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Review

# Tissue-Specific Effects of Leptin on Glucose and Lipid Metabolism

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Received: 6 July 2020; Editorial Decision: 27 October 2020; First Published Online: 5 November 2020; Corrected and Typeset: 11 December 2020.

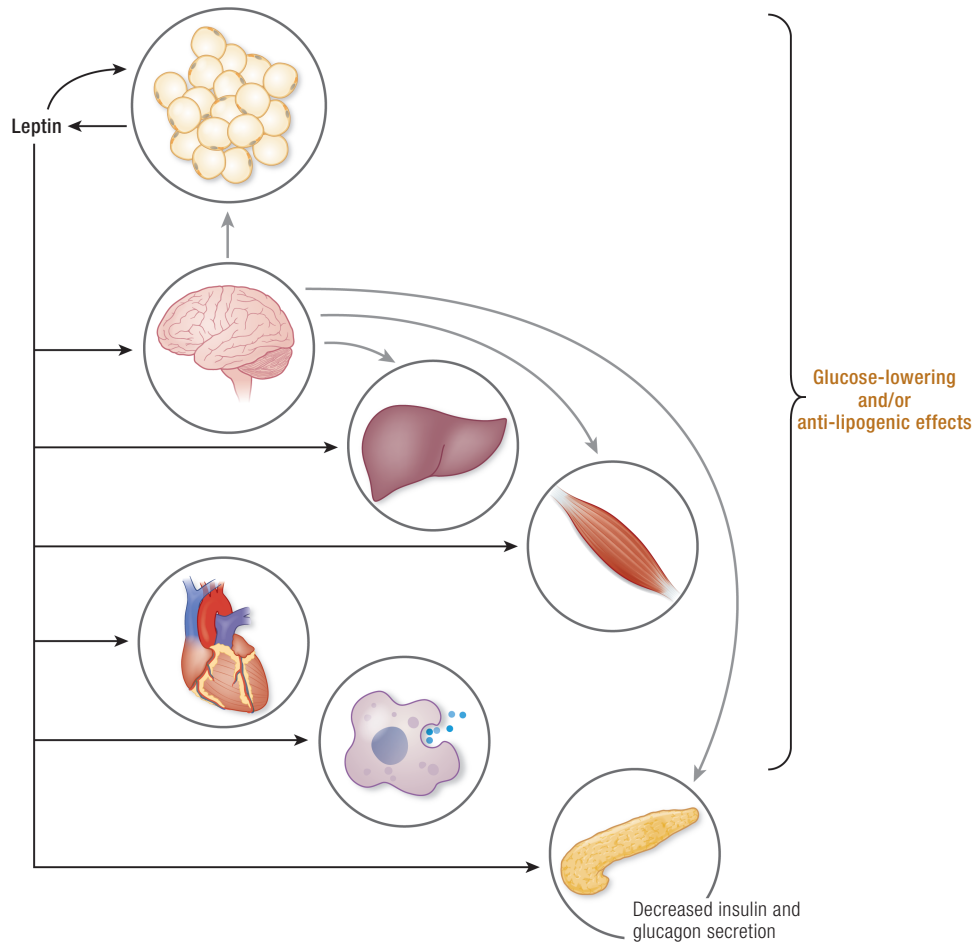
## Abstract

The discovery of leptin was intrinsically associated with its ability to regulate body weight. However, the effects of leptin are more far-reaching and include profound glucose-lowering and anti-lipogenic effects, independent of leptin's regulation of body weight. Regulation of glucose metabolism by leptin is mediated both centrally and via peripheral tissues and is influenced by the activation status of insulin signaling pathways. Ectopic fat accumulation is diminished by both central and peripheral leptin, an effect that is beneficial in obesity-associated disorders. The magnitude of leptin action depends upon the tissue, sex, and context being examined. Peripheral tissues that are of particular relevance include the endocrine pancreas, liver, skeletal muscle, adipose tissues, immune cells, and the cardiovascular system. As a result of its potent metabolic activity, leptin is used to control hyperglycemia in patients with lipodystrophy and is being explored as an adjunct to insulin in patients with type 1 diabetes. To fully understand the role of leptin in physiology and to maximize its therapeutic potential, the mechanisms of leptin action in these tissues needs to be further explored.

**Key Words:** leptin, glucose, lipid

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## Graphical Abstract



### ESSENTIAL POINTS

- Leptin lowers blood glucose and has anti-lipogenic effects.
- Leptin regulates glucose and lipid metabolism centrally and peripherally.
- Leptin's peripheral target tissues include the endocrine pancreas, liver, skeletal muscle, adipose tissues, immune cells, and cardiovascular system.

Since its discovery approximately 25 years ago, leptin has been most extensively studied for its ability to decrease food intake by acting at the level of the central nervous system (CNS) (1). Despite initial disappointment of leptin not being a simple treatment for obesity (1), leptin has emerged as a powerful modulator of glucose, lipid, and protein metabolism, like the hormone insulin. The effects of leptin are complex since they often appear to be tissue- and sex-specific as well as context-dependent. Our objective in this review is to examine the tissue-specific effects of leptin on glucose and lipid metabolism. We focus on the involvement of

the CNS, liver, skeletal muscle, adipose tissues, immune system, and the cardiovascular system.

### Leptin

Leptin is a peptide hormone encoded by the *Lep<sup>ob</sup>* gene and is mainly synthesized in white adipose tissue (2,3), although expression levels and rates of secretion differ between depots (4,5). Leptin concentration in plasma is typically proportional to fat mass (3), but the underlying mechanism is poorly understood. It was recently reported that the link between intracellular lipid content and leptin

gene expression is likely the heterodimerization of nuclear receptors peroxisome proliferator-activated receptor  $\gamma$  and retinoid X receptor  $\alpha$  (6). Intracellular glucose metabolites and circulating factors such as insulin stimulate leptin secretion (7,8), while leptin signaling in hypothalamic proopiomelanocortin (POMC) neurons inhibits leptin secretion during fasting (9). Importantly, leptin's metabolic influence is contingent on the nutritional status of the animal, and therefore many of the leptin-induced changes described during leptin treatment of fasting animals are reduced or absent in fed controls (10). Plasma leptin concentrations have a circadian rhythm (highest during the night in humans) and are higher in women; in obesity, the rhythm is disrupted, and levels are elevated overall (hyperleptinemia) (11-13). Leptin is also synthesized in the gut, where it is released into its lumen and can evidently eventually arrive in the systemic circulation (14,15). Lastly, leptin expression is reportedly induced in skeletal muscle in response to exogenous leptin and to activation of the hexosamine biosynthetic pathway, which can be triggered by factors such as hyperglycemia and hyperlipidemia (7,16).

While this review focuses on mammals, mostly mice, rats, and humans, other animal models are also used to study leptin physiology. Leptin and white adipose tissue are found in various species, including humans, mice, rats, and fish (17-19). The *ob/ob* mouse (*Lep<sup>ob</sup>* mouse), which does not have functional leptin, highlights the importance of leptin in energy balance and metabolism; it is characterized by severe obesity, hyperlipidemia, insulin resistance, and hyperglycemia depending on the genetic background (20,21). Zebrafish (*Danio rerio*) have two leptin genes (*lepa* and *lepb*) and one leptin receptor gene (22-24). Expression of leptin in zebrafish does not occur in adipose tissue, but it is found in other tissues, such as liver, gut, heart, pituitary gland, and ovary (22). Leptin receptor expression occurs in various tissues, including liver, heart, muscle, brain, ovary, and testes (23). Disruption of leptin signaling in zebrafish through leptin isoform A and/or leptin receptor knockdown affects embryonic development in various ways, including diminishing body size and brain differentiation (25). In adult zebrafish, leptin promotes an appetite-suppressing transcriptional profile in the brain (26), and *lepa* knockout adult zebrafish are obese (27). However, adult zebrafish with a mutation in the leptin receptor gene that leads to a premature stop codon in the cytoplasmic region have normal body weight (24), perhaps because this specific leptin receptor mutation is insufficient to alter energy balance. Interestingly, this leptin receptor mutation results in elevated whole-body glucose content at the larval stage, but better glucose tolerance that is associated with augmented insulin expression in adult zebrafish (24). In aggregate, these studies indicate that the role of

leptin in zebrafish physiology depends on its stage of development, with leptin signaling in adulthood being important for maintaining normal body weight and restraining insulin expression. The fruit fly (*Drosophila melanogaster*) does not have white adipose tissue and therefore does not have leptin as an adipokine (17). However, fruit flies do have a leptin analog expressed in neurons, unpaired 1 (*upd1*), and Beshel et al. (28) reported that knockdown of *upd1* causes obesity. *Upd1* binds to the domeless receptor, which, similar to leptin receptors in mammals, activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (28,29). Moreover, human leptin binds to and activates the domeless receptor (30). Taken together, these findings indicate that leptin signaling prevents weight gain and alters glucose metabolism across greatly divergent species. Due to their affordability, zebrafish and drosophila are becoming increasingly more popular in physiological studies, and further study in these models may provide some novel insights regarding the role of leptin signaling in metabolism.

## Leptin Signaling

We direct the reader to excellent reviews of leptin signaling pathways (eg, (31,32)). Briefly, there are various isoforms of the leptin receptor (LepR) that, with exception of the soluble leptin receptor, can be found at the plasma membrane (32). Different isoforms of LepR are the result of alternative splicing of messenger ribonucleic acid (mRNA) (32,33). In humans and mice, the soluble LepR is produced through protein cleavage, and in addition, mice have mRNA for soluble LepR (designated LepRe) (32-37). The soluble leptin receptor may increase the concentration of leptin in the circulation while decreasing leptin bioavailability (38-40).

