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The cellular and molecular bases of leptin and ghrelin resistance in obesity

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Abstract

Obesity, a major risk factor for the development of diabetes mellitus, cardiovascular diseases and certain types of cancer, arises from a chronic positive energy balance that is often due to unlimited access to food and an increasingly sedentary lifestyle on the background of a genetic and epigenetic vulnerability. Our understanding of the humoral and neuronal systems that mediate the control of energy homeostasis has improved dramatically in the past few decades. However, our ability to develop effective strategies to slow the current epidemic of obesity has been hampered, largely owing to the limited knowledge of the mechanisms underlying resistance to the action of metabolic hormones such as leptin and ghrelin. The development of resistance to leptin and ghrelin, hormones that are crucial for the neuroendocrine control of energy homeostasis, is a hallmark of obesity. Intensive research over the past several years has yielded tremendous progress in our understanding of the cellular pathways that disrupt the action of leptin and ghrelin. In this Review, we discuss the molecular mechanisms underpinning resistance to leptin and ghrelin and how they can be exploited as targets for pharmacological management of obesity.

Obesity is associated with increased morbidity and mortality owing to the many medical conditions that are caused by excess fat accumulation[1]. Many factors, such as genetic predisposition, ready availability of calorie-dense food and a sedentary lifestyle, have contributed to the rise in obesity prevalence. The regulatory systems that control body weight homeostasis also often promote positive energy balance, which might also contribute to weight gain and fat accumulation in individuals with obesity. Indeed, whereas a robust biological response is triggered to restore homeo stasis when body fat stores are endangered

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(such as in times of starvation), excess of adiposity is associated with absence of significant response. The regulatory pathways controlling energy balance become dysfunctional and are unable to defend the body against excess energy stores in obesity.

A prototypic defect that occurs in obesity is the development of resistance to the action of key metabolic hormones such as leptin and ghrelin. Leptin and ghrelin have emerged as important players in the neuroendocrine control of energy homeostasis (FIG. 1). These hormones communicate information to the central nervous system (CNS) about the current levels of energy reserves and nutritional status. In this Review, we discuss the molecular signalling pathways and the dietary and nutritional interventions that interfere with the actions of leptin and ghrelin. We also discuss the cross-talk between the 'classic' leptin-resistance phenomenon and the novel concept of ghrelin resistance.

Leptin

Leptin, discovered in 1994 by positional cloning[2], is a 16 kDa cytokine that is produced predominantly by adipose tissue: it is released into the bloodstream and circulates in proportion to body fat mass[3]. Consequently, levels of leptin convey information about the energy reserves of the body to the centres that regulate energy homeostasis[4–6]. Increased adipose depots that are associated with positive energy balance increase leptin production and its circulating levels, which typically trigger a response to reduce feeding and promote energy expenditure[7–14]. Conversely, a fall in circulating levels of leptin, associated with negative energy balance in the body (such as during calorie restriction and/or excessive exercise) triggers a strong motivation to eat and conserve energy[15,16].

Leptin has also been implicated in the regulation of the immune system, autonomic and cardiovascular regulation, reproductive function and bone formation (FIG. 1). For example, in humans and rodent models, leptin deficiency reduces the immune system response, decreases sympathetic nervous system activity and blood pressure, delays puberty and can lead to infertility, in addition to reducing bone density[17–22]. Importantly, the role of leptin in these processes is largely independent of its role in the regulation of body weight, which implies a broader role of this hormone beyond energy homeostasis.

Leptin receptor signalling

The leptin receptor (LepR) was cloned from the mouse choroid plexus by affinity-based screening assay and was found to be a single transmembrane spanning receptor of the class I cytokine receptor family[23]. In mice, six LepR isoforms have been identified and have been classified as secreted (LepRe), short (LepRa, LepRc, LepRd and LepRf) and long (LepRb) forms[24,25]. These isoforms arise from the single *Lepr* gene by alternative mRNA splicing or post-translational cleavage[24]. LepRb is primarily responsible for leptin signalling and seems to mediate most physiological actions of leptin. For example, the phenotypes of mice that lack the LepRb isoform, such as *db/db* mice, are very similar to the phenotypes of mice lacking leptin, such as the *ob/ob* mice or those lacking all isoforms of LepR, such as the db^{3J}/db^{3J} mice[26].

LepRb is expressed at high levels in areas of the brain that are involved in the regulation of feeding and energy expenditure[27]. In the hypothalamus, LepRb is expressed in a subset of neurons in nuclei that are important for metabolic regulation, such as the arcuate nucleus (ARC), ventromedial nucleus of the hypothalamus (VMH) and dorsomedial nucleus of the hypothalamus, and in the lateral hypothalamic area[27] (FIG. 1). Similar to other cytokine receptors, LepRb signals via a non covalently associated kinase, Janus kinase 2 (JAK2)[28–30]. Upon binding of leptin to its extracellular domain, homodimerized LepRb undergoes a conformational change that enables the activation of JAK2, which then phosphorylates other tyrosine residues within the LepRb–JAK2 complex to activate downstream signalling cascades[31,32] (FIG. 2). Three tyrosine phosphorylation sites, Tyr985, Tyr1077 and Tyr1138, have been identified within the carboxy-terminal tail of LepRb[33]. Signal transducer and activator of transcription 3 (STAT3) is recruited to Tyr1138 and phosphorylated by JAK2; this enables STAT3 translocation to the nucleus and transcriptional initiation of target genes, including suppressor of cytokine signalling 3 (SOCS3), which is an inhibitor of LepRb–JAK2 signalling. Likewise, STAT5 is recruited to Tyr1077 and is also phosphorylated and activated by JAK2 (REFS 34,35). Finally, protein tyrosine phosphatase non-receptor type 11 (PTPN11; also known as SHP2) is recruited to Tyr985 and activates extracellular signal-regulated kinase (ERK), which mediates some of the downstream effects of LepRb [36]. Notably, SOCS3 is also recruited to Tyr985 and provides feedback inhibition to suppress LepRb signalling[36] (FIG. 2). Stimulation of LepRb also activates the insulin receptor substrate (IRS)– phosphoinositide 3-kinase (PI3K) pathway; however, the exact mechanism involved is unclear[18,23]. Mechanistic target of rapamycin complex 1 (mTORC1) and its downstream effectors, such as ribosomal S6 kinase (S6K) and 40S ribosomal protein S6, are important targets of the LepRb–PI3K axis and mediate the physiological actions of leptin[37,38].

Leptin resistance in obesity

Differential resistance to endogenous versus exoge- nous leptin.

Obesity is associated with leptin resistance, a phenomenon that is similar to insulin resistance in patients with type 2 diabetes mellitus[4]. Leptin resistance is associated with elevated circulating levels of leptin, as well as with the inability of exogenous leptin to decrease food intake and body weight. However, some data using LepR antagonists suggest that resistance to endogenous leptin does not contribute to high-fat diet (HFD)-induced obesity. In these data, intraperitoneal or intracerebroventricular administration of a LepR antagonist has the same effect on food intake and body weight gain in hyperleptinaemic mice with diet-induced obesity (DIO) and in lean mouse controls[39]. These findings indicate that the action of endogenous leptin is intact in mice with DIO despite hyperleptinaemia and resistance to exogenous leptin, thus challenging the view that leptin no longer suppresses food intake and reduces body weight in obesity. Interestingly, in clinical studies, even individuals with obesity (average BMI: 33 kg/m^2) feel strong hunger in response to moderate (10%) weight loss, and administration of leptin (0.08–0.14 mg per kg of fat mass; which restored plasma concentrations of leptin to those before weight loss) can mitigate this hunger[40]. Although these observations suggest that LepR signalling might be a therapeutic

target for treating obesity, how energy imbalance and obesity develop when endogenous leptin is functional is still puzzling.

