



Review

Direct and indirect effects of leptin on adipocyte metabolism[☆]

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ABSTRACT

Leptin is hypothesized to function as a negative feedback signal in the regulation of energy balance. It is produced primarily by adipose tissue and circulating concentrations correlate with the size of body fat stores. Administration of exogenous leptin to normal weight, leptin responsive animals inhibits food intake and reduces the size of body fat stores whereas mice that are deficient in either leptin or functional leptin receptors are hyperphagic and obese, consistent with a role for leptin in the control of body weight. This review discusses the effect of leptin on adipocyte metabolism. Because adipocytes express leptin receptors there is the potential for leptin to influence adipocyte metabolism directly. Adipocytes also are insulin responsive and receive sympathetic innervation, therefore leptin can also modify adipocyte metabolism indirectly. Studies published to date suggest that direct activation of adipocyte leptin receptors has little effect on cell metabolism *in vivo*, but that leptin modifies adipocyte sensitivity to insulin to inhibit lipid accumulation. *In vivo* administration of leptin leads to a suppression of lipogenesis, an increase in triglyceride hydrolysis and an increase in fatty acid and glucose oxidation. Activation of central leptin receptors also contributes to the development of a catabolic state in adipocytes, but this may vary between different fat depots. Leptin reduces the size of white fat depots by inhibiting cell proliferation both through induction of inhibitory circulating factors and by contributing to sympathetic tone which suppresses adipocyte proliferation. This article is part of a Special Issue entitled: Modulation of Adipose Tissue in Health and Disease.

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1. Introduction

Early animal and human studies indicated that mechanisms are in place to balance energy intake and energy expenditure over periods of days or weeks [1,2]. This slow, but precise regulation allows the body weight of an individual to fluctuate around a stable mean [3,4]. Various hypotheses have been put forward as to the feedback signal that would allow body weight to be controlled including mechanisms that regulate blood glucose [5], amino acids [6] or body temperature [7]. In 1953, Kennedy proposed the lipostatic theory [8] in which food intake is controlled by the hypothalamus to regulate body fat stores and over time it has become accepted that changes in the body weight of an adult are primarily determined by changes in body fat mass. This hypothesis requires that the hypothalamus detects the size of fat stores and parabiosis studies with hypothalamic obese rats [9] and genetically obese mice [10] demonstrated the presence of a humoral factor in the feedback regulation of body fat content. In 1994, Friedman's group [11] identified this circulating factor as ob protein which is now more commonly known as leptin.

1.1. Leptin production

The primary site of leptin production is white adipose tissue [11], although it is also expressed in several other tissues including the stomach [12], lungs [13], placenta [14] and possibly the brain [15]. In rodent adipose tissue leptin expression becomes apparent after differentiation of preadipocytes to adipocytes coinciding with early stages of lipid accumulation in the cells [16]. Expression is low during suckling, but increases rapidly after weaning [17]. The rate of increase in expression is proportional to white fat mass [18,19] and, although leptin expression varies between different fat depots [20,21], it shows a strong correlation with adipocyte size [22]. Leptin is continuously secreted, however, the rate of secretion may be increased or decreased independently of regulation of leptin mRNA expression [23] due to the presence of small vesicular stores of leptin in adipocytes [24,25]. Sustained stimulation of secretion requires a simultaneous increase in leptin mRNA expression to prevent rapid exhaustion of these leptin stores [23].

As noted above circulating concentrations of leptin increase in proportion to the size of white fat depots [18], but release is pulsatile [26] and there are diurnal oscillations in leptin release independent of weight related changes in circulating leptin. In humans leptin is higher at night than during the day [27] and may be entrained by meal consumption [28,29]. The response to a meal is greater at night than in the morning, and also correlates with the glycemic index of the meal [30]. By contrast, fasting [31] or cold exposure [32] inhibit leptin

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production and release. Increases in leptin expression and secretion from adipocytes have been linked to insulin stimulation [23], cell glucose uptake [33] and the availability of energy substrates [34], all of which are indicative of an anabolic state. Activation of β -adrenergic receptors [35], or elevation of intracellular cAMP inhibit leptin expression [36] as does exposure to free fatty acids [37,38], each of which may be indicative of a state of energy mobilization. Detailed reviews of the regulation of leptin expression and secretion have been provided by Szkudelski [39] and by Lee and Fried [40].