Leptin action requires leptin binding to a receptor on the plasma membrane. Leptin receptor isoforms at the plasma membrane consist of the long isoform (LepRb) and multiple isoforms which have shorter intracellular domains compared to LepRb (32). In mice, the short isoforms of LepR are LepRa, LepRc, and LepRd (37, 41), while the short isoforms LepRa, LepRc, and LepRf have been detected in rats (42,43), and LepRa and LepRc have been found in humans (35,44). LepRs are expressed in many tissue/cell types throughout the body, and the hypothalamus of mice, rats, and humans is rich in LepRb (32,37,41,42,44-47). The majority of leptin's actions appear to be mediated by LepRb, as demonstrated by the *db/db* mouse (*Lepr<sup>db</sup>* mouse), which lacks only LepRb and is characterized by severe obesity, hyperlipidemia, and insulin resistance comparable to that of the leptin deficient *Lep<sup>ob</sup>* mouse model (20,37,48,49). The presence of fasting hyperglycemia in the *Lepr<sup>db</sup>* mouse depends on genetic strain,

with the C57BLKS-*Lep<sup>rd</sup>* strain being particularly susceptible to diabetes (49). The cytoplasmic portion of LepRb associates with JAK2, which phosphorylates LepRb on specific tyrosine residues, allowing other mediators to be recruited to LepRb and phosphorylated by JAK2, such as STAT3 (48). Phosphorylated STAT3 dimerizes and translocates to the nucleus where it regulates the transcription of leptin target genes (48,50). Although JAK2/STAT3 is a prominent pathway of leptin-mediated regulation of body weight and glucose homeostasis (48,50), another JAK2-dependent, but STAT3-independent, pathway that has not yet been fully characterized is necessary for leptin's full effects (51). Barnes et al (51) recently generated various LepRb deletion mutant mice using CRISPR/Cas9 and found that maximal obesity, hyperglycemia, hyperleptinemia, and hyperinsulinemia were obtained when proximal LepRb cytoplasmic regions, in addition to LepRb STAT3-binding sequences, were also deleted. The biological significance of the short isoforms of LepR is starting to be unravelled. In mice, knocking out LepRa results in lower fasting glycemia and better glucose tolerance on standard chow (52). These metabolic alterations are associated with elevated LepRb and LepRc mRNA levels in various tissues, although the expression of these leptin receptor splice variants was not affected in the hypothalamus (52). In hepatocytes, LepRa increases lipoprotein assembly through the mitogen activated protein kinase pathway (53). Hence, recent investigations have revealed the importance of pathways other than JAK2/STAT3 in mediating the metabolic effects of activated leptin receptors.

Obesity is associated with hyperleptinemia, and there is debate over whether hyperleptinemia induces leptin resistance (diminished leptin action at a given leptin concentration), or not. Recent studies provide evidence for the former (54) and the latter (55). Moreover, there is crosstalk between leptin and insulin signaling pathways and prolonged hyperleptinemia can cause insulin resistance (56,57). The effect of hyperleptinemia and leptin resistance on various tissues and the pathophysiology of various disorders are largely unexplored topics. This could be highly relevant to deciphering the mechanisms of leptin resistance associated with obesity in humans and could direct promising approaches to enhance leptin sensitivity and new weight loss therapies.

Except for treating lipodystrophy, therapeutic applications of leptin are still controversial due to apparently conflicting lines of evidence and the limitations of the widely used *ob/ob* model. Zhao et al (54) have shown that by genetically reducing the amount of functional leptin secreted by adipose tissue, mice are unexpectedly protected from high-fat diet-induced obesity. This finding suggests that protecting from hyperleptinemia may be important in preventing diet-induced obesity, which seems

at odds with the paradigm that leptin decreases food intake and increases energy expenditure. Low leptin levels are associated with a greater capacity for weight loss in obese human subjects (58), which may indicate that high leptin levels result, in part, from leptin resistance. Thus, there is merit to the idea that lowering leptin levels could be therapeutically beneficial for weight gain prevention. However, the *ob/ob* model used by Zhao et al (54) comes with a number of important caveats relevant to energy metabolism and inflammation. The developmental consequences of reduced leptin in *ob/ob* mice include impaired proliferation and elongation in neurons of the arcuate nucleus (ARC), reduced projections to the dorsomedial nucleus (DMH), and reduced agouti-related protein (AgRP) and  $\alpha$ -melanocyte-stimulating hormone projections to the paraventricular nucleus (59). These nuclei of the hypothalamus are essential sites of action for signals that affect feeding and energy expenditure. Furthermore, liver protease inhibitor levels such as serine protease inhibitor  $\alpha$ 1-antitrypsin can become disrupted in *ob/ob* mice and obese humans, driving inflammation in adipose tissue and liver, insulin resistance, and body weight gain (60). These studies show that models with constitutively lowered leptin suffer from developmental abnormalities and chronic inflammation, which may confound interpretations of leptin lowering therapy.

In contrast with Zhao et al (54), various groups have shown evidence that reducing leptin exacerbates the metabolic consequences of high-fat diet exposure. For example, weight gain resistant A/J mice had a 20-fold increase in leptin concentration in response to 14-week high-fat diet but weight gain susceptible C57BL/6 mice experienced only a 2-fold increase in leptin levels (61). This is not congruent with the idea that lower leptin during high-fat diet is favorable. Another study showed that lowering leptin levels can favor glucose intolerance, lipid metabolism dysregulation, and obesity in high-fat diet fed mice (62). These studies demonstrate that the suppression of leptin to prevent obesity and diabetes requires further study to better understand the mechanisms that underlie this paradoxical result.

## Effects of Leptin on Glucose and Lipid Metabolism

### Effects of peripherally administered leptin on metabolism in rodents

Leptin administered outside the CNS through various routes, namely intraperitoneal (i.p.), intravenous (i.v.), and subcutaneous (s.c.), reaches peripheral tissues and select regions of the CNS (63,64). Alterations in leptin levels in the systemic circulation modulate glucose and

lipid metabolism. Indeed, the ability of leptin to lower the body weight of *Lep<sup>ob</sup>* mice occurs alongside leptin-induced improved glycemic control (65,66). Moreover, a low leptin dose (1 mg kg<sup>-1</sup> day<sup>-1</sup>) improves glycemic control without decreasing body weight in *Lep<sup>ob</sup>* mice (65), and pair-feeding experiments indicate that improved glycemic control can be dissociated from leptin-induced decrease in food intake (67).

Peripherally administered leptin can alter glucose metabolism in various tissues. Although short-term (hours) administration (300 µg kg<sup>-1</sup> h<sup>-1</sup>) of recombinant mouse leptin in rats does not affect basal (fasting) hepatic glucose production (68), leptin increased insulin sensitivity, specifically insulin-induced suppression of hepatic glucose production, and interestingly, while leptin had a robust additive effect on insulin-stimulated suppression of glycogenolysis, leptin blunted insulin-induced suppression of gluconeogenesis (68). In the same study, leptin did not alter insulin-stimulated glucose disposal (glucose uptake). At a lower peripheral infusion rate (180 µg kg<sup>-1</sup> h<sup>-1</sup>) of recombinant mouse leptin in rats, similar effects on glycogenolysis and gluconeogenesis during hyperinsulinemia were found, but leptin did not change insulin-induced suppression of hepatic glucose production (69). In mice, short-term (hours) leptin administration (1 µg h<sup>-1</sup>) boosts hepatic glucose production as well as glucose uptake in skeletal muscle and brown adipose tissue (70). Prolonged (days) administration of recombinant mouse leptin (500 ± 200 µg kg<sup>-1</sup> d<sup>-1</sup>) to rats also augments insulin-induced suppression of hepatic glucose production, and additionally, insulin-induced glycogenesis and glucose disposal are elevated (71). Leptin increases insulin sensitivity, specifically insulin-stimulated glucose utilization or glucose oxidation, in skeletal muscle and brown adipose tissue, but blunts it in white adipose tissue (72-74). Overall, these studies indicate that exogenous peripheral leptin can increase insulin sensitivity of glucose metabolism in the liver (glycogenolysis), skeletal muscle, and brown adipose tissue, but not in white adipose tissue.

It is important to note the impact of nutritional state on leptin's effects. During fasting (24 h), but not the fed state, an i.p. injection of human or murine leptin significantly raises plasma glucose, insulin, and glucagon through sympathetic pathways in mice (75). Nevertheless, leptin-induced elevation of circulating glucose concentrations during the fasting state depends on circulating leptin concentrations. Perry et al (76) found that, in 48 h fasted rats with plasma leptin concentrations of ~30 pM, i.v. leptin infusion boosted circulating levels to ~60 pM and decreased plasma glucose concentrations, but when increased to supraphysiological levels (~1250 pM), circulating glucose was substantially elevated. Hence, the glycemic response to

exogenous leptin depends on the phase of the fed-fasting cycle as well as the circulating leptin concentration that is achieved.

Lipid metabolism in various tissues is also modulated by systemic leptin administration. Systemic leptin increases lipolysis in white adipocytes (77), possibly via hormone-sensitive lipase (HSL) and nitric oxide (NO) synthase (78,79). Leptin also augments breakdown of triglycerides in skeletal muscle, oxidation of fatty acids in skeletal muscle and liver, and ketogenesis in the liver (80-82). Accordingly, leptin decreases the size of adipose tissue depots (71) and reduces lipid content in skeletal muscle as well as liver (81,83). Interestingly, the depletion of hepatic lipid induced by peripheral leptin administration can be mediated by Kupffer cells, which are resident macrophages in the liver (81). Leptin inhibits insulin-stimulated lipogenesis in white adipocytes (72) but enhances the inhibition of very low density lipoprotein (VLDL) synthesis in the liver by insulin (84). Overall, systemic leptin diminishes lipid accumulation in various tissues.

Leptin is a treatment for humans with lipodystrophy, a disorder with various causes that is characterized by diminished adipose tissue stores (85,86). A murine model of congenital generalized lipodystrophy was generated by expressing truncated nuclear sterol regulatory element-binding protein 1c in adipose tissues (nuclear sterol regulatory element-binding protein 1c is driven by the *aP2* promoter) (87). These mice have low circulating levels of leptin, hyperglycemia, and hepatic steatosis, which are improved by peripheral and intracerebroventricular (i.c.v.) leptin administration (87,88). Lipodystrophy can also be induced in mice by dietary consumption of conjugated linoleic acid (89), and peripheral leptin treatment reduces hepatic lipid content, stimulates lipid oxidation, and increases insulin sensitivity (90,91). Hence, leptin improves glucose and lipid metabolism in rodent models of lipodystrophy.