Selective leptin resistance.

In obesity, leptin resistance seems to be selective, in a manner similar to selective insulin resistance[41]. This concept was derived from observations in mouse models that exogenous leptin could cause renal sympathetic nerve activation and increase blood pressure but was unable to exert an anorectic or weight-reducing action[42,43]. These findings also highlight that the cardiovascular sympathetic effects of leptin can be dissociated from its effects on energy homeostasis. Similar to *ob/ob* mice, patients with obesity who lack leptin have low-to-normal sympathetic nerve activity and blood pressure, which highlights the importance of leptin in driving sympathetic overactivity and hypertension in the context of obesity[44]. Currently, the proposed mechanisms of selective leptin resistance include specific brain region and/or cell type actions, as well as differentially regulated intracellular signalling cascades downstream of LepR, or indeed any combination of these factors[45], but these mechanisms need to be further investigated.

Potential mechanisms of leptin resistance

Leptin and blood-brain barrier.

Leptin circulates as either a free protein or in an inactive form that is bound to the circulating form of the receptor (LepRe)[46]. Interestingly, in contrast to lean individuals in whom up to 65% of circulating leptin is bound to LepRe, individuals with obesity predominantly have the active free form of circulating leptin (~85% of total leptin)[47,48] Consequently, individuals with obesity might have chronically elevated levels of active free leptin in the brain, which desensitize LepRb and increase leptin resistance. Indeed, levels of leptin in the cerebrospinal fluid (CSF) strongly correlate with plasma levels of leptin and increased BMI[49]. Whereas absolute amount of leptin in the CSF of individuals with obesity might be higher than in the CSF of lean individuals, the efficiency of movement of leptin from plasma to the CSF (measured as the CSF:plasma leptin ratio) diminished by as much as 80% in individuals with obesity[49]. This finding is further supported by the fact that intravenous administration of leptin caused a dose-dependent increase in CSF levels of leptin in lean mice, but not in mice with DIO[50]. Assessment of leptin transport rate across the blood–brain barrier (BBB) using multiple-time regression analysis and *in situ* brain perfusion showed that obese mice have reduced leptin passage across the BBB[51,52]. These findings might explain why systemic administration of exogenous leptin to suppress food intake and reduce body weight is blunted in individuals with obesity[53,54].

Astrocytes have emerged as potential mediators of leptin resistance in obesity. Astrocytes, which express different isoforms of LepR, participate in the transport of leptin across the BBB[52]. A HFD causes rapid activation of astrocytes, thus inducing inflammation, and hyperleptinaemia induced by a long-term HFD further activates astrocytes and inflammation, reducing delivery of leptin to the brain[55,56]. Moreover, binding of leptin to its receptors in astrocytes interferes with the action of leptin in neurons, and astrocyte-specific deletion of *Lepr* ameliorates leptin resistance in mice with DIO[57]. Tanycytes have also

been involved in obesity-associated leptin resistance and, in the median eminence, form a diet-responsive neurogenic niche for newborn neurons that express LepRb and can integrate the functional circuit later in their life[58]. Tanycytes in the hypothalamus seem to be a conduit for leptin into the brain[59]. Blood-borne leptin is first taken up by tanycytes in the median eminence and then released into the mediobasal hypothalamus, a process that is disrupted in mice with DIO[59]. The passage of leptin through tanycytes is colchicine sensitive, indicating the involvement of intracellular vesicular trafficking and suggesting that the release of leptin by tanycytes requires LepRb–ERK signalling[59]. Importantly, treatment with epidermal growth factor, which activates ERK signalling, can rescue impaired tanycytes-mediated transport of leptin in mice with DIO and increase energy expenditure and locomotor activity, facilitating weight loss[59]. These findings indicate that leptin released from tanycytes has functional consequences on energy balance. Surprisingly, ablation of tanycytes-derived newborn neurons by CT-guided irradiation protects mice from weight gain during HFD feeding[60]. With our current limited knowledge, targeting median eminence tanycytes to treat obesity might not be a realistic therapeutic approach, but developing a long-lasting leptin analogue that can cross the BBB might help to circumvent obesity-associated reduction in leptin transport in the brain[61].

Defective LepRb trafficking.

Impaired trafficking of LepRb to the membrane in neuronal subpopulations of the hypothalamic nuclei that control energy homeostasis has emerged as a novel mechanism of leptin resistance. Bardet–Biedl syndrome (BBS) is a highly pleiotropic autosomal recessive human disorder in which obesity is a common manifestation and that is associated with leptin resistance and energy imbalance[62]. Mice with global loss or mutation of Bbs genes, which are used as a model of BBS, are obese and have leptin resistance[62]. In addition, targeted deletion of the *Bbs1* gene in the nervous system leads to obesity in mice[57]. Interestingly, this phenotype can be reproduced by selective deletion of *Bbs1* in the mediobasal hypothalamus or in LepRb-expressing cells in the brain, but not in adipocytes, thus excluding a contribution of adipocyte Bbs genes to obesity[63]. Mice that are deficient in BBS proteins show impaired trafficking of LepRb to the plasma membrane, which leads to leptin resistance, but this resistance is independent from obesity[63,64]. BBS1 directly interacts with LepRb, which might explain how BBS proteins mediate the trafficking of LepRb[63,64]. Moreover, a 36–41% reduction in the number of leptin binding sites, as determined by autoradiography, occurs in the hypothalamic nuclei of obesity-prone rats[65], which suggests that defects in LepRb trafficking are also present in common forms of obesity.

Suppression of LepRb signalling in obesity

Many molecules and signalling pathways contribute to obesity-associated leptin resistance. Our enhanced understanding of LepRb signalling has highlighted certain molecules as candidates that might restore sensitivity to leptin in obesity.

SOCS3.

SOCS3, a member of a large family of cytokine-inducible inhibitors of signalling, was discovered by an expression cloning approach in mouse monocytic leukaemic M1 cells that

respond to cytokines such as IL-6 (REF. 66). SOCS3 can be rapidly induced by IL-6 and in turn suppresses cytokine signal transduction in a negative feedback loop[66,67]. Expression of *Socs3*, but not of *Socs1* or *Socs2*, is rapidly induced in the hypothalamus of *ob/ob* mice in response to peripheral administration of leptin, and overexpression of *Socs3* blocks leptin-induced signal transduction in cultured mammalian cell lines[68]. These observations suggest that SOCS3 mediates cellular leptin resistance in obesity[68]. Indeed, mice heterozygous for *Socs3* (REF. 69) or lacking SOCS3 specifically in the brain[70] have increased sensitivity to exogenous leptin-mediated suppression of food intake and reduction of body weight, and are resistant to DIO. These findings implicate SOCS3 as a negative regulator of LepR signalling *in vivo*. However, two other studies in mice have shown that deletion or overexpression of *Socs3* in LepRb-expressing cells does not affect HFD-induced weight gain, which questions the causative role of SOCS3 in the development of cellular leptin resistance[71,72].

PTPs.

LepRb signalling transduction relies on initial activation of JAK2 and subsequent phosphorylation of tyrosine residues within LepRb and STAT3 (FIG. 2). The overall protein phosphorylation in a cell depends on the dynamic balance of phosphorylation by kinases and dephosphorylation by PTPs. Consequently, any phosphatase that targets JAK2 and STAT3 could suppress LepRb signalling. Several PTPs have been implicated in LepRb signalling, including PTPN11, which binds to Tyr985 to activate the RAS–mitogen-activated protein kinase (MAPK) pathway; PTP1B (also known as PTPN1), which dephosphorylates JAK2; PTPN2 (also known as TCPTP) and PTPε, which dephosphorylate STAT3; and phosphatase and tensin homologue (PTEN), which dephosphorylates phosphatidylinositol-3,4,5-trisphosphate (PIP3) to regulate PI3K signalling pathway.

