Leptin revisited: its mechanism of action and potential for treating diabetes

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Abstract | Since the discovery of leptin in 1994, we now have a better understanding of the cellular and molecular mechanisms underlying its biological effects. In addition to its established anti-obesity effects, leptin exerts antidiabetic actions that are independent of its regulation of body weight and food intake. In particular, leptin can correct diabetes in animal models of type 1 and type 2 diabetes. In addition, long-term leptin replacement therapy improves glycaemic control, insulin sensitivity and plasma triglycerides in patients with severe insulin resistance due to lipodystrophy. These results have spurred enthusiasm for the use of leptin therapy to treat diabetes. Here, we review the current understanding of the glucoregulatory functions of leptin, emphasizing its central mechanisms of action and lessons learned from clinical studies, and discuss possible therapeutic applications of leptin in the treatment of type 1 and type 2 diabetes.

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Despite the availability of improved antidiabetic drugs, enhanced glycaemia monitoring systems and easier patient-to-physician accessibility, patients with type 2 diabetes[1,2] (BOX 1) are still at a significantly higher risk of developing cardiovascular disease and cancer than non-diabetic individuals[3,4]. The incidence of coronary artery disease in patients suffering from type 1 diabetes[5–7] (BOX 2) is also remarkably high: greater than 90% after the age of 55 years[8,9]. In the United States, 40% of patients diagnosed with diabetes do not achieve the accepted glycaemic targets and 80% fail to achieve blood-pressure- and lipid-lowering goals[10]. Therefore, despite the fact that insulin therapy transformed the previously lethal disease of type 1 diabetes into a manageable condition, and the fact that current type 2 diabetes drugs improve glycaemic control, these interventions do not restore metabolic homeostasis and, when used over long periods of time, may cause serious comorbidities associated with diabetes. Hence, better antidiabetic approaches are urgently needed.

Leptin is a hormone that is produced by adipose tissue and regulates various physiological processes and behaviours, including appetite, body weight, neuroendocrine functions and glycaemia. These effects are mediated via actions on leptin receptors (LEPRs) expressed by neurons in the central nervous system (CNS)[11,12]. Among several splice variants of the *Lepr* gene[13], the long LEPRB isoform is thought to mediate all actions of leptin via the activation of multiple intracellular signalling pathways (FIG. 1). Studies in rodents have identified some of the specific neuronal targets that mediate the hormonal effects of leptin on the aforementioned parameters. For example, the actions of leptin on body weight are mediated mainly by GABA (γ-aminobutyric acid)-ergic neurons[14] (the anatomical locations of which remain unclear), and its role in puberty is mediated by neurons within the ventral premammillary nucleus of the hypothalamus[15]. The potent effects of leptin on glucose homeostasis in the context of severe obesity and insulin resistance are predominantly mediated by pro-opiomelanocortin (POMC)-expressing neurons within the arcuate nucleus of the hypothalamus (ARH)[16,17].

Owing to its potent beneficial effects on glucose metabolism, as demonstrated by the administration of leptin to diabetic rodents and to humans with insulin-resistant diabetes caused by lipodystrophy[18–20] (BOX 3), this 16 kDa polypeptide has the potential to become a novel and effective antidiabetic agent. In this Review, we present available results from leptin-based clinical trials. We also examine data from animal studies that led to the current understanding of the underlying mechanisms of leptin-mediated control of glucose homeostasis. In addition, because patients with type 2 diabetes are commonly obese and have

Box 1 | **Type 2 diabetes: current treatments, complications and limitations**

Type 2 diabetes is caused by a combination of insufficient insulin production by the pancreas and diminished responsiveness to insulin in target tissues and organs. The illness is characterized by insulin resistance, hyperglycaemia and elevated circulating lipid levels; in later stages of the disease the loss of pancreatic β-cells can also occur[1]. There are several treatments available to patients with type 2 diabetes[151]. The most widely prescribed drug for the management of this metabolic defect is metformin, a biguanide that enhances hepatic insulin sensitivity and hence curtails the elevated hepatic glucose production typically observed in patients with type 2 diabetes[152],[153]. Metformin inhibits complex I of the mitochondrial oxidative phosphorylation machinery and causes an increased AMP/ATP ratio[154], an effect that induces the activation of AMP-activated protein kinase (AMPK).

The antidiabetic action of metformin stems from the activation of hepatic liver kinase B1 (LKB1)–AMPK pathways; this leads to inhibition of histone deacetylases (HDACs), which leads to the suppression of forkhead box protein O1 (FOXO1) activity, which causes reduced gluconeogenic gene expression, ultimately resulting in diminished hepatic glucose output[153,155–157]. Metformin causes minor side effects (such as gastrointestinal disturbances) if it is administered to patients who are not prone to developing lactic acidosis[158,159].

Thiazolidinediones (TZDs; for example, rosiglitazone (Avandia; GlaxoSmithKline), pioglitazone (Actos; Takeda) and troglitazone) are synthetic ligands of the nuclear receptor transcription factor peroxisome proliferator-activated receptor-γ (PPARγ)[160] that increase insulin sensitivity[161–163]. Recent studies have unveiled some of the mechanisms underlying the unwanted (for example, augmenting body adiposity) and wanted (alleviation of diabetes) actions of TZDs. For example, increased appetite and body fat mass are frequent undesired effects of TZDs[164–169]. These untoward outcomes are likely to be due to the action of TZDs on PPARγ in the brain[170,171]. TZDs influence PPARγ activity and consequentially glucose metabolism by blocking the phosphorylation of PPARγ by cyclin-dependent kinase 5 (CDK5); CDK5 activity increases in the adipose tissue of obese and diabetic individuals[163]. New compounds tailored to target only the CDK5–PPARγ interaction site have been successfully tested in preclinical studies[172]. These findings may pave the way for the development of better antidiabetic compounds that exert their effects via PPARγ. Indeed, because of its known detrimental effects on the heart, the use of rosiglitazone-containing medicines was recently restricted in the United States and suspended in the European Union[173].

Other classes of approved antidiabetic (type 2) drugs

- Agonists of the glucagon-like peptide 1 (GLP1) receptor (for example, exenatide (Byetta; Amylin) and liraglutide (Victoza; Novo Nordisk) have moderate efficacy; exenatide is now approved in both Europe and the United States as a once-weekly injection, but can cause nausea and vomiting[151].
- Inhibitors of the endogenous GLP1-inactivating protein dipetidyl peptidase 4 (DDP4) also have moderate efficacy but can cause serious side effects such as an increased risk of developing pancreatitis[151].
- Insulin secretagogues (such as sulphonylureas), by lowering the open probability of ATP-sensitive K^+ channels, diminish the threshold for glucose-induced insulin secretion; these compounds have good efficacy but substantially increase the risks of hypoglycaemia (an event that can be life-threatening).
- Insulin also has moderate efficacy but needs to be injected multiple times a day; it can cause increased ectopic lipid deposition and hypoglycaemia.

Other classes of drugs are currently in clinical trials (reviewed in REFS 151,174,175). In addition to the drawbacks mentioned above, the patients' requirement of currently available antidiabetic (type 2) drugs (either alone or in combination) usually augments with the progression of the disease, hence increasing the risks of unwanted effects[175].

Type 2 diabetes
An illness characterized by insulin resistance, elevated blood levels of glucose, insulin and lipids, and estimated to affect more than 300 million people worldwide.

Type 1 diabetes
An illness characterized by the loss of pancreatic β-cells, lack of insulin, hyperglycaemia, cachexia and ketoacidosis, and estimated to affect millions of people worldwide.

Lipodystrophy
A rare condition that can be inherited or acquired, and is typically characterized by varying adipose tissue loss and distribution.

Hyperleptinaemia
A condition in which leptin levels in the blood are elevated, typically in obesity.

Leptin resistance
A condition in which endogenous and exogenous leptin is less effective at mediating its actions: for example, at reducing food intake or lowering glucose and lipids levels in the blood.

Hypoleptinaemia
A condition in which the level of leptin in the blood is below normal owing to reduced fat mass: for example, in patients with anorexia, lipodystrophy or hypothalamic amenorrhea.

hyperleptinaemia, and are thus resistant to the metabolic actions of leptin, we deliberate on the possible mechanisms responsible for leptin resistance. A detailed understanding of this issue is probably crucial for the successful development and therapeutic use of leptin, or compounds targeting downstream pathways engaged by the hormone, for treating diabetes. Finally, we touch upon the therapeutic potential and limitations of leptin in becoming an addition to the pharmacopoeia of antidiabetic agents.

Leptin in clinical settings

Below, we discuss results from leptin-based clinical trials, data from which are available from the ClinicalTrials.gov website and from the PubMed database. Although these results indicate that leptin therapy can effectively improve energy and/or glucose imbalances in individuals who have severe hypoleptinaemia, they also suggest that hyperleptinaemic individuals (who represent the vast majority of the obese and diabetic population) are refractory to therapy (TABLE 1). These observations highlight the need to overcome the 'leptin-resistant' obstacle if leptin therapy is to become an effective and widely used tool against obesity and diabetes.

Effects on obesity. Because of reproducible preclinical results showing that leptin exerted potent anti-obesity effects, the hormone was initially heralded as the panacea for the obesity pandemic. In humans, leptin therapy indeed rescued obesity and several other endocrine defects (for example, pubertal delay and infertility) present in the very few obese people suffering from congenital leptin deficiency[21–23]. However, most of the individuals suffering from obesity have elevated levels of circulating leptin and are therefore likely to be leptin-resistant[24]. Indeed, obese patients with hyperleptinaemia show poor responses (in terms of anorectic effects and body weight suppression) to exogenously administered leptin[25,26]; these results diminished expectations from leptin-based anti-obesity approaches.

