Review

MOLECULAR METABOLISM

The glucoregulatory actions of leptin

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ABSTRACT

Background: The hormone leptin is an important regulator of metabolic homeostasis, able to inhibit food intake and increase energy expenditure. Leptin can also independently lower blood glucose levels, particularly in hyperglycemic models of leptin or insulin deficiency. Despite significant efforts and relevance to diabetes, the mechanisms by which leptin acts to regulate blood glucose levels are not fully understood.
Scope of review: Here we assess literature relevant to the glucose lowering effects of leptin. Leptin receptors are widely expressed in multiple cell types, and we describe both peripheral and central effects of leptin that may be involved in lowering blood glucose. In addition, we summarize the potential clinical application of leptin in regulating glucose homeostasis.
Major conclusions: Leptin exerts a plethora of metabolic effects on various tissues including suppressing production of glucagon and corticosterone, increasing glucose uptake, and inhibiting hepatic glucose output. A more in-depth understanding of the mechanisms of the glucose-lowering actions of leptin may reveal new strategies to treat metabolic disorders.

Keywords Leptin; Diabetes; Glucose metabolism

1. INTRODUCTION

The prevalence of diabetes is rising around the world, with the number of people affected predicted to increase to ~642 million in 2040 [1]. This imposes a substantial economic burden on society with over $100 billion being spent in 2013 in the United States alone [2]. Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-producing β-cells and is treated by delivering insulin by injection or pump. However, insulin therapy is not perfect as periods of hyperglycemia occur, which can result in long-term complications such as retinopathy, nephropathy, and neuropathy, and conversely an excess of insulin can result in hypoglycemic episodes that can be deadly. Type 2 diabetes (T2D), which is more common and highly correlated with obesity, is characterized by insulin resistance and eventual β-cell loss, and can be difficult to manage despite current drug treatments. In recent years, the satiety-regulating hormone leptin has garnered excitement as a potential therapeutic for the treatment of diabetes due to its potent body weight and blood glucose lowering effects.

In humans and rodents, leptin is a 167 amino acid protein secreted primarily from white adipose tissue [3] into the bloodstream and can be transported across the blood-brain barrier [4]. Leptin is also expressed in brown adipose tissue (BAT) [5,6], mammary gland [7], placenta [8,9], skeletal muscle [10], stomach [11], and pituitary gland [12]; however, the relative contribution from these tissues to total circulating leptin levels is negligible [13]. In humans and rodents, leptin levels generally correlate with the total amount of body fat, except during fasting [14]. Circulating leptin levels are similar among lean humans, rats, and mice, typically ranging between ~0.5–15 ng/mL [15–21]. In mice, the leptin receptor gene is alternatively spliced to produce six isoforms, LepRa-LepRf [22]. All isoforms, with the exception of LepRe, have identical extracellular and transmembrane domains but differ in length of the intracellular tail [22]. The long form of the leptin receptor (LepRb) is the only isoform capable of Janus kinase – signal transduction and activators of transcription (JAK-STAT) signaling and is the major mediator of the metabolic effects of leptin [22–25]. JAK2 activation autophosphorylates multiple tyrosine kinase residues in rodents, and activation of these tyrosine residues creates a binding site for STAT molecules [26–28]. Phosphorylation of STAT3 results in dimerization and nuclear translocation such that STAT3 acts as a transcription factor to affect various target genes [29], including suppressor of cytokine signaling 3, which acts in a negative feedback loop to impair leptin signaling after prolonged stimulation [30–32]. The LepRb isoform is distributed in both the central nervous system (CNS) [30,33,34] and the periphery [11,35–38].

Rodents lacking the gene encoding leptin (*ob*/*ob* mice or KiloRat™) [18,39–43] or the leptin receptor (*db*/*db* mice, Zucker diabetic fatty rats, and JCR:LA-cp or SHR/N-cp rats) [41,44–49] are commonly characterized by obesity, hyperphagia, insulin resistance, hyperinsulinemia, impaired glucose tolerance, and, in some cases, chronic hyperglycemia. In humans lacking leptin or its receptor due to rare

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mutations, obesity is also evident [25,50–52], and though impairments to glucose tolerance are not as severe as in rodents, hyperinsulinemia is often present [53]. Leptin therapy in leptin-deficient rodents and humans improves all of their metabolic abnormalities [18,39,53].

The improvement in hyperglycemia following leptin therapy in rodents was initially attributed to the secondary effects of reduced body weight; however, numerous observations suggest that leptin can have metabolic effects independent of reductions in body weight. First, in *ob*/*ob* and *db*/*db* mice, hyperinsulinemia precedes obesity, suggesting that impairments to glucose regulation occur distinctly from weight gain [43,54]. Second, pair feeding *ob*/*ob* mice to consume the same amount of food as leptin treated *ob*/*ob* mice did not improve blood glucose or plasma insulin to the same extent as leptin treatment [55]. Third, a low dose of leptin (1 mg/kg/day) that was unable to lower body weight was still capable of normalizing blood glucose and insulin levels in *ob*/*ob* mice [39]. Fourth, acute disruption of leptin signaling using a leptin antagonist raised blood glucose and plasma insulin levels before altering body weight [56]. Fifth, rodents and humans with lipodystrophy accompanied by loss of fat tissue and extremely low leptin levels also exhibited hyperglycemia, hyperinsulinemia, and insulin resistance which were corrected by leptin therapy [57–59]. Lastly, insulin deficient rodents, which had depleted white adipose tissue (WAT) depots, exhibited hyperglycemia, insulin resistance, and impaired glucose tolerance, all of which were normalized by leptin therapy [19,20,60–66]. Together, these findings demonstrate that leptin signaling can influence glucose regulation independent of its effects on body weight.