When PTP1B was first described, it was found to negatively regulate insulin signalling by dephosphorylating IRS[73–75] but was also found to suppress LepRb signalling *in vivo* by directly dephosphorylating JAK2 at Tyr1007 and Tyr1008 (REF. 76). *Ptp1b*-knockout mice, which are lean and hypersensitive to leptin, display increased energy expenditure and resistance to DIO[77–80] These effects can be recapitulated by ablation of PTP1B in neurons[81], presumably those expressing LepRb[82,83] but not in muscle or liver[81]. Targeting PTP1B activity is a potential therapeutic intervention in obesity[84]. For example, a small molecule inhibitor of PTP1B, trodusquemine, acts in the CNS to promote weight loss in both mice with DIO and genetic mouse models of obesity[85–88]. *Ptp1b*-directed antisense oligonucleotides yielded promising results in a phase II clinical trial[89].

Levels of PTPN2 are increased by as much as twofold in the hypothalamus of mice with DIO, and mice that lack neuronal PTPN2 have increased sensitivity to leptin (indicated by the exaggerated leptin-induced decrease in food intake and body weight and by activation of hypothalamic STAT3) and, on a HFD, remain leptin sensitive and gain less weight than control mice[90]. The mechanisms of action of PTPN2 seem to implicate a direct dephosphorylation of nuclear STAT3 that causes its export from the nucleus[90]. Importantly, intracerebroventricular administration of a specific PTPN2 inhibitor in wild-type mice increased leptin-induced STAT3 phosphorylation, resulting in decreased body weight and increased energy expenditure, which suggests that targeting this molecule

for pharmacological treatment of obesity might be feasible[90]. However, PTPN2 seems to be redundant in LepRb signalling, as *Ptpn2* deletion in a subset of leptin-sensitive neurons expressing pro-opiomelanocortin (POMC) did not promote leptin-induced STAT3 phosphorylation or suppression of food intake and body weight loss[91]. However, expression of PTP1B and PTPN2 in a subset of POMC neurons in the ARC (with some POMC neurons co-expressing both PTP1B and PTPN2) might have differential but synergistic effects on systemic energy metabolism[91]. Single deletion of *Ptp1b* or *Ptpn2* from POMC neurons revealed that PTP1B, but not PTPN2, mediates leptin sensitivity[91], whereas PTPN2, but not PTP1B, seems to be involved in insulin signalling. Notably, ablation of both PTP1B and PTPN2 in POMC neurons leads to a synergistic enhancement of the actions of leptin and insulin that promotes energy dissipation through browning of white adipose tissue[91].

PTPε, which negatively regulates LepRb signalling, exists in two forms: a soluble form and a transmembrane receptor-type form (RPTPε)[92]. PTPε has been implicated in ERK signalling by inhibiting ERK1 and ERK2 kinase activity and by interfering with their downstream events[93]. PTPε also suppresses JAK2–STAT3 signalling through its association with and dephosphorylation of JAK2 (REF. 92). PTPε and RPTPε are co-expressed with LepRb in hypothalamic nuclei such as the ARC, and treatment with leptin induces phosphorylation of RPTPε in the hypothalamus, an effect that is pronounced in mice with DIO[93]. Moreover, female, but not male, mice that lack both RPTPε and PTPε have increased sensitivity to leptin and are protected from fat accumulation induced by HFD, ageing and ovariectomy[93]. However, as male mice are more susceptible to DIO than females, the observation of a female-specific effect of PTPε on leptin sensitivity and DIO has diminished the enthusiasm for its development for therapeutic intervention. Nonetheless, these findings might enhance our understanding of sexual dimorphism of body energy homeostasis.

Exchange proteins directly activated by cAMP pathway.

cAMP mediates the actions of hormones by activating protein kinase A (PKA) and exchange proteins directly activated by cAMP (EPACs)[94]. The role of PKA in regulating energy balance has been well documented[95], but only recently has EPAC, which acts as a cAMP-regulated guanine nucleotide exchange factor for the small G protein RAP1, been found to function as a negative regulator of LepRb signalling[96,97]. In *ex vivo* hypothalamic organotypic cultures, elevated levels of cAMP were found to impair the signalling cascades that are activated by leptin, including STAT3 and S6K pathways, independently of the activation of PKA[97]. By contrast, activation of EPACs is sufficient to impair LepRb signalling with concomitant induction of SOCS3 and also blunts leptin-induced depolarization of hypothalamic POMC neurons. Moreover, intracerebroventricular administration of an EPAC activator blunted the anorexigenic effect of leptin. Taken together, these findings suggest an important role of EPAC in the negative regulation of LepRb signalling[97].

This conclusion is further supported by the observation that mice lacking *Epac1*, which encodes one of the two isoforms of EPAC in mammals, are more resistant to DIO and have improved glucose tolerance and heightened central leptin sensitivity, as measured through

STAT3 activation, compared with control mice[98]. Furthermore, inhibition of EPAC1 using a selective chemical inhibitor (ESI-09) can reduce plasma levels of leptin *in vivo* and resulted in increased leptin sensitivity in organotypic hypothalamic slices[98].

Inflammation and endoplasmic reticulum stress.

Endoplasmic reticulum stress has emerged as a key factor in obesity-associated inflammation and leptin resistance. HFD consumption increases the expression of pro-inflammatory cytokines in the hypothalamus, such as IL-1, IL-6 and tumour necrosis factor (TNF), which activate inflammatory pathways[99]. Surprisingly, only 1 day of HFD feeding, or a single central administration of fatty acid, is sufficient to induce hypothalamic inflammation in rodents[100–102]. A HFD activates nuclear factor-κB (NF-κB) and its upstream regulator inhibitor of NF-κB kinase-β (IKKβ) by increasing endoplasmic reticulum stress in the hypothalamus, which might cause leptin resistance[102] (FIG. 2). A lack of TNF receptor or brain infusion of a TNF antibody improves leptin resistance in HFD-fed mice[103,104]. Furthermore, amelioration of hypothalamic endoplasmic reticulum stress using chemical chaperones such as 4-phenylbutyric acid (4-PBA; which enhances protein folding) or genetic approaches such as expression of a constitutively active IKKβ improves leptin resistance and protects mice from DIO[102,105–108]. POMC neurons seem to be crucial for this effect, as targeted overexpression of XBP1, a transcription factor that modulates endoplasmic reticulum stress response, in this neuronal population is sufficient to protect animals from DIO108. 4-PBA has been used in the clinic for the treatment of urea cycle disorders in children, sickle cell disease, thalassaemia and cystic fibrosis[109], and is clinically safe with few adverse effects[110]. 4-PBA might therefore be a suitable candidate drug for treating the leptin resistance that is induced by endoplasmic reticulum stress.

Celastrol, a small molecule found in the roots of the *Tripterygium wilfordii* plant, has been identified as a candidate to improve sensitivity to leptin in obesity by ameliorating endoplasmic reticulum stress[107]. Systemic, intraperitoneal (100 μg/kg) or oral (10 mg/kg) delivery of celastrol can dramatically decrease body weight (up to 45%) in mice with DIO but not in leptin-deficient *ob/ob* mice, *db/db* mice or lean control mice[107]. A subsequent independent study has shown that celastrol-induced weight loss and energy expenditure involve heat shock factor protein 1 (HSF1) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α)-mediated stimulation of mitochondrial and thermogenic gene programmes, leading to the activation of brown and beige adipose tissues and increasing muscle endurance[111]. However, we need a deeper understanding of the molecular mechanisms underlying the effects of celastrol on leptin resistance, and further studies are needed to clarify whether celastrol can be used in humans to combat obesity.