Box 2 | **Type 1 diabetes: current treatments, complications and limitations**

Type 1 diabetes is caused by the loss of pancreatic β-cells, which results in a lack of insulin and a lethal catabolic outcome if untreated. A classical endocrinological approach for treating an illness resulting from the lack of a given hormone is to replace levels of the hormone therapeutically; type 1 diabetes clinical practice strictly abides by this paradigm. Thus, insulin administration is part of the daily activities of virtually all patients with type 1 diabetes[6,176]. Despite its undisputable life-saving action, insulin therapy does not restore metabolic homeostasis in patients with type 1 diabetes, as these individuals are at a much higher risk of developing challenging morbidities (such as heart disease, blindness, kidney failure, neuropathy and hypertension) than normal unaffected individuals [177–179]. It has yet to be resolved whether these complications are due to the volatility of euglycaemia (wide fluctuations in blood glucose levels are commonly seen in patients with type 1 diabetes), direct consequences of therapy or a combination of both.

In addition to the euglycaemic volatility, drawbacks of insulin therapy stem from its lipogenic actions, as insulin stimulates the transcription of genes that encode enzymes involved in the biosynthesis of lipids[180]. Thus, long-term insulin treatment may underlie the excessive ectopic lipid deposition (that is, in non-adipose tissues)[181] and the high incidence of coronary artery disease observed in patients with type 1 diabetes[8,9]. Of note, these lipogenic actions of insulin are likely to promote a vicious cycle of fatty-acid-induced insulin resistance in insulin target cells (for example, hepatocytes and myocytes) and hence lead to increased insulin requirements in the enduring management of patients with type 1 diabetes[182–184]. In part owing to the potent, fast-acting, glycaemia-lowering effect of the hormone, intensive insulin therapy also substantially increases the risk of hypoglycaemia — an event that can be life-threatening[6,185–188].

Nevertheless, more recent clinical data have indicated that combination treatment with leptin and pramlintide (Symlin; Amylin Pharmaceuticals) (an amylin analogue) significantly reduces body weight (up to 12% of pre-treatment body weight) in leptin-resistant obese individuals[27,28]. Because the duration of therapy was limited to 20 weeks, it is unclear whether a longer treatment period would result in maintenance or further reduction of body weight or cause negative side effects. Although longer-term clinical trials have been initiated (ClinicalTrials.gov identifiers: NCT00819234 and NCT00673387) to address these issues, results are not yet available. In addition, the efficacy of leptin monotherapy or leptin and pramlintide bi-therapy in obese individuals with low or normal levels of circulating leptin (representing ~10% of the obese population)[11,24] is unknown. Therefore, although there are still chances for possible improvements, it seems as though treatment with leptin alone is not an effective approach for the treatment of obesity in the vast majority of obese individuals who also have hyperleptinaemia (fasting serum levels of leptin >15 ng per ml).

Effects on lipodystrophy. Soon after the publication of the remarkable preclinical results obtained by Shimomura and colleagues[18] — who showed that leptin administration corrects the severe insulin resistance and hyperglycaemia displayed by rodents with congenital generalized lipodystrophy (BOX 3) — the clinical effects of the hormone were analysed. The clinical outcomes satisfied, to some extent, the expectations engendered by the preclinical results. For example, the insulin resistance, hyperinsulinaemia, hyperglycaemia and hypertriglyceridaemia present in patients with severe hypoleptinaemia (fasting serum levels of leptin <4 ng per ml) and lipodystrophy were all improved (without adverse effects) following daily subcutaneous administration of recombinant methionyl human leptin[19,20]. Of note, the improvement in diabetes observed in these leptin-treated lipodystrophic patients was achieved even after discontinuation of previously administered antidiabetic therapy, hence ruling out the possibility of a synergistic or additive positive effect of leptin and antidiabetic drugs on glucose and lipid imbalances[20]. Results from additional studies have established the beneficial effects of leptin therapy on glucose and lipid metabolism in lipodystrophic patients with hypoleptinaemia[29,30].

Nevertheless, lipodystrophy refers to a very heterogeneous group of disorders that may be accompanied by diverse changes in the amount of circulating leptin[31] (BOX 3). Indeed, not all patients with lipodystrophy have reduced leptin levels[32]. Thus, concerns pertinent to the efficacy of leptin therapy in all types of lipodystrophies have been raised; more specifically, the antidiabetic potential of leptin administration in lipodystrophic patients who do not have severe hypoleptinaemia has been questioned. Supporting these concerns, recent findings indicate that even though leptin therapy is very effective in ameliorating lipid profiles, it does not improve hyperglycaemia in lipodystrophic individuals with moderately low levels of circulating leptin (fasting serum levels of leptin ~5 ng per ml)[32]. Thus, people who develop lipodystrophy without having severe hypoleptinaemia are expected to respond poorly to the hyperglycaemia-lowering effect of the hormone yet still benefit from its hypertriglyceridaemia-lowering effect. This potential limitation of leptin therapy in the context of lipodystrophy underscores the necessity of developing better approaches to treat metabolic imbalances in individuals affected by this disorder.

To this end, combination therapy may be a better option. For example, the most prevalent type of lipodystrophy is the acquired form, which affects patients with HIV or AIDS who have been taking highly active antiretroviral treatments[33]. These patients usually have subcutaneous fat loss and increased abdominal fat. Results from a small clinical trial indicate that leptin administration improves glucose metabolism but not lipid parameters in lipodystrophic patients with HIV or AIDS[34]. Conversely, the administration of tesamorelin (Egrifta; Theratechnologies) (a growth hormone-releasing factor analogue approved by the US Food and

Amylin
A hormone that is co-secreted with insulin from pancreatic β-cells; amylin slows gastric emptying and promotes satiety.

Euglycaemia
Normal levels of glucose in the blood.

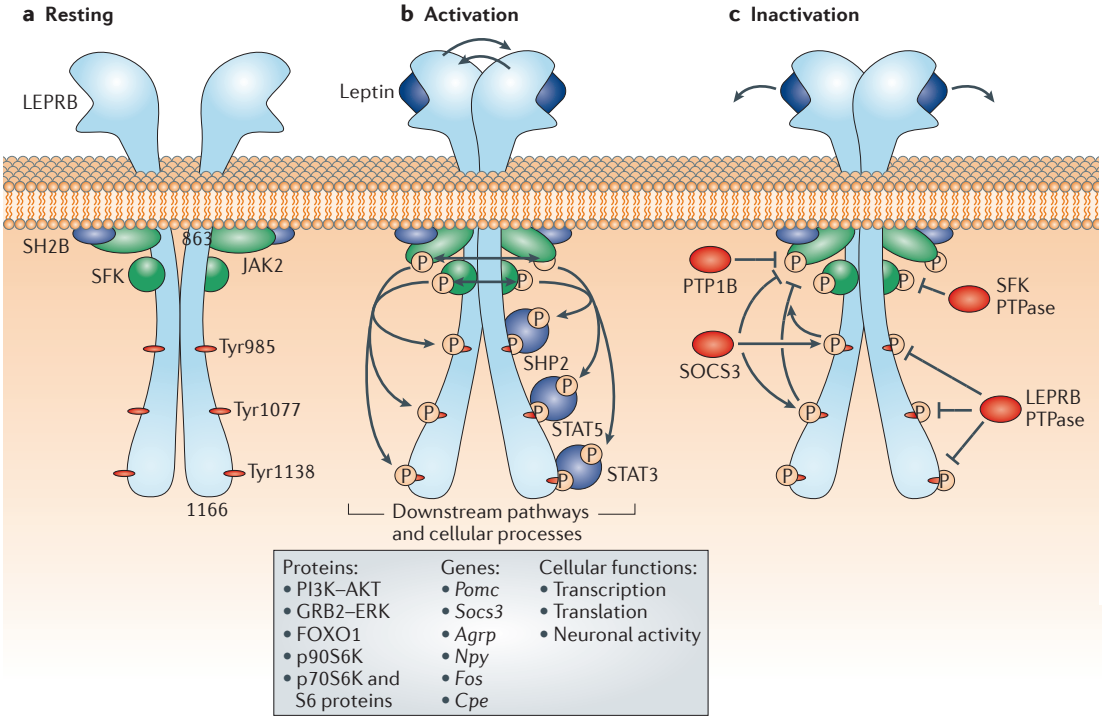


Figure 1 | **Neuronal leptin receptor activation and inactivation. a** | In the resting state, the long isoform of the leptin receptor (LEPRB) exists as a homodimer at the plasma membrane[193]. The tyrosine kinases Janus kinase 2 (JAK2) and SRC family kinases (SFKs), as well as the JAK2-binding protein SH2B, are constitutively associated with membrane-proximal regions of LEPRB[194,195]. **b** | Following the binding of leptin to LEPRB (in a 1:1 stoichiometry)[196], the two receptor subunits undergo a conformational change resulting in transphosphorylation and transactivation of JAK2 and SFK proteins[197,198]. SH2B enhances JAK2 enzymatic activity[199]. Activated JAK2 and possibly SFK enzymes then phosphorylate Tyr residues of LEPRB[58]. Three cytoplasmic Tyr residues of the murine LEPRB act as binding sites for SH2 domain-containing proteins. Specifically, SH2 domain-containing phosphatase 2 (SHP2) principally binds to phosphorylated Tyr985, signal transducer and activator of transcription 5 (STAT5) binds to phosphorylated Tyr1077 and STAT3 binds to phosphorylated Tyr1138 (REFS 200,201). LEPRB-bound SHP2, STAT3 and STAT5 proteins then become phosphorylated by JAK2 and SFKs. Additional downstream signalling proteins and pathways include: the phosphoinositide 3-kinase (PI3K)–AKT pathway; the pathway mediated by growth factor receptor-bound protein 2 (GRB2) and extracellular signal-regulated kinase 1 (ERK1) or ERK2 (REFS 58,200,202); 90 kDa ribosomal protein S6 kinase (p90S6K)[58]; 70 kDa ribosomal protein S6 kinase (p70S6K) and ribosomal S6 proteins; and forkhead box protein O1 (FOXO1)[203,204]. These enzymes and pathways ultimately exert the regulation of cellular processes, including transcriptional control (of the following genes: suppressor of cytokine signalling 3 (*Socs3*)[205,206], pro-opiomelanocortin (*Pomc*)[207,208], *Fos*[58], carboxypeptidase E (*Cpe*)[209], Agouti-related protein (*Agrp*) and neuropeptide Y (*Npy*)[208]), translational control[210–212] as well as neuronal activity and firing[109,213]. **c** | LEPRB is inactivated at proximal sites by protein tyrosine phosphatase 1B (PTP1B), a tyrosine phosphatase that directly dephosphorylates JAK2 (REF. 76). Furthermore, SOCS3 acts to inhibit JAK2 activity either by binding directly to JAK2 or indirectly by first binding to Tyr985 or Tyr1077 of LEPRB[68,80,201]. Finally, protein tyrosine phosphatases (PTPase) are predicted to directly dephosphorylate LEPRB and SFKs, but these proteins have yet to be identified. Residues 863 and 1166 are the first and last amino acid residues in the intracellular domain of LEPRB, respectively.

