Minireview

# Drug targeting of leptin resistance

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## ABSTRACT

Leptin regulates glucose, lipid and energy homeostasis as well as feeding behavior, serving as a bridge between peripheral metabolically active tissues and the central nervous system (CNS). Indeed, this adipocyte-derived hormone, whose circulating levels mirror fat mass, not only exerts its anti-obesity effects mainly modulating the activity of specific hypothalamic neurons expressing the long form of the leptin receptor (Ob-Rb), but it also shows pleiotropic functions due to the activation of Ob-Rb in peripheral tissues. Nevertheless, several mechanisms have been suggested to mediate leptin resistance, including obesity-associated hyperleptinemia, impairment of leptin access to CNS and the reduction in Ob-Rb signal transduction effectiveness, among others. During the onset and progression of obesity, the dampening of leptin sensitivity often occurs, preventing the efficacy of leptin replacement therapy from overcoming obesity and/or its comorbidities.

This review focuses on obesity-associated leptin resistance and the mechanisms underpinning this condition, to highlight the relevance of leptin sensitivity restoration as a useful therapeutic strategy to treat common obesity and its complications.

Interestingly, although promising strategies to counteract leptin resistance have been proposed, these pharmacological approaches have shown limited efficacy or even relevant adverse effects in preclinical and clinical studies. Therefore, the numerous findings from this review clearly indicate a lack of a single and efficacious treatment for leptin resistance, highlighting the necessity to find new therapeutic tools to improve leptin sensitivity, especially in patients with most severe disease profiles.

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## 1. Introduction

The discovery of leptin in 1994, changed the view about adipose tissue, considered originally only a fat depot, as an endocrine organ [1]. Indeed, leptin circulating levels were found to correlate with adipose tissue, its main source, mirroring energy status. Leptin is now recognized as a fundamental regulator of energy homeostasis, modulating glucose and lipid metabolism in peripheral tissues, and acting at the central nervous system (CNS) on feeding behavior and energy expenditure [2]. While patients with congenital leptin deficiency take advantage by leptin replacement therapy in reducing neuroendocrine and metabolic alterations, obesity is generally related to the so called leptin resistance, characterized by elevated leptin circulating levels and disruption of leptin receptor signaling [3]. Indeed, leptin acts through the binding of the functional long isoform leptin receptor (ObR) in CNS, as well as in peripheral tissues, where its signaling is finely regulated. The majority of obese individuals are unresponsive to leptin treatment, and a more subtle approach would be to overcome leptin resistance, rather than to increase the already high leptin levels. However, leptin resistance is not only involved in obesity, but also constitutes a risk factor for the onset of other pathological ailments, such as cardiovascular diseases [4], osteoporosis [5], diabetes [6] and depression [7,8], due to the pleiotropic functions of this hormone. Actually, obesity-induced leptin resistance may have a detrimental role in several peripheral tissues, such as the liver, pancreas, vasculature, and skeletal muscle, inducing not only metabolic dysregulation, but also a low grade inflammation, implicated in the aforementioned associated diseases. The mechanisms underpinning leptin resistance are largely studied; it is the result of many molecular and cellular alterations, such as an increase of leptin synthesis and secretion [9], an impairment of leptin entry into the brain [10–13], a disruption of leptin signal transduction [14–16], a defect of ObR trafficking [17,18], and endoplasmic reticulum (ER) stress [19–21]. All these mechanisms represent potential targets to prevent or overcome leptin resistance for treating obesity and its comorbidities.

The aim of this review is to present leptin signaling pathways and their impairment in leptin resistance in order to highlight the possible approaches aiming to restore leptin sensitivity by changes of life styling or pharmacologic strategies.

## 2. Leptin synthesis and its modulation

Leptin is mainly produced by adipose tissue and secreted into the bloodstream. Interestingly circulating leptin levels, related to fat mass and adipocyte size, target the brain to communicate peripheral energy storage [22,23]. Leptin levels change with circadian rhythm and nutritional state: while fasting decreases leptin levels, feeding and obesity increase levels [24]. Moreover, circulating leptin levels are able to modulate hormone responsiveness, in that leptin deficient mice are very sensitive to exogenous leptin injections, while hyperleptinemia is accompanied by reduced leptin responses [25], since hyperleptinemia is considered a sufficient condition to induce leptin resistance.

It is recognized that leptin crosses the blood brain barrier (BBB) through a specific, saturable, and unidirectional transport system [26], found also in cerebral microvessels and choroid plexus [27,28]. There is accumulating evidence indicating that several short Ob-R isoforms, including ObRa and ObRc, are involved in leptin entry through the BBB or choroid plexus [27–31]. Leptin diffuses to the hypothalamic arcuate nucleus (ARC) neurons by fenestrated capillaries extending from the median eminence during fasting [32]. Moreover, leptin is transported by tanycytes toward target cells through cerebrospinal fluid [33].

Finally, leptin may reach proximal ARC neurons by perivascular routes [34], while other leptin receptor expressing neurons are reached by leptin through the saturable transport across the BBB or by the CSF. ARC neurons, expressing the long form of the leptin receptor, can detect leptin and display an increase in sensitivity to circulating hormone [35]. Furthermore, in 2008, megalin, also known as LRP2, has been identified in the choroid plexus epithelium, as a potential leptin transporter [36].