Ceramide-induced lipotoxicity might also cause hypothalamic endoplasmic reticulum stress and leptin resistance in obesity[112]. Reduction of leptin resistance, leading to weight loss, increases brown adipose tissue thermogenesis and improves metabolic condition, and glucose homeostasis can be achieved by amelioration of endoplasmic reticulum stress by overexpressing 78 kDa glucose-regulated protein (GRP78)[112], an endoplasmic reticulum chaperone that facilitates proper protein folding. This mechanism is also pertinent to LepR-deficient Zucker rats that are characterized by elevated hypothalamic levels of ceramide[112].

Manipulation of GRP78 function in the VMH can induce weight loss in obese Zucker rats[112], demonstrating the potent effects of targeting ceramide-induced lipotoxicity and endoplasmic reticulum stress even in the absence of LepRb signalling. However, this finding raises the possibility that the beneficial effects of endoplasmic reticulum stress inhibition might be independent of leptin signalling.

Neonatal programming.

Excess nutrition and growth during prenatal and/or postnatal life might contribute to the aetiology of obesity and related diseases in later life[113,114]. A classic model of neonatal nutritional programming is the manipulation of rat litter size in the first days of life[115–117]. Rats raised in small litters gain more weight than those raised in litters of a normal size. In addition, rats from small litters have hyperphagia, increased adiposity, hyperleptinaemia, hyperinsulinaemia, impaired glucose tolerance, elevated triglycerides and increased systolic blood pressure[115–118]. This effect can be explained by the increased milk intake of individual rats in small litters. Importantly, overfed rats in this model maintain the obese phenotype and the associated metabolic disturbances throughout their lifespan[115–118].

Leptin resistance in overfed neonatal rats results in impaired sensitivity to leptin of hypothalamic neurons, and the responsiveness to leptin in VMH and ARC neurons is different in rats raised in small litters. Whereas leptin increases the firing rate of VMH neurons of rats raised in normal-sized litters, it reduces the discharge rate of VMH neurons of rats raised in small litters[119]. Conversely, leptin inhibits neurons in the ARC of rats raised in normal-sized litters but has no effect on ARC neurons of small-litter rats[120]. This programmed leptin resistance of overfed neonatal rats is age dependent: in juvenile (24 days old) rats raised in small litters, expression of LepRb is decreased by ~45% in the hypothalamus[115]. These data are also consistent with the increase in expression of agouti-related protein (AgRP) and neuropeptide Y (NPY) in the ARC of rats raised in small litters, despite their marked hyperleptinaemia[115,116]. However, overfed adult (60 days old) rats do not show any change in the hypothalamic expression of LepRb or of any other LepR isoform[116]. Interestingly, adult rats raised in small litters have abnormally low levels of leptin in the CSF compared with controls, despite increased plasma levels of leptin, suggesting that the rate of leptin influx into the CNS is compromised in these animals[116].

Ghrelin

Ghrelin was identified in 1999 as the endogenous ligand of the growth hormone secretagogue receptor (GHSR) from extracts of rat stomach[121–124]. Although first described as a growth hormone (GH) secretagogue[121,125–127], the association of ghrelin with food intake, adiposity and metabolism regulation rapidly became the main focus of ghrelin function-related research[122,123,128–130]. However, the pleiotropic actions of ghrelin have implicated this hormone in different physiological processes (FIG. 1). Ghrelin has also been considered as a target to treat obesity[131,132] and the metabolic disorders that are associated with cachexia[133], sarcopenia[134] and myopenia[135].

GHRL encodes a precursor peptide that is 117 amino acids in length: preproghrelin, which is post-translationally processed into at least five products. Among them, acyl-ghrelin and

desacyl-ghrelin have emerged as the most biologically relevant[121,124]. Acyl-ghrelin accounts for ~10% of the total amount of ghrelin and is acylated at Ser3 in the cytoplasm before secretion[121]. This process is catalysed by ghrelin *O*-acyltransferase (GOAT) and occurs in the endoplasmic reticulum[136–138]. GOAT expression and activity are modulated by nutrient availability, particularly by the availability of medium-chain fatty acids, which are used as acylation substrates and promote acyl-ghrelin production and secretion[139]. Acylation of ghrelin is required for its binding to GHSR and for its endocrine, metabolic and orexigenic actions[121,124,140]. The role of desacyl-ghrelin is still controversial, which is quite remarkable, given that it accounts for 90% of the circulating levels of ghrelin[124,141], with new actions ascribed to this molecule that could be mediated by GHSR or an unidentified mechanism[124,140,142,143]. Obestatin is also translated from the *GHRL* transcript and was first described as a hormone with anorexigenic actions on appetite and body weight[144]; however, follow-up studies have so far failed to confirm the proposed anorexigenic actions of this molecule[145–148].

Ghrelin synthesis and secretion

When nutrient availability is low, levels of ghrelin increase, and, after consumption of a meal, ghrelin levels are decreased, as measured by gene expression, hormone secretion from the stomach and circulating levels in both rodents and humans[149–152]. These findings are consistent with the idea that ghrelin is metabolically more active during negative energy balance than during positive energy balance[153,154]. Ghrelin has an inverse relationship with BMI: it is upregulated in under-nourished states, such as anorexia nervosa, and is downregulated in states of positive energy balance, such as obesity[155–157]. One exception is Prader–Willi syndrome, which is characterized by obesity, severe hyperphagia, GH deficiency and hypogonadism[142] Patients with this syndrome have high circulating levels of ghrelin that could explain the hyperphagia and the consequent obesity[158,159]. However, it should be noted that individuals with Prader–Willi syndrome display hyperghrelinaemia in infancy, before the manifestation of hyperphagia[160,161], and do not lose weight or appetite when the levels of ghrelin are decreased through treatment with octreotide, a somatostatin analogue[162].

Strikingly, the actual physiological role of ghrelin is still unclear. For example, pharmacological doses (up to 5 pmol/kg/min; intravenously) of ghrelin increase food intake in humans and rodents[109,163–166], but whether the orexigenic effect of ghrelin and its chronic obesogenic effects are coupled is unclear. Although ghrelin might provide an acute hunger signal in the preprandial period, little evidence exists to support a role for high levels of ghrelin in inducing feeding. Whereas in humans and rodents plasma levels of desacyl-ghrelin are increased upon long-term starvation, there are conflicting data about the changes in acyl-ghrelin[139,167]. Furthermore, neither mice deficient in ghrelin, GHSR or GOAT nor transgenic mice overexpressing ghrelin and/or GOAT have impaired feeding compared with wild-type animals[139,168,169]. Consistent with this finding, selective ablation of ghrelin-producing cells in adult mice using diphtheria toxin does not promote changes in feeding, body weight or sensitivity to a HFD, despite the substantial reduction (80–95%) in plasma levels of ghrelin. However, this ablation of ghrelin-secreting cells was associated with severe hypoglycaemia, consistent with a role for the hormone in the

control of blood glucose during caloric deficit[170]. Ghrelin-induced stimulation of food intake requires a 10-fold to 100-fold increase in its circulating levels compared with its physiological levels[171]. However, anti-ghrelin vaccines, which were originally developed as antiobesity drugs, did not have chronic anorexigenic properties[132]. The preprandial surge of ghrelin might prepare the organism for incoming food to metabolize and store energy efficiently, and indeed ghrelin activation is highly influenced by dietary lipids[124,139,171]. For example, ghrelin might signal to the brain that abundant calories are available. This notion is consistent with the fact that ghrelin also stimulates expression of genes that are involved in lipogenesis[129,172,173], and ghrelin signalling in the hypothalamus is totally dependent on neuronal fatty acid metabolism[130,174,175]. However, new assays show that total levels of ghrelin increase during fasting, specifically the levels of desacyl-ghrelin, whereas the levels of acyl-ghrelin remain unchanged[139], which indicates that, although extensively studied, the mechanism of ghrelin secretion is still not well understood[124,176].