Drug Administration for the treatment of lipodystrophy in patients with HIV or AIDS) has been shown to improve visceral fat, triglyceride and cholesterol levels but not glucose parameters in these individuals[35]. Thus, combination treatment with leptin and tesamorelin may ameliorate both lipid and glucose parameters in lipodystrophic patients with HIV or AIDS. Future clinical trials aimed at addressing this possibility are warranted.

Effects on non-alcoholic steatohepatitis (NASH). The beneficial effects of leptin replacement therapy on fat deposition in the liver of lipodystrophic patients[36] spurred enthusiasm on the possibility that leptin administration lowers the increased fat content in the liver of non-lipodystrophic patients with non-alcoholic steatohepatitis (NASH). A clinical trial designed to test this possibility (ClinicalTrials.gov identifier: NCT00596934) has enrolled non-lipodystrophic patients with NASH who had relatively low circulating levels of leptin (the results of this trial are not yet available).

Effects on hypothalamic amenorrhea. Hypothalamic amenorrhea is a condition characterized by amenorrhea with anovulatory infertility, moderate to severe hypoleptinaemia and decreased bone mineral density. Leptin administration to lean women who undertake strenuous

Amenorrhea
A condition characterized by the absence of menstrual periods in a woman of reproductive age.

Box 3 | **Lipodystrophy: current treatments, complications and limitations**

Lipodystrophy refers to a heterogeneous group of disorders that are characterized by abnormal adipose tissue homeostasis. Lipodystrophy can either be partial (wherein patients have adipose tissue abnormalities in one or more sites in the body) or generalized (wherein patients have a near-total lack of adipose tissue throughout the body). Both partial and generalized forms can either be congenital or acquired[139]. Congenital lipodystrophy has diverse molecular origins, as mutations in several different genes have been found in people affected by this disorder. Nevertheless, a common thread is the altered function of genes that are known to control adipogenesis and/or lipid storage. Mutations in the gene encoding peroxisome proliferator-activated receptor-γ (PPARγ; a key transcription factor for normal lipid uptake and storage as well as adipocyte differentiation) or the gene encoding perilipin 1 (a crucial protein for normal lipid droplet formation and lipid storage) represent just a few examples of the genetic defects found in the congenital lipodystrophic patient population[189,190].

Acquired lipodystrophy is thought to be caused by autoimmune-mediated destruction or iatrogenic-induced dysfunction of adipose tissue. For example, highly active antiretroviral therapy used in patients with HIV or AIDS is thought to underlie the lipodystrophy syndrome seen in these patients[33]. The adverse effects of this therapy on adipose tissue homeostasis are possibly due to the administration of nucleoside reverse transcriptase inhibitors and/or protease inhibitors[191,192]. Of note, acquired lipodystrophy in patients with HIV or AIDS (a peculiar form of lipodystrophy that can lead to subcutaneous fat loss and increased abdominal fat) has become the most prevalent type of lipodystrophy[139]. Depending on the amount of adipose tissue loss and the region of the body in which this deficiency occurs, lipodystrophy may lead to various degrees of hypoleptinaemia and insulin resistance[139]. In some patients, lipodystrophy can even cause high levels of circulating insulin, lipids and glucose[20]. Treatment of lipodystrophy can be very challenging and it varies depending on the type of lipodystrophy. It may include lifestyle changes (involving changes to the diet and increased physical activity) and/or the administration of drugs (for example, statins and/or metformin and/or insulin). The administration of leptin substantially reduces hyperglycaemia and hyperlipidaemia in patients with lipodystrophy who have very low levels of circulating leptin[20]. Nevertheless, the majority of patients with lipodystrophy do not have severe hypoleptinaemia, and current treatments fall short of restoring metabolic homeostasis in these individuals. Hence, patients with lipodystrophy are at a higher risk of developing cirrhosis, renal disease, retinopathy as well as heart and/or circulatory defects in comparison with normal unaffected individuals[139].

exercise and are affected by this condition leads to recuperation of menstruation and correction of gonadal abnormalities[37]. This treatment also has a clear positive effect on bone mineral density and content[38,39]. The results on bone homeostasis are somewhat unexpected for two reasons: first, leptin deficiency in mice correlates with increased bone mineral density and mass, suggesting that leptin may have a suppressive role on these two parameters; second, humans lacking the hormone usually do not display defects in bone mineral density and mass[23]. However, because the two clinical studies mentioned above enrolled fewer than 20 women, it is currently unclear whether the results are reproducible in large cohorts.

Effects on type 1 diabetes. In mouse models of type 1 diabetes, leptin monotherapy (that is, without the use of exogenously administered insulin and/or other compounds) corrects diabetes and the lethal catabolic consequences of insulin deficiency[40,41]. In two patients with type 1 diabetes and acquired generalized lipodystrophy, 1 year of leptin treatment remarkably improved glucose and lipid profiles[42]. Although insulin therapy was not completely discontinued, leptin treatment improved insulin sensitivity to the extent that insulin doses were significantly reduced (by 30–50% compared to pre-leptin administration doses) in the affected individuals[42]. These preclinical and clinical findings spurred enthusiasm on the antidiabetic (type 1) therapeutic potential of leptin. As a result, a clinical trial is underway (ClinicalTrials.gov identifier: NCT01268644) that is aimed at determining the safety of the hormone and its efficacy in diminishing insulin requirements, hyperglycaemia, glycaemic fluctuations and circulating lipid levels in patients with type 1 diabetes.

Effects on type 2 diabetes. Results from several preclinical studies have indicated that leptin improves insulin resistance as well as glucose and lipid imbalances in mouse models of type 2 diabetes[17,43–45]. However, the results of two recent clinical trials indicate that leptin therapy is ineffective (or only marginally effective) in improving diabetes and insulin resistance in obese patients with type 2 diabetes[46,47]. The antidiabetic (type 2) effect of leptin may, however, be unmasked in patients with type 2 diabetes who are not obese and have either normal or low levels of leptin, including Asian patients with type 2 diabetes who generally have low adipose tissue mass. Future clinical trials aimed at addressing this possibility are therefore warranted.

Summary of clinical studies. Regardless of the diseases in which leptin therapy has been tested, a common thread seems to emerge: the failure of leptin administration to improve metabolic imbalances in individuals who do not have severely low leptin levels. For example, in obesity, leptin therapy improves metabolic imbalances in leptin-deficient individuals but fails to do so in patients with hyperleptinaemia. In lipodystrophic individuals, leptin therapy improves glucose and lipid imbalances in patients with severe hypoleptinaemia but fails to do so in individuals with moderately low levels of circulating leptin.

However, owing to the small number of patients studied and limited results available, caution is needed when drawing conclusions. Nevertheless, leptin therapy seems to be ineffective in people who have hyperleptinaemia. Moving forward, this information would indicate that first, we need to have a better understanding of the mechanisms underlying leptin resistance. Second, ways

Table 1 | **Leptin in clinical settings**

Condition	Basal serum levels of leptin	Intervention	Outcome	Refs
Obesity (caused by genetic leptin deficiency)	Absent (<0.04 ng per ml)	Leptin	Markedly improves obesity and endocrinological imbalances	21–23
Obesity	High (>15 ng per ml)	Leptin	Does not improve obesity	25,26
Obesity	High (>15 ng per ml)	Leptin and pramlintide	Modestly improves obesity	27
Lipodystrophy-induced type 2 diabetes	Very low (<5 ng per ml)	Leptin	Improves lipid and glucose imbalances	19,20
HIV and lipodystrophy-induced type 2 diabetes	Very low (<5 ng per ml)	Leptin	Improves glucose but not lipid imbalances	34
Lipodystrophy-induced type 2 diabetes	Low (~5 ng per ml)	Leptin	Improves lipid but not glucose imbalances	32
Hypothalamic amenorrhea	Very low (<5 ng per ml)	Leptin	Recuperates menstruation and corrects gonadal abnormalities	37–39
Type 2 diabetes and obesity	High (>15 ng per ml)	Leptin	Marginal or no improvement in glycaemic control	46,47
Type 1 diabetes and lipodystrophy	Very low (<5 ng per ml)	Leptin	Improves lipid and glucose imbalances	42

to improve leptin resistance should be investigated. Third, research aimed at identifying the molecular mechanisms underpinning the beneficial effects of leptin therapy must be encouraged, as results from these endeavours are anticipated to provide molecular targets for novel drugs that could circumvent the obstacle of leptin resistance. Below, we discuss the currently available information that could help to address these challenges.

