The role of leptin in glucose homeostasis

Heather C Denroche[1], Frank K Huynh[1], Timothy J Kieffer[1,2*]

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 ago[1,2]. 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 factor[3]. The identity of this satiety factor remained unknown until 1994, when the *ob* gene was identified through positional cloning[4]. *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 levels[4,5]. Injection of wild-type leptin can lower body weight in both *ob/ob* and wild-type mice[5]. Although rare, mutations in the human leptin gene have been identified in several families[6–10], 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 therapy[7,11–14].

The discovery of the leptin receptor (Lepr), encoded by the *db* gene followed soon after leptin was identified[15]. 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 domain[16,17]. Lepr-b has the longest intracellular domain of the leptin receptor isoforms[16,17], and signals through Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathways to influence transcription of target genes[18–21]. When back-crossed to the same genetic background, *ob/ob* and *db/db* mice have nearly identical phenotypes[22,23], 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 fat[24,25], and acts on hypothalamic neurons to inhibit food intake and increase energy expenditure, leading to a reduction in body weight[5,26,27]; 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 diabetes[2,27–32]. 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 independent of its effects on body weight and food intake. First, hyperinsulinemia occurs in *ob/ob* mice[29,31–33], along with transient hypoglycemia[32,33], 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 rats[30,34–37]. 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 antagonist[38]. Adult mice treated with this antagonist developed fasting- and glucose-stimulated hyperinsulinemia, and insulin resistance within 3 days, without

[1]Department of Cellular and Physiological Sciences, The Life Sciences Institute, and [2]Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada
*Corresponding author. Timothy J Kieffer Tel.: +1-604-822-2156 Fax: +1-604-822-2316
E-mail address: tim.kieffer@ubc.ca
Received 12 December 2011; accepted 21 December 2011

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 hypoleptinemic, and have hyperinsulinemia, insulin resistance and hyperglycemia[39,40]. 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[7,11–14,27,39,40]. Leptin administration reduces circulating insulin and glucose levels in *ob/ob* mice, indicative of increased insulin sensitivity, to a greater extent than pair-feeding[41–43], 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[44,45]. We also showed that temporary leptin therapy has longer lasting effects on blood glucose than food intake in *ob/ob* mice[46]. 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[27,47]. 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[48–54] and mice[55–57], non-obese diabetic (NOD) mice[49,58], insulin deficient Akita mice[59,60] and BioBreeding rats with virally-induced β-cell destruction[61]. Leptin therapy also normalizes water intake and urine output, and reverses glycosuria, hyperketonemia and hyperphagia in insulin deficient rodents[50,52,54,58], 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[49,52,58]. 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[48–50,55,58,62]. 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[55]. 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[63]. In addition, leptin therapy decreases levels of counter regulatory hormones[49,50,55,58], 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[50,55]. Leptin also robustly decreases glucagon levels in this model[49,58]. Hyperglucagonemia is a common characteristic of diabetes, and is a requisite for hyperglycemia in several models of insulin deficiency[64]. Interestingly, suppression of endogenous glucagon[65] or antagonism of glucagon receptor signaling decreases hyperglycemia in STZ-diabetic rats[66–68]. Furthermore, mice with genetic knockout of glucagon receptors are resistant to developing STZ-induced diabetes[69]. 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[70]. 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[71]. Interestingly, by continuously administering a dose of leptin that prevents the decrease in leptin levels in STZ-treated rats, hyperphagia[72], insulin resistance[73] and hyperglucagonemia[73] are prevented. It is therefore intriguing to consider that key metabolic disturbances associated with insulin deficiency are actually secondary to the underlying hypoleptinemia. Importantly, despite reversal of hyperphagia, insulin resistance and hyperglucagonemia, restoration of physiological leptin levels does not reverse hyperglycemia in STZ-treated rats[73]. 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 isoform, are widely expressed throughout the central nervous system and peripheral tissues[18]. 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[74–76]. 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.

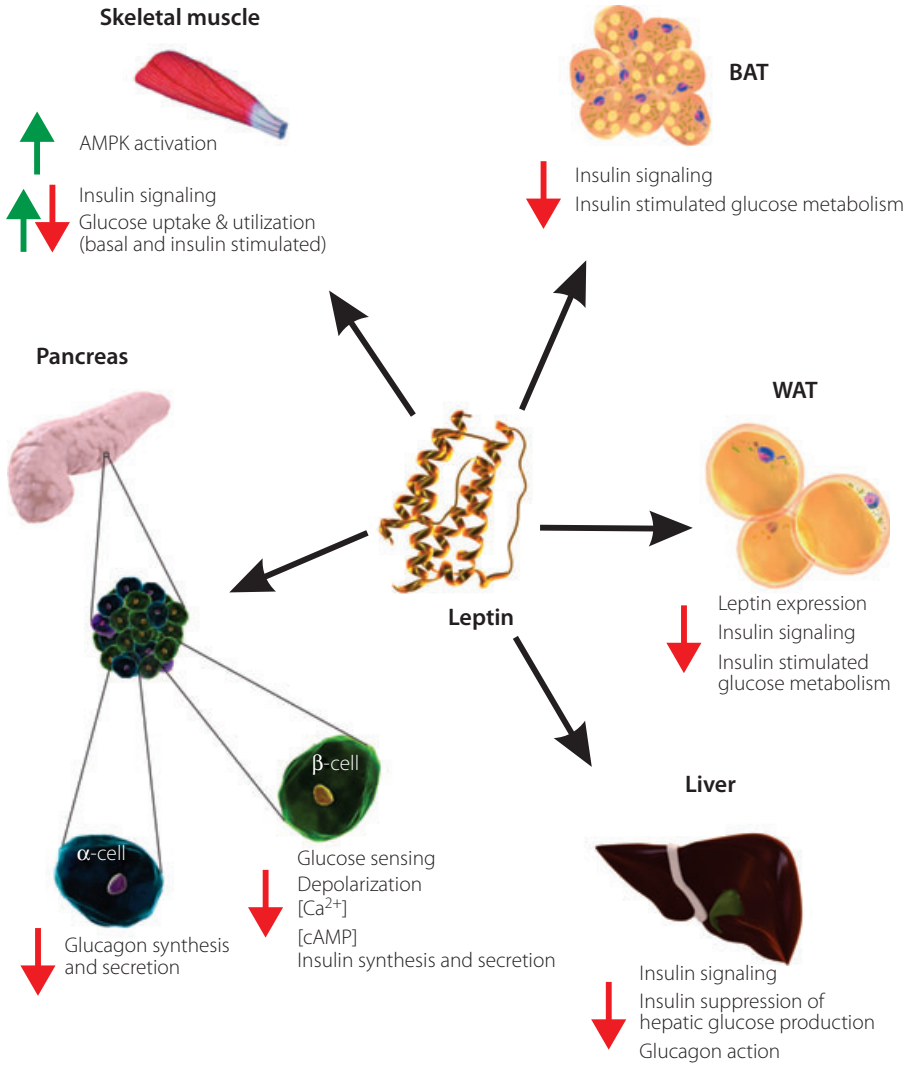

Figure 1 | Direct actions of leptin on tissues that contribute to glucose homeostasis. Leptin acts on peripheral leptin receptor-b isoform expressing tissues, including the endocrine pancreas and insulin-sensitive tissues. Direct leptin action on the endocrine pancreas inhibits insulin secretion from 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.