Ghrelin receptor signalling

The effect of ghrelin on feeding is mediated by GHSR, as the orexigenic effect of this hormone is blunted in *Ghsr*-knockout mice[177]. GHSR is highly expressed in the hypothalamic cell populations that regulate feeding and body weight, such as ARC AgRP-expressing and NPY-expressing neurons and VMH neurons expressing AMP-activated protein kinase (AMPK) and fatty acid synthase[178–181] (FIG. 3).

Ghrelin and hypothalamic fatty acid metabolism.

The binding of ghrelin to GHSR increases intracellular levels of PIP3 and Ca^{2+} via induction of phospholipase C and PKC[182,183]. The rise in intracellular Ca^{2+} activates hypothalamic calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2), which activates the cellular energy sensor AMPK[130,174,184–187]. Ghrelin also specifically activates a hypothalamic sirtuin 1–p53 pathway that promotes AMPK phosphorylation[188,189]. Activation of AMPK in the hypothalamus modulates fatty acid metabolism. When activated by ghrelin, phosphorylated AMPK inactivates acetyl-CoA carboxylase, leading to a decrease in the levels of malonyl-CoA, which disinhibits carnitine palmitoyl transferase 1A (CPT1A)[130,174,175,185–187,190–192]. In addition to the ghrelin-induced phosphorylation of AMPK catalytic subunit-α (AMPKα), AMPKγ2 is involved in the orexigenic action of ghrelin[193]. Chronic activation of AMPK, due to a mutation in the gene encoding AMPKγ2, promotes ghrelin-dependent hyperphagia and obesity in mice and humans[193]. The overall outcome of such effect is increased fatty acid oxidation and accumulation of reactive oxygen species, which are buffered by UCP2 (REF. 174). In addition, hypothalamic CPT1C, a brain-specific isoform that is located in the endoplasmic reticulum[194], was implicated in the orexigenic action of ghrelin, as indicated by the lack of effect of ghrelin on food intake in CPT1C-null mice[195]. This pathway involves an increase in hypothalamic ceramide synthesis[195]. Genetic or pharmacological inhibition of CaMKK2, AMPK, CPT1A, CPT1C or UCP2, as well as increased concentrations of malonyl-CoA or decreased levels of ceramide in the hypothalamus, prevents ghrelin-induced feeding[130,174,184,186,187,195].

Furthermore, ghrelin caused a ~2-fold upregulation in mTORC1 signalling in the ARC, and pharmacological or genetic central inhibition of the mTORC1 pathway decreases the orexigenic action of ghrelin and ghrelin-mediated induction of AgRP and NPY expression[196,197]. This evidence indicates that ghrelin induces feeding by acting on AMPK and mTORC1 in two different hypothalamic nuclei, the VMH and the ARC, respectively[130,175,187,196,197]. However, whether these two networks act in concert or independently is currently unclear[196,198].

The effects of ghrelin on hypothalamic neuropeptides.

Ghrelin ultimately increases the potential firing of AgRP-expressing and NPY-expressing neurons[174], and activates transcriptional events in the ARC by increasing expression and/or activity of key transcription factors, such as cAMP-responsive element-binding protein (CREB) and its phosphorylated isoform, pCREB; FOXO1 and its phosphorylated isoform, pFOXO1; and brain-specific homeobox protein homologue (BSX)[185,199]. These changes underlie, at least in part, the rise in orexigenic AgRP and NPY neuropeptides in the ARC[123,130,185,200,201]. Interestingly, AMPK and its direct downstream targets, as well as the orexigenic neuropeptides, are located in different neuronal populations: whereas AgRP-expressing and NPY-expressing neurons are located in the ARC, AMPK-expressing neurons, which send synaptic projections to ARC neurons[202], are located in the VMH[130,175]. The binding of ghrelin to GHSR increases intracellular levels of Ca^{2+} through the mobilization of Ca^{2+} from intracellular stores and through the opening of Ca^{2+} channels[203]. Ghrelin signalling increases presynaptic AMPK activity by promoting intracellular Ca^{2+} release and the subsequent activation of CaMKK2[184]. The action stimulates the firing rate of AgRP-expressing and NPY-expressing neurons and inhibits the firing activity of POMC neurons by increasing inhibitory GABAergic neurotransmission through postsynaptic inputs[204]. Consequently, the anorectic effect of α-melanocyte-stimulating hormone (αMSH; the cleaved product of POMC) is inhibited, which further enhances orexigenic drive. However, if feeding continues, leptin then stimulates POMC neurons, which exert an anorectic action, and promotes the release of opioids to inhibit AMPK in presynaptic neurons through k-opioid receptors[202,205] (FIG. 3).

Food intake is induced through activation of cannabinoid receptor 1 (CB1) and stimulation of AMPK by both exogenous and endogenous cannabinoids[206] (FIG. 3). A functional CB1 is needed for ghrelin to affect AMPK activity and food intake; these findings are based on studies using CB1-null animals and CB1 antagonists, which do not respond to or block, respectively, ghrelin orexigenic actions or ghrelin-induced AMPK activation[191,207,208] Conversely, the effect of ghrelin on GH release does not require CB1 signalling[207,208]. The increased intracellular levels of Ca^{2+} that are induced by binding of ghrelin to GHSR activate diacylglycerol lipase-α, the enzyme that synthesizes 2-arachidonoylglycerol (an endogenous cannabinoid), directly or through activation of PKC, resulting in increased 2-arachidonoylglycerol synthesis and release in the hypothalamus[191]. This mechanism was implicated in ghrelin-mediated inhibition of glutamate excitatory inputs, originating from ARC neurons, onto parvocellular neurons of the paraventricular nucleus of the hypothalamus that promote feeding[204,209,210].

In addition to the hypothalamus, ghrelin exerts its orexigenic effect in other regions of the brain, such as the ventral tegmental area (VTA), in which the motivational dopaminergic system is located[211,212]. A negative energy balance potentiates the orexigenic effect of ghrelin; for example, the induction of AgRP and NPY expression in response to ghrelin is increased in fasted rats[102]. Furthermore, refeeding, as well as hormonal signals of positive energy balance (insulin and leptin), suppresses ghrelin responsiveness, potential firing rates and *Agrp* and *Npy* gene expression in the ARC[181,213–215]. Therefore the fact that the effects of ghrelin are increased by fasting is probably due to the reduced levels of leptin and insulin that characterize such a state.

Ghrelin resistance in obesity

The role and relevance of ghrelin resistance in obesity are unclear. Ghrelin resistance has been proposed as a mechanism to protect against the higher body-weight set point that is established during times of food availability and to maximize energy reserves during a time of food scarcity[154]. In such cases, inhibition of ghrelin sensitivity might prevent the rebound in body weight that follows dietary restriction-induced weight loss in patients with obesity[216]. Supporting this hypothesis is the observation that ghrelin-null mice with DIO have reduced body-weight regain after calorie-restricted weight loss[216].

Effects of dietary obesity.