Leptin as an anti-obesity agent

The anti-obesity effects of leptin. Leptin is secreted by adipocytes proportionally to the amount of body fat and represents one of the key peripheral cues signalling the status of the body's energy reserves to the brain[11,12]. For example, in fasting rodents, circulating leptin levels fall largely owing to diminished leptin release from the decreasing adipose depots. This reduction in leptin levels is interpreted by the brain as a signal to increase appetite and food-seeking behaviour to ultimately restore the body's fat depots[48]. Consistent with this role of leptin, genetic deficiency of this hormone or of its receptors leads to massive hyperphagia and obesity in both rodents and humans[21,49,50].

Remarkably, daily injection of recombinant leptin into normal mice reduces caloric intake and increases energy expenditure, resulting in near-complete elimination of adipose tissue within a few days, with no apparent signs of toxicity[51]. The disappointing results of initial clinical trials halted progress in using leptin to treat common forms of obesity. Nevertheless, if mechanisms of leptin resistance are understood and diminished through drug therapy, exogenous leptin may become an important adjunct tool for reducing adiposity and maintaining reduced body weight. Currently, leptin therapy is only effective for treating obesity in the very few patients who have congenital leptin deficiency[21,22].

What do we know about mechanisms causing leptin resistance in obesity? An impaired response of exogenous leptin in reducing body weight and food intake demonstrates resistance to the anti-obesity effects of leptin[52]. In addition, hyperphagia and increased adipose mass in the presence of hyperleptinaemia indicate resistance to endogenous leptin. In rodents that are given unrestricted access to a high-fat diet, leptin resistance manifests after only a few weeks[53]. Similarly, common obesity in humans is characterized by hyperleptinaemia and diminished anorectic effects as well as diminished body weight suppression in response to the administration of exogenous leptin[25,26]. Most cases of obesity in humans cannot be attributed to genetic defects in the genes encoding leptin or its receptors. Therefore, because the anti-obesity actions of leptin are mediated by the brain, proposed mechanisms underlying leptin resistance can, in principle, be divided into three categories: first, impaired transport of leptin across the blood–brain barrier (BBB); second, impaired neuronal leptin signalling in target neurons; and third, altered signalling in downstream target cells and neurocircuits (FIG. 2a).

Early evidence supported the possibility of impaired leptin transport across the BBB as a primary defect underlying obesity in humans and rodents. For example, obese humans have only slightly increased leptin levels in the cerebrospinal fluid despite having substantially elevated levels of leptin in the blood[54]. Also, transport of leptin across the BBB is reduced in diet-induced obese (DIO) animals[55]. However, more recent data obtained from rodents suggest that the impaired leptin transport across the BBB is acquired during the development of obesity, and is therefore a secondary defect[56]. Consistent with this notion, the anorectic effect of leptin and its ability to activate neuronal pSTAT3 (phosphorylated signal transducer and activator of transcription 3)

Common obesity
A condition that is characterized by increased body weight due to excess adipose mass, as well as hyperleptinaemia, and is associated with an increased risk of developing type 2 diabetes, cardiovascular disease, cancer and non-alcoholic fatty liver disease.

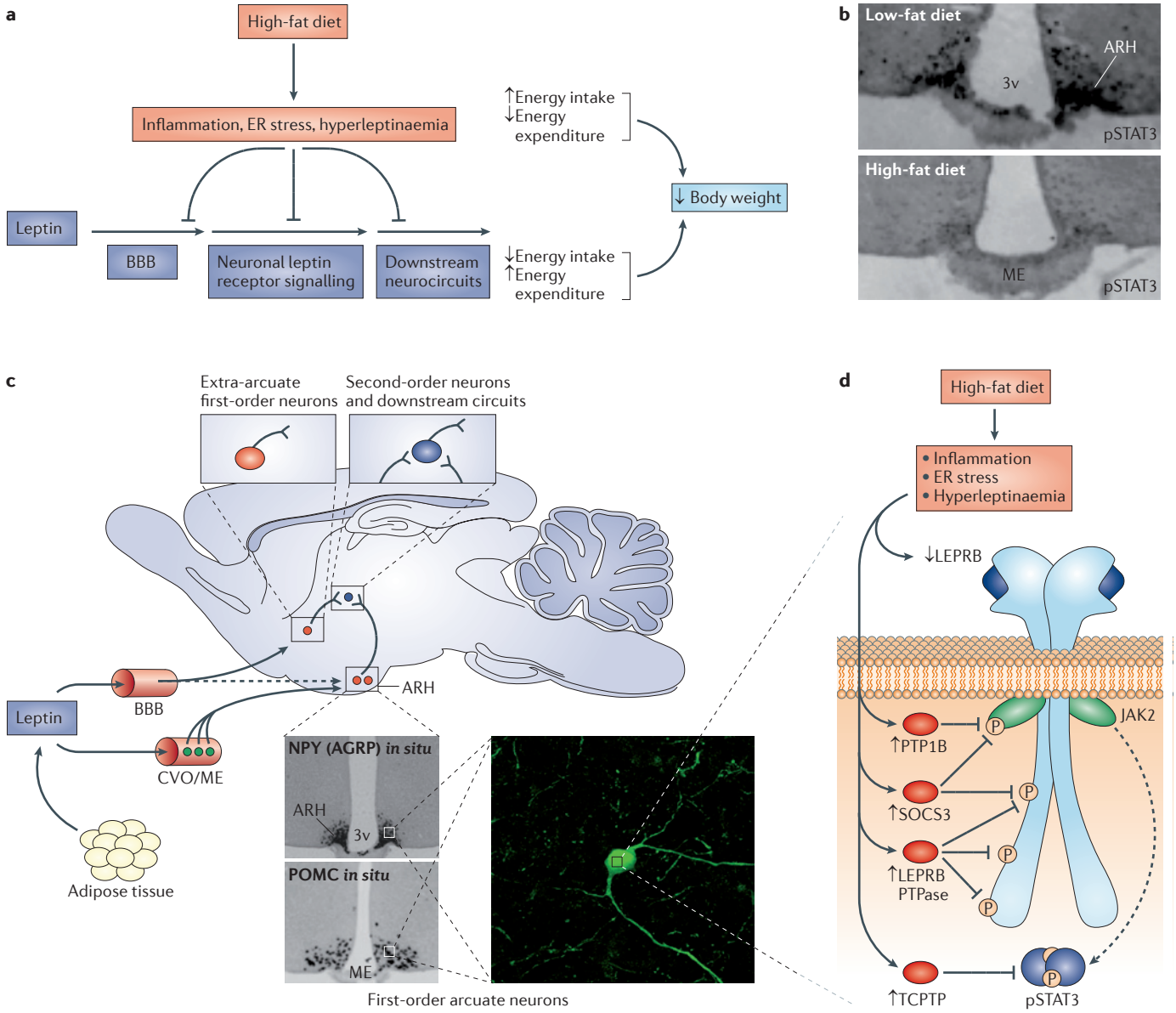


Figure 2 | **Cellular mechanisms that cause leptin resistance in rodents.** **a** | Leptin normally inhibits fat accumulation and weight gain by entering the brain to decrease caloric intake and increase energy expenditure. In rodents, consumption of a high-fat diet causes hyperleptinaemia[78], hypothalamic endoplasmic reticulum (ER) stress[214] and the production of pro-inflammatory cytokines[215], neuronal leptin resistance and diminished anti-obesity actions of leptin. This leptin resistance is caused by a defect in leptin transport across the blood–brain barrier (BBB), leptin receptor (LEPR) signalling in neurons expressing the long isoform (LEPRB) and/or in downstream circuits. **b** | Leptin-activated phosphorylated signal transducer and activator of transcription 3 (pSTAT3) immunoreactivity is reduced in the arcuate nucleus of the hypothalamus (ARH) of mice fed a high-fat diet. Because pSTAT3 is a functional marker for LEPRB signalling in LEPRB-expressing (first-order) neurons, this diminished pSTAT3 response to leptin in mice fed a high-fat diet indicates cellular leptin resistance within these neurons[60,207]. **c** | Leptin normally enters most parts of the brain and reaches its target neurons via transport across the BBB. The ARH, however, is in close anatomical proximity to the median eminence (ME), a circumventricular organ (CVO) with fenestrated capillaries (as illustrated by the green circles), therefore leptin may reach its first-order LEPRB-expressing neurons within the ARH without being actively transported across the BBB[66,67]. The first-order neurons in the ARH include the pro-opiomelanocortin (POMC)- and Agouti-related protein (AGRP)-expressing neurons (as shown in the insets). **d** | Hyperleptinaemia[78], ER stress[214] and/or inflammation[215] induced by a high-fat diet cause leptin resistance within POMC- and AGRP-expressing neurons[61,62]. Because activation of pSTAT3 is diminished in these neurons[61,62], by exclusion the signalling defect must be located upstream of STAT3 phosphorylation. Mice that are given a high-fat diet display increased expression of negative regulators of proximal LEPRB signalling in the ARH: namely, protein tyrosine phosphatase 1B (PTP1B), suppressor of cytokine signalling 3 (SOCS3) and T cell protein tyrosine phosphatase (TCPTP)[60,72]. Additional possibilities that may explain neuronal leptin resistance include the inhibition of LEPRB surface expression or increased expression of as-yet unidentified LEPRB tyrosine phosphatases (induced by a high-fat diet). 3v, third ventricle; NPY, neuropeptide Y; PTPase, protein tyrosine phosphatase. Images courtesy of R.C. and C.B.

signalling remains reduced in DIO rodents following intracerebroventricular (i.c.v.) administration of the hormone[57,216]. Together, these data suggest that intracellular signalling is impaired in neurons that express LEPRs (this is termed neuronal or cellular leptin resistance).