Leptin Action on Pancreatic β-Cells

Leptin, either through gene delivery or recombinant peptide administration, rapidly lowers circulating insulin levels in *ob/ob* mice[44,45,77–79], but is ineffective in *db/db* mice[79,80]. 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[79]. Leptin has an inhibitory effect on insulin synthesis as well; a single leptin injection in *ob/ob* mice decreases preproinsulin messenger ribonucleic acid (mRNA) in islets within 24 h, by alterations in transcription factor binding to the insulin promoter[44]. 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 preproinsulin gene transcription[81]. Leptin administration has also been reported to acutely decrease glucose-stimulated insulin levels in normal rodents[82], albeit the effect is less robust than in *ob/ob* mice[45].

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[80,81,83–85]. *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)[44,79–81,84–88]. Leptin robustly inhibits insulin secretion from isolated islets and the perfused pancreas of *ob/ob* mice[80,84]. Most studies using islets or perfused pancreata from non-leptin

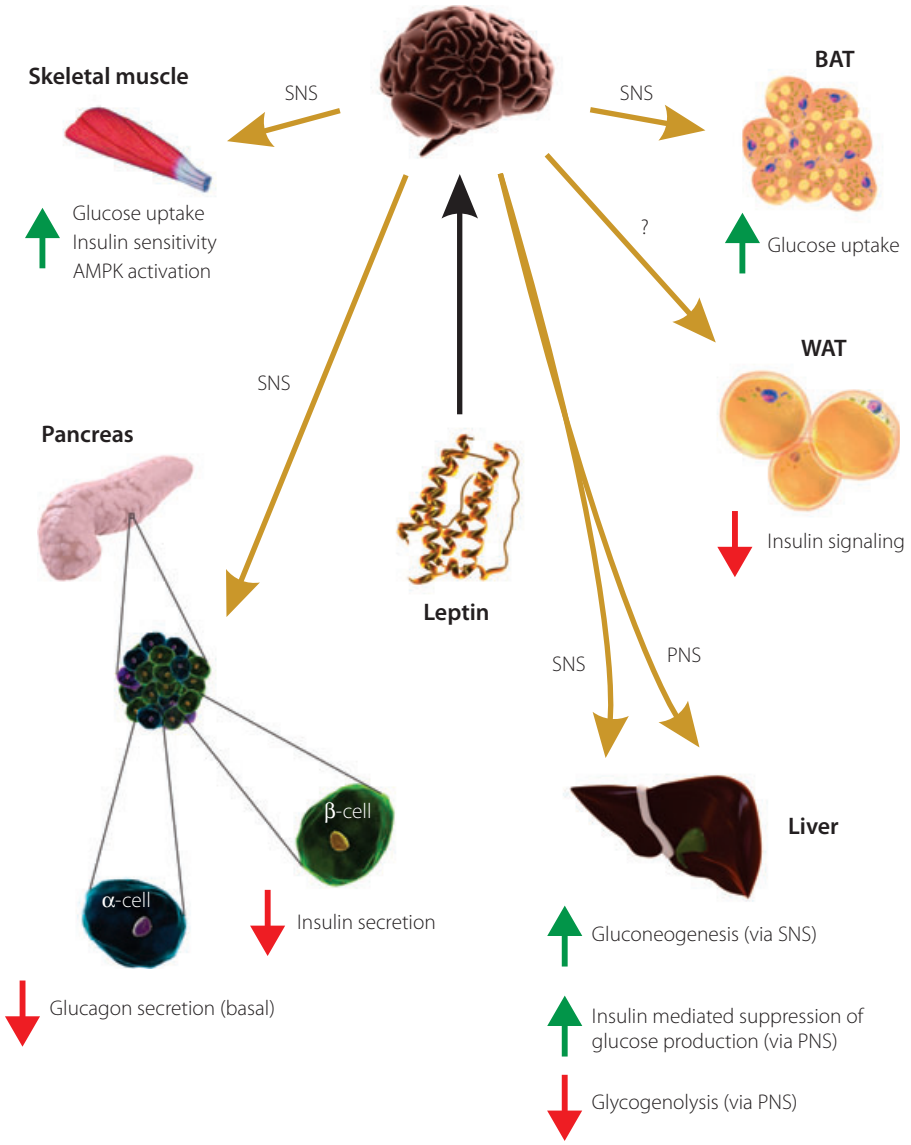

Figure 2 | Centrally-mediated actions of leptin on tissues that contribute to glucose homeostasis. Leptin activates leptin-responsive relays initiating in the hypothalamus that mediate leptin action on the endocrine pancreas and insulin sensitive tissues through autonomic efferents. The sympathetic nervous system has been implicated in central leptin action on insulin secretion, and glucose metabolism in brown adipose tissue, skeletal muscle and the liver. The parasympathetic nervous system might mediate effects of central leptin on hepatic insulin sensitivity and glycogenolysis. It is unclear which autonomic system mediates leptin action on glucagon secretion and the inhibition of insulin signaling in white adipose tissue. AMPK, adenosine monophosphate-activated protein kinase; BAT, brown adipose tissue; PNS, parasympathetic nervous system; SNS, sympathetic nervous system; 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_{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_{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 β-cells *in vivo*, we used the Cre-*lox* system to generate mice with disrupted Lepr-b signaling in β-cells[104]. Mice with a disrupted signaling domain of the *Lepr* gene in β-cells and the hypothalamus showed hyperinsulinemia and fasting hypoglycemia, supporting a physiological role of leptin to inhibit insulin secretion from β-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 hyperinsulinemia in mice with disrupted Lepr signaling in β-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 β-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 extrapancreatic 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 β-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[111–115], although in one study the effect was modest[116], whereas in another, leptin did not lower meal-stimulated insulin levels compared with pair-fed controls[43]. The effect of central leptin action on insulin levels is consistent with increased sympathetic tone to β-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)[113]. One SNS-mediated mechanism of leptin action on the β-cell has been proposed to occur through sympathetic inhibition of bioactive osteocalcin secretion from osteoblasts, which in turn reduces β-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 β-cells, this might be counterbalanced by the parasympathetic nervous system (PNS).

Collectively, these findings indicate that both direct and centrally-mediated leptin action inhibit β-cell insulin secretion. Insulin secretion from pancreatic β-cells promotes lipid storage and leptin synthesis in adipocytes, creating a bidirectional regulatory loop between β-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 α-Cells

Several lines of evidence implicate a role of leptin in inhibiting glucagon secretion from pancreatic α-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[49,55,58,73]. Recent evidence indicates that leptin can suppress glucagon secretion by directly acting on pancreatic α-cells. The expression of *Lepr* transcript has been shown in an α-cell line, and Lepr-b immunoreactivity was shown in mouse and human α-cells[121]. Application of leptin to isolated mouse α-cells induces phosphorylation and nuclear translocation of STAT3[122]. Stimulation of α-tumor cell 1 (TC1) cells and mouse α-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 α-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 β-cell insulin secretion.

In addition to a direct suppressive action of leptin on α-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[57]. 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[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 tonicly inhibits glucagon synthesis and secretion from α-cells, through direct leptin signaling in α-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[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 *ob/ob* mice, leptin administration profoundly alters hepatic gene expression[123]. Short-term leptin administration in normal rodents does not alter basal hepatic glucose production[124], but alters hepatic glucose fluxes under hyperinsulinemic conditions[124,125]. Although the majority of studies show that leptin administration in rodents enhances insulin mediated suppression of hepatic glucose production[38,48,124,126,127], the effects of leptin on hepatic glucose flux pathways are conflicting. Leptin has been found to promote[58,124,125,128] and decrease[129] hepatic glycogen storage, as well as promote[124–126] and suppress[50] hepatic gluconeogenesis. One study reported that leptin administration in *ob/ob* mice increased hepatic glucose production and increased glucose-6-phosphatase (G6Pase) activity while simultaneously inhibiting phosphoenolpyruvate carboxykinase (PEPCK) activity[127]. Thus, the effects of leptin on hepatic glucose metabolism are complex and likely dependent on the current metabolic state.