Since leptin can inhibit insulin secretion (72,92), studies of leptin physiology have also been performed in models in which insulin is severely reduced (93) or completely absent (94) to define the insulin-independent actions of leptin. Peripheral administration of leptin in rodent models of insulin-deficient diabetes, induced for example by the pancreatic β cell-destroying toxin streptozotocin, improves glycemia, represses circulating lipids, and decreases hepatic lipid content (93,95). In addition, whole-body and hepatic insulin sensitivity are improved by leptin in rodent models of insulin-deficient diabetes (96,97). These effects usually occur independently of changes in body weight and thus are not merely secondary to the well-recognized weight-lowering effects of leptin. While the mechanisms for the glucose lowering effect of leptin therapy in insulin deficiency are still being elucidated, the



effect is independent of increased insulin sensitivity (94) and involves decreased glucose production (93). However, these leptin mediated improvements in metabolism are associated with the risk of severe hypoglycemia (93). As well, while some aspects of metabolism in insulin-deficient rodents can be improved with physiological levels of leptin therapy, reduction of fasting blood glucose to normal levels requires pharmacological levels of leptin (98). This is in striking contrast to the glucose lowering effects of leptin in leptin-deficient *ob/ob* mice, which are highly sensitive to very low leptin doses (65). Hypoinsulinemia is associated with hypoleptinemia, and studies in insulin-deficient rodents indicate that leptin therapy is more effective as hypoinsulinemia or hypoleptinemia becomes more severe (99-102). Hence, leptin may be a potential adjuvant treatment for patients with insulin-deficient diabetes, but careful assessment of the risks for hypoglycemia will be needed.

### Effects of peripherally administered leptin on metabolism in humans

The pivotal role of leptin in the regulation of body weight and metabolism in humans has been demonstrated in individuals who are leptin deficient or have impaired leptin signaling. Leptin deficiency is a term that encompasses patients with undetectable or lower than normal levels of leptin in the circulation as well as patients with elevated, but biologically inactive, circulating leptin (103-107). Congenital leptin deficiency, which results from various types of mutations in the leptin gene, and acquired leptin deficiency, which includes anorexia nervosa, have an overall low prevalence (103,106,108,109). Patients with congenital leptin deficiency are massively obese starting early in life and have impairments in immune and reproductive systems (104-106,110). With respect to metabolism, congenital leptin deficiency is associated with hyperinsulinemia, moderate dyslipidemia, and severe liver steatosis (111). Patients have also been identified with various types of mutations in the leptin receptor gene (reviewed in (112)). Dysfunction of the leptin receptor in humans results in similar characteristics to those observed with leptin deficiency, including obesity and dysregulated glucose metabolism (106,110,112-115). While congenital leptin deficiency is rare, numerous studies have reported improvements in metabolism following leptin replacement therapy. Leptin therapy results in significant decreases in body weight and fat mass (reviewed in (116)). Moreover, leptin therapy of congenital leptin deficiency leads to substantial (18%–85%) decreases in plasma insulin levels (117-119). In one patient with congenital leptin deficiency and type 2 diabetes, leptin therapy also decreased blood glucose levels from 131 mg/dL to

86 mg/dL after 18 months of therapy (120). Leptin therapy also improved insulin sensitivity in a patient with congenital leptin deficiency, reducing the HOMA-IR value from approximately 4.5 to 3.6 (121). In addition, the modest dyslipidemia (slightly elevated serum triglycerides and low density lipoprotein [LDL] cholesterol and low high density lipoprotein [HDL] cholesterol) was improved with the leptin therapy (117,119). Leptin replacement therapy for congenital leptin deficiency leads to rapid reversal of hepatic steatosis (121,122). Consistent with this, treatment of congenital leptin deficiency with acute leptin replacement therapy leads to a shift in the metabolome consistent with leptin therapy promoting lipolysis and fatty acid oxidation (123).

Human lipodystrophy is a collection of disorders characterized by loss of adipose tissue and forms with widespread loss of adipose tissue and reduced serum leptin levels. Subjects with congenital generalized lipodystrophy are the most hypoleptinemic with serum concentrations in the range of 0.05 to 3.7 ng/mL (124) compared to normal levels in individuals in the range of 7 ng/mL for males and 15 ng/mL for females (125); note that leptin levels are positively correlated with fat mass (126). Numerous studies of leptin replacement therapy in patients with lipodystrophy effectively raise leptin levels to greater than 10 ng/mL (reviewed in (127)). This increase in leptin is associated with reduced fasting blood glucose and insulin levels, reduced hemoglobin A1c, and lower serum triglycerides and cholesterol (total and LDL) as well as hepatic de novo lipogenesis (85,116,128-130). Metabolomics of blood suggest that leptin treatment in people with lipodystrophy elevates  $\beta$  oxidation of fatty acids and catabolism of amino acids (131). This agrees with the effect of acute leptin administration to healthy humans. Short-term (4 h) i.v. administration of leptin resulting in a ~20-fold increase in circulating leptin concentrations (similar to levels found in obese people), decreased insulin levels during the infusion and augmented systemic fatty acid oxidation as well as specifically in skeletal muscle (132). Interestingly, a proportion of the beneficial metabolic effects of leptin therapy in people with lipodystrophy occur independently of its hypophagic action (133). The success of leptin therapy to improve insulin sensitivity in leptin deficient people led to trials of methionyl leptin in obese patients with type 2 diabetes. However, neither low nor high dose therapy (raising leptin levels 3- or 150-fold, respectively) for 14 days led to weight loss-independent improvements in insulin sensitivity (134).

Leptin treatment may also benefit patients with anorexia nervosa, who have suboptimal body weight, are hypoleptinemic, and have greater lipid concentration in the circulation (135,136). In a study of adolescent girls with anorexia nervosa, leptin levels were in the range of 5 ng/

mL compared to healthy controls that were closer to 17 ng/mL (137). Anorexia nervosa is a psychiatric disorder that causes individuals, mostly females, to chronically reduce their food intake (135). Clinical trials for the use of leptin to treat anorexia nervosa are warranted (138), but for leptin treatment to be successful in anorexia nervosa, leptin should diminish disturbances in metabolism while not further reducing body weight (135).

As previously mentioned, promising studies showing weight loss stimulated by leptin in rodent models have prompted clinical trials trying to replicate this finding in humans. Current evidence suggests that lean and obese individuals under no caloric restriction are not subject to the weight-reducing effects of leptin, but that the effects of leptin are significant under conditions of caloric restriction. In one clinical study that failed to demonstrate weight-reducing effects of leptin in humans, 0.3 mg kg<sup>-1</sup> day<sup>-1</sup> leptin was administered for 6 days in lean individuals, but this led to a large increase in circulating leptin, from 0.5 to 8 ng/mL in phosphate-buffered saline controls to 150 to 500 ng/mL in those receiving leptin (139). It should be noted that this measurement was made 2 h after subcutaneous leptin injection to capture maximum plasma leptin levels, and average leptin levels were thus likely much lower. Weekly 60 mg injections of long-acting pegylated leptin to obese humans for 8 weeks on a mildly hypoenergetic diet (140) also failed to induce any differences in energy expenditure, glucose metabolism, or body weight change relative to placebo. In a longer (24 weeks) study, a 0.3 mg kg<sup>-1</sup> day<sup>-1</sup> leptin dose was sufficient to moderately enhance weight loss in obese subjects under moderate caloric restriction (141). This finding has been replicated in obese individuals experiencing severe caloric restriction (142,143), which supports the idea that falling leptin levels are responsible for initiating energy conservation and certainly warrants further investigation of leptin as an adjuvant weight loss therapy under conditions where fat reduction may occur, such as caloric restriction, exercise, and even liposuction (144).

Based on the success of leptin therapy in normalizing metabolism in rodent models of type 1 diabetes, studies of leptin therapy in type 1 diabetes were initiated. In a small pilot study, patients with type 1 diabetes were given metreleptin therapy and followed for 20 weeks (145). The therapy increased plasma leptin levels modestly from 22 ng/mL to 49 ng/mL and did not significantly change hemoglobin A1c, fasting glucose, or plasma triglycerides. However, the total daily insulin dose was reduced by 50% at the end of study and a 6.6% decrease in body weight was achieved by the end of the therapy. While the leptin therapy did not improve glycemic control (aside from improved insulin sensitivity), it should be noted that, similar

to the comparisons of leptin-induced weight loss in mice *vs* humans, this could be due to differences in the levels of leptin achieved in this study. The 2.1-fold increase in circulating leptin in the trial (145) is substantially less than the 13- to 15-fold increase used in most of the leptin therapy studies with rodent models of insulin-deficient diabetes (93-97). Due to the promising insulin-sensitizing effects of leptin and the discrepancies in leptin dosing in humans *vs* rodents, investigations into higher-dose leptin therapy for type 1 diabetes are warranted.

### How peripheral leptin enters and communicates with the CNS

The mechanisms through which peripheral leptin enters the CNS have not been fully elucidated. Leptin from the periphery enters the CNS by transport through the blood-brain barrier, which consists of endothelial cells in blood vessels of the brain being tightly held together, and by transport through the barrier created by epithelial cells of the choroid plexus, a structure that produces cerebrospinal fluid (CSF) (146-149). Leptin can cross the blood-brain and blood-CSF barriers through a process involving LepRs in endothelial cells of microvessels and in epithelial cells of the choroid plexus, the latter of which transports leptin into CSF (43,150,151). LepR-mediated entry of leptin into the brain involves transcytosis (150,152,153). However, the extent of the importance of LepRs for leptin to enter the CNS is debatable and other receptors have been suggested to facilitate leptin transport (154). Circumventricular organs of the CNS, such as the median eminence in the hypothalamus, lack a blood-brain barrier due to their fenestrated capillaries, thus allowing free passage of leptin to select neurons of the mediobasal hypothalamus, particularly the ARC (150,151). Leptin entrance into the brain at the median eminence is controlled because the extent of fenestration of these capillaries is altered by hypothalamic neurons (155). In addition, some studies suggest an important role of tanycytes, specialized ependymogial cells that line the third ventricle of the hypothalamus, in mediating leptin transport from the median eminence to hypothalamic neurons (151,156). However, their role is controversial because Yoo et al could not detect LepR in murine tanycytes (156,157). Dendrites of LepR-expressing ARC neurons also reach the median eminence, thereby giving these neurons immediate access to leptin leaving fenestrated capillaries, before leptin enters the brain *per se* (158). Binding of leptin to ARC neurons is increased during fasting (159), but the amount of leptin passing the blood-brain barrier may be decreased (160). In high-fat diet-induced obesity in mice, it has recently been reported that the ability of leptin to cross from the periphery to the CNS is not altered (151,161), in contrast to previous studies indicating that it is diminished (43).