The LEPRB isoform has a long intracellular domain that is capable of activating several intracellular signalling pathways, including the STAT3 pathway[58] (FIG. 1). The LEPRB–STAT3 pathway is vital for the anti-obesity actions of leptin[59]. LEPRB is expressed in numerous hypothalamic and extra-hypothalamic regions of the brain. Importantly, activation of STAT3 phosphorylation by LEPRB is impaired in some — but not all — neuronal groups in the brain of DIO rodents. Specifically, neurons within the ARH exhibit reduced STAT3 activation (FIG. 2b), whereas LEPRB-expressing neurons elsewhere in the brain appear to have relatively normal leptin sensitivity[60], indicating that LEPRB-expressing neurons in the ARH have a key role in the development of leptin-resistant obesity. Not surprisingly, the leptin-resistant ARH neurons in DIO mice include the Agouti-related protein (AGRP)- and POMC-expressing neurons[61,62], which mediate at least part of the anti-obesity effects of leptin[63,64].

The ARH is in close anatomical vicinity to the median eminence — a circumventricular organ that lacks a BBB — so ARH neurons may have direct access to blood-borne leptin. Although some studies argue against this possibility[65], others support it because large proteins that cannot pass the BBB can reach the ARH by passive diffusion[66]. In addition, LEPRB-expressing neurons in the ARH appear to be more sensitive to low doses of leptin than LEPRB-expressing neurons located in other regions of the brain that are protected by the BBB[67]. Together, these data suggest that the leptin resistance of ARH neurons (in animals with diet-induced obesity) is not caused by defective leptin transport into the brain, but instead by a defect in LEPRB-mediated signal transduction in first-order neurons (FIG. 2c). By exclusion, the resistant site should be localized upstream of STAT3 phosphorylation. Theoretical possibilities include reduced LEPRB surface expression, downregulation of positive regulators or upregulation of negative regulators of the LEPRB–STAT3 pathway.

The identification of suppressor of cytokine signalling 3 (SOCS3) as a leptin-inducible feedback inhibitor of LEPRB-induced Janus kinase 2 (JAK2) activity[68], and reports demonstrating increased *Socs3* mRNA expression in the hypothalamus of leptin-resistant animals[60,61], provided an attractive molecular mechanism to explain neuronal leptin resistance[69–71] (FIG. 2d). Increased levels of protein tyrosine phosphatase 1B (PTP1B (also known as PTPN1); a JAK2 tyrosine phosphatase) and of the related T cell protein tyrosine phosphatase (TCPTP (also known as PTPN2); a STAT3 tyrosine phosphatase) in rodents with diet-induced obesity may also contribute to neuronal leptin resistance[62,72,73].

Despite these advancements, the specific mechanism (or mechanisms) by which a high-fat diet increases hypothalamic levels of SOCS3, PTP1B and TCPTP are still unclear. Possibilities include the activation of inflammatory pathways[74–76] and endoplasmic reticulum stress[77]. Although paradoxical and not understood, hyperleptinaemia itself may also cause leptin resistance[78], possibly by increasing SOCS3 and PTP1B expression[79,80]. In conclusion, leptin resistance in the neurons that mediate the antidiabetic and anti-obesity actions of leptin may represent a major roadblock for the therapeutic use of leptin in patients who suffer from obesity and type 2 diabetes.

First-order neurons
In a neurocircuitry aimed at orchestrating responses to changes in a circulating cue, first-order neurons are equipped with the molecular tools to monitor the levels of the circulating cue (for example, they express the cognate receptor for the circulating ligand).

Discovery of glucoregulatory actions of leptin

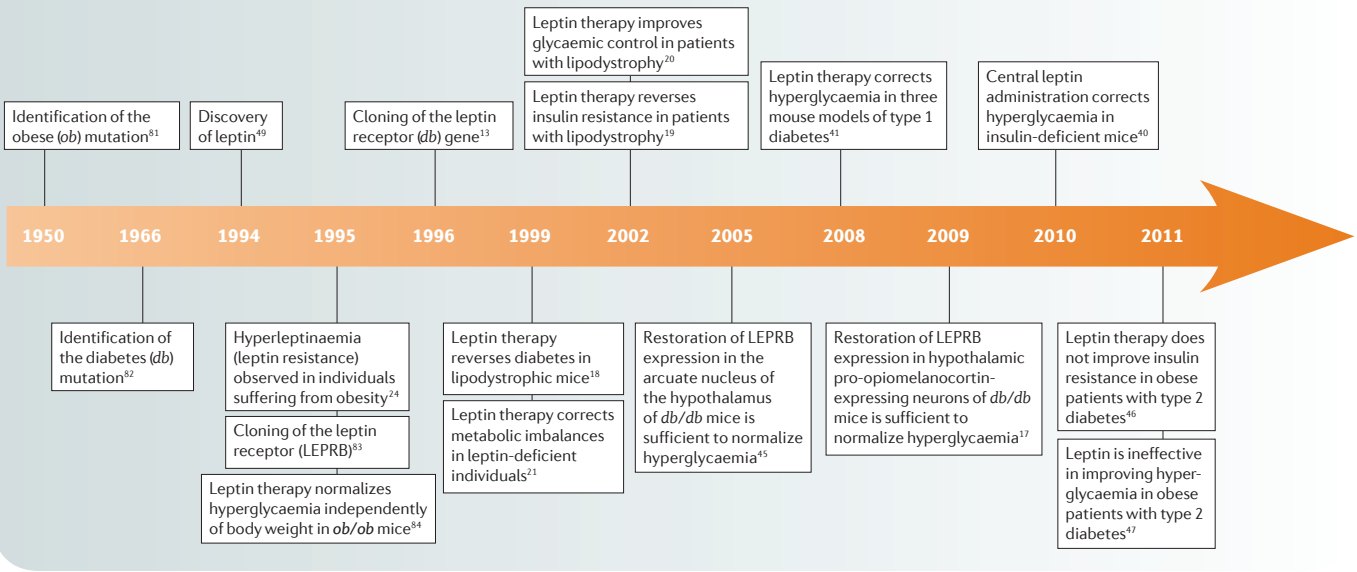
The landmark discoveries of the beneficial effects of leptin on glucose metabolism are shown in the TIMELINE. The phenotypes of mice that are homozygous for the autosomal recessive mutations termed *ob* (for obesity) and *db* (for diabetes) were first described in 1950 and 1966, respectively[81,82]. In the mid-1990s, the gene that is mutated in the highly obese (and diabetic) *ob/ob* mouse[49] was identified and the gene product was named leptin. This was soon followed by the identification of LEPRs[13,83]. Following the production of recombinant leptin, it was shown that low doses of the hormone that did not reduce food intake and body weight almost normalized the severe hyperglycaemia displayed in *ob/ob* mice[84]; this study represents the first demonstration that the effects of leptin on glycaemia can be separated from its effects on body weight and food intake.

This idea was bolstered by the demonstration that restricting untreated *ob/ob* mice to consume the same amount of food as leptin-treated *ob/ob* mice was not sufficient to fully recapitulate the glucose-lowering action mediated by leptin therapy[85]. Additional evidence supporting a direct glucoregulatory action of leptin came from the observation that leptin administration corrects the severe insulin resistance and hyperglycaemia displayed in rodents with lipodystrophy; this effect was also independent of a change in body weight[18]. Importantly, beneficial results of leptin on insulin sensitivity and glycaemic control in humans were observed in 2002 when leptin was first given to insulin-resistant patients with lipodystrophy[19,20]. However, in 2011 two studies reported that leptin was ineffective in improving insulin sensitivity and glycaemic control in obese individuals with type 2 diabetes[46,47].

Interestingly, leptin might have antidiabetic (type 1) actions, as the delivery of leptin to hypoinsulinaemic rodent models of type 1 diabetes diminished hyperglycaemia[86,87]; this apparent insulin-independent action of leptin in rodents was mediated by the CNS[40]. Consistent with this possibility of antidiabetic (type 1) actions of leptin, long-term leptin therapy was reported to be effective in improving glycaemic control in two patients with type 1 diabetes, as described above[42].

Following these discoveries, there has been progress in the identification of specific neurons that are capable of mediating glucose regulation by leptin in hyperinsulinaemic rodent models of type 2 diabetes, and there have been advancements in understanding mechanisms underlying the antidiabetic effects of leptin in mouse models of type 1 diabetes that completely lack insulin; these results are discussed below.

Timeline | **Milestones in the discovery of the antidiabetic actions of leptin**



CNS and efferent antidiabetic mechanisms

Central mechanisms. Specific sites in the brain that are capable of mediating the antidiabetic actions of leptin in models of type 2 diabetes were first identified in 2005, when it was reported that restoration of *Lepr* expression only in ARH neurons normalizes hyperglycaemia in obese, hyperinsulinaemic and severely diabetic *Lepr*-null (*db/db*) mice, without affecting body weight and food intake[45]. Furthermore, selective expression of LEPRB only in the POMC-expressing neurons within the ARH of the LEPRB-deficient *db/db* mice was sufficient to mediate this remarkable glucose normalization[17]. These results identified POMC-expressing neurons in the ARH as principal candidates for mediating antidiabetic actions of leptin in a mouse model of type 2 diabetes (FIG. 3a).

Although the re-expression of LEPRB in POMC-expressing neurons fully corrects hyperglycaemia in very young *db/db* mice, the effect is only partial with increasing age[17]. In addition, selective deletion of LEPRs from POMC-expressing neurons in wild-type mice does not precipitate overt hyperglycaemia[63]. These data suggest that other neurons, in addition to POMC-expressing neurons, have a role in leptin-mediated glycaemic control. The ARH also contains AGRP-expressing neurons that, similarly to POMC-expressing neurons, express LEPRB[64]. AGRP is a key neuropeptide component of the central melanocortin system, and acts as an antagonist of α-melanocyte stimulating hormone (α-MSH) at the melanocortin 4 receptor (MC4R)[88]. This receptor is widely expressed throughout the CNS in neurons that are targeted by axons from POMC- and AGRP-expressing neurons[89,90], and are as such second-order neurons with respect to first-order LEPR-expressing neurons. Loss of MC4Rs causes obesity in both mice and humans[88]. Several studies have implicated the central melanocortin system in glycaemic control[91,92], as discussed below.