Lepr-b expression, has been shown in hepatic cell lines[130,131], isolated hepatocytes from rats and pigs[132], and in mouse liver[18,55,133]. Furthermore, we and others have demonstrated specific leptin binding or functional leptin signaling in hepatocytes[45], hepatocyte cell lines[45,130,131,134], and rat and mouse liver[19,135,136]. Application of leptin to hepatocytes often has simultaneously opposing effects on insulin signaling pathways[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)[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[131]. Furthermore, we found administration of leptin to a hepatic cell line, and to *ob/ob* mice *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[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[131,135,138], and to inhibit glucose production in response to gluconeogenic precursors in the perfused rat liver and isolated hepatocytes[135,139]. Leptin can also inhibit glucagon action in primary rat hepatocytes[140] and the perfused rat liver[139], an effect possibly mediated through activation of PI3K and PDE3B[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[131], species[132] and nutritional status[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[142].

To examine the physiological role of hepatic leptin signaling, Cohen *et al.*[110] used a Cre-*lox* approach to knock out total *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[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[133]. Thus, while *in vitro* effects of leptin on hepatocytes are highly variable, our *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[55].

In normal rats, ICV leptin administration mimics the acute effects of peripheral leptin administration on hepatic glucose flux during hyperinsulinemia[124,125], showing that leptin action on the liver can also be mediated through central mechanisms. ICV leptin administration enhances hepatic insulin sensitivity in *ob/ob* mice, and normal and diet-induced obese rats[125,143,144]. Similarly, reconstitution of Lepr-b in the hypothalamus of *fa/fa* rats enhances insulin-mediated suppression of hepatic glucose production[145]. Central leptin administration or gene delivery also influences hepatic expression of genes controlling glucose flux[50,51,57,59,125]. ICV leptin administration in normal rats inhibits glycogenolysis, but stimulates gluconeogenesis, the net effect resulting in enhanced insulin suppression of hepatic glucose production[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[146].

Furthermore, the stimulatory effect of leptin on gluconeogenesis in normal rodents is consistent with increased SNS tone, and antiglycolytic 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[125], whereas reconstitution of Lepr-b in the hypothalamus of *fa/fa* rats increased hepatic insulin sensitivity, in a manner dependent on hepatic vagal innervation[145]. 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[147].

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[55,124,127,148], or even decreased glucose uptake after acute leptin administration *in vivo*[149], most studies show that leptin stimulates glucose uptake and insulin sensitivity in skeletal muscle[129,149–153]. Several studies have reported *Lepr-b* expression in skeletal muscle[154,155], 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[156,157] and isolated soleus muscle[149,151,158] stimulates glucose uptake and glucose utilization either alone or in the presence of insulin; however, others have reported inhibition of insulin-stimulated glucose metabolism[157,159–161], or no effect on muscle glucose uptake[162,163]. In soleus muscle isolated from *ob/ob* mice, leptin inhibited insulin stimulated glycogen synthesis[160,161], but had no effect on glycogen synthesis in soleus isolated from wild-type mice[162]. 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[157]. 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[156,164], and incubation with leptin alone has been shown to stimulate glucose uptake through a PI3K dependent mechanism[157] in muscle cell lines. Acute leptin administration *in vivo* was shown to directly stimulate skeletal muscle adenosine monophosphate-activated protein kinase (AMPK), which alters lipid partitioning by promoting fatty acid oxidation[148]. Changes in lipid partitioning are also observed after leptin application to muscle cell lines[162,165]. Although acute leptin stimulation of AMPK did not alter glucose uptake[148], chronic hyperleptinemia decreases lipid accumulation in muscle *in vivo*, and simultaneously enhances muscle insulin sensitivity, an effect mimicked by pharmacological stimulation of AMPK[166–168]. 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[129], 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[148,150]. Interestingly, this occurred when leptin was injected directly into the ventromedial hypothalamus[150,169], and was blocked by a melanocortin receptor antagonist[169]. Furthermore, injection of leptin into the lateral hypothalamus alters the expression and activity of metabolic enzymes in skeletal muscle, including Akt and AMPK[170]. The effect of leptin on skeletal muscle is diminished by denervation[129,148] or sympathetic blockade[148,170]. 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[50,57,171]. However, central leptin administration or gene therapy has also been reported to have no effect[59] or to decrease *Glut4* expression[51]. In one study, ICV leptin increased *Glut4* expression in white gastrocnemius, but not soleus muscle[57], whereas the acute stimulation of glucose uptake by hypothalamic leptin injection was more prominent in the soleus than the extensor digitorum longus[150], 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[127,129,152,172]. 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[173,174], and primary BAT and cultured brown adipocytes from rats[175]. 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[175,176]. Isolated white adipocytes

incubated with leptin become desensitized over several hours to insulin-induced glucose uptake and glycogen synthesis[177]. This desensitizing 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 a brown adipocyte cell line[178]. There is significant cross-talk between leptin and insulin signaling in adipocytes; leptin inhibits insulin stimulated phosphorylation of IR and GSK3, and binding of insulin to its receptor in isolated white adipocytes[179,180], and reduces insulin-stimulated IR kinase activity, and IRS-1 phosphorylation in a brown adipocyte cell line[178]. Of note, leptin application also reduces leptin gene expression in adipocytes, possibly through suppression of the stimulatory effect of insulin on leptin synthesis[181]. One *in vivo* study found that mice with a knockdown of adipocyte leptin receptors had increased body weight and adiposity, indicative of enhanced adipogenic action of insulin in adipose tissue. In addition these mice had impaired glucose tolerance and insulin sensitivity[176] suggesting that the direct inhibitory effect of leptin on insulin action in WAT is important for normal glucose homeostasis.

The inhibitory effect of leptin on insulin signaling is also observed in WAT explanted from rats treated with ICV leptin for 7 days, indicating that central leptin action induces prolonged changes to white adipocytes to inhibit insulin signaling[179]. In contrast to WAT, central leptin administration or gene therapy can acutely stimulate glucose uptake in BAT[50,127,129,150], and increase expression of *Glut4* and uncoupling proteins (*UCP*)*-1* and *-3*[51,59]. The effect of leptin on BAT glucose metabolism is abolished by denervation[150], and mediated by the SNS[182,183]. Interestingly, in *ob/ob* mice, ICV leptin acutely stimulated whole body glucose turnover, and this correlated with enhanced glucose uptake in BAT, but not skeletal muscle or WAT, indicating that leptin might acutely stimulate whole-body glucose metabolism through central mediated effects on BAT[127]. Collectively, these studies show that leptin inhibits insulin signaling in adipocytes primarily through direct action (Figure 1), whereas central leptin-responsive relays mediate leptin action on glucose uptake in BAT (Figure 2).