Activation of STAT3 in various brain regions, namely the ARC, DMH, and ventromedial hypothalamus (VMH), and the nucleus of the solitary tract in the brainstem, is proportional to the amount of leptin administered peripherally in rodents (162,163). Notably, Faouzi et al (162) found differences in the kinetics of STAT3 activation in ARC and VMH/DMH by peripheral vs i.c.v. leptin, with i.c.v. leptin having a more immediate pan-activating effect. Hence, while administration of i.c.v. leptin is a reductionist approach to determine central leptin action, the results of such studies may not necessarily translate to the central effects caused by endogenous peripheral leptin. Peripheral leptin also communicates with the CNS via neural afferents. Murphy et al (164) reported that afferent neurons originating in white adipose tissue respond to leptin. In addition, knockout of LepR in vagal afferent neurons causes augmentations in food intake and body weight (165). Taken together, these findings indicate that leptin does not need to enter the CNS from the periphery to exert effects on the CNS.

## Leptin's Tissue-Specific Effects on Glucose and Lipid Metabolism

### Effects of direct leptin action in the CNS

The CNS is currently considered the primary site of leptin action (Fig. 1) and various insightful reviews have been written about the mechanisms through which central leptin action regulates metabolism in the periphery (eg, (166-168)). Here we highlight recent reports and areas that we think require further investigation.

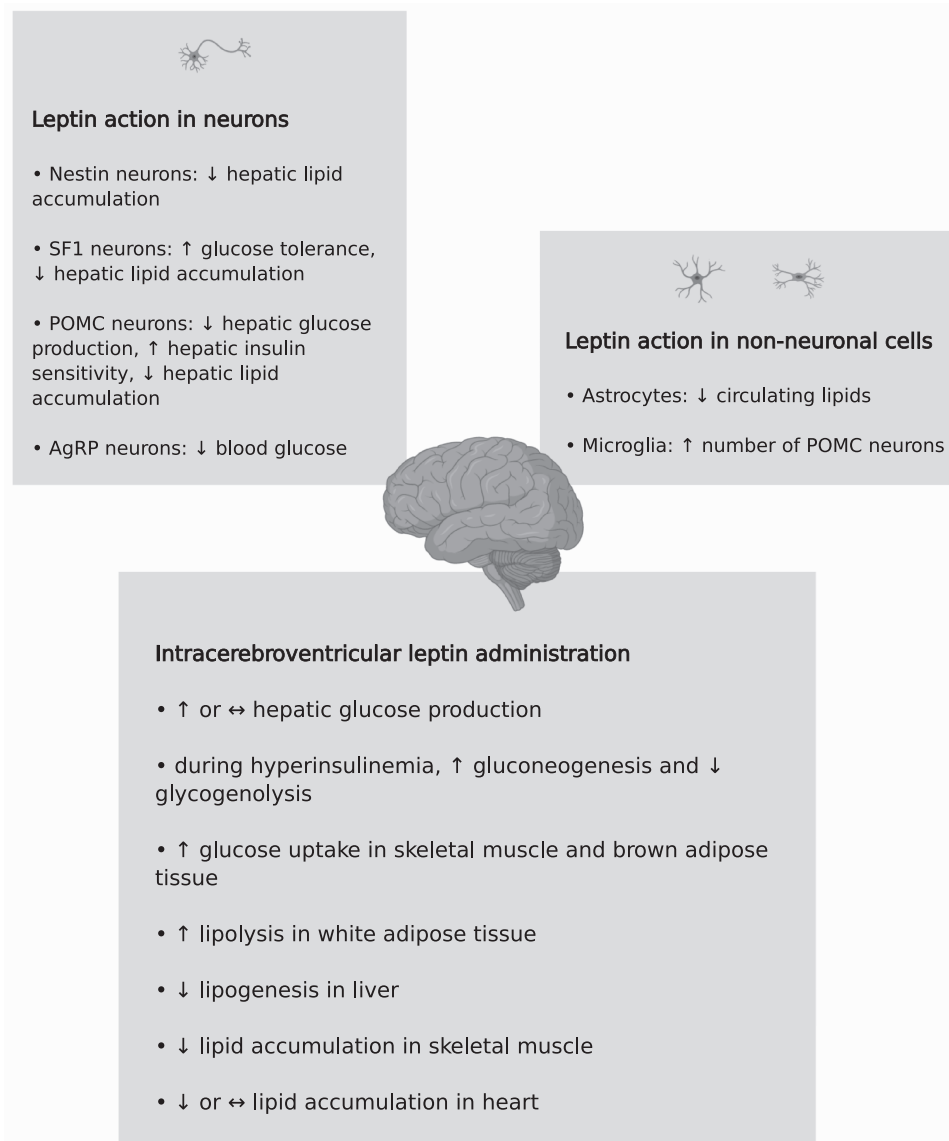
The effects of peripheral vs i.c.v. administered exogenous leptin on metabolism have been compared. Since these effects generally appear to be similar (70,169,170) and the effects of i.c.v. leptin are potent, peripheral leptin may regulate peripheral metabolism predominantly through the CNS. Tamoxifen-inducible periphery-specific LepRb deficient mice do not have discernable changes in phenotype other than hyperleptinemia, which is caused by elevated bound leptin in the circulation (free leptin concentration in the circulation is not affected) (36). It is noteworthy that the hyperleptinemia did not result in an increase in hypothalamic phosphorylated STAT3, suggesting there was no change in bioactive leptin. The protein bound to circulating leptin is likely soluble LepR (36). Interestingly, while leptin secretion rate from white adipose tissue is elevated in mice with tamoxifen-inducible periphery-specific LepRb deletion, LepRe mRNA levels are not altered in white adipose tissue (36). This suggests that increases in LepRe mRNA levels in other tissues, LepRe translation, or LepR protein cleavage (thereby generating soluble LepR) may be necessary for the spike in circulating

bound leptin among mice with peripheral LepRb deletion. It should be noted for this study that the level of LepRb inactivation is modest in several of the peripheral tissues examined, thereby limiting the conclusion as to the role of direct leptin signaling in the periphery. Central and peripheral leptin can communicate with the liver via autonomic nervous system efferents (171,172). Moreover, peripheral leptin increases white adipose tissue lipolysis while central leptin blunts white adipose tissue lipogenesis by activating the sympathetic nervous system (SNS) (173-175). Central leptin acts on skeletal muscle, brown adipose tissue, and heart via the SNS to modulate tissue glucose uptake (176-178), but peripheral leptin can also act directly on these tissues as discussed further below. I.c.v. leptin decreases circulating levels of the pancreatic hormones insulin and glucagon (179,180), a finding that is mirrored in ex vivo studies (92,181,182) (see later discussion of the endocrine pancreas). Although leptin has profound effects on metabolism in the periphery, the contribution of direct peripheral vs central leptin signaling to these outcomes in vivo remains to be clearly defined.

Short-term i.c.v. administration of leptin does not modify glucose production by the liver or glucose utilization, either in basal or hyperinsulinemic states, in rats (169). However, during hyperinsulinemia, gluconeogenesis increases with a proportional decrease in glycogenolysis (169). The effect on gluconeogenesis, but not the effect on glycogenolysis, requires the melanocortin pathway (69). The melanocortin pathway, in which melanocortin 4 receptors are stimulated by  $\alpha$ -melanocyte-stimulating hormone and inhibited by AgRP secreted by neurons in the hypothalamus, mediates various effects of leptin (69). It also mediates the effect of peripherally administered leptin on gluconeogenesis (69). In mice, i.c.v. leptin administration (hours) elevates hepatic glucose production and glucose uptake in skeletal muscle as well as brown adipose tissue (70). Prolonged (days) i.c.v. leptin administration in rats also increases glucose uptake, glycogen content, and markers of insulin sensitivity in skeletal muscle (183). Hence, i.c.v. leptin increases insulin sensitivity of glucose metabolism in the liver (glycogenolysis) and skeletal muscle.

I.c.v. leptin also alters lipid metabolism in various tissues. Similar to what is observed with peripherally administered leptin (see previous discussion), i.c.v. leptin diminishes the size of adipose tissue depots and impedes ectopic fat accumulation (184). I.c.v. leptin stimulates white adipose tissue lipolysis, which is associated with augmented HSL gene expression (185). Recently, it was also found that i.c.v. leptin decreases lipogenesis in the liver (186). I.c.v. leptin also alters the peripheral activity of lipoprotein lipase (LPL), an enzyme that hydrolyzes triglycerides and facilitates fatty acid uptake into tissues (187,188); specifically, LPL activity





**Figure 1.** Metabolic effects of direct leptin action in the central nervous system. Abbreviations: AgRP, agouti-related protein; POMC, proopiomelanocortin; SF1, steroidogenic factor-1.

is stimulated in skeletal muscle and blunted in white adipose tissue (187). I.c.v. leptin administration diminishes levels of triglycerides and free fatty acids in skeletal muscle (183). By way of the SNS ( $\alpha$ -adrenergic receptor), central leptin activates adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle, which in turn restrains intramuscular acetyl coenzyme A carboxylase (189). Inhibition of acetyl coenzyme A carboxylase causes a shift towards fatty acid oxidation (190). However, not all studies have found that central leptin activates AMPK in skeletal muscle (191). I.c.v. leptin decreases (192) or does not change (193) triglyceride content in the heart. Interestingly, the effect of i.c.v. leptin on metabolism in the heart also depends on diet. I.c.v. leptin decreases fatty acid oxidation

in the hearts of mice on a high-fat diet, while no effect is observed in mice fed a low fat diet (193). In various models of obesity and type 2 diabetes, i.c.v. leptin ameliorates glucose metabolism, lipid metabolism, insulin sensitivity, and energy balance, although not all of these parameters may be affected in a given model (194-196).

