FOOD INTAKE, METABOLISM AND THE BRAIN

Leptin and the maintenance of elevated body weight

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Abstract | Obesity represents the single most important risk factor for early disability and death in developed societies, and the incidence of obesity remains at staggering levels. CNS systems that modulate energy intake and expenditure in response to changes in body energy stores serve to maintain constant body adiposity; the adipocyte-derived hormone leptin and its receptor (LEPR) represent crucial regulators of these systems. As in the case of insulin resistance, a variety of mechanisms (including feedback inhibition, inflammation, gliosis and endoplasmic reticulum stress) have been proposed to interfere with leptin action and impede the systems that control body energy homeostasis to promote or maintain obesity, although the relative importance and contribution of each of these remain unclear. However, LEPR signalling may be increased (rather than impaired) in common obesity, suggesting that any obesity-associated defects in leptin action must result from lesions somewhere other than the initial LEPR signal. It is also possible that increased LEPR signalling could mediate some of the obesity-associated changes in hypothalamic function.

Energy expenditure
The burning of calories by an organism on normal metabolism (basal metabolic rate) and activity.

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Obesity, generally defined by a body mass index (BMI) ≥30 kg per m^2, results from the accumulation of adiposity owing to the consumption and storage of calories in excess of metabolic needs. Currently, 70% of the US adult population is overweight (BMI >25 kg per m^2) and 37.7% is obese[1]. This obesity epidemic has far-reaching consequences for health, medical care and economics: individuals with obesity have shorter life expectancy owing to their increased risk of chronic diseases such as diabetes, cardiovascular disease and cancer and thus incur $2,741 higher annual health-care costs than normal-weight people[2].

Individuals (even if weight-stable and within the normal BMI range) differ in basal metabolic rate, exercise and average daily food consumption; furthermore, for any individual, each of these parameters varies on a daily basis. Despite these differences, most people maintain mostly stable body weight and adiposity over the long term as a consequence of homeostatic systems that serve to counter fluctuations in energy consumption and expenditure. When energy stores (that is, adipose mass) decrease, hunger increases and energy expenditure decreases owing to the activation of an anabolic system that tends to increase energy stores to previous levels. A catabolic pathway that decreases hunger and augments energy utilization when energy stores are replete works in opposition to the anabolic system[3].

Whereas the control of energy expenditure is tightly coupled to adiposity in most instances, food intake more commonly becomes uncoupled from its appropriate control by energy stores. Most individuals with obesity demonstrate increased basal metabolic rate compared with lean controls but do not suppress food intake to restore energy stores to within the normal range[3]. Perhaps more importantly, even modest weight loss (to adiposity levels well above those for lean individuals) in individuals with obesity activates the anabolic system, increasing hunger and decreasing energy expenditure in a manner similar to that observed with weight loss in normal-weight subjects[4]. Thus, we require therapies that can countermand the anabolic response to weight loss in order to help patients lose weight and sustain weight loss. This will probably require an understanding of the systems that control energy balance and how they may become dysregulated in obesity.

Leptin and energy balance

The 1949 discovery of the autosomal recessive obese (*ob*; also known as *Lep*) allele in mice (*ob*/*ob* mice are hyperphagic, obese, diabetic and sterile) revealed the existence of a gene crucial for the control of feeding, metabolism and body weight[5]. Positional cloning of the *ob* allele in 1994 revealed the presence of a nonsense mutation in the coding region for a peptide hormone — termed leptin — that is secreted by adipocytes[6].

Hyperphagia
Literally meaning 'eating too much', it is the consumption of more calories than needed to maintain energy homeostasis and results in the deposition of excess calories in adipose tissue.

Energy homeostasis
The process by which the number of calories eaten are matched to the number of calories burned to maintain a constant body weight; also known as energy balance.

White adipose tissue
The tissue commonly thought of as fat; major depots are found under the skin and inside the abdominal cavity.

Adipocytes produce leptin in approximate proportion to their triglyceride stores; leptin thus represents a hormonal signal that circulates in proportion to body energy stores[7] (FIG. 1). Like *ob*/*ob* mice, rare humans lacking leptin owing to genetic mutations display hyperphagic obesity, decreased energy expenditure and hyperinsulinaemia[8].

The diabetic (*db*/*db*) mouse, which is phenotypically similar to the *ob*/*ob* mouse, arises from a splicing mutation that truncates the long isoform of the leptin receptor (LEPR)[9]. LEPR is most highly expressed in brain areas known to be important for the control of feeding and energy expenditure[10] (FIG. 1). Other so-called short isoforms of LEPR are expressed mainly in peripheral tissues and appear to be dispensable for the control of energy balance[11]. Cleavage of the extracellular domain of these short LEPR isoforms generates circulating leptin binding protein, which may stabilize leptin and/or control its bioavailability[12].

The hyperphagia and low energy expenditure exhibited by *ob*/*ob* and *db*/*db* mice, together with the ability of exogenous leptin to ameliorate these defects in *ob*/*ob* mice, suggest the importance of leptin as a regulator of energy balance[10]. However, leptin-deficient and LEPR-deficient animal models and humans display other defects (high glucocorticoid levels, hypothalamic infertility and decreased growth, thyroid and immune function) that speak to the broader physiological function of leptin[10,13]. Indeed, deficiency in leptin action mimics the response to starvation, suggesting that the withdrawal of leptin action plays a crucial role in the anabolic response to decreased fat stores[13]. Consistently, exogenous leptin not only ameliorates these defects in leptin deficiency but also blunts these responses to nutritional deficiency in humans and rodent models[13]. Thus, leptin plays a crucial role in modulating the pathways that maintain energy homeostasis, and low leptin represents a powerful signal to promote the anabolic response to negative energy balance[13].

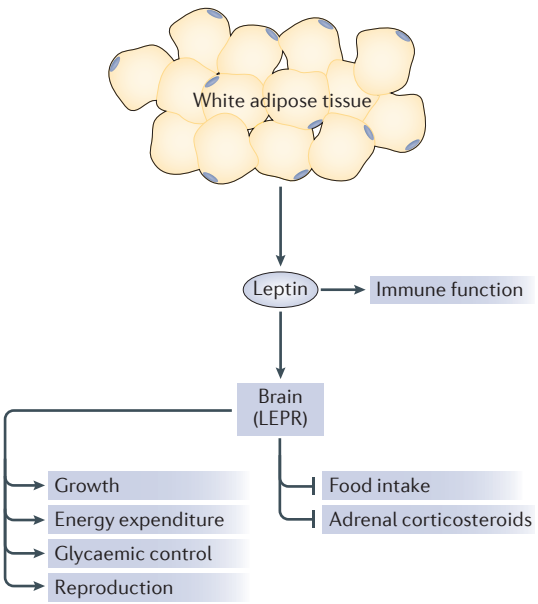


Figure 1 | **Leptin action.** Cells of white adipose tissue secrete leptin in approximate proportion to their triglyceride content. Circulating leptin binds to the long form of the leptin receptor (LEPR) in the brain and, in doing so, promotes growth, energy expenditure, glycaemic control and reproduction. Leptin also suppresses food intake and the production of adrenal corticosteroids. Leptin also influences the production and function of immune cells.