In addition, the virally mediated reactivation of endogenous LEPRs in the ARH of *Lepr*-null mice presumably targeted both POMC- and AGRP-expressing neurons[45]. Because blood glucose levels are completely normalized with increasing age in these mice (in contrast to the partial correction of hyperglycaemia in mice of the same age with only POMC-selective re-expression of LEPRB), the combination of the above data at least suggest that AGRP-expressing neurons may also have a role in mediating glucose regulation by leptin (FIG. 3a). Preliminary experiments (C.B., unpublished observations) that assessed glycaemia in *db/db* mice expressing LEPRB only in AGRP-expressing neurons suggest that these cells also have the capacity to markedly reduce the severe hyperglycaemia of this model.

The above studies raise the question of how leptin acts via one group of neurons (for example, POMC-expressing neurons) to independently influence both peripheral glucose metabolism and energy balance. Several non-mutually exclusive possibilities can be proposed: first, POMC-expressing neurons are a heterogeneous population of cells, each serving distinct functions; second, POMC-expressing neurons produce and secrete different neurotransmitters and neuropeptides, each serving separate metabolic functions; third, different intracellular LEPRB signalling pathways regulate the effects of leptin on glucose versus energy balance.

There is evidence to support the notion that POMC-expressing neurons are highly heterogeneous. For example, leptin activates some (30–90%), but not all, POMC-expressing neurons[93]. Similarly, insulin has been reported to affect (namely inhibit) only subsets of POMC-expressing neurons[93–96]. It remains unclear whether the leptin-responsive POMC-expressing neurons are insulin-sensitive or not, adding further complexity[93,97]. In addition, POMC-expressing neurons produce various different neuropeptides (such as

Second-order neurons
In a neurocircuitry aimed at orchestrating responses to changes in a circulating cue, second-order neurons receive direct synaptic inputs from first-order neurons.

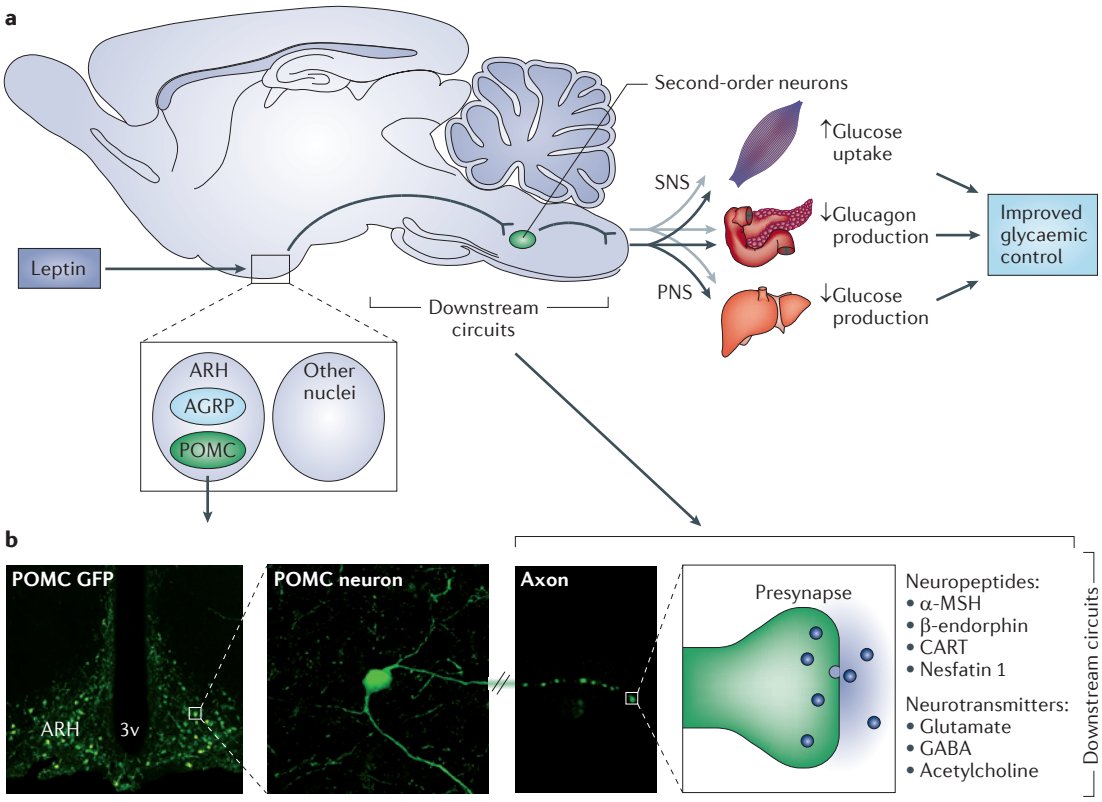


Figure 3 | **Mediators, pathways and mechanisms underlying the antidiabetic actions of leptin. a** | Schematic model of central neuronal pathways and efferent processes wherein leptin exerts its antidiabetic actions in insulin-resistant obese mice (for example, genetically modified *db/db* rodent models of type 2 diabetes). Pro-opiomelanocortin (POMC)-expressing neurons in the arcuate nucleus of the hypothalamus (ARH) have the capacity to mediate improved glucose control by leptin in the *db/db* rodents, although Agouti-related protein (AGRP)-expressing neurons and other hypothalamic and extra-hypothalamic neurons are also likely to have important roles. These neurons act via axonal projections on downstream neurocircuits (for example, the melanocortin system) to engage efferent pathways. This may include the regulation of sympathetic and parasympathetic branches of the autonomic nervous system, ultimately affecting muscle glucose uptake, pancreatic glucagon production and/or hepatic glucose production. **b** | Leptin target neurons in the ARH, including POMC-expressing neurons, produce and secrete various different molecules from axon terminals, including the POMC polypeptide-derived neuropeptides α-melanocortin stimulating hormone (α-MSH) and β-endorphin. Cocaine- and amphetamine-regulated transcript (CART) and nesfatin 1 are also co-expressed with POMC peptides. Finally, these ARH neurons are heterogeneous with regard to neurotransmitter phenotype; different subpopulations produce glutamate, GABA (γ-aminobutyric acid) and acetylcholine. Combined, these neurotransmitters and neuropeptides are the candidate effector molecules for mediating glycaemic control by leptin in the *db/db* model. 3v, third ventricle; GFP, green fluorescent protein; PNS, parasympathetic nervous system; SNS, sympathetic nervous system. Images courtesy of R.C. and C.B.

α-MSH, β-MSH, γ-MSH, adrenocorticotropic hormone and β-endorphin) that are derived from the POMC polypeptide precursor[98], as well as other neuropeptides such as cocaine- and amphetamine-regulated transcript (CART)[99] and nesfatin 1 (REF. 100). Furthermore, POMC-expressing neurons appear to be highly heterogeneous with regard to neurotransmitter phenotype; they can be GABAergic, glutamatergic or cholinergic[14,101,102] (FIG. 3b). Additional studies are clearly needed to understand the specific roles of each of these heterogeneous POMC-expressing neuronal populations and POMC-secreted molecules on glucose metabolism versus energy balance.

LEPRB regulates several downstream intracellular signalling pathways. In particular, four motifs of the murine LEPR (that is, the proximal JAK2-binding box, Tyr985, Tyr1077 and Tyr1138) regulate largely distinct intracellular signalling pathways (FIG. 1). Dissecting the contribution of each motif to specific cellular and whole-body processes is an active area of research. For example, global knock-in of a serine at Tyr1138, the activation site for STAT3, leads to obesity and hyperphagia that is nearly as severe as that of *db/db* mice (which entirely lack LEPRB signalling)[59], suggesting that STAT3 and its nuclear gene targets have a major role in the regulation of appetite and whole-body energy balance. Linear growth and fertility, however, remain intact, indicating that other LEPRB motifs and non-STAT3 pathways regulate these actions of leptin[103].

Global mutation of all three Tyr residues of LEPRB leads to severe hyperphagia and obesity that is similar to that observed in mice with a mutation in Tyr1138 alone[59]. Interestingly, these triple-mutant mice are reportedly

nearly normoglycaemic, indicating that non-tyrosine-mediated signalling events (for example, the JAK2–PI3K (phosphoinositide 3-kinase) pathway) might mediate the effects of leptin on glucose. Consistent with this notion, carboxy-terminal truncation of LEPRB (leaving only the proximal JAK2-binding motif intact) delays the onset of diabetes while increasing adipose mass at a similar rate to that seen in *db/db* mice[104]. Some caution should, however, be taken with regard to the interpretations of the above data because the diabetic phenotype of *db/db* (and *ob/ob*) mice depends greatly on the genetic background[105], which may not be the same between studies and even within studies in some cases. In addition, the identity of the important LEPRB-expressing neurons was not identified in these global mutational studies[59,104].

Despite these limitations, the LEPRB mutational studies suggest that different LEPRB signalling pathways may regulate largely distinct whole-body metabolic functions and that proximal LEPRB signalling pathways may be of particular importance for leptin-mediated glycaemic control.

Neuron-specific genetic manipulations of intracellular proteins in POMC-expressing neurons support an important role of these particular cells in the control of glucose homeostasis. For example, SOCS3 deletion in POMC-expressing neurons in mice lowers blood glucose concentrations without influencing body weight and adiposity[71]. In addition, mice lacking PTP1B in POMC-expressing neurons have normal body weight and fat mass but exhibit increased whole-body insulin sensitivity[106].