#### Effects of direct leptin action in specific regions of CNS

The central effects of leptin on the periphery emanate from specific regions in the CNS. Leptin receptors are expressed throughout the brain, including various locations in the hypothalamus and brainstem (197-199). Leptin signaling in the VMH augments glucose uptake in skeletal muscle, brown adipose tissue, and heart in mice and

rats (176,177,200,201). Administration of leptin into the VMH stimulates glucose uptake in skeletal muscle via the SNS ( $\beta_2$ -adrenergic receptor), but intramuscular AMPK is not involved (177). Moreover, VMH leptin action increases insulin-induced reduction in hepatic glucose production and stimulation of glucose disposal (whole-body and skeletal muscle) (201). Buettner et al (175) found that leptin injected into the mediobasal hypothalamus decreases lipogenesis in white adipose tissue. Recently, it was demonstrated that leptin signaling in the dorsal vagal complex, located in the brainstem, stimulates secretion of VLDL (186). The ARC in the hypothalamus is crucial for leptin's ability to maintain glucose homeostasis and for leptin's effects on body weight (202). Glucose uptake by brown adipose tissue is also augmented following leptin injection in the ARC (200). Moreover, leptin's stimulation of thermogenesis in brown adipose tissue is mediated by the hypothalamus (203,204). In summary, the central effects of leptin on energy balance, glucose metabolism, lipid metabolism, and insulin sensitivity are largely mediated by leptin signaling in the hypothalamus.

#### Effects of direct leptin action in specific neuronal populations in the CNS

The ablation or reconstitution of LepR, usually achieved with Cre-lox methodology in mice, has been used to investigate the role of leptin signaling in various types of neurons. To date, there is no Cre-expressing mouse that selectively targets all LepR-expressing neurons in the CNS. Synapsin1-Cre mice have been used to delete LepR in neurons throughout the CNS, with leptin signaling disrupted in the lateral hypothalamus and ventral premammillary nucleus of the hypothalamus (205), but not in ARC POMC and AgRP neurons (206). These mice do not have impairments in glucose metabolism and are resistant to obesity caused by high-fat diet (205). Nestin-Cre mice have also been used to inactivate LepR in neurons throughout the CNS (207); with Nestin-Cre mice, Cre-mediated recombination occurs centrally, including in the hypothalamus (207), as well as in the peripheral nervous system and some non-neuronal cells (208). LepR deficiency using Nestin-Cre mice causes severe obesity, hyperinsulinemia, hyperglycemia, and hepatic steatosis in male mice (207). Yet, the magnitude of these effects is less than what is observed in male *Lep<sup>ob</sup>* mice (207). In contrast, the extent of hyperglycemia and hyperinsulinemia is similar between female mice with Nestin-specific LepR deficiency and female *Lep<sup>ob</sup>* mice (207). It is important to note that in this study, while male and female mice with Nestin-specific LepR deletion were on a mixed genetic background, male and female *Lep<sup>ob</sup>* mice were on a C57BL/6J background (207).

Studies involving inactivation and reconstitution of LepR in steroidogenic factor-1 (SF1) neurons, which are found in the VMH, indicate that leptin signaling through SF1 neurons has a moderate effect on the regulation of body weight, glucose tolerance, and hepatic lipid content (209-211). POMC neurons, which are activated by leptin, and AgRP neurons, which are inhibited by leptin, are located in the ARC of the hypothalamus (202). Inducible and non-inducible LepR deletion as well as LepR reconstitution selectively in POMC neurons show that leptin signaling in POMC neurons (i) mediates basal and insulin-induced suppression of hepatic glucose production, resulting in improved glycemic control and (ii) moderately reduces body weight and diminishes circulating as well as hepatic lipids (9,212-214). Some of these effects are more pronounced in males (214). Neurons can release various neurotransmitters and widespread neurotransmitters in the CNS are  $\gamma$ -aminobutyric acid (GABA; inhibitory) and glutamate (excitatory) (215). While approximately half of POMC neurons are GABAergic, the majority of AgRP neurons are GABAergic (215). Vong et al (216) reported that inactivation of LepR in neurons capable of releasing GABA throughout the CNS, which includes AgRP neurons, results in a phenotype similar to that of *Lep<sup>rd</sup>* mice. Gonçalves et al (217) found that leptin signaling selectively in AgRP neurons of *Lep<sup>rd</sup>* mice improves glycemic control via the melanocortin pathway. GABA from AgRP neurons does not mediate this outcome, and obesity is mildly reduced (217). In another pivotal study, Xu et al. (218) found that inactivation of LepR in AgRP neurons using CRISPR-Cas9 results in obesity and hyperglycemia similar to *Lep<sup>rd</sup>* mice. Leptin also acts on AgRP neurons indirectly, by increasing release of GABA from the DMH (218). In aggregate, these studies indicate that leptin's ability to lower blood glucose concentrations and maintain normal body weight occurs via leptin signaling in AgRP neurons, independently of GABA release by these neurons.

The role of specific neuronal populations in the control of lipid metabolism is less understood. A recent report demonstrated that leptin acts on AgRP and POMC neurons to activate sympathetic nerves innervating white and brown adipose tissues (219). Hence, AgRP and POMC neurons appear to be important first order neurons in the maintenance of peripheral lipid homeostasis by leptin.

#### Role of central leptin signaling in the beneficial effects of leptin in the context of insulin deficiency

Leptin delivered by various routes improves glucose and lipid metabolism in insulin-deficient diabetes (94,96,100,102,180,220-225). In insulin-deficient rodents, i.c.v. leptin decreases hepatic glucose production

and increases glucose uptake in skeletal muscle, brown adipose tissue, heart, and brain (226). Insulin sensitivity is also augmented (227). I.c.v. leptin lowers blood glucose concentrations via leptin receptors in hypothalamic AgRP neurons in insulin-deficient mice (218). In contrast, inhibition of GABA release from leptin receptor-expressing neurons does not block i.c.v. leptin from normalizing blood glucose in insulin-deficient diabetes (228). Hence, while GABAergic neurons are important for the glucose-lowering effects of central leptin in insulin-deficient diabetes, GABA itself likely is not. As previously mentioned, the mechanisms of leptin action can depend on circulating insulin concentrations. In severe insulin deficiency, leptin signaling selectively in GABAergic and POMC neurons lowers blood glucose levels following i.c.v. leptin administration (229). Furthermore, Singha et al (230) recently reported that in severely insulin deficient mice, central leptin's reduction of blood glucose levels is only partially blunted by LepR deletion in AgRP neurons. Furthermore, LepR deletion in neurons that express rat insulin II gene promoter (RIP) driven Cre, can reverse the leptin-mediated improvements in glycemic control and lipid metabolism (231). This reinforces the concept that leptin's effects via the CNS are impacted substantially by pathological states such as diabetes and that the mechanisms through which central leptin improves glucose metabolism in insulin-deficient diabetes should be further investigated.

Peripherally administered leptin can improve metabolism in insulin-deficient diabetes via the CNS. Perry et al (99,101) found that i.v. leptin lowers concentrations of circulating glucose and lipids through inhibition of the hypothalamic-pituitary-adrenal axis in rats that are substantially insulin-deficient. Denroche et al (93) and Perry et al (101) reported that amelioration of glycemic control in insulin-deficient diabetes by s.c. leptin is accompanied by diminution of metabolites in the circulation and peripheral tissues, especially (i) lipids and glycerol in plasma; (ii) glucose, lipids, and glycerol in the liver; and (iii) lipids in skeletal muscle. Multiple pathways may be involved in the antidiabetic action of leptin and leptin action in the CNS may contribute to the antidiabetic effects of s.c. leptin in insulin-deficient diabetes (232). For example, s.c. leptin induces hypoglycemia after a prolonged fast (93) and leptin signaling in the parabrachial nucleus (brainstem) has been found to sustain hypoglycemia (233). Thus, it is possible that leptin-induced reductions in glucose could be mediated in part via brainstem pathways in addition to the previously described hypothalamic pathways. It is unclear, however, whether central leptin communication with the periphery is altered by insulin-deficient diabetes (222). Additional studies are needed to further understand the mechanisms

through which s.c. leptin improves metabolism in insulin-deficient diabetes.

### Effects of direct leptin action in non-neuronal cells in the CNS

In addition to neurons, the brain consists of several types of glial cells, including astrocytes and microglia, which interact with neurons. Recent studies indicate that leptin signaling in glial cells is important for homeostasis. I.c.v. leptin alters the morphology of astrocytes (234), which play an important role in brain homeostasis and maintenance of the blood-brain barrier. Inactivation of LepRb in astrocytes results in reduced circulating triglycerides, circulating leptin, leptin gene expression in white adipose tissue, and astrogliosis when mice are challenged with a high-fat diet (235). Moreover, uncoupling protein 1 gene expression is elevated in brown adipose tissue of mice with astrocyte-specific LepR knockout fed a high-fat diet (235). However, knocking out all LepR isoforms in astrocytes results in a different phenotype in high-fat feeding conditions; knockout mice are heavier, have elevated circulating leptin concentrations, and increased astrogliosis (236). To circumvent potential developmental effects of non-inducible Cre-lox methodology, mice with tamoxifen-inducible LepRb knockout in astrocytes were generated (237). These mice have mildly increased food intake following leptin administration as well as elevated circulating triglycerides (237,238), thereby indicating that leptin signaling in astrocytes of adult mice promotes energy balance and lipid homeostasis. Microglia act as resident immune cells of the brain and play an important role in synaptic pruning. LepR knockout in microglia causes morphological and functional changes in microglia, diminished count of POMC neurons, and mild obesity (239). Taken together, these studies suggest that leptin signaling in non-neuronal cells of the CNS maintains lipid homeostasis and energy balance.