1.2. Leptin receptors

There are multiple isoforms of the leptin receptor (ObRa-ObRe) [41] that have the structure of Class I cytokine receptors [42]. The different receptor subtypes have different length intracellular sequences and ObRb is the only receptor that includes binding domains necessary for JAK-STAT signaling. This long-form receptor (ObRb) has been shown to activate the transcription factors signal transducer and activator of transcription (STAT) 1, 3, 5 and 6 [43,44] in addition to phosphorylating phosphoinositide 3-kinase (PI3K) [45], mitogen activated protein kinases (MAPK) 1 and 2 [46] and increasing insulin receptor substrate-1 (IRS-1) and IRS-2-associated PI3K [44]. Disruption of the STAT3 binding site on ObRb results in hyperphagia, a reduced energy expenditure and a degree of obesity that is equivalent to that in mice that are deficient in ObRb [47]. Therefore, activation of STAT3 is commonly used as a marker of leptin receptor activation. ObRa, ObRc, and ObRd have short intracellular domains and it is suggested that their primary functions are to transport leptin across the blood brain barrier [48] and to mediate lysosomal degradation of leptin [49]. ObRe is a circulating receptor that is produced by cleavage of the extracellular domain of both long and short-form leptin receptors [50,51]. ObRe sequesters leptin in the circulation [52] and can therefore regulate leptin bioactivity [53,54]. In lean subjects the amount of leptin that is bound to ObRe predominates over free leptin [55], but with the development of obesity the concentration of free leptin increases while bound leptin does not change. Brabant et al. [56] reported that free and bound leptin are released from adipose tissue in proportion to their concentrations in the circulation, and at least some of the leptin secreted from adipocytes is already bound to ObRe.

Leptin receptors are expressed on a majority of tissues and high concentrations have been identified in the arcuate nucleus of the hypothalamus [57], lung, liver, spleen, kidneys, adrenal [58] and reproductive tissues [59]. Lower concentrations have been identified in multiple peripheral tissues including adipocytes [60] where receptors have been detected both as mRNA and long- and short-form protein. These include hypothalamic nuclei, the raphe nucleus, the hippocampus, the amygdala, the ventral tegmental area, the area postrema and the nucleus of the solitary tract [57], and many areas of the brain that contribute to the control of food intake or energy expenditure. Although the level of leptin receptor expression is low in adipocytes, both mRNA [61–63] and protein [64–66] have been detected in various species, demonstrating the presence of long- and short-form receptors [65,66] which were reported to be located on the cell membrane and in small cytoplasmic vesicles in adipocytes [64]. Because adipocytes express leptin receptors [64] and receive sympathetic innervation [67] it is possible for leptin to have direct effects on adipocyte development and function, but also to indirectly modify adipocyte metabolism both through central mechanisms and via modification of release and function of metabolically active hormones, such as insulin.

The expression of ObR on T cells [68], B cells [69], monocytes and macrophages [70] indicates the potential for leptin to play an important role in both innate and acquired immune responses, as discussed in recent reviews [71,72]. Both in vivo [73] and in vitro [74] studies indicate that leptin has pro-inflammatory properties. Of relevance to this review is that obesity is recognized as a chronic low-grade inflammatory condition [75] and that pro-inflammatory cytokines contribute

to the associated development of insulin resistance [76]. Experimental measures in humans [77] demonstrate a correlation between adipose tissue leptin expression and markers of inflammation, therefore the environment within obese adipose tissue appears to be one in which hyperleptinemia has the potential to promote inflammation and indirectly influence adipocyte metabolism.