Although several CNS regions and specific neurons are known to act as glucose sensors, it is interesting that POMC-expressing neurons possess glucose-sensing capabilities[107]. Abolishment of glucose sensing only in POMC-expressing neurons impairs glucose tolerance[108]. Thus, the anatomical location of POMC-expressing neurons — near the median eminence — uniquely places them as a key component of both an acute and long-term homeostatic rheostat, possibly by allowing the direct sensing of hormones (for example, leptin) and metabolites (for example, glucose) in the circulation, combined with the ability of this group of cells to influence glucose balance.

Various studies have investigated the cellular function and whole-body metabolic roles of PI3K in POMC-expressing neurons. For example, deletion of PI3K α-subunits (PI3K regulatory subunit 1 (PIK3R1); also known as p85) specifically in POMC-expressing neurons indicates that the PI3K pathway is required for the acute electrical effect (that is, axonal firing) of leptin (and glucose) on POMC-expressing neurons[109]. Genetic studies of PI3K are, however, complicated by the existence of different isoforms. In addition, alteration of PI3K activity will not only affect LEPRB signalling but also affect other cellular functions and pathways, including those of insulin. With these limitations in mind, it has been reported that genetically mediated alteration of PI3K activity in POMC-expressing neurons affects circulating insulin levels and hepatic insulin sensitivity without causing changes in whole-body energy balance[110]. This result, combined with the metabolic analyses of mice with the various LEPRB mutations (in Tyr residues) described above, supports the possibility that the PI3K pathway may be involved in glycaemic control mediated by POMC-expressing neurons. However, further investigations are required to identify the key downstream PI3K signalling pathways and cellular processes.

Roles of the central melanocortin system, the autonomic nervous system and peripheral target tissues. A substantial body of evidence shows that the autonomic nervous system can exert control on glucose homeostasis via its actions on the endocrine pancreas[111], skeletal muscle[112], liver[113] and adipose tissue[114]. It is therefore tempting to speculate that the antidiabetic actions of leptin in the CNS are mediated via the sympathetic and/or parasympathetic nervous systems. Indeed, ARH neurons (for example, POMC- and AGRP-expressing neurons) send projections to the paraventricular hypothalamic nucleus, where a group of pre-autonomic (sympathetic and parasympathetic) neurons reside[115,116]. In addition, a subset of POMC-expressing neurons project directly to the intermediolateral nucleus of the spinal cord, where sympathetic preganglionic cholinergic neurons reside[117]. Importantly, the neurons in the paraventricular hypothalamic nucleus and the intermediolateral nucleus express MC4Rs[90].

Alternatively, or in addition, leptin may regulate peripheral glucose metabolism via ARH-mediated synaptic actions on parasympathetic preganglionic neurons located in the dorsal motor nucleus of the vagus nerve (these neurons also express MC4Rs)[90]. Pharmacological and genetic studies have shed light on the possible role of the melanocortin system in leptin-mediated glycaemic control[118–120]. However, pharmacologically induced activation of the central melanocortin system is not sufficient to alleviate streptozotocin (STZ)-induced diabetes, suggesting that other circuits are important[121]. Indeed, mice and humans lacking functional MC4Rs are not diabetic[122], demonstrating that if defects in the melanocortin system have a role in the development of blood glucose imbalance (a characteristic of diabetes) then other processes must also be defective.

Increased hepatic glucose production is the major contributor to fasting hyperglycaemia in individuals with diabetes[123]. Hyperinsulinaemic euglycaemic clamp studies in normal rats and in *ob/ob* mice show that i.c.v. administration of leptin primarily inhibits hepatic glucose production without having significant effects on glucose disposal[113,124]. In addition, virally mediated re-expression of LEPRB in unspecified ARH neurons of LEPRB-deficient Koletsky rats inhibits hepatic glucose production without influencing glucose disposal[125]. Interestingly, this effect is lost after hepatic vagotomy, indicating a possible requirement of parasympathetic nerve activity and suggesting the existence of a leptin–ARH–parasympathetic–liver axis[113,125]. However, because nerve branches to other organs may also have been severed in these studies, the possibility of an indirect mechanism cannot be excluded. In addition, the specific neurons involved in linking leptin to parasympathetic control of glucose balance have not yet been identified, although the POMC-expressing neurons (and AGRP-expressing

Koletsky rats
Rats that are homozygous for a nonsense mutation in the leptin receptor gene, causing lack of leptin receptor function and hence altered metabolic homeostasis.

Hepatic vagotomy
Severing of the hepatic branch of the vagus nerve, resulting in lack of vagal efferent and afferent innervations of the liver.

neurons) are attractive candidates. In contrast to this proposed role of parasympathetic nerve activity to the liver, other studies point to the regulation of hepatic sympathetic activity[91]. Although LEPRB in the ARH is required for leptin to stimulate sympathetic nerve activity to renal and brown adipose tissues[126], direct measurements of leptin-dependent regulation of hepatic sympathetic and parasympathetic activity have not been reported, and will need to be measured further in conscious animals.

Although the above studies support a role of autonomic innervation of the liver in whole-body glucose balance, it is important to consider that denervation of the liver does not alter glycaemia in rodents (for example, following vagotomy) or humans (for example, following liver transplants). In addition, hepatic deletion of muscarinic acetylcholine receptors (the receptors for the primary neurotransmitter secreted from postganglionic parasympathetic neurons) does not affect glucose homeostasis[127]. Studies are therefore needed to determine whether other neurotransmitters are important for mediating parasympathetic activity via leptin and whether autonomic control of hepatic glucose metabolism, compared to efferent nerve regulation of other organs and tissues (such as the pancreas, fat or muscle), is of greater relevance in the antidiabetic actions of leptin.

In parallel with the possible direct neural (that is, autonomic) connections listed above, it is well known that the hypothalamus can also affect glycaemia through neuroendocrine mechanisms such as the hypothalamic–pituitary–adrenal axis and the hypothalamic–pituitary–thyroid axis, as well as through sympathoadrenal efferents, hence influencing circulating levels of glucocorticoids, thyroid hormones and catecholamines. Despite considerable progress over the past decade, the relative contributions of neuroendocrine versus autonomic pathways in mediating the glucoregulatory actions of leptin are unknown and warrant further research. Insulin-like growth factor binding protein 2 (IGFBP2) has been reported to mediate a proportion of the antidiabetic actions of leptin[128]. Studies aimed at determining a potential involvement of the autonomic system, and at determining whether IGFBP2 has a role downstream of LEPRB in POMC-expressing neurons, deserve further investigation[128,129]. Finally, additional studies are required to determine under which specific physiological and pathophysiological circumstances the leptin–hypothalamic–peripheral tissue axis influences the liver, endocrine pancreas and/or skeletal muscle to ultimately govern glucose balance.

Glucoregulatory actions in the absence of insulin

Until recently there was a general consensus that circulating insulin is required for the antidiabetic actions of leptin (for example, in *ob/ob* and *db/db* models of type 2 diabetes and in patients with lipodystrophy). Even in STZ-treated rodents, insulin-producing β-cells were not entirely ablated in all studies, leaving a residual level of circulating insulin[87,130,131]. However, because leptin administration improves diabetes and increases the survival of animals that completely lack insulin[40,41,132,133], the view that insulin is required for the antidiabetic effects of leptin needs to be amended. Indeed, many of the metabolic imbalances studied in these papers and the lethal effects of total insulin deficiency were reversed by leptin monotherapy without the need for insulin co-administration. Furthermore, adenovirally or pharmacologically induced hyperleptinaemia rescues hyperglycaemia in various animal models of type 1 diabetes, including the non-obese diabetic mice as well as rat models of STZ- and alloxan-induced diabetes[41,132]. Importantly, these effects were not secondary to the anorectic effects of leptin[40,41,132].

Similar results have been described when leptin was subcutaneously administered in a mouse model of whole-body insulin receptor deficiency; this intervention also led to remarkable beneficial effects on diabetes symptoms[133]. In addition to its glycaemia-lowering action, leptin monotherapy ameliorated several other metabolic defects brought on by insulin deficiency, such as hyperglucagonaemia, polyuria and hyperketonaemia, and normalized reduced hepatic glycogen content[41,132]. It must be noted that some of these changes may be the result of alleviating hyperglycaemia.

Despite the importance of the above-mentioned observations, the mechanism (or mechanisms) underlying the antidiabetic (type 1) actions of leptin still need to be determined. For example, is this effect due to the direct action of leptin on glucagon-producing pancreatic α-cells? This possibility seems to be unlikely because leptin administration does not suppress glucagon secretion from cultured α-cells[134]. Alternatively, does LEPRB signalling in hepatocytes mediate the antidiabetic (type 1) action of leptin? This idea also seems to be unlikely because leptin therapy alleviates hyperglucagonaemia and diabetes in mice with type 1 diabetes that selectively lack LEPRs in hepatocytes[135]. The possibility that LEPRB signalling in the brain is the key mechanism underpinning the antidiabetic actions of leptin is strongly supported by a study in which CNS-restricted leptin administration normalized hyperglycaemia and hyperglucagonaemia (as well as other metabolic defects) in mice with type 1 diabetes; this effect is similar to the effects observed after systemic leptin administration in these mice[40].

Because of the aforementioned findings and the importance of hypothalamic LEPRB in regulating glucose homeostasis in models of type 2 diabetes such as *db/db* mice[17,44,45,125], it is tempting to speculate that LEPRB-expressing neurons within the hypothalamus may also mediate the beneficial effects of leptin in the context of type 1 diabetes. Unfortunately, there is still a lack of evidence supporting this contention and future studies will be required to directly test this hypothesis; these studies would involve testing the antidiabetic (type 1) action of leptin in genetically engineered mice that either lack LEPRB or express it only in discrete hypothalamic neurons. There is also a need to examine the antidiabetic potency of leptin in alternative animal models of type 1 and type 2 diabetes because STZ-treated and *db/db* mice are not generally representative models of the clinical aetiologies of type 1 and type 2 diabetes, respectively.

