DIO not only decreases body weight and leptinaemia to values similar to lean mice but also recovers leptin-induced

pSTAT3 levels in some brain regions to a greater extent than energy restriction (Morabito *et al.* 2017). However, ARH and DMH do not recover leptin sensitivity neither with energy restriction nor with dietary switch (Morabito *et al.* 2017). Diet composition can also affect leptin re sensitisation. When rats become leptin-resistant on HF/high-fructose diet, the removal of fructose from this diet reverses hyperleptinaemia and improves leptin sensitivity (Shapiro *et al.* 2008). Notably, not all studies have found that dietary manipulations restore leptin sensitivity. It is likely that type and duration of HF diet intake, degree of obesity achieved and duration of the dietary intervention impacts on the degree of leptin re sensitisation. Besides, sleeve gastrectomy in obese rats induces not only body weight loss but also an improvement of leptin sensitivity, which is secondary to weight loss since pair-fed groups show similar response to leptin (Stefater *et al.* 2010). Exercise also decreases body weight and leptinaemia and improves leptin sensitivity by activating leptin sensitive neurons in the VMH and increasing pSTAT3 in the VTA. This effect seems to be independent of fat mass loss (Shapiro *et al.* 2011, Kang *et al.* 2013).

Genetically modified mice provide a powerful strategy to study the reversion of obesity and leptin resistance. For instance, *Pomc*-knockout mice display early-onset extreme obesity; however, normal body weight can be restored if POMC is re-expressed in young reactivatable *Pomc*-knockout mice. If POMC re-expression is induced in aged *Pomc*-knockout mice, elevated body weight and leptin resistance are observed (Bumaschny *et al.* 2012, Chhabra *et al.* 2016); nevertheless, normal body weight can be restored only if POMC is re-expressed after leptinaemia is first reduced by calorie restriction. This fact suggests that a critical threshold of leptinaemia exists in order to reverse obesity (Chhabra *et al.* 2016).

In the case of obesity-prone rats, obesity and leptin resistance can be reversed by rearing in large litters, limiting the pups' nutrient supply during the suckling period. Large litter rearing reduces body weight and enhances leptin sensitivity by lowering plasma leptin levels, protecting the animals from becoming obese (Patterson *et al.* 2010). In this obesity model brief postweaning exercise can also increase leptin sensitivity and reduce body weight (Patterson *et al.* 2009).

Neonatal leptin resistance induced by litter size reduction seems to partially reverse in adulthood, as exogenous leptin injection suppresses food intake, despite body weight and circulating leptin remain elevated (Sominsky *et al.* 2017). Interestingly, long-term energy restriction in ARH-ablated animals decreases not only

adiposity and plasma leptin but also normalises peripheral sensitivity to leptin (Perello *et al.* 2009). In contrast, fasted ARH-ablated animals show a reduction of leptin levels but adiposity and leptin sensitivity are not affected, suggesting that a short-term decrease of leptinaemia alone is not sufficient to restore leptin sensitivity (Perello *et al.* 2009). ARH-ablated animals subjected to adrenal enucleation, a strategy to transiently reduce adiposity and leptinaemia due to a reduction of glucocorticoid levels, also display a transient leptin resensitisation that ends when plasma glucocorticoid and leptin return to high levels (Perello *et al.* 2003). In aged animals, food restriction reduces SOCS3 expression and prevents leptin resistance (Moyses *et al.* 2012). Unfortunately, the molecular events mediating leptin resensitisation in these cases are poorly known.

Reversion of leptin resistance in physiological models

The transitory state of leptin resistance caused by pregnancy is spontaneously reversed once the hormonal milieu of pregnant animals returns to basal levels, and leptin sensitivity of postpartum females is normalised. Therefore, the end of pregnancy is a physiological model of leptin resensitisation. Regarding small seasonal animals, the switch from long to short day photoperiod is associated with a state of increased leptin sensitivity subsequent to reduction of SOCS3 and PTP1B following prolactin reduction (Tups *et al.* 2004, Tups 2009).

Leptin-sensitising compounds

Several pharmacological compounds have been reported to modulate leptin sensitivity, and some compounds naturally found in foods also act as leptin sensitisers in DIO models. Phenolic compounds (e.g., resveratrol) suppress leptin expression from adipocytes and attenuate hyperleptinaemia and leptin resistance developed in the context of early weaning or maternal HF diet exposure (Franco *et al.* 2016). Teasaponin and ginsenoside reduce food intake and body weight and increase hypothalamic pSTAT3 levels and *Pomc* expression in rodents fed HF diet (Franco *et al.* 2016). These effects are achieved after 3 or 4 weeks of treatment with these compounds.