Here we assess studies aimed at addressing the mechanisms by which leptin regulates blood glucose. We describe the ability of leptin to lower blood glucose through critical pathways within the CNS, CNS-mediated effects on the periphery, as well as direct effects on peripheral tissues. Studies performed *in vitro* and *ex vivo* have been used to examine the direct effect of leptin on various tissues; however, due to the lack of physiological environment (e.g. innervation and interaction with hormones from other tissues), the outcomes may not reflect the actions of leptin in the whole organism. The use of techniques including central or systemic delivery of leptin, and genetic deletion or overexpression, have helped to elucidate the role of leptin action *in vivo*. In addition, the use of dietary and pharmacological manipulation to mimic metabolic diseases including obesity and insulin-deficient diabetes has provided insight into the role of leptin in disease states. However, many caveats may hamper the analysis of *in vivo* studies, such as promiscuous or ineffective cre recombinases when using cre-lox technology, the inability to distinguish between direct and indirect effects of leptin, lack of reproducibility between studies due to differences in experimental design or facilities, and difficulty in translating results from animal models to human physiology. These caveats should be considered when interpreting studies aimed at elucidating the mechanisms of leptin in regulating glucose homeostasis.

2. ROLE OF LEPTIN IN REGULATING GLUCOSE HOMEOSTASIS

2.1. Effects of leptin on the central nervous system

The expression of the long form of the leptin receptor (LepRb) is higher in the CNS relative to peripheral tissues [30,33,34]. Intracerebroventricular (ICV) injection of leptin that results in negligible peripheral leptin levels restores euglycemia and insulin sensitivity in *ob*/*ob* [67], high fat fed [68], and insulin deficient rodents [60,66,69,70], providing compelling evidence that central leptin signaling alone is sufficient for potent glucose-lowering actions of leptin. To better understand the specific CNS regions involved in leptin mediated glucose regulation, studies have been performed involving leptin injection into specific brain regions or genetic deletion or restoration of the leptin receptor in specific neuron populations.

Leptin receptors are expressed primarily in GABAergic and, to a lesser extent, in glutamatergic neurons, in several regions of the hypothalamus including the ventromedial nucleus of the hypothalamus (VMH), arcuate nucleus of the hypothalamus (ARC), lateral hypothalamic area (LHA), and dorsomedial hypothalamic nucleus (DMH) [35,71–73], as well as extra-hypothalamic regions [34]. Leptin-responsive neurons in these regions consist of heterogeneous populations that have not been entirely characterized. However, the main populations that have thus far been implicated in leptin-mediated effects on glucose homeostasis are well-studied neurons in the VMH and ARC, as discussed below (Figure 1).

Injection of leptin into the VMH of lean rats did not affect blood glucose or plasma insulin levels but stimulated glucose uptake into BAT, muscle and heart [74,75]. However, in rats rendered diabetic using streptozotocin (STZ), injection of leptin into the VMH completely normalized blood glucose levels and hepatic glucose production but had no effect on glucose uptake into BAT or muscle [76]. The differences in the results of these studies may be due to the effect of insulin depletion and hyperglycemia in the diabetic vs lean state. Within the VMH, glutamatergic steroidogenic factor-1 (SF1) expressing neurons have been implicated in body weight and glucose regulation [77]. To investigate the role of leptin receptors in these neurons, leptin receptors were knocked out of SF1 neurons of mice, which resulted in modestly increased body weight and adiposity, as well as hyperinsulinemia and glucose intolerance [78,79]. However, *db*/*db* mice

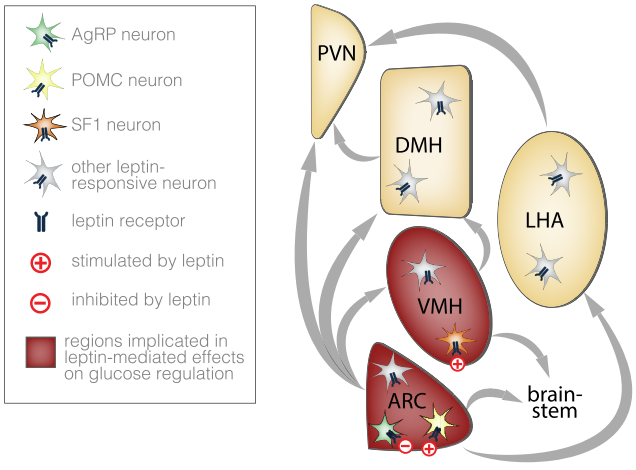

Figure 1: Leptin responsive regions of the hypothalamus. Within the hypothalamus, heterogeneous populations of leptin-responsive neurons are found in the ARC, VMH, DMH, and LHA. The ARC and VMH regions have been implicated in the leptin-mediated control of glucose regulation, including the AgRP and POMC neurons of the ARC that are inhibited and stimulated by leptin, respectively, and the leptin-stimulated SF1 neurons of the VMH. Further research is necessary to determine whether other leptin-responsive neurons in these regions and in the DMH and LHA may also play a role in glucose regulation. There are extensive interconnections among these four hypothalamic regions (arrows) as well as the PVN, a region that mediates downstream effects of AgRP and POMC neurons on food intake and energy expenditure via MC4Rs. There are additional projections to extra-hypothalamic regions including brainstem regions that mediate autonomic outputs. Thus, the full pathways that translate leptin action within specific hypothalamic neuronal populations to a final peripheral effect on glucose homeostasis may be multi-synaptic, and remain to be fully delineated.

with restoration of leptin receptors in SF1 expression neurons had no improvement in hyperglycemia, and knockout of leptin receptors in SF1 neurons in both *ob*/*ob* [80] and STZ-injected mice [61] did not block the glucose lowering action of leptin. These findings suggest that in the lean state, leptin action in SF1 neurons may contribute to glucose homeostasis, however in a hyperglycemic state, other cells within the VMH must be involved in the leptin-mediated reduction of blood glucose.

The ARC contains pro-opiomelanocortin (POMC) and agouti-related protein (AgRP) neurons, both of which express leptin receptors and are stimulated and inhibited by leptin, respectively. POMC and AgRP neuron projections to the paraventricular nucleus of the hypothalamus (PVN) mediate their opposing effects of leptin on food intake and energy expenditure via the balance between secreted α-MSH and AgRP on melanocortin 4 receptors (MC4R) [81]. These circuits have been manipulated to investigate the role of these neuronal populations in leptin-mediated lowering of blood glucose levels. Leptin injection into the ARC increased glucose uptake into the BAT of lean mice [82]. Similarly, in mice with a global mutation of the leptin receptor, re-expression of LepR in the ARC by stereotaxic injection of an adeno-associated virus expressing FLPe-recombinase resulted in improvements in body weight, food intake, insulin levels, and blood glucose levels [83]. When POMC-cre was used to drive recombination of mutated LepR and thus restore LepR function selectively within POMC neurons, there was a complete reversal of hyperglycemia and improved insulin sensitivity [84,85]. However, loss of leptin receptors in POMC neurons of *ob*/*ob* mice did not block the glucose lowering action of leptin [80], and in STZ-injected mice only partially prevented leptin-mediated reversal of hyperglycemia [61]. Therefore, leptin action exclusively on POMC neurons is sufficient but not solely required to lower blood glucose in mice.

Restoring leptin receptor expression in AgRP neurons of *db*/*db* mice completely normalized blood glucose levels, and loss of leptin receptors in AgRP neurons of *ob*/*ob* mice blocked the glucose lowering actions of leptin [80], suggesting that AgRP neurons are sufficient and required for glucose lowering by leptin. AgRP neurons are GABAergic and co-secrete neuropeptide Y (NPY); however, knocking out either GABA or NPY selectively from AgRP neurons in *ob*/*ob* mice did not prevent the glucose lowering effect of leptin, suggesting that AgRP itself may mediate leptin effects [80]. Another study in STZ-injected mice found that knockout of GABA in leptin receptor expressing neurons did not prevent the glucose lowering actions of leptin [86], again suggesting that GABA itself does not mediate the effect of leptin on glucose lowering. Knocking out leptin receptors in GABAergic neurons using cre-lox technology increased blood glucose and insulin levels, which was associated with a dramatic increase in body weight [87], and selectively restoring leptin receptors in GABAergic neurons of LepR null mice was able to partially reverse hyperglycemia [61]. While GABAergic leptin responsive neurons are not limited to the AgRP population and include neurons in the LHA, DMH, as well as other ARC neurons, AgRP neurons likely contribute to these observations. In STZ-diabetic MC4R knockout rats and *ob*/*ob*-MC4R knockout mice, leptin was unable to lower blood glucose levels, suggesting that MC4R, the receptor mediating the downstream effects of α-MSH from POMC neurons and AgRP, contributes to the glucose lowering effects of central leptin signaling [80,88], supporting the potential importance of AgRP itself in the glucose lowering effects of leptin. Finally, deleting leptin receptors from glutamatergic neurons resulted in a minute increase in body weight and no change in fed or fasting blood glucose levels [87]. Leptin-responsive glutamatergic neurons include the SF1 neurons, neurons in the VMH, DMH, LHA, and a small subset of POMC neurons, suggesting that these populations may not be necessary for the glucose lowering effects of glucose. Together, these studies suggest that GABAergic neurons, particularly AgRP neurons, play a role in the glucose lowering effects of leptin, but GABA itself is not required. The identity of other leptin-responsive GABAergic neurons that may contribute to these effects remains to be determined.

2.2. Effects of leptin on the sympathetic and parasympathetic nervous system

The CNS acts through the sympathetic and parasympathetic nervous system to modulate peripheral tissues. In lean rodents, intravenous or ICV injection of leptin increased sympathetic nerve activity in BAT, muscle, liver, kidney, and adrenal gland [89–91] and parasympathetic nerve activity in the liver [91]. Microinjection of leptin into the VMH of lean rats increased glucose uptake into the BAT within hours, which was blocked by surgical sympathetic denervation [74,75]. In STZ-injected mice, neither partial chemical sympathectomy, subdiaphragmatic vagotomy [92], antagonism of β-adrenergic receptors [93], nor deletion of β-adrenergic receptors [61] blocked the glucose lowering actions of leptin, when measured days after initiation of leptin therapy. In contrast, hepatic vagotomy in lean mice with T2D modestly inhibited the ability of leptin, administered for 2 weeks, to improve glucose tolerance [94]. Collectively, these studies provide evidence that leptin stimulates the sympathetic and parasympathetic nervous systems. The time frame required for leptin to lower glucose via the SNS is unclear since acute (less than 24 h) leptin action increases glucose uptake in lean mice, whereas chronic (multiple days) leptin action in mice with T1D lowers blood glucose independent of the SNS. Thus, additional studies are needed to clarify the time frame, mechanism, and tissues involved in the glucose lowering actions of leptin through the CNS.

2.3. Effects of leptin on β-cells

Insulin and glucagon, synthesized by β- and α-cells, respectively, are key regulators of glucose homeostasis. In addition to lowering blood glucose levels, leptin treatment potently reduced circulating insulin in *ob*/*ob* mice [39,95–100]. Moreover, insulin secretion was reduced within minutes of leptin therapy in mice, as well as in rat and human islets [100], and preproinsulin mRNA levels were reduced within 24 h of a single leptin injection in *ob*/*ob* mice [95] indicating that leptin can suppress insulin secretion and production from β-cells. The insulin lowering effects of leptin are not secondary to glucose lowering, since a fall in insulin levels is observed following leptin treatment in euglycemic lean or *ob*/*ob* mice [21,96]. Insulin can act on adipose tissue to stimulate leptin production and secretion [101,102]. This interaction has been named the adipoinsular axis, a dual hormonal feedback loop involving insulin from β-cells and leptin from adipose tissue, which may function to maintain nutrient balance [103,104].

There is conflicting evidence in the literature as to whether leptin signals directly on β-cells to suppress insulin synthesis and secretion. Many studies using islets or perfused pancreas demonstrate that leptin exposure can inhibit insulin secretion [100,105–111], although this has not been replicated in other studies [112–116]. To investigate the role of leptin on β-cells *in vivo*, various mouse lines with a knockout of the LepR in β-cells have been generated. Although $LepR^{fl/fl}$ RIP-cre and $LepR^{fl/fl}$ Pdx1-cre mice were characterized by hyperinsulinemia [117,118], both of these mouse lines exhibit off target recombination in the brain making interpretation of the direct effect of leptin on β-cells challenging. However, $LepR^{fl/fl}$ Ins1-cre mice, which reportedly express cre exclusively in β-cells, did not exhibit hyperinsulinemia [119]. Despite evidence of leptin binding, *LepRb* transcript expression, and

functional LepRb signaling in islets and β-cells of rodents and humans [104,105,120–122], more recent studies have been unable to detect *LepRb* on β-cells using RT-qPCR [119] or leptin receptor promoter activity using tdTomato$^{fl/fl}$ LepR-ires-cre reporter mice [61]. Furthermore, single cell transcriptome analysis of human and mouse islet cell populations indicate that *LepR* is not highly expressed in β-cells [37,123,124]. Although the direct effects of leptin on β-cells are controversial, the indirect effects of leptin on insulin suppression are unambiguous. ICV or systemic leptin reduces insulin levels in rodents [39,125–128], and knocking out LepR in the brain of mice [129,130] results in hyperinsulinemia, suggesting that the regulation of insulin by leptin can be mediated through the CNS. Therefore, leptin can potently reduce hyperinsulinemia through the CNS, but it is unclear whether leptin receptors on β-cells directly affect insulin levels.

2.4. Requirement of insulin for the glucose lowering effects of leptin

Leptin can dramatically lower blood glucose in rodent models of insulin-deficient diabetes; therefore, it has been suggested that leptin can treat diabetes independently of insulin. It could be hypothesized that leptin normalized glucose homeostasis in STZ-injected rodents by increasing insulin levels or promoting β-cell regeneration. However, leptin reportedly had no discernable effect on plasma or pancreas insulin levels [60] or β-cell mass [64]. Further, when leptin therapy was withdrawn, hyperglycemia returned within days confirming no long-term changes such as β-cell regeneration [60]. Although leptin does not increase insulin levels, it can potently increase insulin sensitivity in models of T1D [20,65,70]. Because many of the models used to test leptin therapy use chemical- or immune-mediated β-cell destruction, insulin depletion is not 100% complete. Given that leptin treated STZ-diabetic mice exhibit vastly improved insulin sensitivity [20], even beyond that of healthy, non-diabetic mice, it is possible that leptin improves insulin sensitivity to a level where residual endogenous insulin can act to reverse diabetes. To definitively determine whether leptin requires insulin to lower blood glucose, we tested leptin therapy in mouse models with maximized abrogation of insulin action [131]. First, in mice with combined STZ-injection and insulin receptor antagonism, we found that leptin was still efficacious at lowering blood glucose [131]. Second, in insulin knockout (InsKO) mice, which cannot survive longer than 24 h without insulin therapy, leptin was able to increase survival for up to 3 weeks. In addition, although leptin was capable of lowering blood glucose, mice exhibited overt fed hyperglycemia and severe fasting hypoglycemia [131]. Therefore, in the complete absence of insulin, leptin can reduce blood glucose levels but they are volatile and the length of survival finite.

2.5. Effects of leptin on α-cells

Hyperglucagonemia is present in leptin deficient [18,132,133] and STZ-diabetic rodents [19,20,63,64,131,134] and leptin therapy potently reduces circulating glucagon levels in both models [19,20,63,64,133,134]. Some *in vitro* studies support a direct role of leptin on α-cells. For instance, incubation with leptin decreased calcium oscillations in mouse and human islets [135] and decreased preproglucagon mRNA, intracellular glucagon content [136], and glucagon secretion [135] from mouse islets. However, *in vivo* deletion of LepR from α-cells does not result in perturbed glucose homeostasis. A partial ablation of LepR from ~43% of α-cells using a LepR$^{fl/fl}$ Glg-cre did not alter glucose or lipid metabolism in mice [137], and similar results were found using the more efficient iGlu-cre [119], suggesting that leptin receptors in α-cells (or other pro-glucagon expressing cells) do not play a critical role in glucose homeostasis. Tuduri et al. report co-localization of LepR with glucagon in mouse pancreas by immunofluorescence and *LepRb* mRNA expression in the α-cell line αTC1-9 [135]; however, in more recent studies, *LepRb* was undetectable in isolated mouse α-cells by RT-qPCR [119] and at very low levels in human α-cells by single cell transcriptomics [37,124]. Although it is unclear whether leptin acts directly on α-cells to diminish glucagon, systemic [20,63–65] or ICV [60,66] leptin administration in STZ-diabetic and non-obese diabetic mice potently reduces plasma glucagon levels. ICV leptin delivery can also decrease glucagon content and preproglucagon mRNA levels in the pancreas [60] suggesting leptin can act through the brain to suppress glucagon production. Therefore, while it has been demonstrated that leptin can reduce glucagon levels through the CNS, it is unclear whether leptin receptors exist on α-cells or whether signaling by leptin receptors in α-cells affects glucagon levels.

2.6. Requirement of glucagon suppression for the glucose lowering effects of leptin

Since glucagon contributes to hyperglycemia by promoting hepatic glucose production, it has been postulated that the glucagon lowering effects of leptin may contribute to the glucoregulatory effects of leptin. Indeed, suppressing glucagon action alone can have potent glucose lowering effects in *ob*/*ob* [138], *db*/*db* [139,140], and STZ-diabetic [141–145] mice, although, as insulin deficiency becomes more severe, glucagon blockage is less efficacious [146–149]. In addition, glucagon suppression may not be pivotal for the metabolic actions of leptin. For instance, a low dose of leptin administered to STZ-diabetic mice, enough to restore physiological circulating leptin levels, induced only a slight reduction in blood glucose despite a normalization of plasma glucagon levels [65]. Additionally, in STZ-diabetic rats, leptin therapy normalized blood glucose after 6 h, while glucagon levels were normalized 24 h after leptin therapy, thereby temporally separating the two effects [19]. Therefore, although glucagon suppression may be beneficial for diabetes, it does not appear to contribute to the glucose lowering action of leptin.

2.7. Effects of leptin on δ-cells

Although the focus of leptin action on islets has been on β- and α-cells, LepR expression was detected by PCR in a tumor-derived δ-cell line, and leptin receptor immunoreactivity and leptin binding were detected in primary isolated rat δ-cells [121]. In addition, use of single cell transcriptomics on human islets revealed that while expression of leptin receptors is very low in β- and α-cells, it is highly expressed in δ-cells [37,124]. Somatostatin released from δ-cells has well known inhibitory effects on insulin and glucagon secretion; therefore, it may be hypothesized that leptin effects on δ-cells may influence insulin and glucagon secretion from neighboring β- and α-cells. However, perfusion of leptin in the rat pancreas does not appear to alter somatostatin release [113,150] and further research is required to clarify the role of leptin receptors on δ-cells.

2.8. Effects of leptin on the hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis consists of the hypothalamus and anterior pituitary that release corticotrophin releasing hormone and adrenocorticotrophic hormone (ACTH), respectively, to regulate the release of cortisol in humans or corticosterone in rodents from the adrenal cortex. Cortisol/corticosterone, in turn, increases blood glucose in response to stress and also suppresses further release of ACTH. In *ob*/*ob* [151] and insulin-deficient [19,20,134] mice, circulating ACTH and corticosterone are elevated compared to controls and leptin administration can reduce levels of these hormones [19,65,66,98].

LepRb mRNA and protein expression have been detected in the hypothalamus [22,73,152], anterior pituitary [12,153], and adrenal cortex [154–158] of humans and rodents. Interestingly, leptin suppressed ACTH-stimulated cortisol/corticosterone release from primary cultures of bovine [159], rat, and human [158] adrenocortical cells suggesting that leptin may also have a direct effect on adrenocortical cells within the HPA axis. Additionally, ICV leptin normalized corticosterone levels in STZ diabetic mice [66], suggesting that suppression of the HPA axis could also be mediated through the CNS. It has been hypothesized that leptin normalizes blood glucose by lowering circulating corticosterone levels. Morton et al. found that reducing circulating corticosterone levels in STZ-diabetic rats by adrenalectomy did not prevent STZ-induced elevations in blood glucose [160], while Perry et al. demonstrated that adrenalectomy effectively lowered blood glucose levels in STZ-diabetic rats [134]. The discrepancy between these studies may be due to the effects of sugar water provided by Morton et al. to adrenalectomized rats. To reproduce these conditions, Perry et al. provided sucrose water to adrenalectomized rats, which resulted in an inability of leptin to lower blood glucose levels in the absence of corticosterone [134]. In addition, leptin was still able to lower blood glucose in STZ-diabetic rats when the drop in corticosterone was prevented [160]; however, when the same strategy was used by Perry et al., the glucose lowering actions of leptin were largely blocked [134]. Although both studies used STZ to deplete insulin levels in rats, Perry et al. reported lower insulin levels than Morton et al. When Perry et al. infused insulin into their STZ-diabetic rats to match insulin levels reported by Morton et al., they found that the glucose lowering actions of leptin were prevented, suggesting that the differences in success of leptin therapy between the two studies may be due to the level of insulinopenia. Perry et al. suggest that the HPA axis is critical for the glucose lowering effects of leptin therapy; however, this was assessed 24 h after injection of STZ and following an overnight fast. Therefore, it is unclear whether leptin normalization of blood glucose via the HPA axis would occur in models with established STZ-diabetes. Interestingly, in leptin treated InsKO mice with complete insulin deficiency, corticosterone was normalized in the hyperglycemic fed state, yet the mice exhibited a robust counter-regulatory response to fasting hypoglycemia (3–6 h) by increasing corticosterone, demonstrating that lowering of blood glucose was not accompanied by lower corticosterone levels [131]. Therefore, the ability of leptin to reduce blood glucose by lowering corticosterone levels may be dependent on feeding status, insulin levels, and/or duration of diabetes.

2.9. Effects of leptin on skeletal muscle

Skeletal muscle plays an important role in the maintenance of glucose homeostasis as it is a key site for glucose uptake. *LepRb* expression has been reported in skeletal muscle [36,161]; however, there is conflicting evidence whether or not leptin has a direct effect on muscle. Some studies have reported no effect of leptin treatment on glucose uptake in rat extensor digitorum longus (EDL) or mouse soleus [162,163], or inhibition of insulin-stimulated glucose metabolism in a rat muscle cell line or mouse soleus [164–166]. Other studies demonstrated that leptin can mimic the effect of insulin on glucose transport and glycogen synthesis through phosphoinositide 3-kinase (PI3K) activity [164,167] and insulin receptor substrate (IRS)-2 phosphorylation [168] in muscle cell lines. In addition, treatment of isolated rodent soleus with leptin alone or in combination with insulin resulted in increased muscle glycogen synthesis and glucose uptake, glucose oxidization, and glycogen synthesis [169–171]. Therefore, leptin may have a direct role in affecting insulin sensitivity, glucose uptake and utilization, and glycogen synthesis in muscle.

In vivo, ICV delivery of leptin promotes uptake of glucose and enhances insulin sensitivity in muscle, suggesting a role of the CNS in regulating glucose uptake in muscle. Hypothalamic leptin injection increased 5′ adenosine monophosphate-activated protein kinase (AMPK) activity in mouse soleus and red gastrocnemius [172]. This increased activity of AMPK stimulated fatty acid oxidation and improved insulin sensitivity [173]. In lean rodents, both acute and long-term leptin therapy stimulated glucose uptake into the EDL, soleus, and red and white gastrocnemius while denervation of the EDL and soleus abolished this effect [75,174–176]. Leptin had no effect on glucose uptake into the EDL and soleus of *ob*/*ob* mice [177]. In STZ-diabetic mice, one study found no effect of leptin on glucose uptake in soleus muscle [20] while other studies found an increased glucose uptake into the red gastrocnemius and soleus muscle [61,66,178]. These effects of leptin on muscle glucose uptake are mediated through downstream activation of the melanocortin receptor [82] and the mitogen-activated protein kinase kinase – extracellular signal–regulated kinase pathway (MEK-ERK) [178] in the VMH, and is dependent on sympathetic nervous system activity [74]. Together, these studies suggest that leptin may promote glucose uptake in muscle and this effect is mediated through signaling in the CNS.

2.10. Effects of leptin on brown adipose tissue

In recent years, BAT has garnered interest due to its capacity to uptake glucose and dissipate energy as heat [179]. Leptin may have direct effects on BAT since *LepR* is expressed in BAT of mice [35] and cultured brown adipocytes from rats [180]. In addition, phosphorylation and translocation of STAT-1 and -3 was found in leptin treated brown adipocytes [180,181]. Interestingly, in brown adipocyte cultures, leptin partially inhibited insulin-stimulated glucose uptake, reduced insulin receptor kinase activity, and diminished insulin-induced IRS-1 tyrosine phosphorylation and binding to PI3K, suggesting that there is substantial negative crosstalk between leptin and insulin in BAT [181].
In vivo, systemic and ICV leptin therapy in lean mice [174,180] and rats [175,176], *ob*/*ob* mice [177], and STZ-diabetic mice [61,66] stimulated glucose uptake, increased glucose transporter (Glut)-4 mRNA [175], and increased uncoupling protein (UCP)-1 and –3 mRNA expression [69,182] in BAT. Since leptin increases glucose uptake and UCP-1 expression in BAT, and UCP-1 can mediate non-shivering thermogenesis and energy dissipation as heat, it was hypothesized that UCP-1 may be required for the anti-diabetic effect of leptin. To test this, UCP-$1^{-/-}$ mice rendered diabetic using STZ were treated with exogenous leptin therapy. Leptin was effective in lowering blood glucose to a similar degree in $UCP1^{-/-}$ and $UCP1^{+/+}$ mice, suggesting that the mechanism is UCP-1 independent [92]. Therefore, despite the inhibitory effects of direct leptin signaling in BAT on glucose uptake, it appears that *in vivo*, leptin action through the CNS is sufficient to increase glucose uptake in a UCP-1 independent manner in BAT.

2.11. Effects of leptin on white adipose tissue

LepRb is expressed in WAT of mice [8,35]. In contrast to the brain-mediated effect of leptin on BAT *in vivo*, systemic and ICV leptin does not promote and may even suppress glucose uptake as well as *Glut-4* mRNA expression in WAT in lean rodents and *ob*/*ob* mice [75,174–177]. Despite the opposing effects of CNS-mediated glucose uptake in BAT versus WAT, the direct effect of leptin on WAT is similar to BAT in that insulin action is inhibited. In isolated white adipocytes, incubation with leptin impaired insulin-stimulated glucose uptake and glycogen synthesis [183], phosphorylation of insulin receptors [184], and insulin binding to insulin receptors [185]. Taken together these

reports suggest that leptin directly and indirectly inhibits glucose uptake in WAT.

2.12. Effects of leptin on lipolysis and generation of gluconeogenic substrates

Recent evidence suggests that whole body lipolysis resulting in fatty acid and glycerol release may influence hepatic glucose output and play a role in the glucose lowering effect of leptin [19,62,134]. However, the effect of leptin on lipolysis differs substantially between lean and *ob*/*ob* mice compared to insulin-deficient diabetic mice. In leptin deficient and lean rodents, leptin therapy causes loss of WAT mass [18,39,186]. Adipocytes from lean or *ob*/*ob* mice acutely cultured with leptin had increased glycerol release, indicative of increased lipolysis [180,187]. Furthermore, lean rodents or *ob*/*ob* mice that were injected with a single bolus of leptin exhibited an increase of both plasma glycerol and fatty acids *in vivo* [188,189], as well as increase in glycerol release *in vitro* [190,191]. Conversely, in insulin-deficient rodents (STZ and InsKO mice) with uncontrolled diabetes, leptin therapy decreased plasma glycerol and fatty acid levels [19,20,62,131,134]. Furthermore, leptin therapy in fasted insulin deficient rats reduced whole-body glycerol, palmitic acid, and β-hydroxybutyrate turnover, which suggests reduced lipolysis [19,134]. These changes were reversed by raising corticosterone to levels seen in diabetic rats [134]. Addition of an adipose triglyceride lipase inhibitor prevented the lipolytic effect of corticosterone, suggesting that leptin suppresses lipolysis through reduced corticosterone [134]. However, these observations were made at the onset (24 h) of STZ-diabetes and should be confirmed by studies in models with chronic hyperglycemia. In conclusion, in the presence of insulin, leptin increases lipolysis; however, when insulin levels are depleted, leptin inhibits lipolysis. This reduction in lipolysis decreases the release of glycerol and fatty acids contributing to suppressed gluconeogenesis.

2.13. Effects of leptin on hepatocytes

Given the central role of the liver in glucose homeostasis, it is an obvious candidate tissue to mediate the glucose lowering actions of leptin. Leptin receptors have been found in isolated hepatocytes [97] and rodent liver [20,23,192–194], suggesting that there may be direct effects of leptin on the liver. Within the liver, cross talk exists between leptin and insulin-signaling pathways [195,196]. Whether the insulin signaling pathway is stimulated or suppressed by leptin may depend on various factors including nutritional status or species [193,196,197]. In an attempt to clarify the direct effect of leptin on the liver *in vivo*, mouse lines have been generated to knockout leptin receptors in hepatocytes using mice expressing a floxed leptin receptor and the rat albumin promoter driving cre expression. One study found that knockout of all isoforms of the leptin receptor from hepatocytes did not cause alterations in body weight or glucose homeostasis in the non-fasted state [130]. In another study, mice with knockout of only the long form of the leptin receptor in hepatocytes had normal glucose metabolism under fasted conditions. Furthermore, the mice remained glucose sensitive despite aging or high fat diet feeding and had enhanced hepatic insulin sensitivity [192]. Therefore, the *in vivo* data suggest that under hyperinsulinemic conditions, direct leptin action on hepatocytes antagonizes insulin sensitivity.

In contrast to the direct effects of leptin on hepatocytes, systemic or ICV leptin therapy in lean rodents and *ob*/*ob* mice augments insulin-mediated inhibition of hepatic glucose output [56,177,198,199], yet there are conflicting reports regarding the pathways that are involved. In the liver, leptin has been found to both promote [198,200,201] and decrease [174] glycogen storage as well as promote gluconeogenesis [198–200]. Leptin therapy in *ob*/*ob* mice reduced mRNA levels of Glut-2, aldolase B, and glucose-6-phosphatase in the liver, which suggested a reduction in glucose oxidation and production [202]. Acute systemic or ICV leptin administration in lean rats increased gluconeogenesis while concomitantly suppressing glycogenolysis, resulting in an overall enhanced insulin inhibition of hepatic glucose production [198,199]. In contrast, leptin therapy in STZ diabetic rodents potently suppresses gluconeogenesis [19,65,66] and increases glycogen synthesis [63] resulting in an overall decrease in hepatic glucose output [66,203]. Taken together, the net effect of leptin treatment results in decreased hepatic glucose output.

2.14. Effects of leptin on gluconeogenesis and substrate depletion

It has been hypothesized that the leptin mediated reduction of gluconeogenesis in mice with insulin deficient diabetes is driven by a reduction in WAT lipolysis and thus gluconeogenic substrates, as described above. The main substrates for gluconeogenesis are lactate, alanine, and glycerol. Glycerol is released along with fatty acids upon lipolysis of triacylglycerol (TAG), and glycogen can supply glucose through glycogenolysis. Leptin treated mice have severely depleted energy-yielding substrates in the liver such as glucose, acetyl-CoA, TAG, and glycogen [62,134]. Although hepatic glycogen levels were reduced with leptin therapy, they were depleted prior to blood glucose lowering [62], suggesting that reduced glycogenolysis does not drive glucose lowering in insulin-deficient diabetes. In another study, leptin therapy normalized blood glucose levels but did not affect plasma lactate and alanine levels, suggesting that the glucose lowering actions of leptin are not dependent on reduction of these gluconeogenic substrates [62]. However plasma ketones, glycerol, fatty acids, and TAG were gradually reduced in a manner which mimicked the reduction of blood glucose levels [62], consistent with other studies [19,131]. In addition, in STZ diabetic rats, leptin reduced conversion of glycerol and pyruvate to glucose through hepatic gluconeogenesis [19]. Leptin therapy reduced acetate turnover [19], plasma acetate levels [19], and liver acetyl-CoA concentrations [19,62,134] but not free CoA levels [62] suggesting elevated glucose oxidation compared to lipid oxidation in the liver. The reduction in acetyl-CoA may in turn prevent the flux of pyruvate converted to glucose thereby lowering glucose levels. To investigate whether the glucose lowering of leptin can be blocked by supplying these gluconeogenic substrates, an acute injection of glycerol [62], infusion of a lipid emulsion with heparin (to increase glycerol and fatty acids) [19], or infusion of acetate to increase hepatic acetyl-CoA levels [134] were administered to leptin treated diabetic mice or rats. Injection of glycerol increased blood glucose levels but not to the level of diabetic controls [62]. Similarly, infusion of a lipid emulsion completely reversed the glucose lowering effect of leptin [19], suggesting that depleted glycerol may contribute but depleted fatty acids may play a larger role in the anti-diabetic effect of leptin. Finally, acetate administration completely blocked the glucose lowering effect of leptin [134]. Therefore, leptin may act to reduce the gluconeogenic flux from glycerol and pyruvate to lower blood glucose levels.

Given that leptin depletes gluconeogenic substrates, it may be postulated that leptin could induce fasting hypoglycemia. Indeed, leptin therapy followed by prolonged fasting of STZ-diabetic and InsKO mice resulted in blood glucose levels falling dramatically from hyperglycemia to hypoglycemia within hours [62,131]. We measured gluconeogenic substrates in both the fed and fasted state to determine whether they might be involved in the mechanism behind the glucose lowering effect of leptin upon fasting. We observed that plasma glycerol levels were unchanged from a fed to fasted state, despite a reduction in blood

glucose [131], which suggests that depletion of plasma glycerol may not be the driving force behind the glucose lowering effects of leptin. However, while the typical response to fasting in normal mice is to increase fatty acids and ketones, leptin treated mice have reduced fatty acids in the fed state and are further depleted upon fasting, which, therefore, may contribute to the blood glucose lowering effect of leptin [62,131]. In conclusion, in insulin-deficient diabetic rodents, leptin therapy inhibits lipolysis and decreases plasma glycerol, fatty acids, and ketones, which may play a role in suppressing gluconeogenesis.

3. FROM LAB BENCH TO CLINIC

3.1. Congenital leptin deficiency

Patients with congenital leptin deficiency due to a mutation in the gene encoding for leptin develop morbid obesity and additional metabolic abnormalities including hypogonadotrophic hypogonadism, increased circulating plasma insulin levels, and increased plasma TAG levels [50–52]. Daily subcutaneous injection of recombinant methionyl human leptin to patients with congenital leptin deficiency was sufficient to restore normal circulating leptin levels and reduce body mass index from an average of 51.2 $\pm$ 2.5 kg/m^2 to 26.9 $\pm$ 2.1 kg/m^2 in 18 months without dietary or exercise intervention [204]. Furthermore, recombinant leptin therapy also proved beneficial to glucose homeostasis by improving insulin sensitivity [204] and reducing plasma insulin and TAG levels [51,53,204]. These favorable outcomes along with the relative safety and effectiveness of leptin therapy resulted in its approval for the treatment of rare cases of congenital leptin deficiency.

3.2. Lipodystrophy

The success of leptin therapy *in vivo* in rodents or in humans with impaired leptin signaling prompted the initiation of clinical trials to determine if leptin is an effective therapy for treating metabolic disorders in humans. Patients suffering from lipodystrophies experience reduced body fat, severe insulin resistance, hypertriglyceridemia and hypoleptinemia [58,205]. The depletion of adipose tissue results in excess calories being diverted to the liver and skeletal muscle where they are stored as TAGs that can, in turn, impair insulin action [58,206]. Daily subcutaneous injections of recombinant leptin for four months resulted in significant improvements to glycemic control, hypertriglyceridemia, and hepatic steatosis in patients with generalized lipodystrophy [58]. Similarly, leptin therapy reduced HbA1c levels of patients with acquired generalized lipodystrophy [207]. Because lipodystrophy is a chronic condition, it is important to demonstrate that leptin treatment is safe and effective to use as a long-term treatment. In one study that tracked patients over 3 years, leptin therapy resulted in sustained improvements to blood glucose and lipid levels, as well as improved liver function [208]. Furthermore, in a recent study monitoring patients over one year, leptin therapy improved insulin sensitivity and increased insulin secretion rate in response to glucose infusion, and these benefits were sustained for the duration of the study [209]. Leptin has now been approved to treat people with generalized lipodystrophy.

3.3. Type 1 diabetes

The current treatment for T1D involves multiple daily insulin injections or insulin delivery by pump; however, even with this treatment, glucose levels typically fluctuate dramatically. Periods of hyperglycemia contribute to long-term vascular complications, and hypoglycemia can result in coma or death. Leptin was proposed as a promising therapy due to its glucose lowering effects in insulin deficient rodents [64] and adding leptin as an adjunct to insulin therapy was capable of minimizing

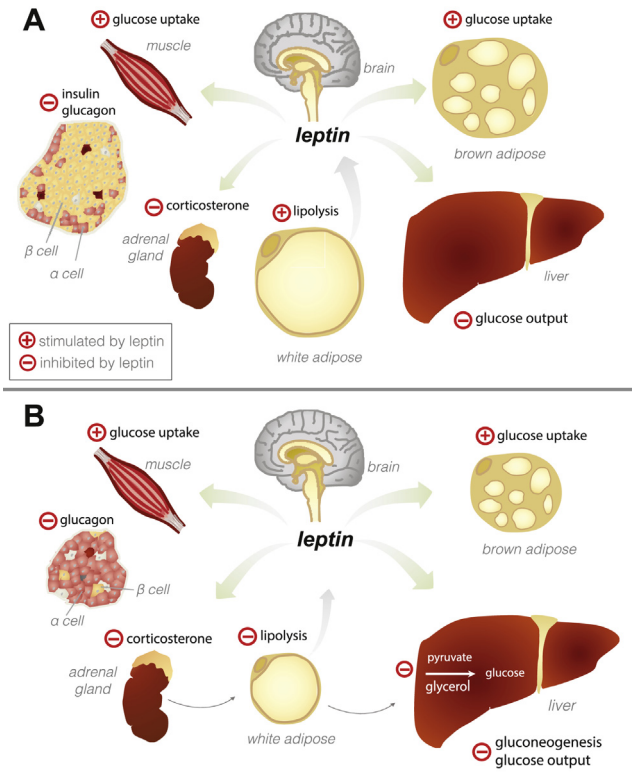

Figure 2: Mechanisms underlying the glucoregulatory effects of leptin in lean or insulin deficient rodents. Leptin is secreted primarily from white adipose tissue and can regulate blood glucose through direct action on peripheral tissues or indirectly through the CNS. Here, we illustrate the main *in vivo* effects of leptin, which are largely mediated by the CNS. The effects of leptin may be inhibitory (red circle with minus sign) or stimulatory (red circle with plus sign). **A)** In lean or leptin deficient rodents, leptin stimulates glucose uptake in muscle and brown adipose tissue and suppresses release of insulin and glucagon secreted from β cells and α cells, respectively. In addition, leptin suppresses release of the counter-regulatory hormone corticosterone, increases lipolysis in white adipose tissue, and causes an overall reduction in glucose output from the liver. **B)** In the insulin deficient state, leptin regulates blood glucose by increasing glucose uptake in muscle and brown adipose tissue. Furthermore, leptin lowers corticosterone levels, resulting in suppression of lipolysis in white adipose tissue, which diminishes fatty acid and glycerol release. Leptin therapy also results in reduced glucagon secretion from α cells, as well as depletion of gluconeogenic substrates, which decreases the flux from pyruvate and glycerol to glucose and results in attenuation of gluconeogenesis and hepatic glucose output.

glucose fluctuations and reducing insulin requirements in non-obese diabetic mice [63]. These exciting findings prompted the initiation of a clinical trial examining the effects of leptin therapy in patients with T1D in the hopes of lowering total insulin requirements and improving blood glucose control. Although body weight, percent body fat, and insulin dose were reduced due to leptin therapy (0.04–0.08 mg/kg/day via subcutaneous injection for 20 weeks), HbA1c, fasting blood glucose and 24 h blood glucose, TAG, fatty acids, and glucagon were unaltered, suggesting that recombinant methionyl human leptin was not efficacious in improving glycemic control in these patients at the doses given [210]. This trial has been terminated and it is unclear whether leptin therapy will continue to be pursued for T1D.

3.4. Type 2 diabetes

T2D is characterized by insulin resistance, hyperglycemia, and elevated lipids and is highly associated with obesity. The most commonly used

therapy to manage T2D is metformin [211], which acts to increase insulin sensitivity, but this intervention does not completely restore metabolic homeostasis [212]. Leptin is an obvious candidate therapy for T2D given its ability to improve insulin sensitivity, lower plasma lipids, and reduce body weight. However, in obese subjects with T2D, recombinant methionyl human leptin was ineffective in improving insulin sensitivity [213], though it did result in marginal improvements to HbA1c levels [214]. The failure of leptin to improve glucose homeostasis in these clinical trials may have been due to the fact that obese individuals are typically hyperleptinemic, which may indicate leptin resistance [16,17]. In light of leptin resistance, recent pre-clinical studies have shifted the focus from exogenous leptin therapy to strategies that enhance endogenous leptin sensitivity [215–219]. Taken together, these findings suggest that leptin monotherapy may not be effective in patients with T2D, but improving leptin sensitivity may be an alternative approach to restore glucose homeostasis.

4. SUMMARY

Leptin has diverse effects on various tissues, which work together to lower blood glucose in lean, *ob*/*ob*, and insulin-depleted rodents (Figure 2). Leptin can potently reduce circulating insulin levels in lean and *ob*/*ob* rodents, and, in insulin-deficient rodents, leptin can act independently of insulin to reduce blood glucose levels. Glucagon is reduced with leptin therapy; however, it appears not to be responsible for reducing blood glucose. Leptin-mediated glucose uptake into BAT and muscle may play a role in depleting glucose from the circulation. In lean rodents and *ob*/*ob* mice, leptin stimulates lipolysis, thereby reducing WAT stores. In addition, hepatic glucose production is reduced as glycogen synthesis is attenuated, while gluconeogenesis is increased. In contrast, in leptin treated rodents with insulin deficient diabetes, lipolysis may be inhibited by lowering corticosterone, resulting in reduced gluconeogenesis through glycerol and pyruvate. The glucose lowering actions of leptin may be partly mediated by direct leptin signaling within peripheral tissues; however, it appears to be largely facilitated through the CNS, particularly through neuronal pathways within the hypothalamus. The pleiotropic effects of leptin on many metabolic pathways suggest that modulation of a single pathway is insufficient to restore glucose homeostasis.

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CONFLICT OF INTEREST

None declared.

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