Various factors can modulate leptin synthesis, such as the distribution of subcutaneous adipose tissue [23,37], and insulin level. In particular, it has been demonstrated a direct effect of insulin on leptin synthesis in adipocytes [38] and in vivo when injected in rodents [39]; furthermore low leptin levels were shown in insulin resistance state, and leptin levels increased after insulin administration [40]. Leptin expression and secretion also depend on glucocorticoids, known to stimulate leptin synthesis and to induce hyperleptinemia. Glucocorticoids increase leptin transcription in adipocytes in vitro [41], and when chronically administered in humans induced leptin level elevation [42]. On the other side, leptin can increase glucocorticoid levels, suggesting endocrine cross-talk between leptin and steroid hormones [43] in humans. In murine obesity, some studies found alterations in steroid metabolism shown in humans. Therefore, while obese mice showed increased level of corticosterone, low cortisol was found in obese humans [44].

Circulating leptin levels also reflect glycemia and glucose metabolites and free fatty acids. Indeed, glucose increases leptin sensitivity in a dose dependent manner [45] and showed a permissive role in leptin expression and secretion [46]. Conversely, free fatty acids reduce leptin secretion [47].

A delicate interaction has been shown among thyroid and sex hormones and leptin levels: thyroid hormones reduce leptin levels in rats [48], while data on the influence of thyroid dysfunction on leptin circulating levels are highly conflicting in humans both in basal conditions and after treatment [49–51]. However, clear evidence showed that in lean subjects the circadian rhythms of TSH and leptin overlap [52]. Loss of estrogen, as in ovariectomized rat, induces an increase in leptin levels, prevented by estradiol or raloxifene treatment [53]. Among other influencing factors, pro-inflammatory cytokines and endotoxin increase leptin synthesis, according with their inflammatory and immunity role [54].

## 3. Leptin receptors and downstream signaling pathways

Leptin controls the energy homeostasis in peripheral tissues both directly, through the modulation of 5′ adenosine monophosphate-activated protein kinase (AMPK) activity and indirectly due to the synthesis of orexigenic/anorexigenic neuropeptides in the hypothalamus [55], leading to satiety, and to an overall negative energy balance.

In the brain, the ARC counteracts the metabolic environmental changes with several mechanisms, including synaptic and mitochondrial plasticity and redox state alterations to preserve energy homeostasis [56]. Leptin, peripherally produced by the adipose tissue, drives the majority of these responses interacting with its cognate receptors in CNS nuclei involved in both glucose and lipid homeostasis.

In particular, leptin modulates the activity of two ARC neural populations: the pro-opiomelanocortin (POMC) and the agouti-related-protein (AgRP) neurons. POMC neurons, in response to leptin-stimulation, secrete anorexigenic neuropeptides, i.e. cocaine- and amphetamine-regulated-transcript, CART, and POMC, the precursor of α-melanocyte-stimulating hormone (MSH), which reduces body weight acting as an agonist of the melanocortin receptors 3 and 4.

Conversely, AgRP neurons are inhibited by leptin, leading to a reduced release of orexigenic neuropeptides, including neuropeptide (NP)Y and AgRP, which exerts its orexigenic effects serving as an α-MSH antagonist. These neural populations act as first-order neurons to take over metabolic signals carried by leptin and then project to other intra-/extra-hypothalamic neurons, constructing a sophisticated neural network responsible for the control of the energy homeostasis [57]. Indeed, this information trafficking between the peripheral tissues and different CNS areas can take place through the expression of the functional isoform of the leptin receptor by these neurons.

Actually, the catabolic and behavioral effects of this adipocyte-derived hormone are mediated by the interaction with the long isoform of the leptin receptor (Ob-R)b, which is expressed in several brain areas [58,59]. Indeed, the importance of leptin binding to its receptor has been demonstrated both in pharmacological studies and genetic models: the administration of leptin antagonists leads to an increase of body weight due to an increase in food consumption in mice [60], as well as Ob-Rb knock-out *db/db* mice show a clear obese phenotype similar to leptin-deficient *ob/ob* mice [61].

Twenty years ago, Ob-R was identified and cloned in the choroid plexus from the mouse brain [62], but the analysis of its mRNA levels suggests that it is expressed not only at central level, such as in the hypothalamus, but also in various peripheral tissues and in different cell populations, including $CD4^+$ T-lymphocytes, macrophages, pancreatic, vascular endothelial, white and brown adipose cells, leading to the leptin pleiotropic functions [63–65].

Indeed Ob-R, homologous to members of the cytokine receptor superfamily, presents six different isoforms, all produced by alternative mRNA splicing: Ob-Ra, b, c, d, e and f [66]. All these isoforms present the same N-terminal extracellular domain, which is necessary for the leptin binding, but differ in their C-terminal intracellular domain. In particular, these isoforms are currently divided into three categories according to the intracellular domain length: short Ob-Ra, c and f, long (Ob-Rb) and secreted isoforms (Ob-Re). Among the short isoforms, Ob-Ra mediates the transport of leptin through the blood–brain barrier (BBB), which is one of the most common mechanisms used by this adipocyte-derived hormone to reach the CNS [67]. On the other hand, Ob-Re, the one and only soluble isoform of Ob-R, characterized by the absence of the transmembrane domain, binds circulating leptin with high affinity, avoiding its central access.

Eventually, Ob-Rb is the only isoform responsible of the biological effects of leptin, since it features a full-length intracellular domain with three tyrosine residues that, after phosphorylation, act as binding sites for downstream signaling molecules, which in turn promote the signal transduction through distinct pathways (i.e. JAK2/STAT3, JAK2/STAT5, PI3K/IRS/AKT, and SHP/ERK pathways) underpinning the hormone's several functions [64,65,68].