Pharmacological leptin sensitisers could be broadly grouped into two categories. The first group includes small molecules that induce marginal weight loss when administered alone but increase the anorectic effect of exogenous leptin. Such compounds include

meta-chlorophenylpiperazine, which is a serotonin receptor agonist and acts as a serotonin reuptake inhibitor (Yan *et al.* 2015), metformin, which is a commonly used anti-diabetic medication (Kim *et al.* 2006) and betulinic acid (Choi *et al.* 2013), which presumably causes leptin sensitisation through PTP1B inhibition.

The second group of molecules induce weight loss in hyperleptinaemic animals even when administered alone, suggesting that they restore leptin signalling and resensitise obese mice to their endogenous hyperleptinaemia. Such molecules include amylin, predominantly secreted from the pancreatic β cells but also produced by hypothalamic neurons (Le Foll *et al.* 2015), and pramlintide, an amylin analogue in clinical use for the treatment of diabetes. The mechanism by which amylin works is unclear but it seems to increase IL-6 production in VMH microglia that in turn activates pSTAT3 signalling in LepR neurons (Le Foll *et al.* 2015). Amylin also exerts a direct effect in enhancing leptin signalling via activation of the ERK/MAPK pathway on POMC neurons (Lutz *et al.* 2018). Glucagon-like peptide-1 (GLP-1) also increases central leptin action and appears to exert its anorectic effects synergistically with leptin. The acute anorectic effect of GLP-1 is attenuated in LepR-deficient rats, and GLP-1 action is also partially mediated by central induction of IL-6 production (Shirazi *et al.* 2013). Accordingly, IL-6 appears to be a common mediator of leptin-sensitising factors in the brain, and central or systemic overexpression of IL-6 attenuates DIO in a leptin-dependent manner. Peripherally restricted cannabinoid receptor 1 (CB1R) inverse agonists act as potent weight loss agents in DIO models and lack the psychiatric effects induced by centrally acting cannabinoid antagonists, such as rimonabant. Peripheral action of CB1R inverse agonists, such as JD5037, work in a leptin-dependent manner since they do not induce weight loss in *ob/ob* or *db/db* mice and their anti-obesity effects in DIO models are attenuated by LepRb antagonists. Notably, CB1R inverse agonists seem to act by decreasing hyperleptinaemia since their effect increasing leptin clearance and suppressing leptin production precedes weight loss. Heat shock protein 90 (HSP90) inhibitors display leptin sensitiser properties (Desarzens *et al.* 2014, Ozcan *et al.* 2017). At least some HSP90 inhibitors were shown to induce weight loss in DIO mice but had a blunted effect in *ob/ob* or *db/db* mice. Some of these HSP90 inhibitors are natural compounds, such as celastrol and gambogic acid, which have pleiotropic effects including PTP1B inhibitory and anti-inflammatory activities (Tan *et al.* 2017). Induction of heat shock response is proposed to exert anti-obesity effects through a peripheral mechanism that involves

adipose tissue and skeletal muscle mediated energy expenditure (Ma *et al.* 2015). Whether and how peripheral heat shock response couples to central leptin sensitisation is currently unknown. HSP90 inhibitors activate ER stress, and some of them also inhibit ER chaperones (Fribley *et al.* 2015). For example, the pentacyclic triterpene celastrol is a potent inducer of ER stress (Fribley *et al.* 2015). However, whether this effect is involved in the leptin sensitising role of these molecules is not known.

Concluding remarks

Experimental evidence shows that leptin resistance can be reversed, at least to some extent, in several animal models of obesity (Fig. 2). Such leptin resensitisation is associated with improvement in endocrine and metabolic disturbances commonly observed in obesity and a sustained decrease of plasma leptin levels, possibly below a critical threshold level. In some cases, leptin resensitisation is not immediate or complete, neither proportional to the decrease of body weight. At a molecular level, the recovery of leptin sensitivity shows a different timing depending on the brain region. Reversion of leptin resistance is total and immediate in physiological conditions, such as during pregnancy and the switch from long to short day photoperiod (Fig. 2). Notably, aged obese mice are less able to fully reverse the leptin-resistant states. Based upon current understandings, several pharmacological compounds are proposed to be promising therapeutic targets for the treatment of leptin resistance associated with DIO.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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