Leptin Action on Counter Regulatory and Insulin Mimetic Hormones

In addition to direct and centrally-mediated effects of leptin on the endocrine pancreas to influence insulin and glucagon levels, leptin might also indirectly regulate glucose metabolism by altering levels of other hormones that regulate glucose metabolism. Leptin is well known to inhibit synthesis and secretion of corticosterone[73,184–187], which could thereby increase insulin sensitivity. Furthermore, a role of leptin in GH secretion has been identified. Although studies indicate that leptin enhances GH secretion[188–191], we found that in STZ-treated mice, leptin therapy robustly suppressed circulating GH levels, which might enhance peripheral insulin sensitivity[55]. Leptin might also improve glycemia indirectly by increasing levels of the glucose-lowering hormone, GLP-1[192]. In addition, leptin administration in STZ-treated rats was found to elevate circulating IGF1, which in turn could have an insulin mimetic effect on peripheral tissues[49]. Finally, leptin therapy has also been found to increase circulating IGF binding protein-2 (IGFBP2) levels in *ob/ob* mice[47], which we have since confirmed[193]. Interestingly, adenoviral-mediated overexpression of IGFBP2 to *ob/ob* mice mimics the effect of leptin administration, by reducing glucose and insulin levels, indicating that some of the metabolic action of leptin might be mediated through IGFBP2[47]. As a whole, it appears that most effects of leptin on altering circulating hormone levels lead to an overall increase in insulin sensitivity.

THE THERAPEUTIC POTENTIAL OF LEPTIN

As a result of the profound ability of leptin to modulate glucose homeostasis, leptin holds therapeutic potential for the treatment of metabolic disorders. Clinical trials showed that leptin had modest weight-reducing effects in some obese individuals[194], and leptin therapy was recently reported to have minimal metabolic benefit in obese, type 2 diabetic patients[195]. 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[196–201]. 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[7,11–14]. Leptin also proved to ameliorate hyperinsulinemia, insulin resistance and hyperglycemia in another subset of leptin-deficient individuals, namely patients with general or partial lipodystrophy[39,202]. Thus, as previously speculated, leptin administration seems to be most effective in conditions with abnormally low circulating leptin levels[203].

Another metabolic disorder that is accompanied by inappropriately low leptin levels is type 1 diabetes[71], and a rapidly increasing number of studies support the fact that leptin has therapeutic properties in rodent models of insulin deficiency[48–55,57–62]. Although insulin therapy restores circulating leptin levels in type 1 diabetic patients[71], 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[58]. 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[55].

Furthermore, given emerging evidence of the inhibitory effect of leptin on α-cell glucagon secretion[121] and circulating glucagon levels[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[204]. NOD mice with a loss of function mutation in the *Lepr* gene have reduced incidence of diabetes[205,206], whereas leptin administration has been reported to accelerate diabetes onset in NOD mice[207]. Nevertheless, the potent 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.

ACKNOWLEDGMENTS

H. C. D. is the recipient of a Canada Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada. F. K. H. is funded by the Canadian Diabetes Association. T. J. K. is the recipient of a senior scholarship from the Michael Smith Foundation of Health Research. Our work in this area is supported by the Canadian Institutes of Health Research. There are no financial conflicts to declare.

REFERENCES

1. Ingalls AM, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *J Hered* 1950; 41: 317–318.
2. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Science* 1966; 153: 1127–1128.
3. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia* 1973; 9: 294–298.
4. Zhang Y, Proenca R, Maffei M, *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425–432.
5. Halaas JL, Gajiwala KS, Maffei M, *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; 269: 543–546.
6. Montague CT, Farooqi IS, Whitehead JP, *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; 387: 903–908.
7. Gibson WT, Farooqi IS, Moreau M, *et al.* Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy. *J Clin Endocrinol Metab* 2004; 89: 4821–4826.
8. Strobel A, Issad T, Camoin L, *et al.* A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 1998; 18: 213–215.
9. Fischer-Posovszky P, von Schnurbein J, Moepps B, *et al.* A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. *J Clin Endocrinol Metab* 2010; 95: 2836–2840.
10. Mazen I, El-Gammal M, Abdel-Hamid M, *et al.* A novel homozygous missense mutation of the leptin gene (N103K) in an obese Egyptian patient. *Mol Genet Metab* 2009; 97: 305–308.
11. Farooqi IS, Matarese G, Lord GM, *et al.* Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002; 110: 1093–1103.
12. Licinio J, Caglayan S, Ozata M, *et al.* Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. *Proc Natl Acad Sci USA* 2004; 101: 4531–4536.
13. Paz-Filho GJ, Babikian T, Asarnow R, *et al.* Leptin replacement improves cognitive development. *PLoS ONE* 2008; 3: e3098.
14. Farooqi IS, Jebb SA, Langmack G, *et al.* Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 1999; 341: 879–884.
15. Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; 83: 1263–1271.
16. Lee GH, Proenca R, Montez JM, *et al.* Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 1996; 379: 632–635.
17. Chen H, Charlat O, Tartaglia LA, *et al.* Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996; 84: 491–495.
18. Ghilardi N, Ziegler S, Wiestner A, *et al.* Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci USA* 1996; 93: 6231–6235.
19. Vaisse C, Halaas JL, Horvath CM, *et al.* Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 1996; 14: 95–97.
20. Bahrenberg G, Behrmann I, Barthel A, *et al.* Identification of the critical sequence elements in the cytoplasmic domain of leptin receptor isoforms required for Janus kinase/signal transducer and activator of transcription activation by receptor heterodimers. *Mol Endocrinol* 2002; 16: 859–872.
21. Bates SH, Stearns WH, Dundon TA, *et al.* STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 2003; 421: 856–859.