1.3. Leptin resistance

Although the first animal experiments clearly demonstrated that leptin could inhibit food intake and weight gain and reduce body fat mass in both lean and obese, leptin deficient ob/ob mice [78–80], it soon became clear that leptin had no effect on food intake or body composition of animals that expressed leptin receptors, but were obese and had elevated circulating concentrations of endogenous leptin [81,82]. This condition is referred to as leptin resistance. More recently it has been shown that the failure to change food intake in response to leptin administration may reflect the development of leptin resistance in specific hypothalamic nuclei [83]. This suggests that only select actions of leptin are abolished by leptin resistance and in support of this Enriori et al. [84] reported that leptin continued to stimulate sympathetic outflow to brown fat in diet induced obese mice that were resistant to the effects of leptin on food intake. In parallel with the development of central leptin resistance Wang et al. [85] have proposed that white adipose tissue develops a local leptin resistance which may partially explain why high circulating concentrations of leptin in obese animals have little effect on white adipose tissue mass. They also [86] reported that activation of STAT3 was diminished in adipose tissue from leptin resistant diet induced obese rats in both basal and leptin stimulated conditions. The resistance was associated with an early elevation of suppressor of cytokine signaling (SOCS) 1 and 3 expression, which inhibits activation of JAK/STAT3 [87], followed by suppression of ObRb expression [86]. Similarly it has been reported that leptin receptor expression is greatly reduced in adipose tissue from morbidly obese women [88], but SOCS3 is also suppressed, possibly because of a reduced need to inhibit cytokine signaling. As discussed below, although leptin has little direct effect on adipocyte glucose metabolism, there is evidence for leptin modifying adipocyte insulin responsiveness [89] and for maintaining basal rates of lipolysis [90], therefore, leptin resistance at the level of the adipocyte may contribute to changes in the ability of fat cells to regulate triglyceride turnover. Thus, the development of leptin resistance is more complicated than just modification of leptin transport into the brain [91] or a change in responsiveness of receptors in specific brain sites [83,84]. Identifying factors associated with the development and consequences of leptin resistance may lead to a better understanding of how leptin influences the metabolic and physiologic state of an animal in conditions of obesity.

2. Evidence for a direct effect of leptin on adipose tissue metabolism

The focus of this review is the effect of leptin on adipocyte metabolism. Relatively little has been published related to an investigation of the effects of leptin on adipocyte metabolism compared with the large number of studies that have examined the effects of central or peripheral leptin administration on whole animal energy balance. Adipose tissue expresses both long- and short-form receptors [60] providing the potential for self-regulation of leptin expression [92,93] in addition to direct metabolic effects of leptin on adipocytes. As discussed below, the change in metabolism may also be achieved indirectly through modification of release of metabolically active hormones, changes in response to these hormones or by increasing the activity of sympathetic afferents to the fat cell. Administration of leptin to leptin-responsive animals reduces body fat mass [94], therefore there must be a shift in the balance between lipolysis and lipogenesis to favor lipid mobilization.

2.1. Direct effect of leptin on lipogenesis and insulin stimulated metabolism

In order to demonstrate that leptin acts directly on adipocytes it is necessary to exclude the involvement of hormonal and neural control by conducting in vitro and cell culture studies. A limitation of these studies is that the normal physiologic concentration of leptin in the local environment of adipocytes is unknown, but can be assumed to be higher than circulating leptin which is typically in the range of 0.2 to 5 nM (3–80 ng/ml). In vitro studies have tested the ability of leptin to directly influence adipocyte metabolism and overall there appears to be a small direct effect on basal activity of lipogenic and lipolytic metabolic pathways, but there is more convincing evidence for leptin inhibiting insulin responsiveness of adipose tissue. Studies examining glucose uptake by white adipose tissue found no response to leptin when it was administered centrally [95], intravenously [96] or directly (30–1000 ng/ml for 30 min) to adipocytes [97]. Similarly, acute exposure to even high concentrations (800 ng/ml) of leptin on isolated rat adipocytes had no effect on metabolism although there was a small increase in basal glucose transport and lipogenesis after 16 h [89]. This prolonged exposure to leptin did, however, cause a substantial dose dependent inhibition of insulin-stimulated glucose uptake by the adipocytes [89] and the time taken for loss of sensitivity to insulin was inversely proportional to leptin concentration. Physiological concentrations of 16 ng/ml (1 nM) leptin caused a significant loss of responsiveness after about 1 h, suggesting that the loss of insulin sensitivity did not result from simply interfering with insulin binding on its receptor. The inhibition of insulin-dependent glucose uptake is paralleled by decreases in lipogenesis, esterification of lipids, and glycogen synthase activity [89]. Leptin does not inhibit the stimulation of glucose metabolism by vanadate [89,98], which increases IRS-1 associated PI3K independent of insulin receptor activation [99], implying that leptin interferes with an early stage of insulin signaling. In support of this notion a 45-minute exposure to high concentrations of leptin (800 ng/ml) has been reported to inhibit insulin binding in isolated rat adipocytes [100] and a 20-minute exposure to high (80–800 ng/ml), but not low (8 ng/ml) concentrations of leptin significantly increases adipocyte SOCS3 expression [98], which can potentially inhibit autophosphorylation of the insulin receptor [101] in addition to down-regulating leptin responsiveness of the cells [102]. Leptin also inhibits activation of the signaling proteins MAPK and GSK3 in isolated adipocytes, although it has no effect on basal levels of activation of these proteins [98].