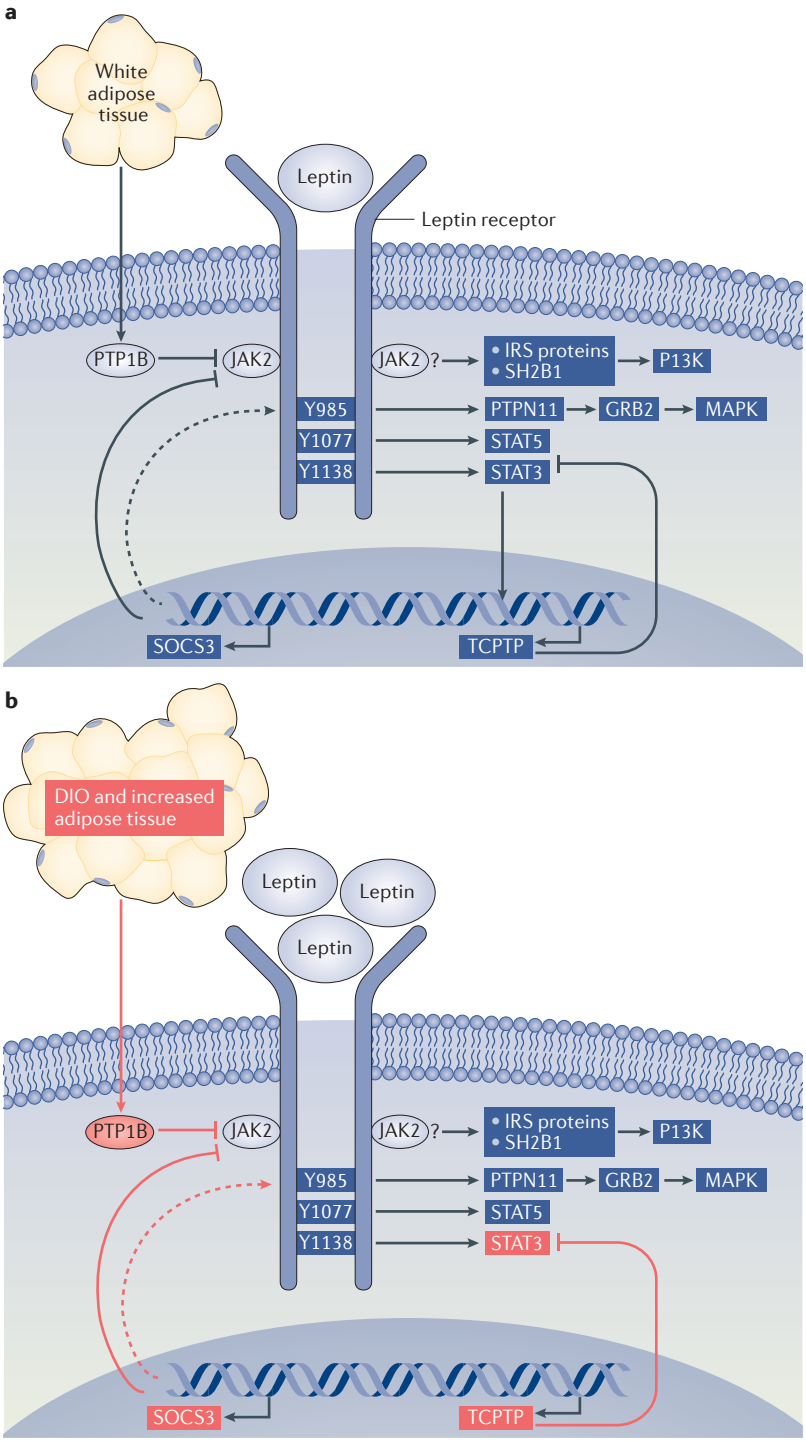
Molecular mediators of leptin action. LEPR is a type I cytokine receptor, which signals via an associated tyrosine kinase, Janus kinase 2 (JAK2)[10,14]. Leptin binding to LEPR activates JAK2, which phosphorylates three conserved tyrosine residues (Y985, Y1077 and Y1138) on the LEPR intracellular domain[14] (FIG. 2). When phosphorylated, each of these tyrosine residues recruits distinct downstream signalling proteins: Y985 binds to tyrosine-protein phosphatase nonreceptor type 11 (PTPN11) and suppressor of cytokine signalling 3 (SOCS3) to mediate mitogen-activated protein kinase (MAPK) signalling and feedback inhibition of LEPR signalling, respectively[14]. Y1077 recruits signal transducer and activator of transcription 5 (STAT5), whereas Y1138 recruits STAT3. STAT proteins are latent transcription factors that become tyrosine-phosphorylated upon their recruitment to the receptor, permitting their nuclear translocation and modulation of gene expression[14]. Leptin promotes the tyrosine phosphorylation of STAT3 (pSTAT3) in LEPR-expressing cells, and most, if not all, hypothalamic pSTAT3 under normal conditions results from direct leptin action via LEPR[14].

LEPR is expressed at low levels and in a low number of neurons. Thus, the detection and quantification of most LEPR signals *in vivo* remain problematic; the detection of pSTAT3 represents a notable exception to this rule, providing a convenient and sensitive histochemical marker for the activation of LEPR[10,14]. Also, mice with mutations at Y1138 or lacking STAT3 in LEPR-expressing neurons (LEPR neurons) display a dramatic hyperphagic obese phenotype, revealing that STAT3 signalling plays a crucial role in leptin action[15,16]. Thus, given the importance of STAT3 signalling to leptin action and the facile detection of leptin-stimulated pSTAT3 *in vivo*, most studies treat pSTAT3 detection as tantamount to the detection of LEPR intracellular signalling.

Additional physiologically important leptin–LEPR signals must exist, as the phenotype of mice mutated for LEPR–STAT3 signalling is not identical to that of *ob*/*ob* or *db*/*db* mice[15,16]. However, mutational analysis reveals that Y985 and Y1077 (and their binding partners) do not mediate this additional signal, suggesting that a signal independent of LEPR-tyrosine phosphorylation must contribute to the leptin–LEPR-dependent control of energy balance[17]. Leptin also controls phosphoinositide 3-kinase (PI3K), and it is possible that the currently undefined LEPR moiety that recruits the putative Src homology domain 2 (SH2)-domain-containing protein 1B (SH2B1)–insulin receptor substrate (IRS)–PI3K pathway could represent the missing LEPR signal[18].