Unexpectedly for an orexigenic hormone, a positive energy balance induces ghrelin resistance (FIG. 4) in humans and rodent models, and obesity is associated with reduced secretion and plasma levels of ghrelin[155–157,217]. Moreover, circulating levels of ghrelin do not fall in response to a meal in humans with obesity[218]. These changes in ghrelin release might result from ghrelin-secreting cells in the stomach that do not respond to noradrenaline-mediated stimulatory actions of the sympathoadrenal system on β1-adrenergic receptors in obesity[219]. Furthermore, mice lacking β1-adrenergic receptors in ghrelin-expressing cells have diminished ghrelin release upon severe caloric deficit[220]. Ghrelin-producing cells from mice with DIO also have a blunted response to the inhibitory actions of glucose on ghrelin release[219]. Furthermore, ghrelin transport through the BBB and the expression of *Ghsr* are reduced in obese mice[221,222], leading to reduced sensitivity to ghrelin[217,223], which might promote hypothalamic ghrelin resistance. Consistently, central and peripheral injections of ghrelin in obese mice fail to induce AgRP and NPY expression and secretion[221,224], raising the possibil that impaired response of AgRP-expressing and NPY-expressing neurons contributes to ghrelin resistance in the hypothalamus.

Interestingly, ghrelin resistance in response to a HFD can occur rapidly and almost independently from the length of the nutritional intervention. Indeed, short-term (12 h) exposure to a HFD is enough to alter the orexigenic effects of ghrelin[173]. Notably, the capacity of ghrelin to modulate lipogenesis in white adipose tissue is unaffected by a HFD, indicating that different neuronal circuitries mediate ghrelin-specific regulation of food intake and lipid metabolism[173].

Neonatal programming.

Blocking ghrelin in neonatal mice can increase αMSH and AgRP projections from the ARC to the paraventricular nucleus of the hypothalamus and can promote increases in body weight, visceral fat and blood levels of glucose and a decrease in sensitivity to leptin[225]. In addition, chronic administration of ghrelin postnatally (from day 4 to day 12) impairs the normal development of ARC projections, leading to metabolic dysfunction. Chronic exposure to ghrelin also led to attenuation of leptin-induced STAT3 activation in ARC neurons[225]. These results indicate that ghrelin has an inhibitory role in the development of hypothalamic neural circuits and that ghrelin action during neonatal life is required for optimal metabolic regulation.

Small-litter mice have lower levels of total ghrelin and acyl-ghrelin in the serum and decreased expression of *Ghrl* mRNA in the stomach[226]. Notably, normalization of the levels of ghrelin by chronic neonatal injection in small-litter mice does not improve the metabolic phenotype. This finding indicates that central ghrelin resistance in mice raised in small litters might arise from altered transport in the mediobasal hypothalamus[226].

Leptin and ARC neuropeptides.

Leptin has been implicated in ghrelin resistance, as indicated by the antagonistic physiological roles of leptin and ghrelin systems[227,228]. Anatomical data show that GHSR and LepR are co-expressed in more than 90% of neurons in the ARC[229] This notion is also exemplified by leptin-mediated inhibition of AgRP and NPY expression, an effect opposite to that of ghrelin[230–232]. Furthermore, *ob/ob* mice have normal responses to ghrelin, and these responses are suppressed after leptin administration[224]. Notably, *ob/ob* mice fed a HFD remain sensitive to ghrelin, further supporting the idea that hyperleptinaemia, instead of obesity or a HFD, causes ghrelin resistance[224].

Inflammation and endoplasmic reticulum stress.

Hypothalamic inflammation has been suggested as a potential mechanism of ghrelin resistance[100,233,234]. Obese *ob/ob* mice and lean mouse controls fed a HFD have similar responses to ghrelin, despite the *ob/ob* mice having a higher level of gliosis, a marker of central inflammation, which indicates that hypothalamic gliosis is not a cause of ghrelin resistance[224]. However, ghrelin resistance was linked to local inflammation in nodose ganglia and to the subsequent dysregulation of vagus afferents[234]. This is based on the observation that upregulation of macrophage and/or microglia markers and of inflammatory cytokines in nodose ganglia of mice with DIO was associated with decreased expression and signalling of nodose ganglion GHSR, and a blunted ghrelin-induced decrease in vagal afferent nerve activity[234]. Another proposed mechanism of ghrelin resistance in obesity relates to endoplasmic reticulum stress[102,105,234]. In mice, treatment with ghrelin increases hypothalamic concentration of ceramide via modulation of CPT1C in the endoplasmic reticulum[195], and, as described earlier in the text, ceramide synthesis is induced by obesity, promoting hypothalamic lipotoxicity and endoplasmic reticulum stress[112]. However, as the levels of ghrelin are decreased in obesity, this hormone is not likely to have a role in the production of ceramides in this context. In addition, ghrelin can reduce hypothalamic expression of endoplasmic reticulum stress markers such as CCAAT/

enhancer-binding protein-ε (CEBPε) and phosphorylated eukaryotic translation initiation factor 2α (pEIF2α)[185]. Thus, the relationship between endoplasmic reticulum stress, ghrelin and ghrelin resistance in obesity remains unclear, warranting further research.

AMPK.

Despite the evidence suggesting that AgRP and NPY mediate the development of ghrelin resistance in obesity, the underlying molecular mechanism is unclear. AMPK in the hypothalamus, which does not respond to leptin in mice with DIO, might mediate this effect. For example, contrary to what happens in normal chow-fed mouse controls, in mice with DIO, leptin does not inhibit AMPK activity in the ARC, which might contribute to leptin resistance[235,236]. Whether the ghrelin-induced increase in hypothalamic AMPK is also blunted by a HFD is currently unclear, but is an interesting possibility.

Ghrelin resistance in extra-hypothalamic regions.

Ghrelin resistance is also found in areas of the brain other than the hypothalamus, such as the VTA, where ghrelin acts on dopaminergic neurons to modulate feeding reward responses[211,212]. Notably, in mice with DIO, although ghrelin retains its orexigenic properties when administered specifically in the VTA[237], it fails to induce the same reward response when injected intraperitoneally[238]. These data indicate that obesity differentially affects the homeostatic (hypothalamic-based) and motivational (VTA-based) actions of ghrelin on food intake. However, these mechanisms in the extra-hypothalamic regions remain to be defined.

Mutations in GHSR.

Several mutations and singlenucleotide polymorphisms of *GHSR* have been identified in humans that contribute to obesity and short stature[239] For example, the nonsense *GHSR* mutation Ala204Glu, which prevents the normal constitutively active GHSR from functioning, is associated with familial short stature syndrome, which can be partially reversed with GH treatment[240,241]. Functional analyses of mutated *GHSR* have shown multiple defects, including altered cell surface expression and changes in ligand binding and basal and stimulated signalling[239–243]. These defects might be considered as a state of ghrelin resistance, although currently the physiological relevance to obesity in humans is unclear.

Conclusions

Resistance to the metabolic actions of leptin and ghrelin is thought to contribute to the development and maintenance of obesity. However, recent evidence challenges the notion that DIO-related leptin resistance exists[39]. The reasons behind the differential resistance to exogenous versus endogenous leptin remain to be determined. Nonetheless, in our opinion, the definition of leptin resistance should be revisited so as to refer to the inability of exogenous leptin to affect food intake and body weight.