Deciphering the cellular and molecular mechanism (or mechanisms) by which leptin therapy suppresses hyperglycaemia and permits the survival of insulin-deficient rodents is likely to be instrumental for developing better

Insulin-like growth factor binding protein 2
(IGFBP2). A protein that is primarily produced by the liver and that may mediate a proportion of the antidiabetic actions of leptin.

Alloxan
A pyrimidine derivative taken up by cells via facilitated transport through glucose transporter 2; this compound is used in research laboratories to destroy insulin-producing cells in animals.

Polyuria
Abnormally elevated levels of urine production; a condition that can be seen in patients with uncontrolled diabetes.

Hyperketonaemia
A condition characterized by a high level of ketone bodies in the blood; this can be seen after prolonged fasting, the use of a ketogenic diet or due to lack of insulin, typically in individuals with type 1 diabetes.

Pancreatic α-cells
Endocrine cells of the pancreas that secrete the hormone glucagon; the proper functionality of these cells is crucial for preventing a life-threatening reduction in blood glucose levels.

antidiabetic approaches. As discussed below, there are several potential drawbacks of leptin use in patients with diabetes; for example, such patients often also suffer from obesity and are leptin-resistant, and thus poorly responsive to exogenous leptin[46]. Thus, a detailed understanding of the mechanisms by which leptin exerts its glucose-lowering effect in type 1 and type 2 diabetes will be crucial for eventually circumventing these expected shortcomings of leptin therapy in patients. For example, an important role of hyperglucagonaemia in the pathophysiology of diabetes has been suggested[136] — an idea bolstered by the fact that mice that lack the glucagon receptor are protected from developing diabetes[137]. Therefore, if LEPRB on POMC-expressing neurons was to mediate the antidiabetic action of leptin by suppressing glucagon secretion, once identified, this POMC-neuron-dependent pathway could be exploited for the development of more effective antidiabetic agents. This idea is tangible as emerging evidence indicates that LEPRs in POMC-expressing neurons may have a role in regulating glucagon secretion[16].

Regardless of these issues, the aforementioned findings in rodents with type 1 diabetes spurred enthusiasm on the therapeutic antidiabetic potential of leptin. As mentioned above, a clinical trial (ClinicalTrials.gov identifier: NCT01268644) is underway.

Leptin as an antidiabetic drug: pros and cons

Leptin is remarkably effective in improving hyperglycaemia in some animal models of type 1 diabetes[40,41,132] and type 2 diabetes[43,45,138], and it substantially increases insulin sensitivity in patients with lipodystrophy[18,20]. Whether leptin will be able to exert similar beneficial effects in models that better represent the human aetiology and, more importantly, in non-lipodystrophic patients with diabetes (who represent the majority of the diabetic population) is the subject of ongoing discussions, with both optimistic and cautious points of view.

Based on the conspicuous data obtained from preclinical investigations (mainly studies in rodents) and results from clinical studies (mainly in individuals with lipodystrophy), the expected benefits of leptin therapy in type 1 and type 2 diabetes can be recapitulated in the following ways: first, leptin has not been reported to induce hypoglycaemia in rodent models of diabetes[40] and patients with diabetes[139] and, as such, its slow-acting glycaemia-lowering action would be preferred over the fast-acting glycaemia-lowering action of insulin that is known to trigger hypoglycaemic events; second, because leptin administration decreases lipid contents in adipose and extra-adipose tissues[40], its effects on lipid metabolism would also be preferred over the ones exerted by insulin, which is known to promote lipid accumulation in adipose and extra-adipose tissues; third, leptin suppresses appetite[40] and this effect is desirable in individuals with diabetes. Therefore, if leptin was to overcome the potential problems discussed below, it could become an effective adjuvant or alternative treatment against diabetes without having the risk of inducing hypoglycaemia and/or lipid-induced cardiovascular defects.

However, there are several potential pitfalls that will need to be overcome if leptin therapy is to become an attractive addition to the antidiabetic pharmacopoeia. For example, some pharmacological studies in rodents show that leptin increases arterial pressure[140], although this does not appear to happen when leptin is co-administered with amylin[141]. Also, people who have leptin deficiency suffer from hypotension despite being highly obese[140]. Hence, it cannot be ruled out that prolonged leptin administration may cause increased blood pressure in humans, which is an undesirable effect in individuals with diabetes who are already prone to hypertension. In addition, leptin has been reported to accelerate autoimmune diabetes in the non-obese diabetic model of type 1 diabetes, possibly by promoting pro-inflammatory cell responses[142].

Another obstacle is probable leptin resistance. Obesity and type 2 diabetes go hand in hand; many obese people are also affected by glucose and/or insulin imbalance. Because the majority of patients with type 2 diabetes are overweight or obese — and have hyperleptinaemia — leptin therapy might be ineffective, as indicated by the results of recent clinical trials[46]. These potential problems may also apply to individuals with type 1 diabetes. It is also possible that leptin therapy may cause leptin-induced leptin resistance in humans. If this happens, then ways to first dampen leptin resistance and hyperleptinaemia may be needed. Finally, not all people with type 2 diabetes suffer from obesity and have hyperleptinaemia[24]. This subgroup of patients is therefore presumably relatively normal in this aspect to leptin action (that is, they are not leptin-resistant) and might benefit from leptin therapy.

However, leptin resistance may not necessarily prevent the use of leptin as a drug for type 2 diabetes. For example, data suggest that insulin resistance negatively affects some insulin receptor signalling pathways (for example, the pathway mediated by AKT and forkhead box protein O1), whereas others appear to be unaffected (for example, the sterol regulatory element-binding protein 1 signalling pathway)[1]. Although currently speculative, this bifurcation may also exist downstream of LEPRB signalling. For example, leptin resistance may selectively affect LEPRB signalling pathways that mediate body weight reduction without affecting the pathways that are important for mediating glucose regulation. The idea that different intracellular components mediate the distinct actions of leptin on body weight versus glucose homeostasis is supported by experimental data, as discussed above. For example, although LEPRB-mediated phosphorylation of STAT3 is crucial for leptin-mediated suppression of food intake and body weight[59], this pathway seems to be less important for the ability of leptin to improve glucose homeostasis[59]. This concept is further bolstered by results showing that pharmacological blockade of hypothalamic PI3K signalling impairs the insulin-sensitizing effect of leptin[44]. Data from rodents also show that low doses of leptin can correct hyperglycaemia without affecting body weight or food intake. These results indicate that the pathways underlying the glucose-lowering actions of leptin are more sensitive than the ones underpinning its anti-obesity effects.

Therefore, it is tantalizing to insinuate that leptin resistance may not represent an insurmountable obstacle for the antidiabetic actions of leptin. Although results from a recently completed clinical trial seem to refute this hypothesis, the patients enrolled in this trial were highly obese (and probably had exaggerated leptin resistance) and the duration of leptin treatment was very short (2 weeks)[46]. As such, it will be important to test the effectiveness of leptin therapy in patients with diabetes who are less obese and to also extend the treatment for several months.

In addition to the aforementioned potential problems of leptin therapy in diabetes, the known stimulatory effects of the hormone on PI3K signalling should not be overlooked. The PI3K pathway is important for normal cellular proliferation[143] and crucial for the growth of certain types of cancers[143,144]. Therefore, leptin (as well as insulin) therapy has the potential to facilitate tumour growth by impinging on this signalling cascade[145]. If leptin was to be widely prescribed for the management of diabetes, either as an alternative or adjunct approach, a careful analysis of tumour progression in patients who also have cancer will be important. Other potential shortcomings of leptin therapy include the generation of neutralizing autoantibodies against the exogenous hormone[29,146,147], increased immune system function and inflammation[148] as well as loss of bone mass[149,150].

Concluding remarks

Originally heralded as the anti-obesity panacea, followed by doubts about its effectiveness, leptin could still become an important asset in the armamentarium against diabetes for patients with type 1 diabetes, type 2 diabetes or lipodystrophy-induced diabetes. As discussed above, the failure of leptin as a general anti-obesity medicine probably rests on the fact that most people with increased adipose mass are resistant to the actions of the hormone on suppressing food intake and lowering body weight. However, not all patients with diabetes are obese and hence some may respond to the metabolic actions of exogenously administered leptin.

The development of leptin-based approaches to treat diabetes has generated considerable enthusiasm because leptin therapy has remarkable efficacy in ameliorating or entirely correcting diabetes in some animal models of type 1 and type 2 diabetes, and also because of the findings that long-term leptin replacement therapy is well tolerated and markedly improves glycaemic control, insulin sensitivity and plasma triglyceride levels in patients with severe insulin resistance resulting from generalized lipodystrophy. Nevertheless, results from recent clinical trials seem to reject the hypothesis that leptin can effectively improve insulin sensitivity in patients with type 2 diabetes who are also highly obese. Because a proportion of the type 2 diabetes population is not highly obese, it will be important to ascertain whether leptin therapy improves insulin sensitivity in non-obese, leptin-sensitive individuals who suffer from type 2 diabetes. In addition, it will be important to find out whether leptin therapy alleviates symptoms of type 1 diabetes.

In the event that leptin fails to improve diabetes symptoms in the vast majority of patients affected by either form of diabetes, there should be ongoing research aimed at uncovering the central circuits, efferent pathways and peripheral processes responsible for mediating the antidiabetic action of the hormone. If leptin resistance proves to be an obstacle for the efficacy of leptin as an antidiabetic therapy, further research aimed at unravelling the underlying molecular mechanisms of the leptin–CNS–glycaemia pathway is clearly warranted, as this may provide opportunities for the identification of new drug targets and therapeutics that can circumvent the blockade in leptin action and ultimately help to improve the quality and length of life of patients with diabetes.

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