Orexigenic
A type of stimuli that increases feeding.

Neural mediators of leptin action. Leptin acts via LEPR-expressing cells in the brain to control energy balance, and neurons appear to make up most, if not all, LEPR-expressing cells in the brain. Although the direct examination of pSTAT3 (REF. 19) along with transcriptional profiling of hypothalamic and brainstem LEPR cell-derived mRNAs and single-cell sequencing from the medial basal hypothalamus revealed no evidence for LEPR expression in glial populations in adult animals[20,21], some studies have suggested the importance of direct leptin action on astrocytes[22].



Most LEPR neurons lie in brain regions that have known roles in the control of energy balance, and hypothalamic LEPR is required for the control of energy homeostasis by leptin[10]; the midbrain and hindbrain also contain substantial populations of LEPR neurons that play known roles in leptin action, however (FIG. 3), and additional sites may also be relevant[23]. Of the populations of LEPR neurons, those of the arcuate nucleus (ARC; which lies adjacent to the median eminence), a circumventricular organ with fenestrated capillaries that permit the passage of circulating factors such as leptin, are best known. Many ARC LEPR neurons express pro-opiomelanocortin (POMC), which produces the ligand for the CNS melanocortin receptors (MCRs) that decrease feeding and promote energy expenditure[10,21]. Other ARC LEPR neurons express agouti-related protein (AGRP), the endogenous antagonist to CNS MCRs, along with the inhibitory neuropeptide Y (NPY) and the inhibitory neurotransmitter GABA[10,21]. POMC neurons decrease food intake and increase energy expenditure, whereas the so-called NAG (NPY, AGRP and GABA) neurons act oppositely[10]. Not surprisingly, leptin plays crucial roles in modulating the function of POMC and NAG neurons, increasing the activity of POMC neurons and the expression of POMC while inhibiting NAG cells and decreasing their expression of orexigenic peptides[10].

Figure 2 | **Leptin signalling and mechanisms that mediate its inhibition. a** | In individuals with normal body weight, circulating leptin binds to its receptor, which activates tyrosine-protein kinase JAK2, resulting in the phosphorylation of leptin receptor (LEPR) tyrosine residues Y985, Y1077 and Y1138. Phosphorylated Y985 recruits and permits the phosphorylation of tyrosine-protein phosphatase nonreceptor type 11 (PTPN11), which recruits growth-factor-receptor-bound protein 2 (GRB2) and activates the mitogen-activated protein kinase (MAPK) pathway in cultured cells. Phosphorylated Y1077 recruits signal transducer and activator of transcription 5 (STAT5), which could contribute to aspects of leptin-regulated gene expression. Phosphorylated Y1138 engages STAT3, resulting in its phosphorylation and translocation into the nucleus to mediate important aspects of gene expression. In addition to mediating changes in gene expression that contribute to the control of energy balance by leptin, STAT3 induces the expression of suppressor of cytokine signalling 3 (SOCS3), which binds to phosphorylated Y985 and blunts leptin signalling. The tyrosine phosphatases protein-tyrosine phosphatase 1B (PTP1B) and T cell protein-tyrosine phosphatase (TCPTP) dephosphorylate JAK2 and STAT3, respectively. Although LEPR–STAT3 represents a major means by which leptin regulates energy balance, leptin also recruits insulin receptor substrate (IRS) proteins and Src homology domain 2 (SH2)-domain-containing protein 1B (SH2B1) by a poorly defined mechanism (indicated by question mark). LEPR also mediates important, but not mechanistically understood, signals that operate independently of LEPR-tyrosine phosphorylation. **b** | In obesity, increased adipose mass increases leptin production and thus circulating leptin concentrations. The consequent increase in LEPR signalling promotes increased expression of SOCS3 and TCPTP; obesity also increases PTP1B expression. These mechanisms blunt the amplitude of the response to increased leptin concentrations. DIO, diet-induced obesity; P13K, phosphoinositide 3-kinase.

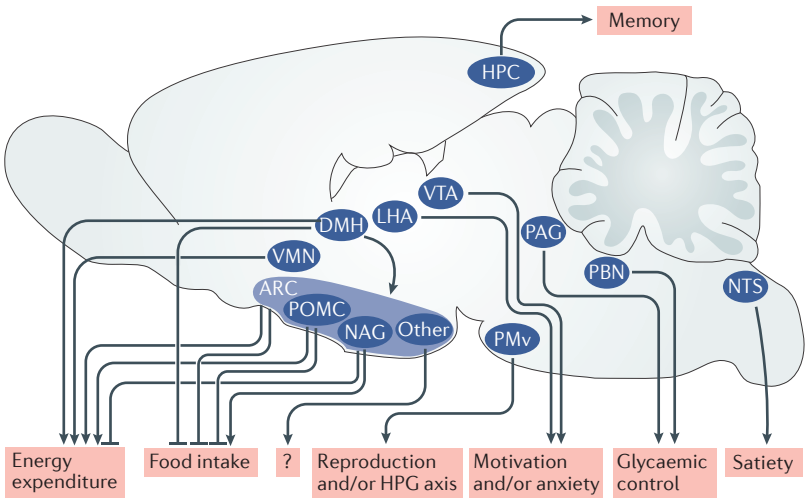


Figure 3 | **CNS leptin action.** Leptin acts on leptin receptor (LEPR)-expressing neurons (LEPR neurons) in various brain regions to mediate distinct actions. Leptin promotes the function of arcuate nucleus (ARC) pro-opiomelanocortin (POMC)-expressing cells to increase energy expenditure and decrease feeding and attenuates the activity of oppositely acting ARC neuropeptide Y (NPY), agouti-related protein (AGRP) and GABA (NAG)-containing neurons. Other ARC LEPR neurons presumably also modulate energy expenditure and food intake in response to leptin. Outside of the ARC, roles for LEPR neurons have been examined in a variety of regions. In the ventromedial hypothalamic nucleus (VMN), *Lepr* deletion decreases basal metabolic rate and other determinants of energy expenditure, resulting in mild obesity independently of leptin action in the ARC. LEPR neurons in the large and dispersed dorsomedial hypothalamic nucleus (DMH) play roles in the control of thermogenesis and feeding, and poorly characterized subpopulations of DMH LEPR neurons play important roles in the control of POMC and NAG neurons. The ventral premammillary nucleus (PMv) has been shown to be involved in the hypothalamic control of reproduction. Lateral hypothalamic area (LHA) LEPR neurons directly innervate the ventral tegmental area (VTA) to modulate the mesolimbic dopamine system and motivation; these cells play a modest role in energy balance; other LEPR neurons in the VTA may play distinct roles in modulating the mesolimbic dopamine system and/or anxiety. LEPR-expressing neurons in the hippocampus (HPC) may also be involved in memory. In the brainstem, leptin acts on LEPR neurons in the nucleus tractus solitarius (NTS) to modulate gastrointestinal satiety signals, while LEPR neurons in the parabrachial nucleus (PBN) and nearby periaqueductal grey (PAG) modulate the sympathetic response to metabolic emergencies appropriately for the status of energy stores. Note that although LEPR neurons in the ARC and DMH appear to mediate the largest effects of leptin on food intake, other areas (including the VTA, HPC, NTS and LHA, among others) may also contribute.