### Effects of direct leptin action in CNS on intracellular metabolism and pathways

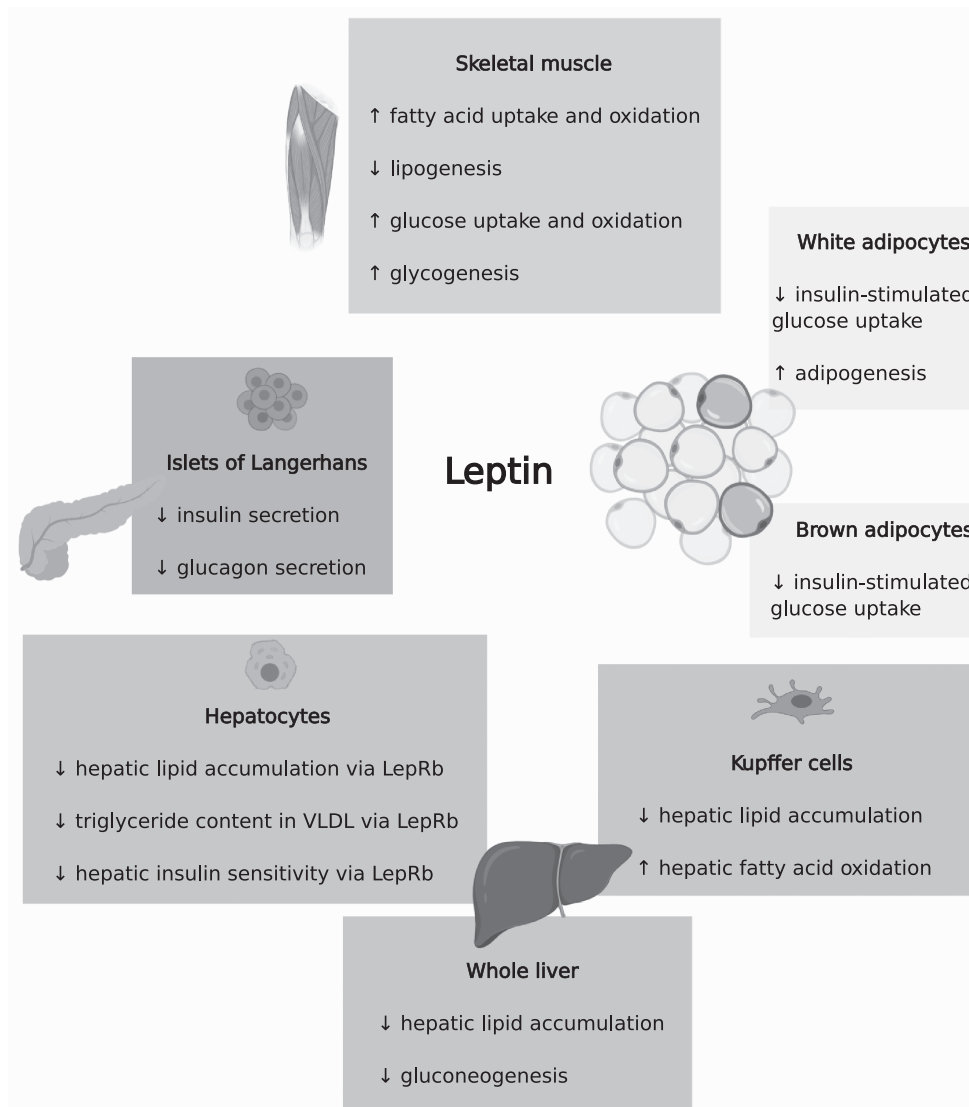
It has been reported that activation/inhibition of POMC and AgRP neurons only partially explains the effect of leptin on energy balance and peripheral metabolism via these neurons (240) and that the intracellular mediator of leptin signaling phosphatidylinositol-3-kinase is important in certain types of neurons (241,242). The effect of leptin signaling on glucose and lipid metabolism may begin within CNS cells themselves. Leptin modulates glucose uptake by astrocytes (243), and central leptin promotes glucose sensing by neurons, a process that involves the conversion of glucose to lactate in astrocytes (244). Synthesis of lipids in astrocytes is also important for glucose metabolism in

the brain and periphery (245), but whether leptin regulates lipid anabolism or catabolism centrally remains largely unexplored.

### Effects of direct leptin action in the endocrine pancreas

Leptin directly modulates the secretion of hormones from the endocrine pancreas (Fig. 2). The islets of Langerhans in the pancreas are clusters of endocrine cells, predominantly  $\beta$  cells and  $\alpha$  cells. While  $\beta$  cells secrete insulin, a hormone that stimulates glucose uptake, reduces hepatic glucose production, and increases lipid accumulation,  $\alpha$  cells secrete the hormone glucagon, which reduces lipid accumulation and increases hepatic glucose production directly, but reduces hepatic glucose production via the brain

(recently reviewed in (246,247)). Leptin decreases insulin secretion by islets from mice, rats, and humans as well as by  $\beta$  cell lines (92,181,247). The underlying mechanisms include activation and membrane translocation of  $K_{ATP}$  channels, which hyperpolarize the  $\beta$  cell membrane and thereby reduce insulin secretion (92,249). However, the role of  $\beta$  cell-specific LepR signaling in vivo is unclear, largely due to issues with the various Cre drivers (250). Deletion of LepRb from  $\beta$  cells and the hypothalamus using RIP-Cre mice results in hyperinsulinemia and glucose intolerance (251). However, these results are potentially confounded by Cre itself because RIP-Cre mice can be glucose intolerant (252). Subsequently, all isoforms of LepR were knocked out of  $\beta$  cells using pancreatic and duodenal homeobox 1-Cre mice (253), a Cre line that is also not specific for  $\beta$  cells (250). On standard chow, the knockout mice had



**Figure 2.** Metabolic effects of direct leptin action in adipose tissues, endocrine pancreas, liver, and skeletal muscle. Abbreviations: LepRb, long isoform of the leptin receptor; VLDL, very low density lipoprotein.

better glucose tolerance and higher insulin secretion, but the opposite was found on a high-fat diet (253). The Ins1-Cre mouse is a more recent model that is  $\beta$  cell-specific, but it is also characterized by inefficient recombination in some cases (254). Glucose-stimulated insulin secretion from perfused islets of female mice with  $\beta$  cell-specific LepRb deletion, achieved with Ins1-Cre mice, was elevated and this is consistent with loss of leptin signaling leading to increased insulin secretion (255). However, it is noteworthy that  $\beta$  cell-specific restoration of LepRb signaling using Ins1-Cre mice in an otherwise LepRb null setting was not sufficient to reduce plasma insulin levels (256). Similarly, while leptin decreases glucagon secretion from islets (182), mice that have a partial deficiency in LepRb in  $\alpha$  cells do not have an altered phenotype (255,257). The reasons for the discrepancies between ex vivo (islet) and in vivo (Cre-lox methodology in mice) studies are unclear but likely include poor efficiency of Cre-mediated recombination. Furthermore, islets express short isoforms of LepR, in addition to LepRb (182), but their individual contributions to  $\beta$  cell function in vivo remains unexplored. Leptin may also act directly on islet cells other than  $\beta$  or  $\alpha$  cells to alter insulin and glucagon secretion. Interestingly, somatostatin-secreting  $\delta$  cells in pancreatic islets also express LepR but the role of leptin in these cells is unknown (258). Lastly, it is possible that leptin robustly inhibits insulin secretion only in conditions of maximal insulin secretion in vivo, including hyperglycemia (259). In aggregate, studies indicate that leptin impedes secretion of insulin and glucagon by isolated islets, but additional investigations are necessary to clarify the in vivo role of leptin signaling in islet cells.

### Effects of direct leptin action in the liver

The liver receives instructions directly from circulating leptin for the regulation of lipid and glucose metabolism (Fig. 2). Exposure of isolated livers to leptin impedes gluconeogenesis via insulin receptor substrate-2 (260) and depletes triglyceride content in livers from rodents fed a standard chow but not in livers from high-fat diet-fed rodents (261). Accordingly, triglyceride levels in the liver and plasma are diminished following adenovirus-mediated restoration of functional LepR expression in the liver of *fal/fa* rats, which have a mutated LepR and therefore are obese and have hepatic steatosis (262). The liver is composed mainly of hepatocytes but also other cells that express LepR, such as Kupffer cells and hepatic stellate cells (262). Hepatocyte-specific LepR knockout in mice does not induce a robust phenotype (264), but Huynh et al (265) found that with aging, mice with hepatocyte-specific LepRb deficiency exhibit improved glucose tolerance and insulin-stimulated suppression of hepatic glucose production. Furthermore,

mice with hepatocyte-specific LepRb deficiency have elevated hepatic triglyceride and cholesterol content, and enhanced hepatic activity of LPL and VLDL with greater triglyceride content (188,265). A recent report suggests that leptin promotes lipoprotein assembly from stored lipids in the liver but inhibits it from dietary sources in the intestine (53). As previously mentioned, leptin administration can also decrease triglyceride content and augment fatty acid oxidation in the liver by directly acting on Kupffer cells (81). Hence, direct leptin signaling in the liver promotes catabolism of lipids, similar to the effects of leptin on the liver via the CNS. However, in contrast to leptin's action on the liver through the CNS, hepatic leptin signaling reduces hepatic insulin sensitivity of glucose metabolism.

### Effects of direct leptin action in skeletal muscle

The metabolic effects of leptin on skeletal muscle have been reviewed elsewhere (166,266-268), and herein we will focus on recent developments (Fig. 2). The extent of the response to leptin in a given skeletal muscle, either via the CNS or directly, depends on the (i) predominant type of fibers in the skeletal muscle, with type 1 fibers being more responsive, and (ii) phase of the fed-fasted cycle (189,269-272). Leptin acts on skeletal muscle directly via LepRb and short LepR isoforms (270, 273-276). In skeletal muscle, leptin activates AMPK, raises fatty acid uptake, increases fatty acid oxidation, reduces triglyceride formation, and boosts thermogenesis (269-272,277,278). Analogous results have been obtained with various types of muscle cells (189,273,279,280) and some of these pathways are impaired in obesity (272,277). Moreover, Koo et al (274) recently found that elevation of fatty acid oxidation caused by leptin requires AMPK in the short-term and STAT3-mediated expression of fatty acid oxidation genes in the long-term. Various, but not all (281), studies show that exposure of skeletal muscle and muscle cells to leptin augments glucose uptake, glycogenesis, and glucose oxidation (275,282,283). In isolated skeletal muscle, C<sub>2</sub>C<sub>12</sub> myotubes, and L6 muscle cells, leptin either does not lessen insulin sensitivity of glucose metabolism (269,283) or causes a short-term impairment (275,281). However, leptin inhibits insulin sensitivity of lipid metabolism in skeletal muscle (insulin promotes intramyocellular lipid accumulation) (269). In aggregate, studies indicate that direct leptin signaling in skeletal muscle has anti-lipogenic effects and promotes glucose disposal.