22. Hummel KP, Coleman DL, Lane PW. The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains. *Biochem Genet* 1972; 7: 1–13.
23. Coleman DL, Hummel KP. The influence of genetic background on the expression of the obese (Ob) gene in the mouse. *Diabetologia* 1973; 9: 287–293.
24. Frederich RC, Hamann A, Anderson S, *et al.* Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1995; 1: 1311–1314.
25. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996; 334: 292–295.
26. Campfield LA, Smith FJ, Guisez Y, *et al.* Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; 269: 546–549.
27. Pelleymounter MA, Cullen MJ, Baker MB, *et al.* Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; 269: 540–543.
28. Genuth SM, Przybylski RJ, Rosenberg DM. Insulin resistance in genetically obese, hyperglycemic mice. *Endocrinology* 1971; 88: 1230–1238.
29. Genuth SM. Hyperinsulinism in mice with genetically determined obesity. *Endocrinology* 1969; 84: 386–391.
30. Coleman DL, Hummel KP. Hyperinsulinemia in pre-weaning diabetes (db) mice. *Diabetologia* 1974; 10(Suppl): 607–610.
31. Grundleger ML, Godbole VY, Thenen SW. Age-dependent development of insulin resistance of soleus muscle in genetically obese (ob/ob) mice. *Am J Physiol Endocrinol Metab* 1980; 239: E363–E371.
32. Dubuc PU. The development of obesity, hyperinsulinemia, and hyperglycemia in ob/ob mice. *Metabolism* 1976; 25: 1567–1574.
33. Dubuc PU, Willis PL. Postweaning development of diabetes in ob/ob mice. *Metabolism* 1979; 28: 633–640.
34. Rohner-Jeanrenaud F, Hochstrasser AC, Jeanrenaud B. Hyperinsulinemia of preobese and obese fa/fa rats is partly vagus nerve mediated. *Am J Physiol Endocrinol Metab* 1983; 244: E317–E322.
35. Russell JC, Ahuja SK, Manickavel V, *et al.* Insulin resistance and impaired glucose tolerance in the atherosclerosis-prone LA/N corpulent rat. *Arteriosclerosis* 1987; 7: 620–626.
36. Schwarzer K, Doring H, Schmidt I. Different physiological traits underlying increased body fat of fatty (fa/fa) and heterozygous (+/fa) rats. *Am J Physiol Endocrinol Metab* 1997; 272(1 Pt 1): E100–E106.
37. Zarjevski N, Doyle P, Jeanrenaud B. Muscle insulin resistance may not be a primary etiological factor in the genetically obese fa/fa rat. *Endocrinology* 1992; 130: 1564–1570.
38. Levi J, Gray SL, Speck M, *et al.* Acute disruption of leptin signaling *in vivo* leads to increased insulin levels and insulin resistance. *Endocrinology* 2011; 152: 3385–3395.
39. Oral EA, Simha V, Ruiz E, *et al.* Leptin-replacement therapy for lipodystrophy. *N Engl J Med* 2002; 346: 570–578.
40. Shimomura I, Hammer RE, Ikemoto S, *et al.* Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 1999; 401: 73–76.
41. Schwartz MW, Baskin DG, Bukowski TR, *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes* 1996; 45: 531–535.
42. Levin N, Nelson C, Gurney A, *et al.* Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci USA* 1996; 93: 1726–1730.
43. Mistry AM, Swick AG, Romsos DR. Leptin acts peripherally to limit meal-induced increases in plasma insulin concentrations in mice: a brief communication. *Exp Biol Med (Maywood)* 2004; 229: 1033–1037.
44. Seufert J, Kieffer TJ, Habener JF. Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci USA* 1999; 96: 674–679.
45. Lam NT, Lewis JT, Cheung AT, *et al.* Leptin increases hepatic insulin sensitivity and protein tyrosine phosphatase 1B expression. *Mol Endocrinol* 2004; 18: 1333–1345.
46. Oosman SN, Lam AW, Harb G, *et al.* Treatment of obesity and diabetes in mice by transplant of gut cells engineered to produce leptin. *Mol Ther* 2008; 16: 1138–1145.
47. Hedbacker K, Birsoy K, Wysocki RW, *et al.* Antidiabetic effects of IGFBP2, a leptin-regulated gene. *Cell Metab* 2010; 11: 11–22.
48. Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin deficient diabetes in the rat. *Diabetes* 1999; 48: 1487–1492.
49. Yu X, Park BH, Wang MY, *et al.* Making insulin deficient type 1 diabetic rodents thrive without insulin. *Proc Natl Acad Sci USA* 2008; 105: 14070–14075.
50. German JP, Thaler JP, Wisse BE, *et al.* Leptin Activates a Novel CNS Mechanism for Insulin-Independent Normalization of Severe Diabetic Hyperglycemia. *Endocrinology* 2010; 152: 394–404.
51. Hidaka S, Yoshimatsu H, Kondou S, *et al.* Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats. *FASEB J* 2002; 16: 509–518.
52. da Silva AA, Tallam LS, Liu J, *et al.* Chronic antidiabetic and cardiovascular actions of leptin: role of CNS and increased adrenergic activity. *Am J Physiol Regul Integr Comp Physiol* 2006; 291: R1275–R1282.
53. da Silva AA, do Carmo JM, Freeman JN, *et al.* A functional melanocortin system may be required for chronic CNS-mediated antidiabetic and cardiovascular actions of leptin. *Diabetes* 2009; 58: 1749–1756.
54. do Carmo JM, Hall JE, da Silva AA. Chronic central leptin infusion restores cardiac sympathetic-vagal balance and

baroreflex sensitivity in diabetic rats. *Am J Physiol Heart Circ Physiol* 2008; 295: H1974–H1981.
55. Denroche HC, Levi J, Wideman RD, *et al.* Leptin therapy reverses hyperglycemia in mice with streptozotocin-induced diabetes, independent of hepatic leptin signaling. *Diabetes* 2011; 60: 1414–1423.
56. Kojima S, Asakawa A, Amitani H, *et al.* Central leptin gene therapy, a substitute for insulin therapy to ameliorate hyperglycemia and hyperphagia, and promote survival in insulin deficient diabetic mice. *Peptides* 2009; 30: 962–966.
57. Fujikawa T, Chuang JC, Sakata I, *et al.* Leptin therapy improves insulin deficient type 1 diabetes by CNS-dependent mechanisms in mice. *Proc Natl Acad Sci USA* 2010; 107: 17391–17396.
58. Wang MY, Chen L, Clark GO, *et al.* Leptin therapy in insulin deficient type I diabetes. *Proc Natl Acad Sci USA* 2010; 107: 4813–4819.
59. Ueno N, Inui A, Kalra PS, *et al.* Leptin transgene expression in the hypothalamus enforces euglycemia in diabetic, insulin deficient nonobese Akita mice and leptin-deficient obese ob/ob mice. *Peptides* 2006; 27: 2332–2342.
60. Naito M, Fujikura J, Ebihara K, *et al.* Therapeutic impact of leptin on diabetes, diabetic complications, and longevity in insulin deficient diabetic mice. *Diabetes* 2011; 60: 2265–2273.
61. Kruger AJ, Yang C, Lipson KL, *et al.* Leptin treatment confers clinical benefit at multiple stages of virally induced type 1 diabetes in BB rats. *Autoimmunity* 2010; 44: 137–148.
62. Lin CY, Higginbotham DA, Judd RL, *et al.* Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab* 2002; 282: E1084–E1091.
63. Miyanaga F, Ogawa Y, Ebihara K, *et al.* Leptin as an adjunct of insulin therapy in insulin deficient diabetes. *Diabetologia* 2003; 46: 1329–1337.
64. Dobbs R, Sakurai H, Sasaki H, *et al.* Glucagon: role in the hyperglycemia of diabetes mellitus. *Science* 1975; 187: 544–547.
65. Brand CL, Rolin B, Jorgensen PN, *et al.* Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats. *Diabetologia* 1994; 37: 985–993.
66. Johnson DG, Goebel CU, Hruby VJ, *et al.* Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist. *Science* 1982; 215: 1115–1116.
67. Unson CG, Gurzenda EM, Merrifield RB. Biological activities of des-His1[Glu9]glucagon amide, a glucagon antagonist. *Peptides* 1989; 10: 1171–1177.
68. Van Tine BA, Azizeh BY, Trivedi D, *et al.* Low level cyclic adenosine 3′,5′-monophosphate accumulation analysis of [des-His1, des- Phe6, Glu9] glucagon-NH2 identifies glucagon antagonists from weak partial agonists/antagonists. *Endocrinology* 1996; 137: 3316–3322.
69. Lee Y, Wang MY, Du XQ, *et al.* Glucagon receptor knockout prevents insulin deficient type 1 diabetes in mice. *Diabetes* 2011; 60: 391–397.
70. Havel PJ, Uriu-Hare JY, Liu T, *et al.* Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. *Am J Physiol Regul Integr Comp Physiol* 1998; 274(5 Pt 2): R1482–R1491.
71. Soliman AT, Omar M, Assem HM, *et al.* Serum leptin concentrations in children with type 1 diabetes mellitus: relationship to body mass index, insulin dose, and glycemic control. *Metabolism* 2002; 51: 292–296.
72. Sindelar DK, Havel PJ, Seeley RJ, *et al.* Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* 1999; 48: 1275–1280.
73. German JP, Wisse BE, Thaler JP, *et al.* Leptin deficiency causes insulin resistance induced by uncontrolled diabetes. *Diabetes* 2010; 59: 1626–1634.
74. Morton GJ, Schwartz MW. Leptin and the central nervous system control of glucose metabolism. *Physiol Rev* 2011; 91: 389–411.
75. Marino JS, Xu Y, Hill JW. Central insulin and leptin-mediated autonomic control of glucose homeostasis. *Trends Endocrinol Metab* 2011; 22: 275–285.
76. Gautron L, Elmquist JK. Sixteen years and counting: an update on leptin in energy balance. *J Clin Invest* 2011; 121: 2087–2093.
77. Stephens TW, Basinski M, Bristow PK, *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 1995; 377: 530–532.
78. Weigle DS, Bukowski TR, Foster DC, *et al.* Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *J Clin Invest* 1995; 96: 2065–2070.
79. Kulkarni RN, Wang ZL, Wang RM, *et al.* Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, *in vivo*, in mice. *J Clin Invest* 1997; 100: 2729–2736.
80. Emilsson V, Liu YL, Cawthorne MA, *et al.* Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 1997; 46: 313–316.
81. Laubner K, Kieffer TJ, Lam NT, *et al.* Inhibition of preproinsulin gene expression by leptin induction of suppressor of cytokine signaling 3 in pancreatic beta-cells. *Diabetes* 2005; 54: 3410–3417.
82. Cases JA, Gabriely I, Ma XH, *et al.* Physiological increase in plasma leptin markedly inhibits insulin secretion *in vivo*. *Diabetes* 2001; 50: 348–352.
83. Kieffer TJ, Heller RS, Habener JF. Leptin receptors expressed on pancreatic beta-cells. *Biochem Biophys Res Commun* 1996; 224: 522–527.
84. Kieffer TJ, Heller RS, Leech CA, *et al.* Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* 1997; 46: 1087–1093.

85. Seufert J, Kieffer TJ, Leech CA, *et al.* Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *J Clin Endocrinol Metab* 1999; 84: 670–676.
86. Zhao AZ, Bornfeldt KE, Beavo JA. Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. *J Clin Invest* 1998; 102: 869–873.
87. Poitout V, Rouault C, Guerre-Millo M, *et al.* Inhibition of insulin secretion by leptin in normal rodent islets of Langerhans. *Endocrinology* 1998; 139: 822–826.
88. Tsiotra PC, Tsigos C, Raptis SA. TNFalpha and leptin inhibit basal and glucose-stimulated insulin secretion and gene transcription in the HIT-T15 pancreatic cells. *Int J Obes Relat Metab Disord* 2001; 25: 1018–1026.
89. Fehmann HC, Peiser C, Bode HP, *et al.* Leptin: a potent inhibitor of insulin secretion. *Peptides* 1997; 18: 1267–1273.
90. Ishida K, Murakami T, Mizuno A, *et al.* Leptin suppresses basal insulin secretion from rat pancreatic islets. *Regul Pept* 1997; 70: 179–182.
91. Ookuma M, Ookuma K, York DA. Effects of leptin on insulin secretion from isolated rat pancreatic islets. *Diabetes* 1998; 47: 219–223.
92. Pallett AL, Morton NM, Cawthorne MA, *et al.* Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochem Biophys Res Commun* 1997; 238: 267–270.
93. Roduit R, Thorens B. Inhibition of glucose-induced insulin secretion by long-term preexposure of pancreatic islets to leptin. *FEBS Lett* 1997; 415: 179–182.
94. Leclercq-Meyer V, Malaisse WJ. Failure of human and mouse leptin to affect insulin, glucagon and somatostatin secretion by the perfused rat pancreas at physiological glucose concentration. *Mol Cell Endocrinol* 1998; 141: 111–118.
95. Leclercq-Meyer V, Considine RV, Sener A, *et al.* Do leptin receptors play a functional role in the endocrine pancreas? *Biochem Biophys Res Commun* 1996; 229: 794–798.
96. Karlsson E, Stridsberg M, Sandler S. Leptin regulation of islet amyloid polypeptide secretion from mouse pancreatic islets. *Biochem Pharmacol* 1998; 56: 1339–1346.
97. Tanizawa Y, Okuya S, Ishihara H, *et al.* Direct stimulation of basal insulin secretion by physiological concentrations of leptin in pancreatic beta cells. *Endocrinology* 1997; 138: 4513–4516.
98. Shimizu H, Ohtani K, Tsuchiya T, *et al.* Leptin stimulates insulin secretion and synthesis in HIT-T 15 cells. *Peptides* 1997; 18: 1263–1266.
99. Lam NT, Cheung AT, Riedel MJ, *et al.* Leptin reduces glucose transport and cellular ATP levels in INS-1 beta-cells. *J Mol Endocrinol* 2004; 32: 415–424.
100. Harvey J, McKenna F, Herson PS, *et al.* Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. *J Physiol* 1997; 504(Pt 3): 527–535.
101. Ahren B, Havel PJ. Leptin inhibits insulin secretion induced by cellular cAMP in a pancreatic B cell line (INS-1 cells). *Am J Physiol Regul Integr Comp Physiol* 1999; 277(4 Pt 2): R959–R966.
102. Lee JW, Swick AG, Romsos DR. Leptin constrains phospholipase C-protein kinase C-induced insulin secretion via a phosphatidylinositol 3-kinase-dependent pathway. *Exp Biol Med (Maywood)* 2003; 228: 175–182.
103. Chen NG, Swick AG, Romsos DR. Leptin constrains acetylcholine-induced insulin secretion from pancreatic islets of ob/ob mice. *J Clin Invest* 1997; 100: 1174–1179.
104. Covey SD, Wideman RD, McDonald C, *et al.* The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis. *Cell Metab* 2006; 4: 291–302.
105. Gray SL, Donald C, Jetha A, *et al.* Hyperinsulinemia precedes insulin resistance in mice lacking pancreatic beta-cell leptin signaling. *Endocrinology* 2010; 151: 4178–4186.
106. Morioka T, Asilmaz E, Hu J, *et al.* Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *J Clin Invest* 2007; 117: 2860–2868.
107. Song J, Xu Y, Hu X, *et al.* Brain expression of Cre recombinase driven by pancreas-specific promoters. *Genesis* 2010; 48: 628–634.
108. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 2002; 129: 2447–2457.
109. Hinoi E, Gao N, Jung DY, *et al.* An Osteoblast-dependent mechanism contributes to the leptin regulation of insulin secretion. *Ann N Y Acad Sci* 2009; 1173(Suppl 1): E20–E30.
110. Cohen P, Zhao C, Cai X, *et al.* Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001; 108: 1113–1121.
111. Amstalden M, Garcia MR, Stanko RL, *et al.* Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. *Biol Reprod* 2002; 66: 1555–1561.
112. Bagnasco M, Dube MG, Katz A, *et al.* Leptin expression in hypothalamic PVN reverses dietary obesity and hyperinsulinemia but stimulates ghrelin. *Obes Res* 2003; 11: 1463–1470.
113. Park S, Ahn IS, Kim da S. Central infusion of leptin improves insulin resistance and suppresses beta-cell function, but not beta-cell mass, primarily through the sympathetic nervous system in a type 2 diabetic rat model. *Life Sci* 2010; 24: 854–862.
114. Boghossian S, Dube MG, Torto R, *et al.* Hypothalamic clamp on insulin release by leptin-transgene expression. *Peptides* 2006; 27: 3245–3254.
115. Muzumdar R, Ma X, Yang X, *et al.* Physiologic effect of leptin on insulin secretion is mediated mainly through central mechanisms. *FASEB J* 2003; 17: 1130–1132.
116. Park S, Hong SM, Sung SR, *et al.* Long-term effects of central leptin and resistin on body weight, insulin resistance,