Interestingly, however, deletion of *Lepr* from POMC and/or NAG neurons only modestly alters food intake and body weight[24], and leptin mediates a substantial component of its control of POMC and NAG neurons indirectly, including via non-POMC, non-NAG LEPR neurons[25–27]. Furthermore, recent single-cell sequencing analysis of ARC cells reveals the existence of additional non-POMC, non-NAG ARC LEPR neurons[21].

LEPR neurons in the ventral portion of the dorsomedial hypothalamic nucleus (DMH), including populations of neurons that may co-express *SLCA32A1* (which encodes solute carrier family 32 member 1, a marker for GABAergic neurons), *NOS1* and/or *PDYN* contribute to the control of ARC POMC and NAG cells and may play crucial roles in the modulation of energy balance[25–27]. Other LEPR neurons in the dorsal DMH and dorsal hypothalamic area promote energy expenditure and heat generation[28,29].

The nearby ventromedial hypothalamic nucleus contains a large population of LEPR neurons that express the transcription factor steroidogenic factor 1 (SF1; encoded by *NR5A1*) and the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP; encoded by *ADCYAP1*). Ablation of LEPR in SF1–PACAP neurons provokes little change in energy balance at baseline[30,31] but increases body weight and adiposity on a palatable high-fat diet (HFD) owing to a failure to increase energy expenditure with weight gain.

LEPR neurons of the lateral hypothalamic area, subpopulations of which express neurotensin and/or galanin, contribute to the control of the mesolimbic dopamine (DA) system and to activity and motivation[32,33]. A subpopulation of ventral tegmental area (VTA) neurons (including some DA and some SLCA32A1 cells) also contains LEPR. Although deletion of *Lepr* from DA neurons modifies measures of anxiety[34] rather than motivation[32], short hairpin RNA (shRNA)-mediated suppression of LEPR expression in the VTA influences hedonic feeding and body weight[35], suggesting a potential role for the SLCA32A1 VTA LEPR neurons in the control of motivation.

Within the brainstem, leptin action via nucleus tractus solitarius (NTS) LEPR neurons increases the response of these NTS cells to gut-derived satiety signals to promote meal termination[36–38], whereas RNAi-mediated suppression of NTS LEPR expression increases food intake and body weight gain[39,40]. Other brainstem and midbrain regions also contain substantial populations of LEPR neurons, including the parabrachial nucleus (PBN), periaqueductal grey (PAG) and dorsal raphe (DR), among others[41,42]. Although roles for leptin action via DR LEPR neurons have not been directly examined (note that the large phenotype previously reported for DR LEPR neurons represents an artefact)[43,44], PBN and PAG LEPR neurons serve to augment the autonomic response to metabolic emergencies (such as pain and hypoglycaemia) when energy reserves are depleted[45,46].

Intriguingly, many genetic reporter strains suggest the presence of LEPR neurons in some regions of the hippocampus and in a few cortical areas. Although leptin does not detectably activate pSTAT3 in these areas, a number of studies suggest roles for hippocampal LEPR in the control of neuronal function and memory[47].

Common obesity and leptin resistance

Most obesity in humans does not result from a single genetic lesion but rather appears to represent a common response to the availability of inexpensive, plentiful, tasty calories (that is, calorie-dense or highly palatable foods) and the sedentary nature of modern society[1]. Furthermore, most humans with obesity, as well as rodents with diet-induced obesity (DIO) made obese by the provision of a palatable high-calorie diet (HCD), exhibit high circulating leptin concentrations commensurate with their elevated adiposity[7]. This observation, coupled with the failure of treatment with exogenous leptin to decrease food intake and provoke weight loss in humans with obesity and DIO animals[48], has led to the promulgation of the notion of leptin resistance (FIG. 4).