### Effects of direct leptin action in adipose tissue

Adipocytes express LepR (284) and, being the main source of leptin (285), are exposed to high local leptin



concentrations. In mice with LepR deficiency in peripheral tissues, the rate of leptin secretion by white adipose tissue is increased, suggesting that leptin exerts negative feedback on its secretion from adipocytes (36). Although the majority of leptin's effects on adipose tissues *in vivo* are likely controlled indirectly via sympathetic activity (174) or other signals, which are reviewed elsewhere (3), multiple *in vivo* and *in vitro* models support a role for adipocyte leptin signaling in modulating glucose and lipid metabolism as well as adipogenesis (Fig. 2).

The intersection of leptin and insulin signaling pathways in adipocytes is an important determinant of leptin's metabolic effects in adipocytes. It has been reported that *in vitro*, leptin increases lipolytic activity of white adipocytes from rodents (286) but not from humans (287). Most reports agree that leptin blunts the insulin response in adipocytes (288), resulting in reduced insulin-induced glucose uptake and lipogenesis in white adipocytes (287,289). In contrast, one study indicated insulin-sensitizing effects on lipogenesis in white adipocytes *in vitro* (72). In brown adipocytes, leptin signaling dampens insulin-stimulated glucose uptake via reduced insulin receptor kinase activity (290). Some cases demonstrating a lack of leptin action on adipocytes utilized supraphysiological leptin levels (291,292). This disparity highlights the importance of the bioactivity of leptin, dosing of leptin, and the biological context of the model in determining the direct effect of leptin on adipocyte lipid metabolism (293).

*In vivo* studies of preadipocytes or adipose tissue transplanted from *LepR<sup>Δb</sup>* mice to mice with intact leptin signaling show that adipocytes in the implants shift over time to mirror the morphology of endogenous adipocytes despite being insensitive to leptin (294,295). This further suggests that the overall biological context is at least as important as leptin signaling directly at adipose tissue. Huan et al (284) found that knockdown of all LepR isoforms in white adipose tissue, using a LepR antisense sequence driven by the phosphoenolpyruvate carboxykinase promoter, results in obesity, glucose intolerance, insulin resistance, and ectopic fat accumulation. It is of note that phosphoenolpyruvate carboxykinase is expressed in cells other than adipocytes, such as cells of myeloid lineage (296), that express LepR and can modulate metabolism (see following discussion on the immune system). Using Cre-lox methodology, modest deficiency or reactivation of LepRb signaling in both mature white and brown adipocytes results in a mild phenotype (297). First, mice with adipocyte-specific LepR deficiency have lower plasma insulin concentrations during a glucose challenge; accordingly, plasma insulin concentrations are elevated during a glucose challenge in mice with adipocyte-specific LepR reconstitution (297). Second, in obesity, reconstitution of LepR in adipocytes improves blood glucose

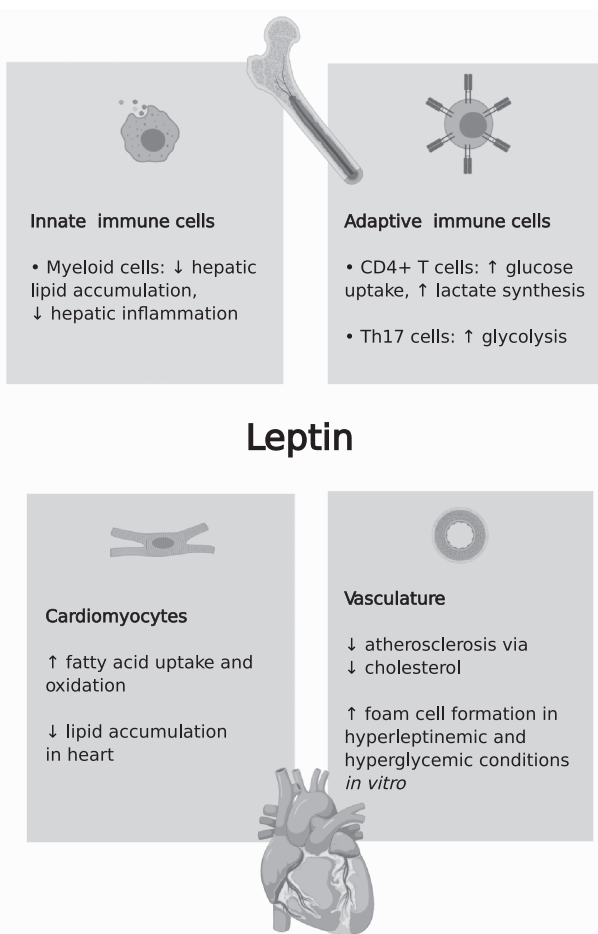
concentrations in an age-dependent manner (297). Third, adipocyte leptin signaling also mildly increases body weight in males (297). Fourth, surprisingly, the ability of leptin to lower blood glucose in insulin-deficient diabetes is faster in mice with adipocyte-specific LepR deficiency (297). It is possible that achieving greater recombination of LepR, knocking out LepR separately in white and brown adipocytes, or knocking out LepR in adipocytes at all stages of development (not just mature adipocytes) results in a more robust or different phenotype. Regarding the last possibility, a recent study demonstrated that leptin is beneficial in the maturation process of adipocytes (adipogenesis) (298), suggesting that inhibition of leptin signaling at earlier stages of adipocyte development may have a more detrimental effect on whole-body metabolism.

Recent studies in white adipose tissue continue to provide support for a role of leptin directly at adipocytes, where lipid metabolism is likely altered through inflammation mediated via NO synthases (299,300), altered HSL and adipose triglyceride lipase action in white adipose tissue (78,301), and mammalian target of rapamycin-mediated adipogenesis (298). Although the importance of these pathways *in vivo* needs to be further studied, lipase regulation by leptin in adipose tissue holds particular promise in diabetes. For example, suppression of lipolysis is crucial in *i.v.* leptin's remarkable ability to restore normal blood glucose in streptozotocin diabetic rodents (101,302). Thus, the investigation of leptin's direct role in adipose tissue maintenance of glucose and lipid metabolism *in vivo*, and the molecular mechanisms responsible, may provide valuable insights.

### Effects of direct leptin action in the immune system

The immune system is broadly divided into the innate and adaptive immune systems. The innate immune system includes cells such as macrophages, neutrophils, and natural killer cells, while the adaptive immune system consists of T cells and B cells (303). Cells of the adult immune system mainly originate in the bone marrow, except for most tissue resident macrophages (304,305). Bone marrow is an important target for leptin (306), and there is evidence that leptin can promote or block immune responses (303,307). As a clinical example, leptin treatment of hypoleptinemic women with hypothalamic amenorrhea increases circulating CD4<sup>+</sup> T cells (108). We refer the reader to multiple reviews on how leptin regulates immunology (eg, (303,307)); the focus of our current discussion will be on how leptin signaling through immune cells affects glucose and lipid metabolism (Fig. 3).

Immune cells can support homeostasis or impair it, depending on cell type, activation status, and disease stage



**Figure 3.** Metabolic effects of direct leptin action in the cardiovascular and immune systems.

(308). Macrophages are needed for appropriate adipose tissue remodeling in obesity (309). Conversely, recruitment of macrophage precursors from the circulation (monocytes) into tissues is a feature of obesity (308,310). Moreover, it is well established that inflammation in immune cells can disrupt glucose and lipid metabolism (311,312). It is likely that chronicity and type of inflammatory cytokines determine whether or not inflammation has an adverse effect on metabolism. The trigger for inflammation in immune cells can be metabolic, such as free fatty acids (313), or both metabolic and pathogenic, for example nucleotide-binding oligomerization domain 1 activation (314).

Bone marrow transplantation studies involving wild-type and *Lepr<sup>db</sup>* mice entail the ablation of immune cells followed by replenishment of the bone marrow, and consequently, cells of both the innate and adaptive immune system. In *Lepr<sup>db</sup>* mice, intact LepRb signaling in bone marrow-derived cells (ie, bone marrow from wild-type mice) reduces circulating leptin, while elevating circulating adiponectin (315). However, the reverse experiment, namely the transplantation of bone marrow from *Lepr<sup>db</sup>* mice into

wild-type mice, does not alter these parameters (315). On a high-fat diet, the lack of LepRb in bone marrow-derived cells (ie, bone marrow from *Lepr<sup>db</sup>* mice) in wild-type mice reduces body weight, restrains adipose tissue inflammation, and ameliorates insulin sensitivity (316). No phenotypic differences were found in a similar experiment with a shorter high-fat diet regimen (317). Thus, results of bone marrow transplantation studies are inconsistent. Limiting factors of bone marrow transplantation studies include their invasiveness (315), different study designs, and likely their inability to substantially alter immune cell leptin signaling in tissues. For instance, the extent of leptin signaling in tissue resident macrophages may not be greatly affected by bone marrow transplantation because a proportion of tissue resident macrophages are not bone marrow-derived (304,305).

Another approach to investigating how leptin signaling in immune cells affects metabolism is to expose specific immune cells to leptin. The effect of leptin on cytokine expression in immune cells is not completely clear, perhaps due to different experimental designs, such as different leptin exposure durations, leptin concentrations, and species. Leptin stimulates proinflammatory cytokine release by natural killer cells acutely but not chronically (318). Classification of macrophages as pro-inflammatory (classically activated; M1) or anti-inflammatory (alternatively activated; M2) is associated with the cytokines they express (308,319). Macrophages from *Lep<sup>ob</sup>* mice have elevated expression of proinflammatory cytokines (320), but it is unclear if this is due to the absence of leptin *per se* or due to the metabolic disturbances, such as obesity, resulting from blunted leptin signaling. In monocytes and macrophages, leptin stimulates (321,322) or does not alter (323,324) proinflammatory cytokine expression and secretion; others have found that leptin exposure causes a mixed M1/M2 phenotype (325). Moreover, recent investigations suggest that we may need to look beyond M1 and M2 cytokines. For example, insulin-resistant macrophages exhibit enhanced baseline glucose uptake and secretion of lactate (326); the latter can cause white adipose tissue browning (327). Hence, it would be of interest to determine if leptin affects glucose metabolism, especially glycolysis, in macrophages themselves.