Unfortunately, the progress in deciphering the molecular processes underlying leptin and ghrelin resistance in obesity has not yet translated into novel and effective obesity

treatments, which highlights the need for better understanding of the mechanisms of obesity-associated leptin and ghrelin resistance. The pleiotropic actions of ghrelin and leptin (FIG. 1) have led to an interest in understanding the consequences of resistance to these hormones beyond metabolic disorders. Indeed, several pathological conditions, including cardiovascular and reproductive dysfunction, might result from defects in the actions of these hormones and in their signalling pathways. For example, substantial evidence suggests that selective leptin resistance underlies obesity-related hypertension and sympathetic nervous system overactivity[244]. This notion is supported by the ability of leptin to increase blood pressure and cardiovascular sympathetic outflow in obesity in spite of the resistance to the anorexigenic effects of this hormone. Despite this finding, the molecular and cellular bases of selective leptin resistance remain elusive. Similarly, the current knowledge about ghrelin resistance is scarce. Several possibilities have been proposed, but more work is needed to fully understand the molecular basis and physiological significance of ghrelin resistance. Decoding the mechanisms underlying selective resistance to metabolic hormones will greatly enhance our understanding of the diseases that are commonly associated with obesity.

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Key points

- The anorexigenic hormone leptin and the orexigenic hormone ghrelin are crucial for metabolic regulation and energy homeostasis
- Obesity-associated resistance to leptin and ghrelin promotes adiposity and might contribute to the diseases that are associated with this condition beyond metabolic disorders
- Resistance to leptin and ghrelin is a multifactorial process that involves changes at several levels: from disturbed hormonal production to altered receptor trafficking and signalling in the brain
- Several molecules and signalling pathways associated with leptin and ghrelin receptors have been identified as potential targets to overcome resistance to these hormones, but none has reversed the energy imbalance in the long term
- The identification of novel molecular targets and pathways that can be modulated to enhance sensitivity to leptin and ghrelin and restore energy homeostasis is necessary for the development of efficient pharmacological treatments for obesity

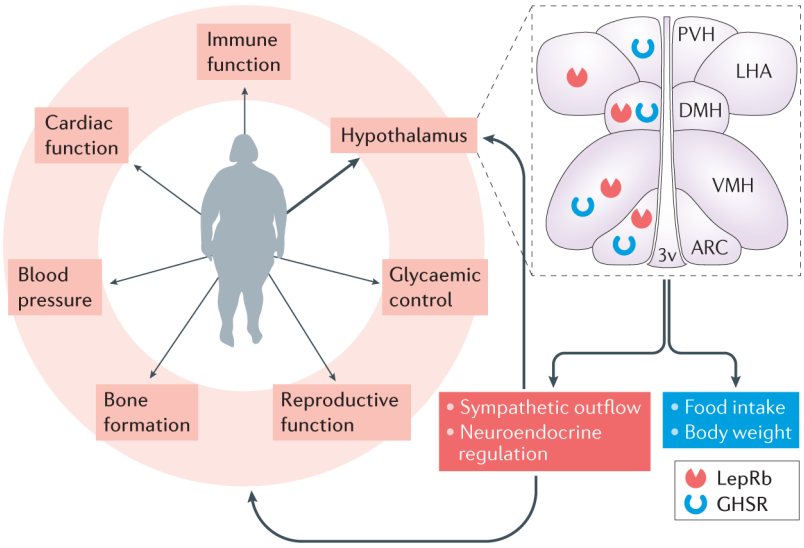

Figure 1 |. Physiological functions of leptin and ghrelin.

Leptin and ghrelin are secreted from adipose tissue and from the stomach, respectively, enter the circulation and affect a wide range of physiological processes. In addition to direct peripheral targets, these hormones exert their actions in different regions of the brain, including several nuclei of the hypothalamus that are important for energy homeostasis, where both the 'long' form of the leptin receptor (LepRb) and the ghrelin receptor growth hormone secretagogue receptor (GHSR) are broadly expressed. The actions of the two hormones on their receptors regulate food intake and body weight, sympathetic nervous system tone and neuroendocrine responses, which in turn regulate physiological function of peripheral organs to coordinate homeostasis. 3v, third ventricle; ARC, arcuate nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; LHA, lateral hypothalamic area; PVH, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus.

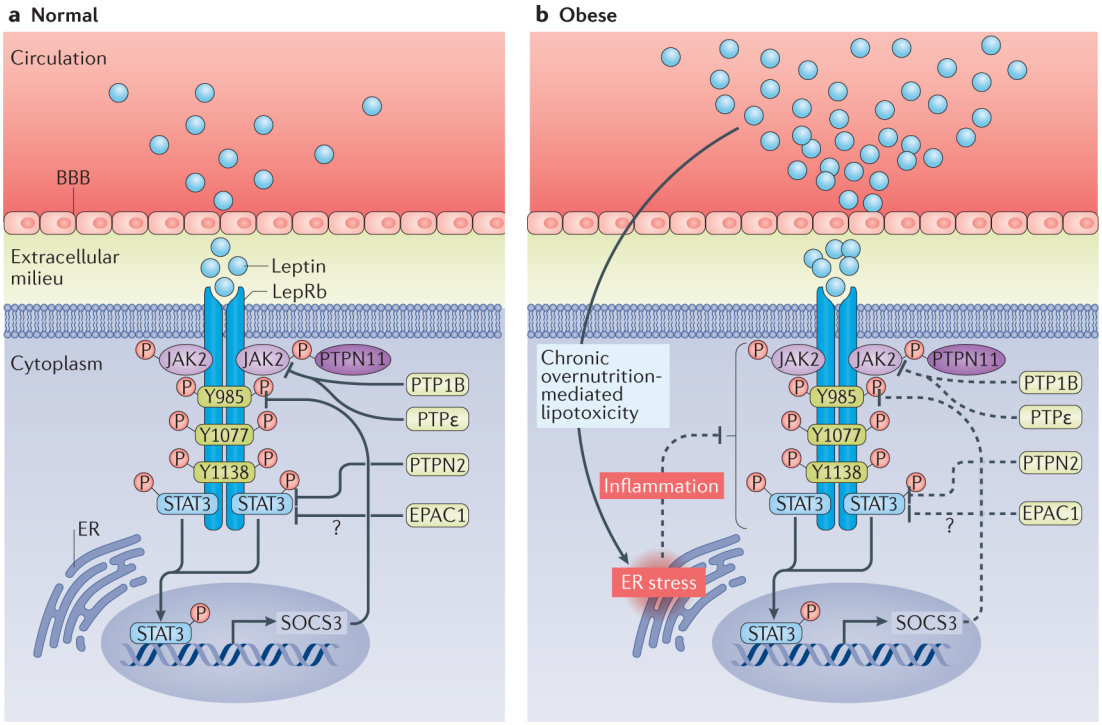

Figure 2 |. LepRb signalling and the molecular mechanisms contributing to leptin resistance in obesity.

a | In individuals with normal body weight, circulating leptin crosses the blood–brain barrier (BBB) and binds to the ‘long’ form of the leptin receptor (LepRb), which induces phosphorylation of Janus kinase 2 (JAK2) and of multiple tyrosine residues in the LepRb intracellular domain. LepRb also receives inhibitory signals from multiple negative feedback loops (such as suppressor of cytokine signalling 3 (SOCS3), protein tyrosine phosphatase 1B (PTP1B), PTP non-receptor type 2 (PTPN2), PTPe and exchange protein directly activated by cyclic AMP 1 (EPAC1)), ensuring that activation of LepRb does not go beyond a physiologically necessary point. **b** | In obesity, circulating levels of leptin increase, which is associated with diminished leptin transport across the BBB and activation of the inhibitory negative feedback systems that eventually lead to diminished LepRb signalling. Increased free fatty acids and chronic overnutrition cause lipotoxicity and endoplasmic reticulum (ER) stress, and trigger inflammatory responses that might contribute to a blunted physiological response to leptin in obesity. STAT3, signal transducer and activator of transcription 3.