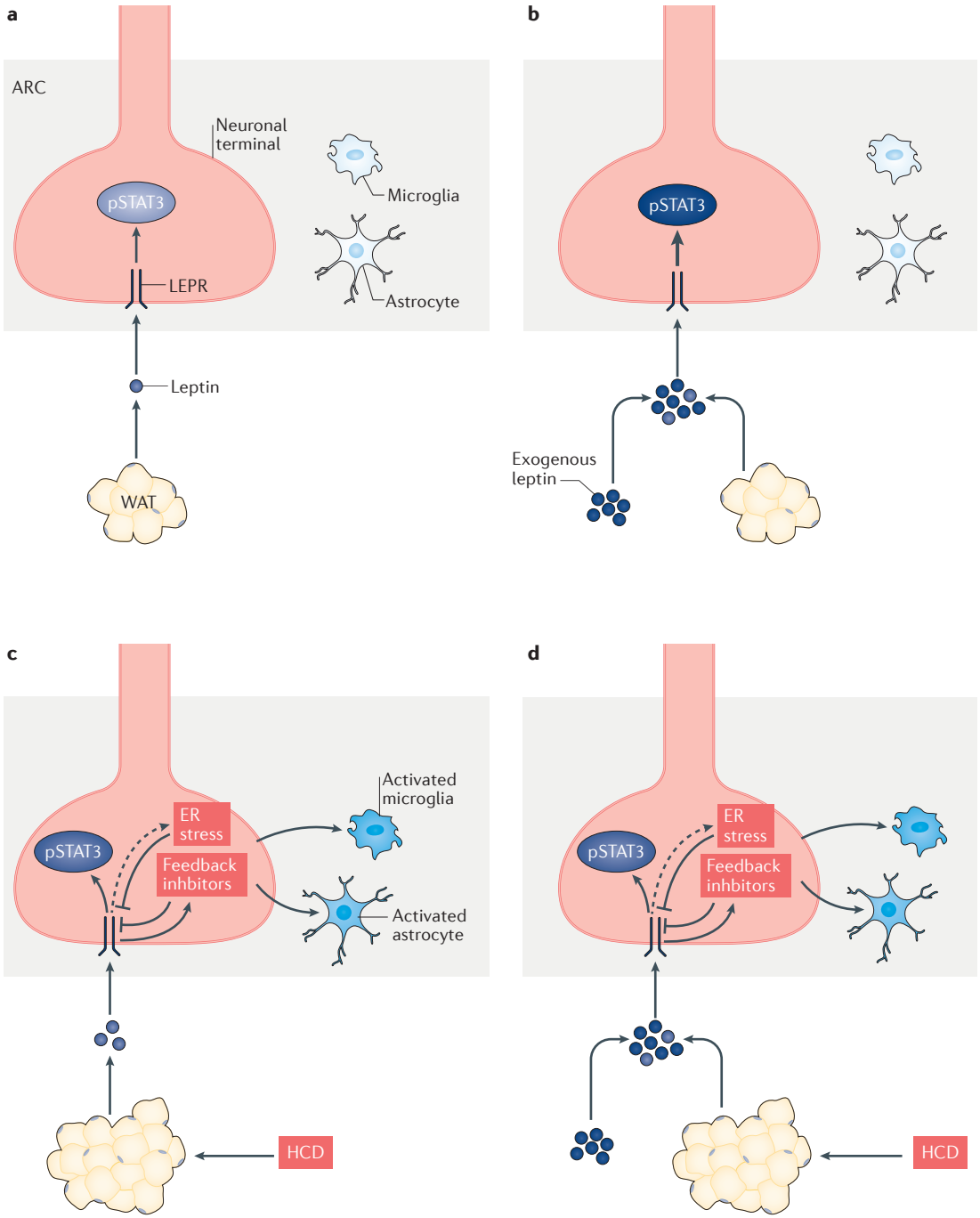


Figure 4 | **Hypothalamic leptin action and changes during diet-induced obesity.** In lean individuals (parts **a**,**b**), white adipose tissue (WAT) produces leptin in proportion to the triglyceride content of the WAT cells and binds to leptin receptor (LEPR) in the arcuate nucleus (ARC), resulting in a baseline level of phosphorylated signal transducer and activator of transcription 3 (pSTAT3) activation and quiescent microglia and astrocytes (part **a**). Acute exogenous leptin injection adds to the endogenous leptin from WAT, augmenting LEPR–STAT3 signalling (part **b**). In diet-induced obesity (DIO) (parts **c**,**d**), a high-calorie diet (HCD) increases WAT and leptin production. The increased endogenous leptin production increases baseline LEPR activation (part **c**), leading to increased pSTAT3 and other changes in neuronal function and activity. This increased LEPR signalling also increases the expression of feedback inhibitors including suppressor of cytokine signalling 3 (SOCS3) and T cell protein-tyrosine phosphatase (TCPTP). Increased leptin action may also increase endoplasmic reticulum (ER) stress, synaptic inputs and potentially other functional parameters in specific subsets of LEPR neurons; this could result in the activation of neighbouring microglia and astrocytes. The presence of feedback inhibitors or other modifiers of LEPR signalling limits the further amplification of LEPR signalling following the administration of exogenous leptin in DIO (part **d**). Dotted lines show potential, but not previously examined, effects of high leptin.

Classical hormone resistance syndromes result from impaired receptor signalling stemming from genetic lesions, circulating antagonists (for example, anti-receptor antibodies) or uncoupling of the receptor from its downstream effectors[49]. Because there is no evidence for genetic lesions in LEPR nor for circulating antagonists in DIO animals or most humans with obesity, any failure of leptin signalling presumably derives from the uncoupling of LEPR from its intracellular signalling mediators or from other mechanisms that impair the function of leptin-controlled neural circuits.

Importantly, high-dose exogenous leptin not only fails to decrease food intake and body weight in humans with obesity and DIO animals[48] but also poorly increases hypothalamic pSTAT3 in rodents[50], potentially consistent with the uncoupling of LEPR from its intracellular signalling cascade in common obesity. The pSTAT3 response to exogenous leptin in DIO animals is most impaired in the ARC[50]. Hence, many consider the ARC to be the major site affected by leptin resistance; consequently, most studies that examine leptin action or potential mediators of leptin resistance have focused on the ARC.

Negative regulators of leptin receptor signalling. Many potential mechanisms have been proposed to explain the limited response to exogenous leptin during obesity, including cellular signalling pathways that inhibit LEPR signalling (FIG. 2). During leptin–LEPR signalling, pSTAT3 translocates into the nucleus to modulate the expression of target genes, including *SOCS3* (REFS 51,52). SOCS3 binds to JAK2 and to phosphorylated Y985 on LEPR, thereby inhibiting JAK2 activity and LEPR signalling[53]. Consequently, mice with a mutation affecting Y985 of LEPR or with ablation of *Socs3* in the brain exhibit increased leptin-stimulated pSTAT3 and anorectic signalling by leptin, resulting in a mildly lean phenotype in chow-fed mice[54,55]. Although Y985 mutation of *Lepr* or CNS ablation of *Socs3* tends to decrease body weight, these animals still gain substantial weight on an HCD. Thus, whereas SOCS3 limits the maximum amplitude of leptin action and interference with SOCS3 function decreases body weight, increased SOCS3 expression cannot explain DIO.

The action of protein-tyrosine phosphatases (PTPases) also limits leptin signalling by dephosphorylating LEPR, JAK2 and STAT3 (REF. 56). In particular, protein-tyrosine phosphatase 1B (PTP1B; also known as PTPN1) dephosphorylates and deactivates JAK2 to suppress leptin–LEPR signalling *in vitro,* and mice with *Ptpn1* (which encodes PTP1B) deleted exhibit exaggerated responsiveness to leptin[56]. Furthermore, for *Socs3*, an HCD increases the expression of *Ptpn1* in the ARC, suggesting that PTP1B plays a role in promoting DIO[57]. Indeed, mice lacking *Ptpn1* in the brain, such as whole-body null animals, are lean and gain less weight than controls when fed an HCD[58]. However, these mice still gain a substantial amount of weight on an HCD[58].

In addition to PTP1B, T cell protein-tyrosine phosphatase (TCPTP; also known as PTPN2), which directly dephosphorylates STAT3, contributes to the attenuation of LEPR signalling. Furthermore, obesity and elevated leptin increase the expression of *Ptpn2* (which encodes TCPTP), and the deletion of neuronal *Ptpn2* decreases body weight, increases leptin sensitivity and blunts weight gain in DIO animals[59]. Moreover, combined deletion of *Ptpn1* and *Ptpn2* in the brain augments leanness and further attenuates weight gain in DIO mice[59]; these mice still gain a substantial amount of weight on an HCD, however. The finding that an HCD increases body weight and adiposity in mice lacking *Socs3* or *Ptpn1* and *Ptpn2* in the brain suggests that none of these mediators of LEPR signal attenuation suffice to explain leptin resistance, although the result of ablating all three of these has not yet been examined[55,58,59].