The effects of leptin on whole-body metabolism via innate immune cells have also been studied *in vivo* using Cre-lox methodology. Cre-mediated recombination of LepR in myeloid cells, which includes macrophages, neutrophils, and microglia, has been achieved with LysM-Cre and Cx3cr1-Cre mice. Cx3cr1-Cre mice appear to induce more recombination in microglia than LysM-Cre mice (239). Myeloid-cell specific LepR deficient mice are, or show a trend toward being, moderately heavier (81,239,328), with effects more pronounced in male mice (239,328), or do not exhibit alterations in body weight (329,330). Although

differences in blood glucose concentrations and glucose tolerance were not found (81,239,330), mice with deleted LepR in cells of myeloid lineage have been found to have elevated circulating leptin levels (330) and increased adipose tissue expression of the proinflammatory cytokine tumor necrosis factor- $\alpha$  (239). Studies that examined the liver of mice with myeloid cell-specific LepR deficiency consistently found abnormalities, namely hepatic triglyceride accumulation (81) and inflammation (328).

Macrophage leptin signaling also regulates cholesterol metabolism. Reverse cholesterol transport is the process whereby cholesterol picked up by macrophages is delivered to the liver via HDL and ultimately excreted from the body (331,332). Exposing J774.2 macrophages (mouse cell line) to leptin promotes cholesterol efflux by stimulating activity of HSL, which participates in the degradation of cholesterol esters (333). In hyperglycemic conditions, however, leptin favors intracellular accumulation of cholesterol (in esterified form) because the stimulatory effect on HSL is abolished and activity of acyl-CoA:cholesterol *O*-acyltransferase, which participates in the synthesis of cholesterol esters, is enhanced (334). Nevertheless, treating murine peritoneal macrophages or human monocytes/macrophages with leptin stimulates the accumulation of lipid (includes cholesteryl esters) in macrophages through various mechanisms, including elevated acyl-CoA:cholesterol *O*-acyltransferase activity and blunted efflux to HDL (335-337). Lipid-filled macrophages, known as foam cells, play a pathogenic role in atherosclerosis (see following discussion on the cardiovascular system). These *in vitro* studies suggest that exposure of macrophages to a hyperleptinemic and diabetic environment might contribute to foam cell formation.

Mice with T cell-specific LepR deficiency have been generated using LepR<sup>flox/flox</sup> mice crossed to lymphocyte protein tyrosine kinase-Cre mice, which target all T cells (338,339), and CD4-Cre mice, which express Cre only in CD4<sup>+</sup> (helper) T cells (340,341). Mice with CD4<sup>+</sup> T cell-specific LepR inactivation have normal body weight (342). CD4<sup>+</sup> T cells from pan-T cell-specific LepR deficient mice have impaired glucose uptake, synthesis of lactate, and production of the proinflammatory cytokine interferon- $\gamma$  (341,343). CD4<sup>+</sup> T cells can differentiate into other T cell types, such as Th17 and Treg (341). Using T cells from CD4<sup>+</sup> T cell-specific LepR deficient mice, it was found that Th17 cells, but not Treg cells, with inactivated LepR have a blunted rate of glycolysis (341). Hence, the effect of leptin signaling on intracellular T cell metabolism and, consequently, function depends on the cell type (343), but overall, leptin signaling appears to stimulate intracellular T cell glucose metabolism. T cells play a part in obesity-associated insulin resistance, but the involvement of T cell leptin signaling in this process is unknown (344). Taken

together, studies indicate that leptin signaling in myeloid cells has a modest impact on whole-body lipid metabolism and energy balance, while the impact of T cell leptin signaling on whole-body metabolism has not been thoroughly described.

### Effects of direct leptin action in the cardiovascular system

The direct effects of leptin on the heart and vasculature is of mounting interest because of the ramifications for cardiovascular health (Fig. 3). In *in vivo* studies of cardiomyocyte leptin signaling using tamoxifen-inducible Cre-lox methodology, cardiac function is worsened by LepR deletion (345-347). With respect to metabolism, leptin does not directly alter glucose oxidation in the heart (348). *In vitro* studies demonstrate that leptin enhances fatty acid uptake and oxidation by cardiomyocytes in an AMPK-dependent manner (278). In isolated heart, leptin contributes to fatty acid oxidation via a STAT3-NO-p38 mitogen activated protein kinase pathway, resulting in diminished accumulation of triglycerides (348,349). However, insulin blocks these effects (348). Consistent with direct anti-lipogenic effects of leptin on the heart, Hall et al (350) created mice that selectively express LepRb in cardiomyocytes on a LepR<sup>db</sup> background and found that they had diminished triglyceride content in the heart.

The arterial wall consists of various cell types, including endothelial cells and smooth muscle cells (351). A principal function of the cardiovascular system is perfusion, a process that depends on arterial blood pressure. Arterial blood pressure is determined by cardiac output and, consequently, heart rate, as well as resistance to flow in blood vessels, which is increased by vascular smooth muscle contraction. Most reports indicate that central leptin signaling, with the exception of certain regions in the CNS (352), increases heart rate, blood pressure, and SNS activity, even in obesity and insulin-deficient diabetes (221,222,353-357). Leptin signaling in the periphery also increases blood pressure, through the carotid body (358) and, especially in females, through the leptin-aldosterone pathway (359,360). It has been known for decades that blood pressure modulates the function of blood vessels (361), and leptin-induced increases in blood pressure could be an indirect way through which leptin causes vascular endothelial cell dysfunction. Additionally, leptin can act directly on the vasculature. In vascular endothelial cells, leptin boosts production of NO, which can be a vasodilator, (362) and it increases production of detrimental reactive oxygen species (ROS) due to heightened fatty acid oxidation (363). Leptin increases the proliferation of vascular smooth muscle cells (364,365). Tamoxifen-inducible LepR deletion in vascular smooth muscle cells does not alter blood pressure or body weight,

but arterial relaxation is elevated (366). Taken together, these studies indicate that leptin increases blood pressure, appears to increase vascular smooth muscle contraction, and increases ROS in vascular endothelial cells.

The role of leptin in the development and progression of atherosclerosis is still being defined and may occur through several distinct mechanisms. Atherosclerosis, defined as lipid accumulation in the intima of arterial walls, is characterized by disturbed lipid metabolism (351). Monocytes from the circulation cross the endothelial barrier and invade the intima, where they engulf LDL and become stationary lipid-laden macrophages (foam cells) (351). Oxidized LDL is especially pro-atherogenic (351) and LDL is oxidized perhaps due to increased ROS, which could be due to increased leptin signaling in endothelial cells (363). The atherosclerotic lesion also contains dysfunctional endothelial cells, vascular smooth cells, and T cells (351). Obesity, which is characterized by hyperleptinemia, is a risk factor for cardiovascular disease (367). In vivo studies using models of atherosclerosis, namely the apolipoprotein-E knockout (ApoE<sup>-/-</sup>) mouse and the LDL receptor knockout (LDLR<sup>-/-</sup>) mouse, suggest that deficiency in leptin signaling promotes atherosclerosis mainly by altering cholesterol metabolism. Deficiency in leptin signaling on a pro-atherogenic background (ApoE<sup>-/-</sup>; Lep<sup>rd</sup> mice and LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice) accelerates development of atherosclerosis (368-370). Accordingly, leptin treatment of LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice (371) or Ins2<sup>+/Akita</sup>; ApoE<sup>-/-</sup> mice, a model of type 1 diabetes and atherosclerosis, (372) mitigates the development of atherosclerosis. Plasma cholesterol is higher in ApoE<sup>-/-</sup>; Lep<sup>rd</sup> mice than ApoE<sup>-/-</sup> mice (368), leptin decreases plasma cholesterol in LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice (371), and leptin simulates reverse cholesterol transport in vivo at the level of the liver through elevated expression of scavenger receptor class B type I, an HDL receptor (373). When plasma cholesterol concentrations are matched between LDLR<sup>-/-</sup> mice and LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice by feeding LDLR<sup>-/-</sup> mice a high-fat diet and high cholesterol diet, while LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice are on standard chow, LDLR<sup>-/-</sup>; Lep<sup>ob</sup> mice have decreased atherosclerosis (374). Therefore, the anti-atherogenic effects of leptin appear to depend on its ability to lower circulating cholesterol. Bone marrow transplantation studies indicate that LepR signaling in immune cells does not alter development of atherosclerosis (375). Since atherosclerotic cardiovascular disease is a main cause of death (351) and the prevalence of obesity is high worldwide (376), it is worthwhile to explore the effect of leptin on the incidence of atherosclerosis.

## Conclusions and Future Directions

Leptin has profound effects on glucose and lipid metabolism, at the whole-body and cellular levels. The effect of leptin on glucose metabolism can depend on whether leptin is acting centrally or directly on peripheral tissues and on

whether the insulin signaling pathway is activated. Leptin therapy has therapeutic potential in insulin-dependent diabetes since it reduces insulin dose requirements and can independently improve hyperglycemia and other metabolic parameters. Central and peripheral leptin reduce ectopic fat accumulation and hence, leptin could also be used therapeutically to alleviate ectopic fat accumulation associated with obesity and insulin resistance (377). Leptin increases blood pressure, and tissue/cell-specific targeting of leptin action may be needed to maximize the beneficial metabolic effects of leptin while minimizing increases in this cardiovascular parameter. Although the relative importance of excessive leptin signaling vs leptin resistance has not been clarified in obesity and related disorders, differential leptin signaling across tissues may add another degree of complexity to the treatment of such disorders. Exciting work remains to further delineate the pathways and circuits throughout the body that result in leptin's metabolic effects.

## Acknowledgments

BioRender.com was used to make the graphical abstract and figures.

**Financial Support:** Research work on leptin was supported by a grant from the Canadian Institutes of Health Research to T.J.K.

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**Disclosure Summary:** The authors have nothing to declare.

**Data Availability:** Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. Color versions of the figures are available upon request.

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