Interestingly, this receptor lacks an intrinsic kinase activity, so leptin binding is necessary to obtain the initial dimerization of its cytoplasmic portions, leading thus to the recruitment and activation of the tyrosine kinase JAK2, which in turn triggers the downstream signal transduction. Indeed JAK2, when activated, mediates the phosphorylation of JAK2 itself and aforementioned three tyrosine residues of Ob-Rb: TyrY985, TyrY1077 and TyrY1138. While the phosphorylation of signal-transducer-and-activator-of-transcription (STAT)5 is mediated by the activation of the TyrY1077, the phosphorylation of STAT3 (pSTAT3), which not only mediates the central effects of leptin but is also an useful marker of leptin-sensitivity, is induced by the activation of the TyrY1138 [69,70]. It is noteworthy that the weight loss effects of leptin are strictly related to the JAK2/STAT3 pathway, as demonstrated by a growing body of evidence [71,72].

Indeed, pSTAT3 dimerizes and translocates into the nucleus where it modulates the transcription of several hypothalamic neuropeptides, such as POMC, CART, AgRP and NPY, as well as numerous genes including c-fos, and the suppressor of cytokine signaling (SOCS)3 [73]. This latter phenomenon ends in the binding of SOCS3 peptides to JAK2 and

TyrY985, blocking thus the leptin-induced STAT3 phosphorylation in a classic negative feedback pathway [74,75].

Furthermore, TyrY985, when phosphorylated, serves also as binding site for the Src homology 2 (SH2) domain of protein tyrosine phosphates 2 (SHP2), leading to the extracellular signal-regulated kinase (ERK) activation. As demonstrated by Rahmouni et al. [76], the SHP2/ERK pathway also mediates the anorectic and weight-reducing effects of leptin at central level, since a reversal of these effects, as well as a reduction of thermogenic sympathetic outflow by leptin, occurs after pharmacological blockade of hypothalamic ERK1/2. Besides the JAK2/STAT3 and SHP2/ERK pathways, it is well known that Ob-Rb in the hypothalamus signals also via another mechanism involving the insulin receptor substrate (IRS)/phosphoinositol-3 kinase (PI3K) pathway, as demonstrated by in vitro experiments, pharmacological studies and genetic models [77–80].

## 4. Mechanisms underpinning leptin resistance

It is worth noting that leptin resistance occurs when there is an impairment of the effectiveness of the Ob-Rb downstream signaling transduction [81], although in the presence of hyperleptinemia, a lack of anti-obesity action of leptin appears. Therefore, leptin resistance is one of the most frequent features in the onset and progression of obesity [82]. This condition is very common in obese humans and occurs after only few weeks of HFD in rodents (Fig. 1) [83].

Since the hypothalamus mediates the anti-obesity actions of leptin, three mechanisms are currently accepted to mediate central leptin resistance, such as the reduction in leptin access to CNS through the BBB, the impairment of leptin signaling in first-order neurons expressing Ob-Rb, or in second-order leptin-targeted neurons and neural circuits. It has been recently suggested that other mechanisms, such as the onset of hypothalamic inflammation, autophagy deficiency or ER stress, can also mediate the obesity-associated central leptin resistance [84–86]. Indeed, since leptin exerts its biological effects not only in CNS but also in peripheral tissues, parallel to central leptin resistance a peripheral dampening in leptin sensitivity can occur.

### 4.1. Reduction in leptin access to CNS

As it is well-known, OB-Ra, which is highly expressed by capillary cells of the choroid plexus, actively transports leptin across the BBB to reach the majority of Ob-Rb expressing neurons in the CNS. This transport system is saturable and recently it has been shown to be flanked by another transport mechanism involving the endocytic receptor megalin, as demonstrated by a decrease in leptin cerebrospinal fluid (CSF) levels in megalin deficiency [36].

In obese individuals the saturation of leptin transport can occur due to the hyperleptinemia, resulting in an only slightly increase in the CSF leptin levels [10]. Oh-I et al. [87] showed that the impairment of leptin transport across BBB can also be caused by the higher plasmatic levels of cytokines and fatty acids in obese individuals relative to lean subjects.

To date, it is still unclear the extent to which the impairment of leptin transport to CNS can contribute to the leptin resistance. Indeed, leptin can reach the CNS through the median eminence, which lacks the BBB. Therefore, ARC first-order neurons can sense leptin trough their projections into the median eminence [88].

### 4.2. Impairment of leptin signaling in hypothalamic neurons

Leptin resistance can occur by alterations in each component of the Ob-Rb downstream signaling cascade. In particular, previous findings have highlighted three potential mechanisms: a reduced expression of Ob-Rb at plasma membrane level, an upregulation of negative regulators of leptin signaling, and a downregulation of its positive regulators.

Diano et al. [89] have demonstrated that Ob-Rb has a predominant localization in the Golgi apparatus in hypothalamic neuronal and glial
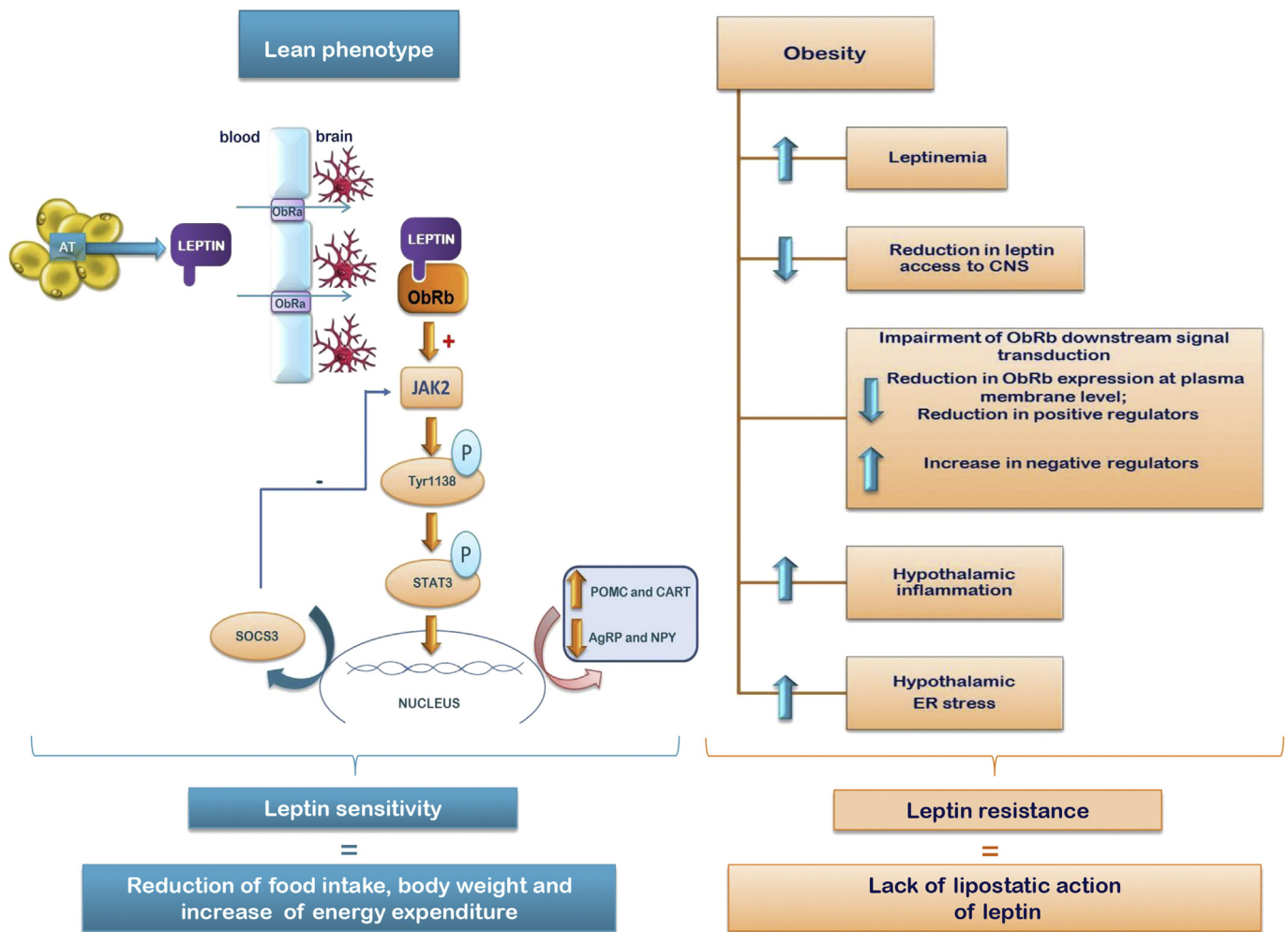
**Fig. 1.** A schematic overview of leptin downstream signaling pathways, underpinning its physiological effects in lean condition, and their main alterations in obese phenotype, leading to leptin resistance. List of abbreviations: adipose tissue (AT); short isoform of the leptin receptor (ObR)a; long isoform of the leptin receptor (ObR)b; Janus kinase (JAK) 2; tyrosine (Tyr) 118; signal transducer and activator of transcription (STAT) 3; suppressor of cytokine signaling (SOCS) 3; pro-opiomelanocortin (POMC); agouti-related protein (AgRP); cocaine- and amphetamine-regulated-transcript (CART); neuropeptide (NP)Y; central nervous system (CNS); endoplasmic reticulum (ER).

cells, thus its trafficking to the plasma membrane is necessary to obtain a physiological response to leptin stimulation. The Ob-Rb expression pattern at cell surface depends on a delicate balance between the activity of the Bardet–Biedl syndrome (BBS) proteins, which mediate its transport to the plasma membrane, and the rate of the ligand-independent endocytosis, a process that promotes the Ob-Rb internalization. Indeed, as previously demonstrated by Rahmouni et al. [90], BBS deficiency can cause leptin resistance, allowing us to speculate that also an endocytosis increase can be involved in the dampening of leptin sensitivity.

Regarding the second molecular mechanism underpinning leptin resistance, the Ob-Rb physiological signaling is under control of two negative regulators: SOCS-3 and phosphotyrosine phosphatase (PTP) 1B [29,15].

Interestingly, a restoration of leptin sensitivity has been shown in neural cell-specific SOCS3 conditional knockout mice, generated using the Cre–loxP system [91]. As known, SOCS3 is involved in the onset of leptin resistance at central level, as well as at peripheral level. It has been demonstrated that an increase in SOCS3 mRNA expression is involved in the development of leptin resistance in skeletal muscle from rats on high-fat diet (HFD) [92]. Moreover, SOCS3 inhibits AMPK activation in peripheral metabolically active tissues, such as the liver, white adipose tissue (WAT) and skeletal muscle, contributing to abnormalities of fatty acid metabolism [93]. AMPK is a fuel-sensing enzyme, whose activity is finely regulated by leptin: in peripheral tissues, i.e. WAT, leptin increases its enzyme activity, promoting the catabolic pathways toward the fatty acid oxidation and glucose internalization, exceeding the anabolism rate. On the contrary, leptin inhibits AMPK activity at central level, where this enzyme is involved in food intake regulation, since it controls the release of hypothalamic neuropeptides [94].

In addition to SOCS3, PTP1B is a negative regulator of both leptin and insulin signaling. This phosphatase dephosphorylates JAK2, dampening the leptin sensitivity. Actually, an increased expression of SOCS3 and PTP1B at hypothalamic level has been found in leptin-resistant mice [15,95,96]. In agreement with this evidence, PTP1B (−/−) mice are more sensitive to leptin and resistant to obesity compared to wild-type littermates [97,98]. Due to its key role in regulating metabolism, PTP1B should be a useful target for developing new drugs for the treatment of obesity and obesity-related pathologies, including the type II diabetes.

Eventually, leptin resistance is also associated with a downregulation of positive regulators of Ob-Rb signal transduction. In particular, the leptin-induced STAT3 phosphorylation is essential to obtain a clear activation of hypothalamic neurons, releasing thus the anorexigenic neuropeptides. In agreement, our previous data have shown that hypothalamic STAT3 phosphorylation is significantly decreased in ovariectomized rats, paralleled to the onset of leptin resistance [81].

To date, in addition to STAT3, another positive regulator, SH2B1, a Src homology 2 (SH2) and pleckstrin homology (PH) domain-containing protein, involved in alterations of leptin responsiveness has

been recognized [99]. While deletions of SH2B1 gene result in leptin unresponsiveness, hyperphagia and obesity, neuronal overexpession of this gene protects mice from HFD-induced obesity, supporting its crucial role in leptin resistance [100,101].

### 4.3. Impairment of MC4R downstream signal transduction in neural circuits

The melanocortin system controls the energy balance, especially trough the MC4R, which is mostly expressed in the brain. Therefore, alterations in the MC4R-dependent brain-derived neurotrophic factor (BDNF)/Tropomyosin receptor kinase (Trk) B signaling pathway, in the ventromedial nucleus (VMN), can affect leptin resistance. As demonstrated by Liao et al. [102], mice harboring a truncated long Bdnf 3′ UTR develop a severe hyperphagy and a clear obese phenotype. In this genetic model, leptin is unable to activate hypothalamic neurons and reduce food intake.

### 4.4. Inflammation as a potential contributor to leptin resistance

We have recently demonstrated that HFD induces a low-grade inflammation in peripheral tissues (i.e. liver), leading to an increase of inflammatory cytokines, such as the interleukin (IL)-6 and the tumor necrosis factor (TNF) α [103,104]. Indeed, HFD feeding as well as obesity promote inflammation not only in peripheral metabolically active tissues but also in the hypothalamus, determining a clear overexpression of these aforementioned cytokines and a strong activation of inflammatory pathways in the CNS [105,84]. Consistently, the hypothalamic deletion of IκB kinase complex (IKK) β (i.e. the enzyme that regulates the translocation of NF-κB p65 subunit into nucleus) or the i. c.v. administration of a IKKβ inhibitor has been found to make animals diet induced obesity (DIO) resistant, while a dampening of leptin sensitivity has been shown to occur as a consequence of the activation of IKKβ in the hypothalamus [85,85]. Therefore, genetic models as well as pharmacological approaches allow us to assert that obesity-related leptin resistance is also strongly associated with hypothalamic and peripheral inflammation.

Toll-like receptor (TLR) 4 signals through the NF-κB/IKKβ pathway, and also hypothalamic alterations in the expression/activation of this receptor or its adaptive proteins, such as Myd88, have been recognized as contributors to central leptin resistance [106].

In vivo studies, showing hypothalamic upregulation of SOCS3 in neuronal IKKβ knockout mice or an increased expression of PTP1B as a consequence of systemic TNFα administration have strengthened the involvement of hypothalamic inflammation in leptin resistance, suggesting that these two negative regulators of leptin signaling serve as a connection between these two processes [84,107].

### 4.5. Role of hypothalamic ER stress in central leptin resistance

The ER is responsible for folding nascent proteins and this process is possible until there is a perfect balance between the ER capability to fold these macromolecules and the amount of loaded proteins. As soon as an imbalance between these folding and loading processes occurs, ER stress appears, leading to the activation of several pathways (i.e. unfolded protein response (UPR), inositol-requiring protein (IRE)-1 and protein kinase RNA (PKR)-like kinase (PERK) pathways), which collectively attempt to counteract the ER stress itself, restoring the ER homeostasis. To date, a growing body of evidence has demonstrated the involvement of the hypothalamic ER stress in central leptin resistance and obesity [84,108,109]. Consistently, pharmacological approaches, consisting in the central administration of ER stress inducers or chemical ER chaperons, are able to modulate leptin responsiveness in an opposite manner, identifying a role of ER stress in leptin resistance [108–110].

### 4.6. Defective autophagy as a contributor of leptin resistance

It has been recently emphasized a key role for the autophagy in regulating the overall energy balance, since the inhibition of this process, by a neuron-specific deletion of autophagy related protein (Atg) 7, can alter the phenotype in mice. In particular, mice show an obese phenotype when this well-known autophagy component is selectively knocked down in POMC neurons, probably because this deletion is associated also with a reduction of leptin-induced STAT3 phosphorylation; in agreement with these results, the deletion of this gene in AgRP neurons causes a reduction in fat mass [111,112].

Collectively, these data highlight that the hypothalamic autophagy deficiency is involved in leptin resistance and obesity.

## 5. Leptin in therapy and leptin sensitizers

Due to its role in energy balance, leptin was first used to treat obese children with Ob gene mutation. This treatment led to a reduction in hyperphagia and weight loss [113,114]. Further, leptin has been used in lipodystrophic animals or patients, where low leptin levels were associated with hyperphagia and increase of fat storage in the liver and skeletal muscle, and finally to type II diabetes and metabolic disorders [115,116]. Leptin or metreleptin (the recombinant methionyl form of the human hormone) administration is able to improve glucose homeostasis and plasma lipid profile, and reduce steatosis in metabolic disorders associated with lipodystrophy or human virus-associated immunodeficiency [117,118]. In 2014, the U.S. Food and Drug Administration approved metreleptin, for injection, as replacement therapy to treat the complications of leptin deficiency, in addition to diet, in patients with congenital or acquired generalized lipodystrophy. Patients with both types of lipodystrophy often develop severe insulin resistance at a young age and may have diabetes mellitus or severe hypertriglyceridemia. Moreover, hypothalamic amenorrhea, characterized by energy deprivation and low leptin levels, is somewhat corrected by exogenous hormone treatment, as well as improved bone mineral density and bone formation [119,120]. However, leptin treatment of common obese or type 2 diabetes patients failed to induce weight loss and improve insulin action [121,122].

Animal studies demonstrated that prolonged maintenance of an elevated body weight alters energy homeostatic systems to defend a higher level of body fat [123]. It seems that leptin reduction after prolonged caloric restriction is one of those metabolic adaptations, that can be prevented by leptin replacement [124]. In this context, leptin combined with a reduction of caloric intake seems to further maintain weight loss avoiding diet-induced compensatory metabolic adaptations, that induce resistance to weight loss [125]. An optimization of this approach has been to combine leptin administration with other molecules able to improve leptin sensitivity, in order to reinforce body weight loss, the so called "leptin sensitizers". Among these, amylin, an endogenous hormone co-secreted after feeding with insulin by β-pancreatic cells, regulates energy balance and glucose homeostasis, interacting with its receptors in the brain [126]. Several studies demonstrated that the co-treatment of leptin with amylin reduced food consumption and body weight of DIO leptin-resistant mice or overweight/obese subjects, compared to leptin or amylin treatment alone, indicating a synergic reversal effect on leptin resistance [127–129]. This synergic effect was also shown in long-term experiments, determining an improvement in plasma insulin and lipid profile and glucose homeostasis in obese rats [130].

Similar findings were obtained by the combination of pramlintide acetate (an amylin analog) and metreleptin in humans [131,132]: this combination in fact had an additive effect on weight loss, implying the activation of common signaling pathways in peripheral tissues [133]. Anyway the clinical study with pramlintide and metreleptin was interrupted due to the induction of anti-metreleptin antibodies.

Cholecystokinin, added to the combination amylin/leptin and administered as subcutaneous tri-infusion, induced a higher effect on body weight and food intake compared to amylin/leptin in DIO rats, indicating its role in increasing leptin sensitivity [134]. Among other possible combinations, exendin-4, acting as glucagon like peptide 1 (GLP-1) on GLP1 receptor, and fibroblast growth factor 21 were able to revert leptin resistance in DIO mice once refed with standard diet [135]. Similarly, clusterin, an LDL receptor-related protein-2 (LPR2), combined with leptin, increased hypothalamic leptin signaling [136].

## 6. Strategies to overcome leptin resistance

### 6.1. Caloric restriction and exercise

Caloric restriction is the first approach for the treatment of obesity able to reduce circulating leptin levels, as an alternative to pharmacologic reversal of leptin resistance [137]. It has been reported that long term exercise, not only decreased leptin levels [41], but also increases the activation of STAT3 and AMPK signaling pathways in the hypothalamic arcuate nucleus [138]. Prevention of leptin resistance by exercise was also demonstrated by Zhou [139], who showed a reduction in hypothalamic SOCS3 mRNA expression and JAK2/STAT3 signaling pathway in rats fed a high fat diet by exercise. However, when exercise was combined with caloric restriction the effect was more evident compared to those obtained by exercise or diet approach alone [140].

### 6.2. Reversal of the inhibition of SOCS3 and PTP1B

As already depicted, SOCS3 and PTP1B are negative regulator proteins of leptin receptor signaling. Therefore their down-regulation can be considered a useful approach to revert leptin resistance. The inhibition of SOCS3 expression and/or activity could possibly lead to an interruption of the negative feedback loop related to leptin resistance and restore leptin activity. Accordingly, ObRb mutation in transgenic mice, disabling SOCS3 binding, reduced food intake, increases leptin sensitivity and reduced weight gain [141]. Moreover, evidence showed that rats with hypothalamic SOCS3 silencing are resistant to diet induced obesity, did not develop leptin resistance, indicating that JAK2/STAT pathway restoration improves leptin physiological activity in terms of body weight and metabolic profile control [142]. Moreover, using SOCS3 short hairpin RNA lentivirus interference, a decrease in lipogenesis and an increase in fat oxidation were found in SOCS-3-knockdown adipocytes after leptin stimulation [143]. These authors conclude that the inhibition of SOCS-3 in adipocytes of rats with DIO increased the leptin-mediated in situ fatty acid oxidation.

All these data support the concept of a new therapeutic strategy, based on the prevention of ObR/SOCS3 binding to reduce the inhibition of JAK2 and ObR phosphorylation, considering that no specific inhibitors of SOCS3 exist.

Besides SOCS3, also PTP1B inhibition seems to be an attractive target to overcome leptin resistance. In fact, deletion of PTP1B in mice increases leptin sensitivity, reduces body weight and increases energy expenditure [144]. These animals also showed an improvement in glucose metabolism and uptake, and they were protected by DIO [145]. The selective inhibition of PTP1B resulted in dampening of STAT3 activation by leptin in HEK cells [146].

To date, inhibitors of PTP1B based on capability to bind PTPB1 active site (without hydrolysis) have been designed. In particular, thiazolidinedione compounds have been shown to exert antiobesity effects as PTP1B inhibitors and PPAR-α activators, ameliorating blood lipid profile in mice on high fat diet [147].

Actually trodusquemine and PTP1B antisense nucleotides represent a new approach as potential therapeutics for diabetes and obesity. Trodusquemine, a selective allosteric inhibitor of PTP1B, induced a reduction of body weight and food intake in DIO mice [148]. Once crossed the BBB, this compound also ameliorates insulin sensitivity, through an increase of insulin-induced phosphorylation of insulin receptor and STAT3 [148]. Recently, intraperitoneal administration of claramine, a synthetic polyaminosteroid derivative, or trodusquemine effectively restored glycemic control in diabetic mice as determined by glucose and insulin tolerance tests and by the activation of key components of insulin signaling (i.e. increased phosphorylation of insulin receptor-β, Akt and GSK3β) [149].

### 6.3. POMC neuron activation

Last molecular targets of leptin effect on energy balance are POMC neurons, therefore their activation seems another attractive strategy to overcome leptin receptor signal to induce α-MSH-mediated suppression of food intake and weight gain together with an increase in energy expenditure. In this context, MT-II, a direct activator of POMC neurons was reported to bypass central leptin resistance, even if leptin effect is not solely mediated by those group of neurons [150]. More recently, it has been reported that teasaponin treatment reduced obesity, peripheral and hypothalamic inflammation, and central leptin resistance in HF diet-induced obese mice. Chronic teasaponin treatment also suppressed energy intake and increased the expression of the anorexigenic neuropeptide POMC in the hypothalamus [151].

### 6.4. Increase in leptin receptor expression and cell surface localization

An increase in ObR expression and its localization at the cell surface are key determinants for cell sensitivity to leptin. We demonstrated that in leptin resistant ovariectomized obese rats, estradiol replacement therapy or long-term raloxifene treatment, reduced leptin levels and body weight and restored leptin receptor expression both in adipose tissue and hypothalamus [53]. Unfortunately, differently from rodent studies, a recent review of the literature regarding estrogen effect on leptin levels in post-menopausal women, did not show evident beneficial effects by hormonal intervention in modulating leptin levels and attenuating weight gain. Therefore the authors discourage the hormonal intervention in relation to cardiovascular and neoplastic risk associated with the replacement therapy [152]. Previously, clinical studies showed an increase in leptin levels and body fat content in post-menopausal woman, corrected by hormone therapy [153–155], in contrast, other studies reported an increase in leptin levels in treated woman, not related to change in fat mass [156,157]. It has also been shown that metformin, acting at ObRb hypothalamic gene level, is able to increase receptor expression and leptin sensitivity, and exert an anorectic effect [158]. Moreover, the inverse agonist of cannabinoid receptor 1, JD5037, is able to overcome leptin resistance and reduce weight gain [159]. Recently, we also showed that palmitoylethanolamide, a PPARα agonist, induces a reversal of leptin resistance, reducing leptin levels, and increasing ObRb expression and signaling in obese ovariectomized rats, reducing food intake and body weight [81].

It has been shown that the majority of ObR is localized into the cytosol, rather than on cell surface [160]. Actually ObR on cell surface is constitutively internalized through ligand independent fashion and clathrin-mediated mechanism [161]. After internalization, ObR is found at (EEA1 and Rab5-positive) endosome level and then it underwent lysosomal degradation [160]. The internalization and retention into the cytoplasm, together with low recycling rate, reduced the cell surface expression of ObR [162]. Therefore, an alteration of ObR trafficking, reduces its signaling [163,164], since ObR expression on cell surface depends on its interaction with trafficking proteins [165]. RNF41, the ubiquitin ligase, controls ObR trafficking, increasing ObR recycling [166], while clusterin and LRP2, increase ObR and leptin endocytosis, leading to an increase in hypothalamic STAT3 activation [136]. An alternative splicing product of db gene encodes for endospanin 1, that retains ObR in the cytoplasm and is involved in the modulation of ObR localization at cell surface [164,167]. In fact, endospanin 1 knock-down induced an increase in ObR cell surface localization and STAT3 activation in the hypothalamus, reverting obesity in

mice fed HFD [168]. Therefore, another approach to treat leptin resistance and obesity would be to increase leptin sensitivity, modulating ObR expression on cell surface in the ARC neurons through small molecules able to modify ObR distribution [169].

## 6.5. Improvement of leptin transport through the BBB

Leptin resistance has been often associated with a defect in leptin entry into the brain through the BBB. In fact central, and not peripheral, administration of leptin was able to reduce food intake and body weight in DIO mice, indicating that at least in part leptin resistance was related to BBB impairment [11]. Several factors modify leptin entry through the BBB to reach the hypothalamus. In particular, it has been shown that when leptin transport through the BBB is impaired, leptin resistance can be reverted by a reduction in triglyceride levels, indicating their role in leptin resistance [12]. Similarly the acute phase CRP has a role in leptin resistance, limiting leptin crossing through the BBB. Anyway, it has been shown that high dosage of CRP increased leptin entry through an improvement of paracellular permeability in the CNS [170]. Moreover, the increase in leptin affinity to its binding proteins can induce an increase in leptin concentration in the brain [36]. To improve leptin passage through the BBB, new ObR agonists or leptin analogs have been designed [171]. Pegylated leptin, although with a longer half-life, does not cross the BBB [172], therefore it does not modify body weight in humans [173]. Other modifications showed to improve BBB transport and influence energy balance, such as the conjugation with a carbohydrate moiety [174], or the modification with trans-activating transcriptional activator or with PluronicR [171, 175]. Further investigation about ERK-dependent release of leptin in the CSF has highlighted another possible approach attempting in the restoration of ERK signaling when leptin resistance is mainly due to a diminished leptin transport in the brain.

## 6.6. Reduction of ER stress

Alteration in ER functions, due to dysregulation in synthesis, folding, maturation, and trafficking of proteins, leads to ER stress. This state is accompanied by the activation of a rescue strategy known as "unfolded protein response" (URP) [176]. Anyway, the overactivation of URP can induce cell death. It has been shown that ER stress plays a role in the development of leptin and insulin resistance [19–21].

Therefore, chaperon compounds able to reduce ER stress have been proposed as a new possible therapeutics against leptin resistance. Drugs, stabilizing protein folding and reducing protein aggregation could reduce ER stress and hence leptin resistance. 4-Phenylbutyrate (4-PBA) and tauroursodeoxycholic acid (TUDCA) reduce hypothalamic ER stress and revert leptin resistance, reducing food intake and body weight in DIO mice [20]. These two molecules, already approved by FDA, improve insulin signaling and glucose homeostasis in insulin resistant patients [177,178], confirming that ER stress reduction constitutes a new strategy for metabolic disorders.

Hosoi et al. [179] have demonstrated that the inhibitor of serotonin uptake, fluvoxamine, attenuated ER stress-induced leptin resistance, increasing STAT3 phosphorylation, and reducing food consumption in mice. Moreover, it has been shown that also the gaseous transmitter carbon monoxide was able to revert leptin resistance induced by thapsigargin or tunicamycin in human cells [180].

Several other treatments were reported to reduce ER stress-induced leptin resistance: a sweet tea leaf extract demonstrated to have an anti-hyperlipidemic activity, reducing oxidative stress [181]; IL-6 and IL-10 combined with exercise are able to inhibit IKKβ/NFκB pathway [182]; and the low molecular weight, fucoidan, an activator of AMPK, that is used for metabolic disorders and obesity [183]. Very recently, it has been reported that the non-steroidal anti-inflammatory drug, flurbiprofen, reduces ER stress and attenuates leptin resistance, inducing weight loss in obese mice [184].

**Table 1**
Currently available strategies to restore leptin sensitivity and their beneficial effects.

| Strategies to overcome leptin resistance | Experimental and clinical beneficial effects |
|---|---|
| Combination of leptin with "leptin sensitizers" | Improvement of glucose homeostasis, plasma lipid profile and reduction of food consumption and body weight in DIO animals. Similar results were obtained in humans [126,127,129–135]. |
| Caloric restriction and exercise | Reduction of hyperleptinemia and SOCS3 transcription, restoration of STAT3 and AMPK activation in ARC nucleus in HFD animals [41,137–140]. |
| Improvement of leptin access to CNS | Modifications of energy balance [171–174]. |
| Increase in ObRb expression | Reduction of hyperleptinemia and body weight, restoration of leptin sensitivity in ovariectomized obese rats and in other mouse model of obesity [53,81,136,158,159,168]. |
| Reversal of SOCS3 and PTP1B | Reduction of food intake and body weight, increase in leptin sensitivity, resistance to DIO in transgenic mice with hypothalamic SOCS3 or PTP1B deletion. The inhibition of PTP1B reduces food intake and body weight in DIO mice, together with an increase in insulin sensitivity [141–145,147–149]. |
| Reduction of ER stress | Reversal of leptin resistance and reduction of food consumption and body weight in DIO mice. Improvement of insulin signaling and glucose homeostasis. Increase in STAT3 phosphorylation [177–179,181–184]. |

## 7. Conclusions

The efficacious interaction between leptin and its functional receptor in the brain, as well as in all peripheral target organs and tissues, is fundamental for body weight control and maintenance of adequate energy balance. Leptin resistance, instead, may affect this homeostasis, inducing long term detrimental adaptation leading to obesity and obesity-induced ailments (Fig. 1). The efforts made to study the mechanisms underlying leptin resistance led to identify the possible targets to restore leptin sensitivity. This review, indeed, shows the complexity of the mechanisms that sustain leptin resistance (i.e. hyperleptinemia, failure of central transport of the hormone, ER stress, and receptor signaling impairment) in common forms of obesity, and how these could be targeted specifically to treat obesity, and related metabolic diseases (Table 1). The numerous findings from this review clearly indicate a lack of single, efficacious and definitive treatment for leptin resistance. Although novel strategies to restore leptin sensitivity have been recognized, and many of them are promising in preclinical studies, these approaches have been hampered by the limited efficacy or elevated adverse effects in patients. In the long-term, new drugs to overcome leptin resistance, acting at multiple levels, should be included in a therapeutic setting in order to modulate food intake and energy expenditure and therefore to lead to steady reversal of obesity.

**Conflict of interest statement**

The authors declare that there are no conflicts of interest.

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