Other processes

In addition to the systems that directly inhibit LEPR signalling, a variety of hypothalamic responses to high-fat feeding or DIO have been proposed to limit leptin–LEPR action. These include hypothalamic inflammation (including the activation of pro-inflammatory cellular signalling pathways and the production of cytokines), inflammatory-appearing alterations in glia surrounding ARC LEPR neurons and alterations of endoplasmic reticulum (ER) stress in LEPR neurons.

Hypothalamic inflammation. DIO is associated with a state of low-grade inflammation (so-called metabolic inflammation) in peripheral tissues (such as adipose tissue), including the production and secretion of pro-inflammatory cytokines, such as tumour necrosis factor (TNF) and interferons, and the recruitment and activation of immune cells, including macrophages and various subtypes of T cells[60]. It makes teleologic sense that this metabolic inflammation might cause insulin resistance, as pathological inflammatory insults (such as infection) interfere with insulin action, and a variety of data support the notion that inflammatory cytokines and cellular signals associated with inflammation play roles in metabolic dysfunction in obesity[60].

As in peripheral tissues, exposure to an HCD and the onset of positive energy balance promote the expression of inflammatory cytokines in the hypothalamus[61]. DIO has also been reported to activate intracellular signalling cascades associated with inflammation within the hypothalamus, including the nuclear factor- kB (NF-kB) pathway (which promotes cytokine expression), c-Jun N-terminal kinases (JNKs) and protein kinase C θ-type (PRKCQ)[62,63].

Thus, DIO-associated hypothalamic inflammatory signalling might play a role in promoting positive energy balance and/or attenuating leptin action (much as it is proposed to promote peripheral insulin resistance). Indeed, intracerebroventricular TNF promotes food intake, decreases energy expenditure and increases body weight[64]. Furthermore, anti-TNF antibody treatment reduces weight gain during an HFD feeding, and mice without TNF receptor superfamily member 1A (TNFRSF1A) are resistant to DIO[65]. Also, viral approaches that block NF-kB signalling throughout the hypothalamus reduce food intake and body weight[62]. Similarly, deletion of JNKs throughout the brain blunts DIO, whereas the constitutive activation of JNKs in NAG neurons results in hyperphagic obesity[66,67], and ARC-specific knock down of PRKCQ attenuates DIO[63].

By contrast, however, the genetic activation of NF-kB in hypothalamic neurons in mice does not increase hypothalamic cytokine levels, and mice deficient in inhibitor of NF-kB kinase subunit-β (IKKB; the endogenous inhibitor of NF-kB signalling) in the ARC are not obese or hyperphagic[68,69]. Thus, although complete ablation of the NF-kB pathway in the hypothalamus produces weight loss, the activation of this pathway is not sufficient to promote obesity.

Further confusing the picture of how inflammatory hypothalamic signals affect energy balance, many cytokines (for example, interleukin-4 (IL-4) and IL-6) act in the brain to reduce food intake[70,71]. Moreover, genetic and pharmacological interventions that block a variety of inflammatory cytokine signals and pro-inflammatory signals result in increased susceptibility to DIO[72]. Indeed, systemic inflammation (for example, from infection or cancer) promotes cachexia, a state of negative energy balance characterized by decreased feeding and increased energy expenditure[73].

Thus, although manipulations of some cytokines and/or inflammatory cellular signalling pathways involved in the response to inflammation suggest an anabolic role for these pathways, most inflammatory stimuli in the hypothalamus decrease food intake, increase energy expenditure and decrease body weight. It is possible that some manipulations of the various signalling pathways that are activated by inflammatory stimuli (for example, NF-kB, JNKs and PRKCQ) interfere with the roles that these signals play in normal cellular physiology at baseline rather than in response to inflammation. Additionally, many of the manipulations undertaken to examine the function of inflammatory signals within the hypothalamus do not target particular cell types but rather affect multiple types of neurons and, in some cases, non-neuronal cells (for example, glia). To resolve the contradictory findings regarding hypothalamic inflammation and obesity, it will be important to learn more about cell-type specific roles for each cytokine and signalling pathway that may participate in the hypothalamic control of energy balance.

Hypothalamic gliosis. The onset of DIO also increases the number and histologic activation state of microglia (the resident macrophage-like cells of the brain) in the ARC[61,74]. Although it is possible that these microglia represent the source of the increased cytokine expression observed in the hypothalamus of DIO animals, the proliferation and histological activation of ARC microglia seen in DIO require the production of fractalkine (CX_3CL1) by neurons[75]. It is not clear whether neurons produce other cytokines in DIO hypothalami as well.

Increased numbers of astrocytes that appear activated in the ARC accompany the microgliosis observed in DIO (FIG. 4); these glial changes are observed in humans as well as rodents[61,69]. The microgliosis and astrocytosis observed in the ARC of DIO animals might play a role in hypothalamic inflammation or otherwise limit the function of hypothalamic neurons involved in energy balance (for example, LEPR neurons). Alternative interpretations exist, however: microglia play important physiologic roles in remodelling neurological circuits and/or synaptic pruning and in otherwise modulating neurons[76]. Similarly, reactive astrocytes play key roles in maintaining synaptic plasticity and supplying neurons with nutrients[77]. Thus, the gliosis observed in DIO could mediate changes in synaptic function and plasticity during DIO (and/or with chronic leptin administration) and could reflect a homeostatic response rather than a pathophysiologic process. Indeed, although the macrophage and immune cell infiltration into adipose tissue in obese animals was initially hypothesized to represent a pathophysiologic process tied to insulin resistance, a variety of data now suggest the importance of these cells for adipocyte remodelling to maintain metabolic homeostasis during positive energy balance[78].

Endoplasmic reticulum stress. Concomitant with increased cytokine production, the activation of inflammatory signals and immune infiltration observed in adipose and other peripheral tissues in DIO mice, ER stress is also increased in these tissues. Similarly, a variety of observations suggest that hypothalamic neurons experience ER stress during DIO[79]. ER stress occurs when protein or lipid synthesis in the ER outstrips the ability of the organelle to complete the processing and export of fully functional molecules. This can occur owing to environmental (for example, heat and infection) or other alterations associated with increased misfolding of proteins or owing to elevated rates of synthesis that augment demand on the ER. The misfolding of proteins in the ER activates the unfolded protein response by activating serine/threonine-protein kinase/endoribonuclease IRE1 (ERN1) and the ERN1-dependent transcription factor, X-box-binding protein 1 (XBP1); these limit protein synthesis and increase the expression of proteins (such as heat shock proteins) that increase the protein folding capacity of the ER[80]. The failure of cells to mitigate ER stress effectively can impair cellular function and even induce apoptosis[80,81].