and beta-cell function and mass by the modulation of hypothalamic leptin and insulin signaling. *Endocrinology* 2008; 149: 445–454.
117. Mizuno A, Murakami T, Otani S, *et al.* Leptin affects pancreatic endocrine functions through the sympathetic nervous system. *Endocrinology* 1998; 139: 3863–3870.
118. Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 2000; 278: E1–E14.
119. Dubuc PU, Mobley PW, Mahler RJ, *et al.* Immunoreactive glucagon levels in obese-hyperglycemic (ob/ob) mice. *Diabetes* 1977; 26: 841–846.
120. Della-Fera MA, Choi YH, Hartzell DL, *et al.* Sensitivity of ob/ob mice to leptin-induced adipose tissue apoptosis. *Obes Res* 2005; 13: 1540–1547.
121. Tuduri E, Marroqui L, Soriano S, *et al.* Inhibitory effects of leptin on pancreatic alpha-cell function. *Diabetes* 2009; 58: 1616–1624.
122. Marroqui L, Vieira E, Gonzalez A, *et al.* Leptin down-regulates expression of the gene encoding glucagon in alphaTC1-9 cells and mouse islets. *Diabetologia* 2011; 54: 843–851.
123. Liang CP, Tall AR. Transcriptional profiling reveals global defects in energy metabolism, lipoprotein, and bile acid synthesis and transport with reversal by leptin treatment in ob/ob mouse liver. *J Biol Chem* 2001; 276: 49066–49076.
124. Rossetti L, Massillon D, Barzilai N, *et al.* Short term effects of leptin on hepatic gluconeogenesis and *in vivo* insulin action. *J Biol Chem* 1997; 272: 27758–27763.
125. Liu L, Karkanias GB, Morales JC, *et al.* Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes. *J Biol Chem* 1998; 273: 31160–31167.
126. Barzilai N, Wang J, Massilon D, *et al.* Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* 1997; 100: 3105–3110.
127. Burcelin R, Kamohara S, Li J, *et al.* Acute intravenous leptin infusion increases glucose turnover but not skeletal muscle glucose uptake in ob/ob mice. *Diabetes* 1999; 48: 1264–1269.
128. O'Doherty RM, Anderson PR, Zhao AZ, *et al.* Sparing effect of leptin on liver glycogen stores in rats during the fed-to-fasted transition. *Am J Physiol Endocrinol Metab* 1999; 277 (3 Pt 1): E544–E550.
129. Kamohara S, Burcelin R, Halaas JL, *et al.* Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 1997; 389: 374–377.
130. Briscoe CP, Hanif S, Arch JR, *et al.* Leptin receptor long-form signalling in a human liver cell line. *Cytokine* 2001; 14: 225–229.
131. Szanto I, Kahn CR. Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. *Proc Natl Acad Sci USA* 2000; 97: 2355–2360.
132. Raman P, Donkin SS, Spurlock ME. Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R206–R216.
133. Huynh FK, Levi J, Denroche HC, *et al.* Disruption of hepatic leptin signaling protects mice from age- and diet-related glucose intolerance. *Diabetes* 2010; 59: 3032–3040.
134. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science* 1996; 274: 1185–1188.
135. Anderwald C, Muller G, Koca G, *et al.* Short-term leptin-dependent inhibition of hepatic gluconeogenesis is mediated by insulin receptor substrate-2. *Mol Endocrinol* 2002; 16: 1612–1628.
136. Ceccarini G, Flavell RR, Butelman ER, *et al.* PET imaging of leptin biodistribution and metabolism in rodents and primates. *Cell Metab* 2009; 10: 148–159.
137. Lam NT, Covey SD, Lewis JT, *et al.* Leptin resistance following over-expression of protein tyrosine phosphatase 1B in liver. *J Mol Endocrinol* 2006; 36: 163–174.
138. Aiston S, Agius L. Leptin enhances glycogen storage in hepatocytes by inhibition of phosphorylase and exerts an additive effect with insulin. *Diabetes* 1999; 48: 15–20.
139. Ceddia RB, Lopes G, Souza HM, *et al.* Acute effects of leptin on glucose metabolism of *in situ* rat perfused livers and isolated hepatocytes. *Int J Obes Relat Metab Disord* 1999; 23: 1207–1212.
140. Zhao AZ, Shinohara MM, Huang D, *et al.* Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. *J Biol Chem* 2000; 275: 11348–11354.
141. Brabant G, Muller G, Horn R, *et al.* Hepatic leptin signaling in obesity. *FASEB J* 2005; 19: 1048–1050.
142. Nemecz M, Preininger K, Englisch R, *et al.* Acute effect of leptin on hepatic glycogenolysis and gluconeogenesis in perfused rat liver. *Hepatology* 1999; 29: 166–172.
143. van den Hoek AM, Teusink B, Voshol PJ, *et al.* Leptin deficiency per se dictates body composition and insulin action in ob/ob mice. *J Neuroendocrinol* 2008; 20: 120–127.
144. Pocai A, Morgan K, Buettner C, *et al.* Central leptin acutely reverses diet-induced hepatic insulin resistance. *Diabetes* 2005; 54: 3182–3189.
145. German J, Kim F, Schwartz GJ, *et al.* Hypothalamic leptin signaling regulates hepatic insulin sensitivity via a neurocircuit involving the vagus nerve. *Endocrinology* 2009; 150: 4502–4511.
146. Gutierrez-Juarez R, Obici S, Rossetti L. Melanocortin-independent effects of leptin on hepatic glucose fluxes. *J Biol Chem* 2004; 279: 49704–49715.
147. Li X, Wu X, Camacho R, *et al.* Intracerebroventricular leptin infusion improves glucose homeostasis in lean type 2 diabetic MKR mice via hepatic vagal and non-vagal mechanisms. *PLoS ONE* 2011; 6: e17058.
148. Minokoshi Y, Kim YB, Peroni OD, *et al.* Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002; 415: 339–343.

149. Harris RB. Acute and chronic effects of leptin on glucose utilization in lean mice. *Biochem Biophys Res Commun* 1998; 245: 502–509.
150. Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes* 1999; 48: 287–291.
151. Ceddia RB, William WN Jr, *et al.* Comparing effects of leptin and insulin on glucose metabolism in skeletal muscle: evidence for an effect of leptin on glucose uptake and decarboxylation. *Int J Obes Relat Metab Disord* 1999; 23: 75–82.
152. Wang JL, Chinookoswong N, Scully S, *et al.* Differential effects of leptin in regulation of tissue glucose utilization *in vivo*. *Endocrinology* 1999; 140: 2117–2124.
153. Yaspelkis BB 3rd, Davis JR, Saberi M, *et al.* Leptin administration improves skeletal muscle insulin responsiveness in diet-induced insulin-resistant rats. *Am J Physiol Endocrinol Metab* 2001; 280: E130–E142.
154. Guerra B, Santana A, Fuentes T, *et al.* Leptin receptors in human skeletal muscle. *J Appl Physiol* 2007; 102: 1786–1792.
155. Ramsay TG, Richards MP. Leptin and leptin receptor expression in skeletal muscle and adipose tissue in response to *in vivo* porcine somatotropin treatment. *J Anim Sci* 2005; 83: 2501–2508.
156. Berti L, Kellerer M, Capp E, *et al.* Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. *Diabetologia* 1997; 40: 606–609.
157. Bates SH, Gardiner JV, Jones RB, *et al.* Acute stimulation of glucose uptake by leptin in l6 muscle cells. *Horm Metab Res* 2002; 34: 111–115.
158. Ceddia RB, William WN Jr, *et al.* Leptin increases glucose transport and utilization in skeletal muscle *in vitro*. *Gen Pharmacol* 1998; 31: 799–801.
159. Sweeney G, Keen J, Somwar R, *et al.* High leptin levels acutely inhibit insulin-stimulated glucose uptake without affecting glucose transporter 4 translocation in l6 rat skeletal muscle cells. *Endocrinology* 2001; 142: 4806–4812.
160. Liu YL, Emilsson V, Cawthorne MA. Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (ob/ob) mice. *FEBS Lett* 1997; 411: 351–355.
161. Muoio DM, Dohm GL, Tapscott EB, *et al.* Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice. *Am J Physiol Endocrinol Metab* 1999; 276(5 Pt 1): E913–E921.
162. Muoio DM, Dohm GL, Fiedorek FT Jr, *et al.* Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* 1997; 46: 1360–1363.
163. Zierath JR, Frevert EU, Ryder JW, *et al.* Evidence against a direct effect of leptin on glucose transport in skeletal muscle and adipocytes. *Diabetes* 1998; 47: 1–4.
164. Kellerer M, Koch M, Metzinger E, *et al.* Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* 1997; 40: 1358–1362.
165. Akasaka Y, Tsunoda M, Ide T, *et al.* Chronic leptin treatment stimulates lipid oxidation in immortalized and primary mouse skeletal muscle cells. *Biochim Biophys Acta* 2009; 1791: 103–109.
166. Olsen GS, Hansen BF. AMP kinase activation ameliorates insulin resistance induced by free fatty acids in rat skeletal muscle. *Am J Physiol Endocrinol Metab* 2002; 283: E965–E970.
167. Shimabukuro M, Koyama K, Chen G, *et al.* Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci USA* 1997; 94: 4637–4641.
168. Chen G, Koyama K, Yuan X, *et al.* Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. *Proc Natl Acad Sci USA* 1996; 93: 14795–14799.
169. Toda C, Shiuchi T, Lee S, *et al.* Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues. *Diabetes* 2009; 58: 2757–2765.
170. Roman EA, Reis D, Romanatto T, *et al.* Central leptin action improves skeletal muscle AKT, AMPK, and PGC1 alpha activation by hypothalamic PI3K-dependent mechanism. *Mol Cell Endocrinol* 2010; 314: 62–69.
171. Cusin I, Zakrzewska KE, Boss O, *et al.* Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. *Diabetes* 1998; 47: 1014–1019.
172. Rouru J, Cusin I, Zakrzewska KE, *et al.* Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue. *Endocrinology* 1999; 140: 3688–3692.
173. Fei H, Okano HJ, Li C, *et al.* Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc Natl Acad Sci USA* 1997; 94: 7001–7005.
174. Hoggard N, Hunter L, Duncan JS, *et al.* Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. *Proc Natl Acad Sci USA* 1997; 94: 11073–11078.
175. Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, *et al.* Direct effects of leptin on brown and white adipose tissue. *J Clin Invest* 1997; 100: 2858–2864.
176. Huan JN, Li J, Han Y, *et al.* Adipocyte-selective reduction of the leptin receptors induced by antisense RNA leads to increased adiposity, dyslipidemia, and insulin resistance. *J Biol Chem* 2003; 278: 45638–45650.
177. Muller G, Ertl J, Gerl M, *et al.* Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J Biol Chem* 1997; 272: 10585–10593.
178. Kraus D, Fasshauer M, Ott V, *et al.* Leptin secretion and negative autocrine crosstalk with insulin in brown adipocytes. *J Endocrinol* 2002; 175: 185–191.

179. Perez C, Fernandez-Galaz C, Fernandez-Agullo T, *et al.* Leptin impairs insulin signaling in rat adipocytes. *Diabetes* 2004; 53: 347–353.
180. Walder K, Filippis A, Clark S, *et al.* Leptin inhibits insulin binding in isolated rat adipocytes. *J Endocrinol* 1997; 155: R5–R7.
181. Wang MY, Lee Y, Unger RH. Novel form of lipolysis induced by leptin. *J Biol Chem* 1999; 274: 17541–17544.
182. Enriori PJ, Sinnayah P, Simonds SE, *et al.* Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. *J Neurosci* 2011; 31: 12189–12197.
183. Haque MS, Minokoshi Y, Hamai M, *et al.* Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. *Diabetes* 1999; 48: 1706–1712.
184. Ahima RS, Prabakaran D, Flier JS. Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. *J Clin Invest* 1998; 101: 1020–1027.
185. Bornstein SR, Uhlmann K, Haidan A, *et al.* Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* 1997; 46: 1235–1238.
186. Heiman ML, Ahima RS, Craft LS, *et al.* Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 1997; 138: 3859–3863.
187. Pralong FP, Roduit R, Waeber G, *et al.* Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. *Endocrinology* 1998; 139: 4264–4268.
188. Clement K, Vaisse C, Lahlou N, *et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; 392: 398–401.
189. Carro E, Senaris R, Considine RV, *et al.* Regulation of *in vivo* growth hormone secretion by leptin. *Endocrinology* 1997; 138: 2203–2206.
190. Vuagnat BA, Pierroz DD, Lalaoui M, *et al.* Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. *Neuroendocrinology* 1998; 67: 291–300.
191. Tannenbaum GS, Gurd W, Lapointe M. Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. *Endocrinology* 1998; 139: 3871–3875.
192. Anini Y, Brubaker PL. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* 2003; 52: 252–259.
193. Levi J, Huynh FK, Denroche HC, *et al.* Hepatic leptin signalling and subdiaphragmatic vagal efferents are not required for leptin-induced increases of plasma IGF binding protein-2 (IGFBP-2) in ob/ob mice. *Diabetologia* 2011; 55: 752–762.
194. Heymsfield SB, Greenberg AS, Fujioka K, *et al.* Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 1999; 282: 1568–1575.
195. Mittendorfer B, Horowitz JF, DePaoli AM, *et al.* Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes. *Diabetes* 2011; 60: 1474–1477.
196. Rosenbaum M, Murphy EM, Heymsfield SB, *et al.* Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab* 2002; 87: 2391–2394.
197. Rosenbaum M, Nicolson M, Hirsch J, *et al.* Effects of weight change on plasma leptin concentrations and energy expenditure. *J Clin Endocrinol Metab* 1997; 82: 3647–3654.
198. Rosenbaum M, Goldsmith R, Bloomfield D, *et al.* Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest* 2005; 115: 3579–3586.
199. Baldwin KM, Joanisse DR, Haddad F, *et al.* Effects of weight loss and leptin on skeletal muscle in human subjects. *Am J Physiol Regul Integr Comp Physiol* 2011; 301: R1259–R1266.
200. Conroy R, Girotra M, Shane E, *et al.* Leptin administration does not prevent the bone mineral metabolism changes induced by weight loss. *Metabolism* 2011; 60: 1222–1226.
201. Rosenbaum M, Sy M, Pavlovich K, *et al.* Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. *J Clin Invest* 2008; 118: 2583–2591.
202. Chong AY, Lupsa BC, Cochran EK, *et al.* Efficacy of leptin therapy in the different forms of human lipodystrophy. *Diabetologia* 2010; 53: 27–35.
203. Savage DB, O'Rahilly S. Leptin: a novel therapeutic role in lipodystrophy. *J Clin Invest* 2002; 109: 1285–1286.
204. Lord GM, Matarese G, Howard JK, *et al.* Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; 394: 897–901.
205. Lee CH, Reifsnyder PC, Naggert JK, *et al.* Novel leptin receptor mutation in NOD/LtJ mice suppresses type 1 diabetes progression: I. Pathophysiological analysis. *Diabetes* 2005; 54: 2525–2532.
206. Lee CH, Chen YG, Chen J, *et al.* Novel leptin receptor mutation in NOD/LtJ mice suppresses type 1 diabetes progression: II. Immunologic analysis. *Diabetes* 2006; 55: 171–178.
207. Matarese G, Sanna V, Lechler RI, *et al.* Leptin accelerates autoimmune diabetes in female NOD mice. *Diabetes* 2002; 51: 1356–1361.