In addition to suppressing insulin-stimulated glucose metabolism, leptin has been shown to directly inhibit lipid synthesis. Adipocytes exposed to physiological concentrations of leptin (20 ng/ml) show a time-dependent suppression of fatty acid synthetase expression such that there is a 90% inhibition after 24 h [103]. This inhibition of enzyme activity also has been reported for cultured 30A5 preadipocytes in which there was a leptin-dependent inhibition of insulin stimulated acetyl co-A carboxylase expression and lipid synthesis [104]. Concurrent with the suppression of lipogenic enzymes is an increase in expression of enzymes associated with fatty acid oxidation [103,105], increased fatty acid oxidation [105] and increased glucose oxidation due to a stimulation of citrate synthase expression and activity of the citric acid cycle [105,106]. Transcriptional control of enzymes required for mitochondrial fatty acid oxidation is regulated by the fatty acid-activated nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR α) [107] and exposure of isolated adipocytes to leptin for 6 h stimulates the expression of PPAR γ coactivator-1 (PGC-1) [108], a coactivator that is required for activation of PPAR α [109].

2.2. Direct effect on lipolysis

A more detailed examination of the effects of leptin on fatty acid metabolism in isolated rat adipocytes [110] showed that a 6-hour exposure to 10 ng/ml leptin inhibited basal and insulin-stimulated

rates of de novo fatty acid synthesis from glucose. By contrast, leptin stimulated fatty acid esterification to triglycerides, and also caused an even greater increase in hydrolysis so that there was a net efflux of fatty acids from the cells. Interestingly, fatty acid oxidation was only increased when fatty acids were present in the incubation media and tracking the fate of fatty acids released from triglycerides showed that they were released from the cell, suggesting that leptin had differential effects on the metabolism of different pools of fatty acid substrate [110]. Recently it has also been reported that leptin stimulates the production of nitric oxide which in turn inhibits glycerol synthesis, reducing the opportunity for fatty acid re-esterification in adipocytes [111]. Thus, leptin inhibits accumulation of lipid in adipocytes by increasing the turnover of triglycerides, inhibiting basal and insulin-stimulated de novo lipogenesis, but stimulating oxidation of glucose and free fatty acids.

Although the studies described above demonstrate that leptin can stimulate lipolysis, this effect may be small compared with the lipolytic activity of adrenergic receptor agonists. In 1997, Fruhbeck et al. [90] reported that leptin directly stimulated lipolysis in adipocytes isolated from fasted wild type or *ob/ob* mice. The response was exaggerated in cells from *ob/ob* mice compared with lean wild type mice and cells from *db^{Lepr}/db^{Lepr}* mice which do not express the long-form leptin receptor [41] were unresponsive. A maximal response was induced in cells from wild type mice with the lowest dose of leptin tested which was below that found in the circulation (0.16 ng/ml). This represented a 28% increase in glycerol release, which was small compared with the 300% stimulation induced by the adrenergic agonists isoproterenol or epinephrine. Others [112] reported a weak effect of leptin on glycerol release from fat cells taken from different fat depots of young or adult Sprague Dawley rats, requiring as much as 1 μ M (16,000 ng/ml) leptin to produce a significant, threefold stimulation. The increase in lipolysis of isolated cells exposed to low concentrations of leptin, compared with those that are incubated in media totally devoid of leptin suggests that under normal conditions leptin contributes to basal triglyceride turnover, but the failure of higher doses to increase lipolysis in cells from wild type mice brings into question whether leptin can produce an acute increase in lipid mobilization through a direct action on fat cells. A more substantial increase in lipolysis has been reported for intact fat pads exposed to leptin in vitro, which may imply that paracrine factors released from non-adipocytes contribute to leptin-stimulated lipolysis [113].