Consistent with a potential role for hypothalamic ER stress in obesity, pharmacologic induction of ER stress in the brain promotes obesity, and mice that lack XBP1 in nestin-positive neurons experience increased neuronal ER stress, dramatic hyperleptinaemia and weight gain[79]. Conversely, treatment with chemical chaperones that decrease ER stress or constitutive expression of XBP1 in POMC neurons protects against DIO[79,82]. Furthermore, two compounds identified in a transcriptional screen to identify molecules that diminish ER stress have been shown to decrease feeding and promote weight loss in DIO animals[83,84]. Thus, a variety of data are consistent with the notion that decreasing ER stress in the hypothalamus enhances the catabolic arm of the energy balance system, potentially by augmenting leptin action.

Defective leptin signalling in obesity?

With the exception of rare cases of leptin deficiency, individuals with obesity and most animal models of obesity exhibit appropriately elevated circulating leptin for their adipose mass[7]. Although this observation has been used to suggest impaired leptin action in obesity, treatment of DIO mice with a leptin–LEPR antagonist

Systemic inflammation
An immune response to infection or other insults that increases the activity of immune cells in the body.

increases food intake and body weight, demonstrating that leptin action continues to restrain food intake and body weight in obesity[85]. Furthermore, DIO animals and most models of obesity (except for animals with mutant leptin, LEPR or STAT3) actually exhibit elevated pSTAT3 at baseline (especially in the ARC)[50], suggesting that LEPR–STAT3 signalling is augmented in response to increased endogenous leptin (FIG. 4). Thus, endogenous leptin accesses LEPR neurons and activates pSTAT3 in obesity, suggesting that obesity does not result from decreased leptin–LEPR signalling. (Note that this observation also argues against a causative role for defective leptin transport in obesity).

Thus, obesity occurs not because of decreased leptin–LEPR signalling but rather despite increased leptin–LEPR signalling. If DIO represents a state of increased LEPR signalling in response to elevated endogenous leptin, however, how then do we explain the failure of exogenous leptin to promote pSTAT3 and weight loss in DIO, and why should elevated LEPR signalling in response to elevated endogenous leptin not reverse obesity?

The failure of exogenous leptin to augment leptin action in diet-induced obesity. Leptin promotes the expression of *Socs3* and *Ptpn2* in obesity[14,59]. Thus, the increased expression of these genes in obese animals is consistent with increased LEPR signalling in response to the increased circulating leptin that accompanies increased adiposity. Although the increased expression of these inhibitors reflects increased LEPR signalling, their increased expression (along with the expression of *Ptpn1*) also limits the maximal amplitude of LEPR signalling, blunting further increases in pSTAT3 and leptin-mediated anorexia. Thus, leptin-stimulated inhibitors of leptin action may not be able to decrease the amplitude of LEPR signalling below the amplitude observed in lean animals with normal leptin levels, but they can limit the incremental response to further increases in leptin concentrations. Importantly, the same would be true for any other leptin–LEPR-driven process that attenuates LEPR signalling.

Thus, elevated leptin limits the magnitude of LEPR signalling and catabolic responses to further increases in leptin concentrations. Indeed, Knight *et al.* demonstrated that *ob*/*ob* mice that were implanted with leptin minipumps to normalize energy balance and 'clamp' their leptin levels to those found in lean mice exhibited both an appropriately robust induction of pSTAT3 and suppression of food intake and body weight following leptin injection — even after being rendered obese by the provision of an HCD (which could not alter their leptin concentrations). These data suggest that nothing about HCD or obesity *per se* interferes with leptin action but that chronically elevated leptin concentrations and consequent increased LEPR signalling limit further increases in the amplitude of LEPR action during treatment with exogenous leptin[86].

As a side note, this finding also highlights an important limitation of many publications that propose leptin resistance as a mechanism by which a manipulation in a specific pathway promotes obesity — the analysis of leptin responsiveness is often carried out in obese (hyperleptinaemic) animals. Instead, this analysis must be carried out in pre-obese animals with demonstrably normal leptin levels to understand whether the pathway in question directly alters LEPR signalling rather than promoting obesity independently of altered leptin action and causing leptin insensitivity secondary to hyperleptinaemia.

The failure of elevated endogenous leptin to promote weight loss in diet-induced obesity. Why should obesity remain in the face of increased LEPR signalling in DIO[50,85]? Explanations include the potential inability to sufficiently increase LEPR signalling to promote long-term catabolic signalling[50]. For instance, although leptin-stimulated (and other) attenuators of LEPR signalling do not decrease endogenous leptin action in DIO animals relative to lean controls, they may limit the ability of elevated leptin to reduce adiposity in obese mice by blunting the increase in LEPR signalling that would otherwise occur in response to elevated leptin levels. In this case, it would be possible to reverse obesity by elevating LEPR signalling sufficiently. Indeed, a recent perspective argued in favour of this notion and suggested that we have not sufficiently tested this possibility (especially in humans)[87]. However, given that increased leptin–LEPR signalling blunts further augmentation of LEPR signalling and leptin action, it may be difficult in practice to test unrestrained LEPR signalling.

It is also possible that even maximal (unrestrained) LEPR signalling would not mediate a sufficiently strong signal to the correct neurons to counteract the augmentation of feeding with palatable, high-calorie foods. Indeed, leptin–LEPR signalling mainly promotes changes in neuronal gene expression rather than directly altering the activity of neurons. Thus, stronger activators of specific LEPR-regulated pathways might reverse obesity. Indeed, many of the currently available CNS-acting molecules that produce weight loss (for example, agonists of the glucagon-like peptide 1 receptor (GLP1R), the amylin receptor and the 5-hydroxytryptamine receptor 2C (5HT2C)) tend to directly activate neurons or circuits that overlap with those controlled by LEPR[10,88–90].

Note that it is also possible (even likely) that both mechanisms that limit maximal leptin action and the somewhat modest modulation of neuronal function by leptin contribute to the insufficiency of the response to elevated endogenous leptin in obesity to promote weight loss. Indeed, the existence of mechanisms to limit the catabolic function of leptin makes evolutionary sense: although it is likely that there would have been selective pressure during evolutionary times to permit adipose accrual when food was plentiful (to permit survival during periods of calorific insufficiency), there was likely little to no selective pressure for leptin (or other signals) to strongly limit food intake and body weight, as there was not enough palatable, high-calorie, readily available food in the environment to promote obesity. Hence, because death by famine was a much more likely event than an obesity-related demise, limiting the amplitude of LEPR signalling and the potential anorectic potency of leptin presumably provided a long-term survival benefit.