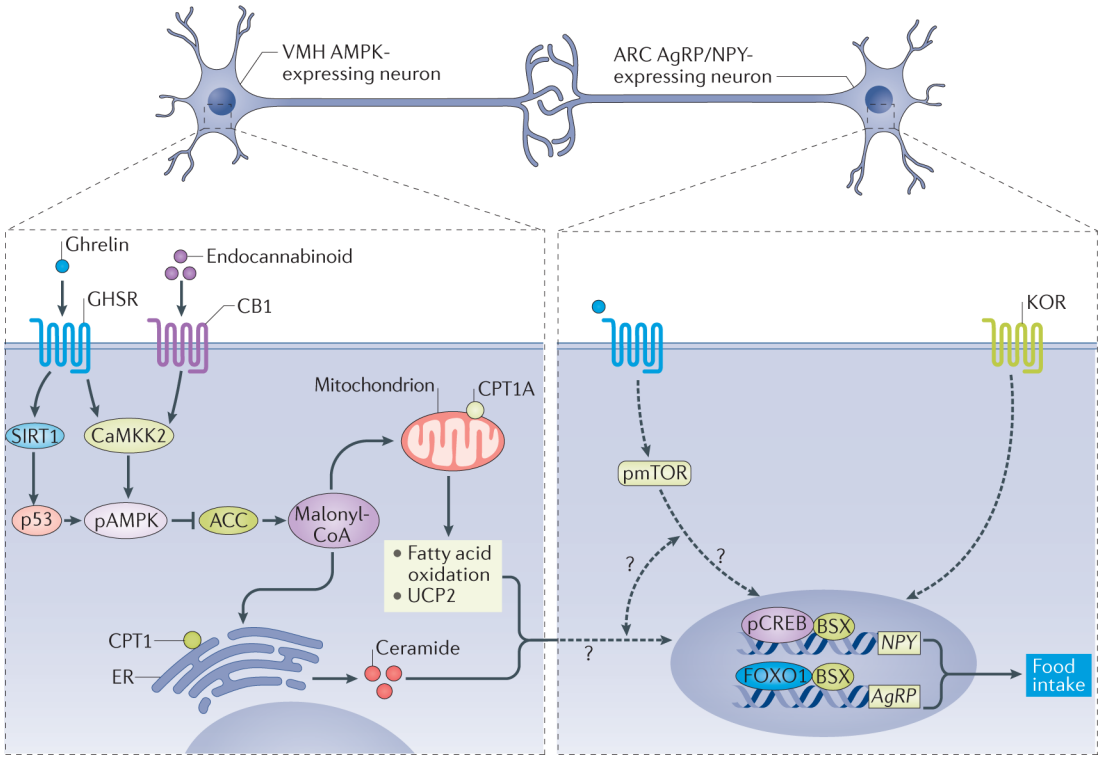

Figure 3 |. Hypothalamic ghrelin signalling.

Ghrelin binds to growth hormone secretagogue receptor (GHSR) and stimulates hypothalamic sirtuin 1 (SIRT1)–p53 and calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2)–AMP-activated protein kinase (AMPK) axes in the ventromedial nucleus of the hypothalamus (VMH). This action requires cannabinoid receptor 1 (CB1). Consequently, hypothalamic levels of malonyl-CoA, the physiological inhibitor of the enzyme carnitine palmitoyltransferase 1 (CPT1) isoforms A and C, are elevated. This effect promotes disinhibition of CPT1A, increases fatty acid oxidation, alters the levels of reactive oxygen species, increases expression of UCP2 and promotes the CPT1C-mediated increase in levels of ceramide. These metabolic changes activate the nuclear transcription machinery by increasing expression and/or activity of key transcription factors, such as cyclic AMP-responsive element-binding protein (CREB) and its phosphorylated isoform, pCREB, FOXO1, and brain-specific homeobox protein homologue (BSX) in the arcuate nucleus (ARC), increasing mRNA expression of *AgRP* and *NPY*, which induces feeding. Mechanistic target of rapamycin (mTOR) and k-opioid receptor (KOR) also mediate the effects of ghrelin in the ARC; however, the association between these two events is unclear. ACC, acetyl-CoA carboxylase; AgRP, agouti-related protein; ER, endoplasmic reticulum; NPY, neuropeptide Y; pAMPK, phosphorylated AMPK; pmTOR, phosphorylated mTOR.

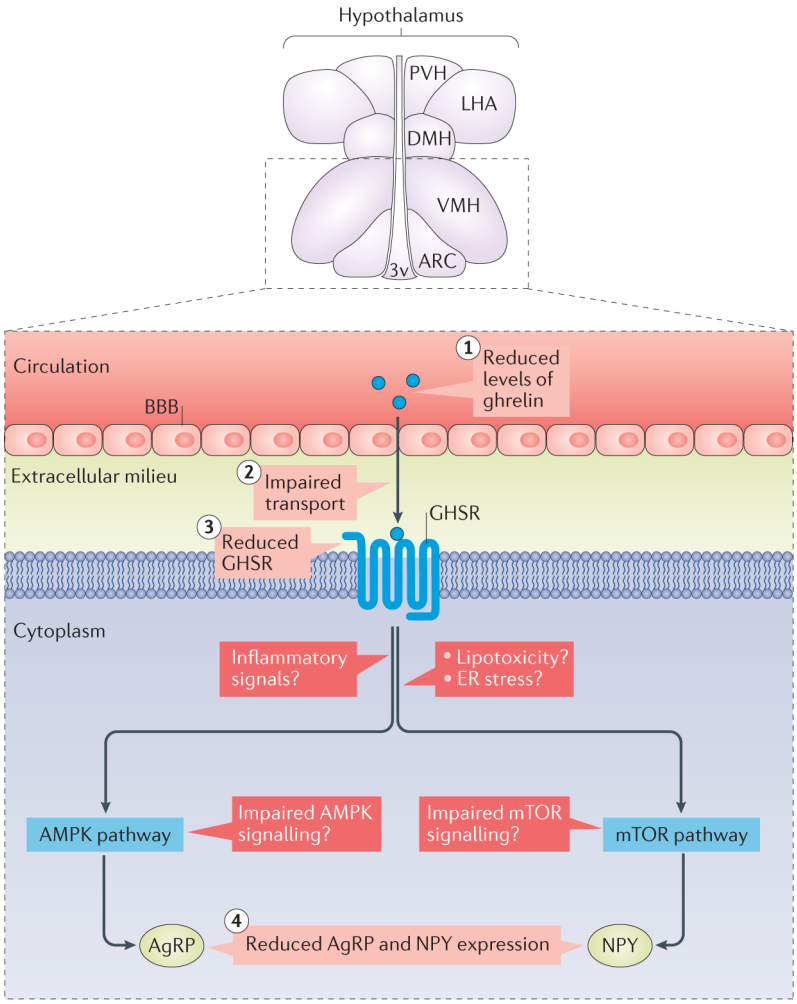

Figure 4 |. Hypothalamic ghrelin resistance.

Obesity-associated ghrelin resistance might develop via different mechanisms, such as decreased circulating levels of ghrelin (1); impaired transport of ghrelin through the blood–brain barrier (BBB) (2); reduced expression of growth hormone secretagogue receptor (GHSR) (3); and reduced expression of agouti-related protein (AgRP) and neuropeptide Y (NPY) (4), which reduces the orexigenic action of ghrelin. The molecular mechanisms leading to the reduction of neuropeptide expression are unclear, but possible candidates include hypothalamic inflammation, lipotoxicity, endoplasmic reticulum (ER) stress and impaired AMP-activated protein kinase (AMPK) or mechanistic target of rapamycin (mTOR) pathways. 3v, third ventricle; ARC, arcuate nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; LHA, lateral hypothalamic area; PVH, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus.