The role of leptin in glucose homeostasis

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Abstract

The fat-derived hormone, leptin, is well known to regulate body weight. However, there is now substantial evidence that leptin also plays a primary role in the regulation of glucose homeostasis, independent of actions on food intake, energy expenditure or body weight. As such, leptin might have clinical utility in treating hyperglycemia, particularly in conditions of leptin deficiency, such as lipodystrophy and diabetes mellitus. The mechanisms through which leptin modulates glucose metabolism have not been fully elucidated. Leptin receptors are widely expressed in peripheral tissues, including the endocrine pancreas, liver, skeletal muscle and adipose, and both direct and indirect leptin action on these tissues contributes to the control of glucose homeostasis. Here we review the role of leptin in glucose homeostasis, along with our present understanding of the mechanisms involved. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00203.x, 2012)

Key Words: Adipokine, Diabetes, Glucose metabolism

Introduction

The inheritable obese phenotype in the ob/ob and db/db mouse lines were discovered several decades ago¹,². Through cross-circulation experiments between ob/ob and db/db mice, Coleman postulated that ob/ob mice lacked a circulating satiety factor, whereas db/db mice lacked a functional responsive site to this factor³. The identity of this satiety factor remained unknown until 1994, when the ob gene was identified through positional cloning⁴. ob/ob mice possess a single nonsense mutation in the ob gene, resulting in the production of a truncated form of the protein product, leptin, and undetectable circulating leptin levels⁵. Injection of wild-type leptin can lower body weight in both ob/ob and wild-type mice⁶. Although rare, mutations in the human leptin gene have been identified in several families⁷–¹⁰, and similar to ob/ob mice, humans with homozygous null mutations in the leptin gene have undetectable circulating leptin levels, and are obese with a plethora of metabolic, reproductive and immune dysfunctions; these patients can be effectively treated with leptin replacement therapy¹¹–¹⁴.

The discovery of the leptin receptor (Lepr), encoded by the db gene followed soon after leptin was identified¹⁵. The db gene encodes an alternatively spliced transcript, capable of producing six leptin receptor isoforms (Lepr-a to Lepr-f)¹⁶. db/db mice have an insertion mutation in the db gene that prevents the normal splicing of the Lepr-b isoform, resulting in a truncated intracellular signaling domain¹⁶,¹⁷. Lepr-b has the longest intracellular domain of the leptin receptor isoforms¹⁶,¹⁷, and signals through Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathways to influence transcription of target genes¹⁸–²². When back-crossed to the same genetic background, ob/ob and db/db mice have nearly identical phenotypes²²,²³, indicating that Lepr-b is primarily responsible for carrying out leptin action. Thus, Coleman’s prediction regarding the defective production of a satiety factor in ob/ob mice, and an unresponsive satiety centre in db/db mice was confirmed. It is now well known that leptin normally circulates in proportion to body fat²⁴,²⁵, and acts on hypothalamic neurons to inhibit food intake and increase energy expenditure, leading to a reduction in body weight²⁶,²⁷, thereby, the loss of leptin action results in hyperphagia, decreased energy expenditure and profound obesity.

The role of leptin in glucose homeostasis

Although leptin was originally recognized for its role as a satiety factor, it is now implicated in a wide variety of biological functions, including the regulation of glucose homeostasis. In addition to obesity, ob/ob and db/db mice have a phenotype similar to human type 2 diabetes, including hyperglycemia, hyperinsulinemia and insulin resistance, and thus have been widely used for decades as animal models of diabetes²⁸–³². It can be postulated that the perturbed glucose metabolism that accompanies leptin or leptin receptor deficiency is secondary to obesity and hyperphagia. However, several lines of evidence show that leptin regulates glucose metabolism independently of its effects on body weight and food intake. First, hyperinsulinemia occurs in ob/ob mice³⁹–³⁵, along with transient hypoglycemia³²,³³, before the onset of insulin resistance, hyperglycemia and obesity. Likewise, early hyperinsulinemia is also common to rodents with disrupted leptin receptor function, including db/db mice, Zucker fatty (fa/ fa) rats and corpulent rats⁴⁰,³⁴–³⁷. To further examine the chronology of events in leptin deficiency, we examined the effect of acutely disrupting endogenous leptin action in wild-type mice using a leptin antagonist³⁸. Adult mice treated with this antagonist developed fasting- and glucose-stimulated hyperinsulinemia, and insulin resistance within 3 days, without

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significant accompanying changes in body composition or body weight. Thus, when leptin action is disrupted or absent, perturbations in glucose homeostasis chronologically precede obesity. Similar to leptin deficient ob/ob mice, rodents and humans with a near or complete loss of adipose tissue (lipodystrophy) are also hypo leptinemic, and have hyperinsulinemia, insulin resistance and hyperglycemia. Thus, regardless of adiposity, inappropriately low leptin levels result in perturbed glucose homeostasis.

Further supporting the body weight independence of leptin action on glucose homeostasis, leptin replacement therapy can improve glucose metabolism in mice and humans with either congenital leptin deficiency or lipodystrophy. Leptin administration reduces circulating insulin and glucose levels in ob/ob mice, indicative of increased insulin sensitivity, to a greater extent than pair-feeding, showing that the actions of leptin on glucose homeostasis cannot simply be explained by reduced food intake. Furthermore, we have shown that leptin can lower circulating insulin and glucose levels in ob/ob mice within 1 or 2 days, before changes in body weight occur.

We also showed that temporary leptin therapy has longer lasting effects on blood glucose than food intake in ob/ob mice. Finally, low doses of leptin that do not alter body weight or food intake can normalize circulating insulin and glucose levels in ob/ob mice. These studies firmly establish that leptin replacement has a more potent effect on glucose metabolism than body weight in leptin deficient animals.

Perhaps the most compelling evidence of the profound effect of leptin on glucose homeostasis is that leptin administration can normalize blood glucose levels in non-obese rodent models of insulin deficient, type 1 diabetes. Leptin infusion or gene therapy, can reverse hyperglycemia without a detectable rise in circulating insulin levels in streptozotocin (STZ)-treated rats and mice, non-obese diabetic (NOD) mice, insulin deficient Akita mice and BioBreeding rats with virally-induced β-cell destruction. Leptin therapy also normalizes water intake and urine output, and reverses glycosuria, hyperketonemia and hyperphagia in insulin deficient rodents, indicating improved overall health of these animals. Despite the anorexigenic effect of leptin, pair-feeding studies have shown that the glucose lowering in response to leptin therapy cannot be explained by decreased food intake. Furthermore, the reversal of glycosuria with leptin therapy rules out euglycemia induced by increased glucose output in urine, and supports a direct antidiabetic effect of leptin on glucose metabolism.

The normalization of blood glucose levels by leptin therapy in insulin deficient rodents correlates with increased insulin sensitivity. We found that STZ-diabetic mice treated with leptin have heightened insulin sensitivity, even compared with non-diabetic controls, and thus postulated that the profound insulin sensitizing effect of leptin in this model might compensate for residual insulin levels in STZ-treated rodents. Indeed, low-dose insulin administration that was ineffective alone was found to dramatically reduce glucose levels in STZ-diabetic mice when combined with a dose of leptin that was ineffective alone. In addition, leptin therapy decreases levels of counter regulatory hormones, which could contribute to the glucose-lowering action of leptin. In insulin deficient rodents leptin administration has been found to decrease corticosterone and growth hormone (GH) levels, hormones which impair insulin sensitivity. Leptin also robustly decreases glucagon levels in this model, which is a common characteristic of diabetes, and is a requisite for hyperglycemia in several models of insulin deficiency. Interestingly, suppression of endogenous glucagon or antagonism of glucagon receptor signaling decreases hyperglycemia in STZ-diabetic rats. Furthermore, mice with genetic knockout of glucagon receptors are resistant to developing STZ-induced diabetes. Thus, the potent suppressive effect of leptin on glucagon levels might contribute to restoration of euglycemia in leptin-treated insulin deficient rodents.

Circulating leptin levels rapidly fall after the decrease in insulin levels induced by STZ administration in rats, and are acutely restored by insulin injection. Low plasma leptin levels are also observed in people with newly-diagnosed type 1 diabetes, before the initiation of insulin therapy, and are subsequently elevated by insulin therapy. Importantly, despite reversal of hyperphagia, insulin resistance and hyperglucagonemia, restoration of physiological leptin levels does not reverse hyperglycemia in STZ-treated rats. Thus, the glucose lowering effect of leptin in the context of insulin deficient diabetes seems to require supraphysiological leptin levels. Taken together, the profound effect of leptin deficiency and leptin administration on glucose metabolism in ob/ob mice and rodent models of type 1 diabetes and lipodystrophy provide ample evidence that leptin plays a primary role in glucose homeostasis.

MECHANISMS OF LEPTIN ACTION ON GLUCOSE HOMEOSTASIS

Leptin receptors, including the Lepr-b isoformal, are widely expressed throughout the central nervous system and peripheral tissues. Numerous studies have been carried out to try to identify the specific target tissues that mediate leptin action on glucose metabolism. From this body of work, both the hypothalamus and several extra-hypothalamic sites within the brain have emerged as major targets of leptin. Others have recently provided thorough reviews focused on how these specific leptin-activated neural pathways regulate glucose homeostasis. In addition, direct leptin signaling in peripheral tissues can also modulate glucose metabolism. The direct and indirect actions of leptin on peripheral target tissues that are likely to contribute to glucose homeostasis are reviewed here, and summarized in Figures 1 and 2, respectively.
Leptin Action on Pancreatic β-Cells

Leptin, either through gene delivery or recombinant peptide administration, rapidly lowers circulating insulin levels in ob/ob mice, but is ineffective in db/db mice. In fact, leptin administration lowers insulin secretion in vivo within minutes, and simultaneously causes an acute rise in blood glucose levels, indicating that decreased insulin levels are not secondary to increased insulin sensitivity. Leptin has an inhibitory effect on insulin synthesis as well; a single leptin injection in ob/ob mice decreases proinsulin messenger ribonucleic acid (mRNA) in islets within 24 h, by alterations in transcription factor binding to the insulin promoter. A potential mechanism is the induction of JAK/STAT-mediated expression of suppressor of cytokine signaling 3 (SOCS3) in β-cells by leptin, which subsequently inhibits proinsulin gene transcription. Leptin administration has also been reported to acutely decrease glucose-stimulated insulin levels in normal rodents, albeit the effect is less robust than in ob/ob mice.

Leptin binding, Lepr-b transcript expression, and functional Lepr-b signaling have been shown in pancreatic islets or β-cells from mice, rats and humans. In vitro studies support a direct suppressive action of leptin on basal- and glucose-stimulated insulin gene expression and secretion in β-cells (Figure 1)44,79–81,54–88. Leptin robustly inhibits insulin secretion from isolated islets and the perfused pancreas of ob/ob mice. Most studies using islets or perfused pancrea from non-leptin

Figure 1 | Direct actions of leptin on tissues that contribute to glucose homeostasis. Leptin acts on peripheral leptin receptor-b isoform expressing tissues, including the endocrine pancreas and insulin-sensitive tissues. Direct leptin action on the endocrine pancreas inhibits insulin secretion from β-cells, and glucagon secretion from α-cells. Leptin acts on adipocytes to suppress insulin signaling and action, and in vivo studies indicate that leptin directly antagonizes hepatic insulin sensitivity. Direct leptin action on skeletal muscle can either increase or decrease glucose uptake and insulin stimulated glucose metabolism, and the overall effect remains controversial (combined up and down arrow). AMPK, adenosine monophosphate-activated protein kinase; BAT, brown adipose tissue; cAMP, cyclic adenosine monophosphate; WAT, white adipose tissue.
deficient animals, and β-cell lines show an inhibitory effect of leptin on insulin secretion\(^79,80,86,89–93\), although in a few reports leptin administration had no effect\(^87,94–96\), or even stimulated\(^97,98\) insulin secretion. The reasons for these apparent discrepancies are not clear.

Our group previously showed that in vitro leptin can reduce glucose transporter 2 (GLUT2) phosphorylation, glucose transport and intracellular adenosine triphosphate (ATP) levels\(^99\). In addition, leptin activates ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels and hyperpolarizes β-cells, thereby decreasing intracellular calcium concentrations\(^84,85,100\). Leptin has also been shown to suppress cyclic adenosine monophosphate (cAMP)-induced insulin secretion, through activation of phosphodiesterase-3B (PDE3B)\(^86,87,101\). Leptin also inhibits protein kinase C (PKC)-induced insulin secretion\(^91,102\). Supporting this, leptin can inhibit acetylcholine\(^103\), and glucagon-like peptide 1 (GLP-1)-stimulated insulin secretion\(^86\). Thus, leptin might directly suppress insulin secretion by inhibiting glucose sensing and K\(_{\text{ATP}}\) channel closure, and by inhibiting cAMP and PKC-mediated insulin secretion in β-cells.
To uncover the physiological role of direct leptin action on \( \beta \)-cells in vivo, we used the Cre-lox system to generate mice with disrupted Lepr-\( b \) signaling in \( \beta \)-cells\(^{104} \). Mice with a disrupted signaling domain of the Lepr gene in \( \beta \)-cells and the hypothalamus showed hyperinsulinemia and fasting hypoglycemia, supporting a physiological role of leptin to inhibit insulin secretion from \( \beta \)-cells\(^{104} \). Furthermore, these mice showed glucose intolerance, impaired glucose-stimulated insulin secretion, insulin resistance and mild obesity. Despite partial hypothalamic recombination of the Lepr gene, these mice maintained normal anorexigenic responses to leptin administration, indicating that the phenotype was not a result of increased food intake. Interestingly, we found that insulin resistance was secondary to the phenotype was not a result of increased food intake. Interestingly, we found that insulin resistance was secondary to hyperinsulinemia in mice with disrupted Lepr signaling in \( \beta \)-cells; administration of metformin to these mice improved insulin sensitivity, but was unable to ameliorate hyperinsulinemia, whereas administration of diazoxide to inhibit insulin secretion ameliorated both hyperinsulinemia and insulin resistance\(^{105} \). In a study carried out by Morioka et al.\(^{106} \), mice with a pancreatic and duodenal homeobox-1 (Pdx1)-cre-mediated loss of Lepr expression in the pancreas showed hyperinsulinemia, but in contrast to our studies, had elevated glucose-stimulated insulin secretion, and improved glucose tolerance. Thus, whether the loss of inhibitory leptin signals in \( \beta \)-cells alone results in impaired or improved glucose homeostasis is unclear. Because the Pdx1-cre used by Morioka et al. was recently found to be expressed in the brain\(^{107} \), and the Pdx1 promoter is active in the gut\(^{108} \), Lepr expression might have been disrupted in endocrine sites of their mice, potentially contributing to differences between our two mouse models. Another possible explanation is the presence of mild obesity in our mice, suggesting that an additional insult is required to observe deleterious effects of disrupted \( \beta \)-cell leptin signaling. Interestingly, when the mice of Morioka et al.\(^{106} \) were fed a high-fat diet, they showed impaired glucose tolerance and glucose stimulated insulin secretion, in agreement with our mouse model.

Evidence suggests that leptin also inhibits insulin secretion through central mechanisms (Figure 2). Mice with neuronal disruption of leptin signaling show hyperinsulinemia, whether body weight is unaltered\(^{109} \) or increased\(^{110} \). Intracerebroventricular (ICV) administration of leptin either as a peptide or as a gene therapy lowers insulin levels\(^{111,113} \), although in one study the effect was modest\(^{114} \), whereas in another, leptin did not lower meal-stimulated insulin levels compared with pair-fed controls\(^{115} \). The effect of central leptin action on insulin levels is consistent with increased sympathetic tone to \( \beta \)-cells. Indeed, a recent study showed that acute ICV leptin administration suppressed glucose-stimulated insulin secretion in a manner dependent on activation of the sympathetic nervous system (SNS)\(^{115} \). One SNS-mediated mechanism of leptin action on the \( \beta \)-cell has been proposed to occur through sympathetic inhibition of bioactive osteocalcin secretion from osteoblasts, which in turn reduces \( \beta \)-cell insulin secretion\(^{109} \). Interestingly, one study found that an inhibitory effect of leptin on glucose stimulated insulin secretion was observed in vagotomized, but not intact rats, and was abolished by sympathectomy\(^{117} \). Thus, although studies indicate that the SNS partly mediates the inhibitory action of leptin on \( \beta \)-cells, this might be counterbalanced by the parasympathetic nervous system (PNS).

Collectively, these findings indicate that both direct and centrally-mediated leptin action inhibit \( \beta \)-cell insulin secretion. Insulin secretion from pancreatic \( \beta \)-cells promotes lipid storage and leptin synthesis in adipocytes, creating a bidirectional regulatory loop between \( \beta \)-cells and adipocytes, previously termed the adipoinsular axis\(^{83,118} \). The in vivo studies carried out by our group and by others show that disruption of this feedback loop results in hyperinsulinemia, which can lead to perturbations in glucose homeostasis. Thus, the adipoinsular axis might physiologically act to protect normal glucose homeostasis from environmental triggers that promote hyperinsulinemia.

**Leptin Action on Pancreatic \( \alpha \)-Cells**

Several lines of evidence implicate a role of leptin in inhibiting glucagon secretion from pancreatic \( \alpha \)-cells. Circulating glucagon levels are elevated in leptin-deficient ob/ob mice\(^{119,120} \), and this is corrected by leptin administration\(^{120} \). Furthermore, leptin administration or gene therapy reverses the hyperglucagonemia present in animal models of type 1 diabetes\(^{49,55,56,73} \). Recent evidence indicates that leptin can suppress glucagon secretion by directly acting on pancreatic \( \alpha \)-cells. The expression of Lepr transcript has been shown in an \( \alpha \)-cell line, and Lepr-\( b \) immunoreactivity was shown in mouse and human \( \alpha \)-cells\(^{121} \). Application of leptin to isolated mouse \( \alpha \)-cells induces phosphorylation and nuclear translocation of STAT3\(^{122} \). Stimulation of \( \alpha \)-tumor cell 1 (TC1) cells and mouse \( \alpha \)-cells under low-glucose conditions in the presence of leptin hyperpolarizes membrane cell potential, leading to decreased electrical activity\(^{121} \). Furthermore, leptin suppresses calcium oscillations in mouse and human \( \alpha \)-cells from intact islets, and decreases glucagon secretion from mouse islets\(^{121} \). Administration of leptin in vivo and in vitro reduces islet preproglucagon mRNA and intracellular glucagon content of mouse islets\(^{122} \). Leptin did not suppress glucagon secretion in the presence of a phosphoinositide-3-kinase (PI3K) inhibitor, or in db/db islets, indicating a Lepr-\( b \) mediated PI3K dependent mechanism. As insulin can inhibit glucagon secretion, it can be postulated that the suppressive effect of leptin on glucagon secretion could be indirectly mediated through changes in insulin secretion; however, this is unlikely due to the inhibitory effect that leptin has on \( \beta \)-cell insulin secretion.

In addition to a direct suppressive action of leptin on \( \alpha \)-cell glucagon secretion, ICV leptin administration also reverses hyperglucagonemia in STZ-treated rodents\(^{50,57} \), and suppresses glucagon content and preproglucagon transcript levels in pancreata of STZ-treated mice\(^{54} \). In contrast, leptin administration has also been shown to enhance hypoglycemia-induced glucagon secretion in rats through activation of the sympathetic nervous system\(^{117} \). This effect was not observed when leptin was perfused in the rat pancreas, supporting an indirect mode of
action\textsuperscript{117}. Therefore, central leptin action might have differential effects on glucagon secretion when under different metabolic stressors, such as hypoglycemia.

Taken together, the evidence indicates that leptin tonically inhibits glucagon synthesis and secretion from \( \alpha \)-cells, through direct leptin signaling in \( \alpha \)-cells (Figure 1), and through leptin-responsive hypothalamic relays (Figure 2). However, whether suppression of glucagon levels alone can account for the antidiabetic action of leptin is unclear. One study giving a low dose of leptin that reversed hyperglucagonemia in STZ-treated rats did not substantially improve glycemia\textsuperscript{73}, suggesting that glucagon suppression is not sufficient for the glucose-lowering effect of leptin therapy in insulin deficient rodents, but this warrants further investigation.

**Leptin Action on Hepatocytes**

In \textit{ob/ob} mice, leptin administration profoundly alters hepatic gene expression\textsuperscript{123}. Short-term leptin administration in normal rodents does not alter basal hepatic glucose production\textsuperscript{124}, but alters hepatic glucose fluxes under hyperinsulinemic conditions\textsuperscript{124,125}. Although the majority of studies show that leptin administration in rodents enhances insulin mediated suppression of hepatic glucose production\textsuperscript{38,48,124,126,127}, the effects of leptin on hepatic glucose flux pathways are conflicting. Leptin has been found to promote\textsuperscript{38,124,125,128} and decrease\textsuperscript{129} hepatic glycogen storage, as well as promote\textsuperscript{124–126} and suppress\textsuperscript{30} hepatic gluconeogenesis. One study reported that leptin administration in \textit{ob/ob} mice increased hepatic glucose production and increased glucose-6-phosphatase (G6Pase) activity while simultaneously inhibiting phosphoenolpyruvate carboxykinase (PEPCK) activity\textsuperscript{27}. Thus, the effects of leptin on hepatic glucose metabolism are complex and likely dependent on the current metabolic state.

\textit{Lepr-b} expression, has been shown in hepatic cell lines\textsuperscript{130,131}, isolated hepatocytes from rats and pigs\textsuperscript{132}, and in mouse liver\textsuperscript{18,55,133}. Furthermore, we and others have demonstrated specific leptin binding or functional leptin signaling in hepatocytes\textsuperscript{46}, hepatocyte cell lines\textsuperscript{45,110,133,134}, and rat and mouse liver\textsuperscript{39,133,136}. Application of leptin to hepatocytes often has simultaneously opposing effects on insulin signaling pathways\textsuperscript{131,134,135}. As an example, in one study, perfusion of leptin in isolated rat livers enhanced insulin stimulated insulin receptor (IR) phosphorylation, and promoted insulin receptor substrate-2 (IRS-2) binding to PI3K, while simultaneously inhibiting that of insulin receptor substrate-1 (IRS-1)\textsuperscript{135}. In contrast, another study showed that application of leptin to rat hepatoma cells enhanced insulin stimulated IRS-1 association with PI3K, while inhibiting that of IRS-2\textsuperscript{131}. Furthermore, we found administration of leptin to a hepatic cell line, and to \textit{ob/ob} mice \textit{in vivo} enhanced hepatic insulin stimulated IR phosphorylation and paradoxically increased protein tyrosine phosphatase-1B (PTP1B) expression, a negative regulator of both insulin and leptin action\textsuperscript{45,137}.

Alone, leptin has been reported to promote glycogen storage in hepatocytes by inhibiting glycogen phosphorylase and glycogen synthase kinase 3 (GSK3) in perfused rat liver and hepatic cell lines\textsuperscript{131,135,138}, and to inhibit glucose production in response to gluconeogenic precursors in the perfused rat liver and isolated hepatocytes\textsuperscript{125,139}. Leptin can also inhibit gluconeogenesis in primary rat hepatocytes\textsuperscript{140} and the perfused rat liver\textsuperscript{139}, an effect possibly mediated through activation of PI3K and PDE3B\textsuperscript{140}. Collectively, these studies show that leptin directly modulates insulin signaling and glucose flux, but the effects of leptin are highly variable. Perhaps contributing to these seemingly contradictory findings is that the effect of leptin on hepatocytes appears to be dependent on duration of leptin pretreatment\textsuperscript{131}, species\textsuperscript{132} and nutritional status\textsuperscript{141,142}. Indeed, one study found that perfusion of livers during the postprandial state inhibited epinephrine-stimulated glucose production, whereas perfusion of livers during the postabsorptive phase stimulated glucose release\textsuperscript{142}.

To examine the physiological role of hepatic leptin signaling, Cohen \textit{et al.}\textsuperscript{110} used a Cre-lox approach to knock out total \textit{Lepr} expression in livers of mice; they found no discernible differences in body weight or glucose metabolism in non-fasted conditions. We subsequently used a similar Cre-lox approach to generate mice with a liver-specific disruption of the signaling domain of Lepr-b, and examined whether these mice showed perturbations in glucose metabolism under varied metabolic conditions\textsuperscript{133}. Although these mice had normal glucose homeostasis under basal, fasted conditions, during an oral glucose tolerance test these mice were protected from glucose intolerance induced by aging or high-fat feeding. Furthermore, during a hyperinsulinemic-euglycemic clamp, these mice showed enhanced hepatic insulin sensitivity when compared to wild-type littermate controls\textsuperscript{133}. Thus, while \textit{in vitro} effects of leptin on hepatocytes are highly variable, our \textit{in vivo} evidence suggests that under hyperinsulinemic conditions, leptin has a direct antagonizing effect on hepatic insulin sensitivity (Figure 1). Interestingly, in insulin deficient STZ-treated mice, the loss of hepatic leptin signaling has no effect on the glucose lowering ability of leptin therapy\textsuperscript{117}.

In normal rats, ICV leptin administration mimics the acute effects of peripheral leptin administration on hepatic glucose flux during hyperinsulinemia\textsuperscript{124,125}, showing that leptin action on the liver can also be mediated through central mechanisms. ICV leptin administration enhances hepatic insulin sensitivity in \textit{ob/ob} mice, and normal and diet-induced obese rats\textsuperscript{125,143,144}. Similarly, reconstitution of Lepr-b in the hypothalamus of \textit{fa/fa} rats enhances insulin-mediated suppression of hepatic glucose production\textsuperscript{145}. Central leptin administration or gene delivery also influences hepatic expression of genes controlling glucose flux\textsuperscript{90,31,57,39,125}. ICV leptin administration in normal rats inhibits glycogenolysis, but stimulates gluconeogenesis, the net effect resulting in enhanced insulin suppression of hepatic glucose production\textsuperscript{125}. In agreement with this, the effect of ICV leptin on hepatic gluconeogenesis was shown to occur through a melanocortin dependent pathway, whereas the effect of leptin on glycogenolysis was unaffected by melanocortin blockade\textsuperscript{146}.
Furthermore, the stimulatory effect of leptin on gluconeogenesis in normal rodents is consistent with increased SNS tone, and antglycolytic action of leptin on liver metabolism is consistent with increased PNS tone. In support of this, the effect of ICV leptin on hepatic glucose fluxes in normal rats was mimicked by pharmacological stimulation of the SNS, whereas reconstitution of Lepr-b in the hypothalamus of fa/fa rats increased hepatic insulin sensitivity, in a manner dependent on hepatic vagal innervation. Similarly, the ability of leptin to improve glucose tolerance was mildly attenuated by hepatic vagotomy in mice with a muscle-specific overexpression of a dominant negative insulin-like growth factor-1 (IGF-1) receptor (MKR mice), which is a model of type 2 diabetes.

Collectively, these studies show that the physiological effects of leptin on the liver are complex. It appears that under hyperinsulinemic conditions, direct leptin action on the liver antagonizes hepatic insulin signaling (Figure 1). However, indirect hepatic actions of leptin through autonomic efferents from the brain enhance insulin-mediated suppression of glucose production, but can have differential effects on glycogenolysis and gluconeogenesis (Figure 2). Thus, the net direct and indirect effects of leptin on the liver in vivo might depend on factors, such as the current metabolic state and the level of SNS or PNS stimulation.

Leptin Action on Skeletal Muscle
In skeletal muscle, a major contributor to insulin-stimulated glucose disposal, the effect of leptin is unclear. Although some studies have found no change, or even decreased glucose uptake after acute leptin administration in vivo, most studies show that leptin stimulates glucose uptake and insulin sensitivity in skeletal muscle. Several studies have reported Lepr-b expression in skeletal muscle, yet the direct action of leptin on skeletal muscle is also controversial (Figure 1). Some studies indicate that application of leptin to muscle cell lines stimulates glucose uptake and glucose utilization either alone or in the presence of insulin; however, others have reported inhibition of insulin-stimulated glucose metabolism, or no effect on muscle glucose uptake. In soleus muscle isolated from ob/ob mice, leptin inhibited insulin stimulated glycogen synthesis, but had no effect on glycogen synthesis in soleus isolated from wild-type mice. Thus, the direct action on skeletal muscle appears dependent on metabolic status. Further adding to the complexity of leptin action, in a skeletal muscle cell line, the response to leptin was dependent on the duration of leptin exposure. There is a substantial degree of insulin and leptin cross-talk in skeletal muscle. Leptin exposure has been shown to induce IRS-2 phosphorylation and PI3K activation, and incubation with leptin alone has been shown to stimulate glucose uptake through a PI3K dependent mechanism in muscle cell lines. Acute leptin administration was shown to directly stimulate skeletal muscle adenosine monophosphate-activated protein kinase (AMPK), which alters lipid partitioning by promoting fatty acid oxidation. Changes in lipid partitioning are also observed after leptin application to muscle cell lines. Although acute leptin stimulation of AMPK did not alter glucose uptake, chronic hyperleptinemia decreases lipid accumulation in muscle in vivo, and simultaneously enhances muscle insulin sensitivity, an effect mimicked by pharmacological stimulation of AMPK. Thus, chronic exposure of skeletal muscle to leptin might have long-term effects on insulin sensitivity.

Both ICV and intravenous short-term leptin administration stimulate glucose uptake in skeletal muscle in normal mice, indicating that leptin action on skeletal muscle can also be indirectly mediated through central pathways (Figure 2). Leptin injection in the hypothalamus acutely stimulates glucose uptake, utilization and fatty acid oxidation in skeletal muscle. Interestingly, this occurred when leptin was injected directly into the ventromedial hypothalamus, and was blocked by a melanocortin receptor antagonist. Furthermore, injection of leptin into the lateral hypothalamus alters the expression and activity of metabolic enzymes in skeletal muscle, including Akt and AMPK. The effect of leptin on skeletal muscle is diminished by denervation or sympathetic blockade. When leptin is administered chronically through an ICV route in insulin deficient rodents, glucose utilization is increased, as well as the expression of Glut4 in skeletal muscle. However, central leptin administration or gene therapy has also been reported to have no effect or to decrease Glut4 expression. In one study, ICV leptin increased Glut4 expression in white gastrocnemius, but not soleus muscle, whereas the acute stimulation of glucose uptake by hypothalamic leptin injection was more prominent in the soleus than the extensor digitorum longus, indicating that leptin might differentially regulate glucose metabolism in oxidative and glycolytic muscle fibres. Collectively, these studies suggest that leptin enhances insulin sensitivity and glucose uptake in skeletal muscle through central relays, whereas direct leptin action on skeletal muscle might enhance or oppose insulin action depending on metabolic state.

Leptin Action on Adipocytes
Unlike the hepatic and muscular actions of leptin, leptin administration is well known to inhibit insulin action in white adipose tissue (WAT). Paradoxically, both prolonged and acute administration of leptin in vivo have been shown to stimulate glucose uptake in brown adipose tissue (BAT), but not WAT. The effect of leptin on WAT and BAT are thereby likely to partially mediate the effect of leptin on glucose homeostasis.

Expression of Lepr-b has been shown in BAT and WAT from mice, and primary BAT and cultured brown adipocytes from rats. Furthermore, leptin-induced STAT phosphorylation and translocation has been shown in brown and white adipocytes, the effect of which is absent in fa/fa brown adipocytes and attenuated in white adipocytes after Lepr-b knockdown by antisense RNA. Isolated white adipocytes

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Incubation of adipocytes with leptin results in desensitization over several hours to insulin-induced glucose uptake and glycogen synthesis. This desensitization effect of leptin is dose- and time-dependent, and reversible after removal of leptin. A similar inhibition of insulin-stimulated glucose uptake was reported in isolated adipocytes, indicating that leptin might acutely stimulate whole-body glucose uptake in BAT, but not skeletal muscle or WAT, suggesting that the direct inhibitory effect of leptin on insulin action in WAT is important for normal glucose homeostasis. In addition, these mice had impaired glucose tolerance and insulin sensitivity, indicative of enhanced adiopgenic action of insulin in adipose tissue. Inclusion of insulin-stimulated IR kinase activity, and IRS-1 phosphorylation in a brown adipocyte cell line. Of note, leptin application also reduces leptin gene expression in adipocytes, possibly through suppression of the stimulatory effect of insulin on leptin synthesis. One in vivo study found that mice with a knockdown of adipocyte leptin receptors had increased body weight and adiposity, indicative of enhanced adiopgenic action of insulin in adipose tissue. Furthermore, a role of leptin in GH secretion has been observed in STZ-treated mice, leptin therapy was recently reported to have minimal metabolic benefit in obese, type 2 diabetic patients. It appears as though obesity is typically associated with elevated leptin levels and leptin resistance, thereby limiting the effectiveness of exogenous leptin. However, leptin therapy might be useful in combination with other means of weight loss, such as diet and exercise, to help reduce regaining weight. In contrast, leptin therapy in humans with rare congenital leptin deficiency markedly reduces body weight and adiposity, and ameliorates the metabolic dysfunction of these patients. Leptin also proved to ameliorate hyperinsulinemia, insulin resistance and hyperglycemia in another subset of leptin-deficient individuals, namely patients with general or partial lipodystrophy. Thus, as previously speculated, leptin administration seems to be most effective in conditions with abnormally low circulating leptin levels.

Another metabolic disorder that is accompanied by inappropriate low leptin levels is type 1 diabetes, and a rapidly increasing number of studies support the fact that leptin has therapeutic properties in rodent models of insulin deficiency. Although insulin therapy restores circulating leptin levels in type 1 diabetic patients, studies in rodents show that higher doses of leptin might have additional therapeutic effects on glycemia. Leptin is now being tested clinically as an adjunct to insulin therapy for type 1 diabetes. Interestingly, occasional leptin injections in NOD mice receiving continuous insulin infusion were found to improve glycemia, compared with insulin alone, even when leptin injections were combined with a lower insulin dose. This indicates that the addition of leptin to insulin therapy regimens for patients with type 1 diabetes might allow for tighter glycemic control, with less-frequent insulin dosing. However, with the potential benefit of combined leptin and insulin cotherapy, is a potential danger of hypoglycemia. Our work has shown that as a result of enhanced insulin sensitivity, a dose of insulin that was only modestly effective in lowering blood glucose in STZ-treated mice was nearly lethal when combined with leptin therapy.
Furthermore, given emerging evidence of the inhibitory effect of leptin on β-cell glucagon secretion\textsuperscript{121} and circulating glucagon levels\textsuperscript{49,50,55,57,58,73}, leptin might interfere with counter-regulatory responses to hypoglycemia, a mechanism that is already impaired in patients with long standing type 1 diabetes. Thus, caution should be used when testing leptin with insulin in patients with type 1 diabetes. Also of potential concern are the immune-related actions of leptin\textsuperscript{204}. NOD mice with a loss of function mutation in the \textit{Lepr} gene have reduced incidence of diabetes\textsuperscript{205,206}, whereas leptin administration has been reported to accelerate diabetes onset in NOD mice\textsuperscript{207}. Nevertheless, the potential glucose-lowering effect of leptin in insulin deficient rodents justifies the careful assessment of leptin therapy in humans with diabetes.

Approximately two decades since its discovery, the so-called anti-obesity hormone, leptin, has now been established as a key regulator of glucose homeostasis, both in rodents and humans. Both leptin deficiency and leptin resistance have a profound impact on metabolism in rodents and humans. Leptin replacement can dramatically improve metabolism in cases of leptin deficiency, and now even shows promise as a therapy for insulin deficient type 1 diabetes. Leptin has multiple, complex actions on insulin-sensitive tissues and the hormones of the endocrine pancreas, all of which likely contribute to glucose homeostasis. Further investigation of the mechanisms of leptin action on metabolism is warranted, both to enhance our understanding of metabolic regulation, and to fully exploit the therapeutic potential of leptin.

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