Hypothalamic changes: roles in obesity

Beyond the increased expression of LEPR signalling attenuators, DIO provokes the activation of signalling pathways associated with inflammatory stimuli (for example, NF-kB) and the production of pro-inflammatory cytokines (such as TNF), gliosis (microglia and astrocytes) and ER stress in the ARC. Understanding the roles for the many potential mediators (for example, hyperleptinaemia, obesity, saturated fatty acids, endotoxaemia and so on) that may underlie these DIO-associated hypothalamic changes (for example, inflammation, gliosis, ER stress and negative regulators), as well as deciphering the roles for these changes in the pathophysiology of obesity, will be crucial as we seek to develop effective treatments for obesity.

Lipids and lipopolysaccharide. One mechanism that has received a great deal of attention is the influx of lipids, especially those that contain saturated fatty acids (FAs), during HFD feeding. Saturated FAs have been shown to increase inflammatory signalling pathways and increase cytokine production as well as to activate JNKs and PRKCQ[63,74,91–93]. However, although free FA levels rise in obesity, they are more elevated after fasting, which sensitizes the hypothalamus to exogenous leptin[7,13]. Furthermore, brain-specific deletion of lipoprotein lipase decreases FA uptake into the brain and increases food intake and body weight, which is consistent with the notion that FAs in the brain mediate anorectic signalling[94]. Thus, the data that support potential roles for increased hypothalamic FAs in the genesis of obesity are somewhat mixed.

Some studies also propose a role for the gut microbiome, which is altered by an HFD, in the genesis and/or maintenance of obesity, potentially by elevating circulating bacterial lipopolysaccharide[95]. To date, however, the observed changes in food intake and body weight with manipulations of the microbiome have been too small to account for the preponderance of weight gain in DIO[96].

Potential mechanisms underlying hypothalamic changes associated with diet-induced obesity. Although we understand well the consequences of decreased leptin–LEPR signalling (during starvation or owing to genetic lesions), we have less information regarding the responses to and consequences of hyperleptinaemia. Some studies suggest that the attenuation of elevated LEPR signalling in response to increased leptin in DIO is less pronounced in non-ARC sites and that this unrestrained elevation in LEPR signalling can produce potentially pathogenic results[97]. Because ARC LEPR signalling, although not as elevated as it might otherwise be, increases in DIO, hyperleptinaemia is likely to mediate changes in ARC physiology as well.

Indeed, it is possible that increased leptin–LEPR signalling could underlie some of these obesity-associated hypothalamic changes. For instance, leptin is a cytokine of the IL-6 superfamily (and LEPR is a member of the IL-6 receptor (IL-6R) superfamily), and leptin increases inflammatory immune function[14,98] (FIG. 1). Hence, increased leptin–LEPR signalling in the hypothalamus might promote local cytokine production, secondarily increasing inflammatory signalling pathways in local cells. Such a mechanism could also potentially underlie the ARC gliosis that is observed in obesity. Because most data suggest that microglia and astrocytes do not express LEPR (see above), this could imply that leptin-induced changes in LEPR neurons would elaborate signals to neighbouring glia. Consistent with this notion, the proliferation and histological activation of ARC microglia with DIO require the production of CX_3CL1 by non-microglial cells[75].

Similarly, increased leptin–LEPR signalling could augment hypothalamic ER stress in obesity as the result of increased demand for the production of anorexigenic peptides (for example, by POMC neurons and/or other LEPR-expressing cells)[86,99]. Indeed, fasting (which increases the production of AGRP and NPY and augments the activity of NAG neurons) produces a cell-autonomous transcriptional signature consistent with ER stress in NAG cells[100].

Thus, while this hypothesis requires extensive testing, many of the hypothalamic perturbations observed in DIO are at least consistent with the theoretical consequences of increased LEPR signalling owing to elevated leptin levels. Additionally, continued research on the consequences of hyperleptinaemia and increased LEPR signalling in common (hyperleptinaemic) obesity will be crucial as we seek to understand the pathophysiology of obesity.

Summary and conclusions

The ongoing worldwide epidemic of obesity represents a serious threat to human health and economic productivity; current obesity therapies are inadequate, and new medicines are required to combat this disease. Identifying such therapies will require a detailed understanding of the mechanisms that maintain adipose stores at mostly constant levels over the long term and of how these may be dysregulated to permit the establishment and maintenance of elevated adiposity.

Leptin, which is produced by adipose tissue in approximate proportion to triglyceride stores, controls the major CNS systems that modulate food intake and energy expenditure and plays an important role in maintaining constant energy stores; low leptin augments food intake and suppresses energy expenditure, both of which tend to restore depleted energy stores. Conversely, adequate leptin suppresses feeding and normalizes energy expenditure.

Because obesity is defined by elevated adipose mass, leptin concentrations are increased in obesity. The failure of high endogenous (and exogenous) leptin to normalize body weight in obese individuals has suggested the possibility of leptin resistance in obesity, spurring a great deal of research aimed at understanding this notion. A number of potential mechanisms that limit leptin–LEPR signalling in obesity (for example, obesity-induced inhibitors of the LEPR signalling pathway, the activation of inflammatory signalling pathways and cytokines or ER stress in the hypothalamus, or hypothalamic gliosis) have been suggested to impair LEPR signalling in obesity and thus permit positive energy balance.

Gut microbiome
The bacteria and other microorganisms that colonize the lumen of the gut.

Hypothalamic LEPR signalling is elevated in obesity; however, this would be expected given increased endogenous leptin. Indeed, because increased LEPR signalling augments the expression of a variety of inhibitors of LEPR signalling, increased LEPR signalling in DIO may limit the amplitude of further increases in LEPR signalling. Thus, although these limitations cannot underlie the development of obesity, they limit the potential maximal amplitude of LEPR signalling such that the strength of the leptin signal in hyperleptinaemic obesity is less than it would be otherwise; the response to exogenous leptin would also be blunted (as is observed). Increased LEPR signalling could also underlie other DIO-associated changes in hypothalamic function as well.

Thus, important questions for future research include not only the mechanisms and relative roles for each potential process proposed to limit leptin action but also the roles for leptin–LEPR signalling in the genesis of each process. Furthermore, it will be crucial to understand whether augmenting the leptin–LEPR signal and/or whether other means of more strongly activating leptin-regulated neural pathways can reverse obesity.

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Author contributions
M.G.M. was involved in researching data for the article, made a substantial contribution to discussion of content and wrote, reviewed and edited the manuscript before submission. W.W.P. was involved in researching data for the article, made a substantial contribution to the discussion of content and wrote, reviewed and edited the manuscript before submission.

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