All of the experiments described above involved a short exposure of adipocytes to leptin which lasted only a couple of hours, but a failure of leptin to change basal rates of lipolysis also has been reported for isolated adipocytes exposed to leptin for as long as 15 h. With this longer exposure leptin did reverse insulin inhibition of isoproterenol-stimulated lipolysis [89]. The small effect of leptin on non-stimulated lipolysis is not limited to cells isolated from rodents as similar results have been reported for ovine [114] and human adipocytes [62,115]. High doses of leptin (50–150 ng/ml) stimulated lipolysis in porcine adipocytes [116] or 3T3 L1 cells [117] that were differentiated in culture, but there was no effect of 5 ng/ml leptin on 3T3-L1 cells during either a 48-hour or 14-day exposure [117].

The studies described above measured only glycerol release as the index of lipolysis, but Wang et al. [103] examined the effect of leptin on both glycerol and free fatty acid release from adipocytes isolated from lean Zucker rats. They found a small effect on glycerol release from the cells that was similar to that reported in the previous studies and that plateaued once leptin in the media exceeded 20 ng/ml, but also reported that free fatty acids were not released from the fat cells. These results have since been confirmed using higher doses of leptin with primary cultures of porcine adipocytes [118] in which the increase in lipolysis was associated with an increase in adipose triglyceride lipase (AGTL), an enzyme that is critical for the initial stages of triglyceride hydrolysis. The increase in glycerol release was also accompanied by an upregulation of cellular enzymes required for fatty

acid oxidation [103], therefore it was proposed that leptin induced a unique type of lipolysis in which the fatty acids were oxidized within the cell. These observations are the opposite to those that would be expected if leptin selectively released fatty acids hydrolyzed from triglycerides as reported by Ceddia [105], but the time course of the two studies was different in that Wang et al. [103] examined fatty acids release over a period of 6 h and Ceddia's study [105] involved a 15-hour incubation. It is possible that fatty acids released by hydrolysis of triglycerides were re-esterified, consistent with the report by William et al. [110] that adipocytes exposed to leptin for 6 h showed increased fatty acid esterification in addition to hydrolysis of triglycerides. The strain of rat was different for each study and it is possible that the age of the animals was also different because Ceddia [105] used young animals, but Wang et al. [103] did not report the age or size of their animals.

2.3. In vivo evidence for direct effect of leptin on adipocyte metabolism

The in vitro studies described above provide evidence for a direct effect of leptin on adipocyte metabolism, but in vivo studies suggest that the impact on body fat mass that these changes would produce is small compared with the indirect effect of leptin on tissue metabolism. Development of mice in which leptin receptor expression is selectively deleted in wild type mice, or selectively expressed in db^{3j}/db^{3j} mice that do not express any functional leptin receptor [119] provide conflicting data on whether, or not, expression of leptin receptors in fat is essential for control of body fat mass. Down-regulation of both long- and short-form leptin receptors exclusively in white fat with antisense RNA increased weight gain, doubled body fat mass and induced insulin resistance in wild type mice [66] implying an important role for these receptors in the maintenance of energy balance. The increased adiposity was attributed to the down regulation of β -adrenergic receptors on adipocytes and appeared to be associated with a decline in energy expenditure because food intake was not changed and body temperature was reduced. Contrary to these results, deletion of only the long-form leptin receptor from all peripheral tissues had no effect on the body composition or metabolic state of mice [120] although it did result in hyperleptinemia, presumably due to a failure of leptin to down-regulate its own production by adipocytes. A simultaneous increase in the amount of leptin bound to the ObR_e resulted in no net change in the concentration of free leptin in the circulation [120]. By contrast, a high level of expression of ObR_b in neural tissue with some low level of expression in adrenals testes and white fat of db^{3j}/db^{3j} almost normalized the body composition of male, but not female mice and diabetes was reversed in both sexes [121]. Similarly, selective removal of ObR_b from neural tissue resulted in obesity and diabetes, but did not affect fertility or cold tolerance of mice [122,123]. The discrepancy in the outcome of the studies that used different technologies to remove or express receptors, is difficult to explain based on evidence for a critical role for long-form leptin receptors in mediating leptin activity [41,124] and leptin's effects on energy balance [47]. One possibility is that the down-regulation of β -adrenergic receptors in adipocytes of mice in which leptin receptors were knocked down with antisense RNA decreased sympathetic tone and that this indirectly caused the enlargement of the fat depots [125–127]. A role for leptin modifying adipocyte metabolism via short-form leptin receptors cannot be entirely excluded because Bates et al. [128] reported that 10 nM (160 ng/ml) leptin produced a rapid onset short-lived stimulation of glucose uptake that was initiated within 10 min, but lost after 4 h in cultured muscle cells that do not express ObR_b via a PI3K-dependent mechanism. The extreme obesity of db^{Lepr}/db^{Lepr} mice which express short-form, but not the long-form leptin receptors [41], however, makes this possibility unlikely.

Fat transplantation is an alternate in vivo technique that provides some insight into the relative importance of leptin acting directly to influence adipocyte metabolism. The classic transplant studies by

Ashwell's group found that small pieces of fat from wild type or *ob/ob* mice transplanted under the kidney capsule took on the phenotype of their host [129,130]. Because *ob/ob* mice do not express leptin, but their fat cells express leptin receptors and wild type mice express leptin, the results from these studies could be interpreted as leptin inhibiting lipid accumulation in adipocytes. More recent studies, however, have involved the transplant of cells from db^{Lepr}/db^{Lepr} mice into wild type mice. Guo et al. [131] found that the morphology of adipocytes that developed from stromal vascular cells obtained from inguinal fat of db^{Lepr}/db^{Lepr} mice transplanted into Ncr Nude mice was indistinguishable from that of adipocytes that developed from wild type cells and was similar to that of the host animal endogenous fat. Because the cells from db^{Lepr}/db^{Lepr} mice do not express the long-form leptin receptor [41] these data suggest that the direct effect of leptin from the host animal has little impact on the differentiation or maturation of adipocyte progenitor cells. The cells obtained from female donors developed into smaller adipocytes than those from males [131], which indicates that genetic influences over fat cell development were detectable if they were present.

3. Evidence for an indirect effect of leptin on adipocytes function through modification of insulin action

3.1. Leptin and insulin secretion

The interaction between leptin and insulin occurs at multiple levels in that prolonged exposure to insulin stimulates leptin expression [40,132], leptin directly inhibits insulin secretion from the pancreas, but leptin also is required for normal whole animal insulin sensitivity [133]. Soon after the discovery of leptin, both long and short-form leptin receptors were identified in pancreatic beta cells and it was determined that leptin inhibits insulin secretion [134]. Since then it has been reported that pancreatic beta, delta and alpha cells express long-form leptin receptors [135,136], but the impact of leptin on beta cells continues to receive the most attention in terms of pancreatic function. Consistent with the inhibition of circulating concentrations of insulin in leptin-treated rats and mice, physiological concentrations of leptin inhibit insulin secretion from beta cells [137] by opening ATP-sensitive potassium channels and lowering intracellular calcium concentrations [138]. Leptin has also been shown to inhibit glucose transport into isolated beta cells and this lack of stimulation may also contribute to disruption of normal functioning of the ATP potassium channels that is observed with physiologic concentrations of leptin (10 ng/ml) [139].

In addition to inhibiting insulin secretion leptin inhibits insulin synthesis by down-regulating mRNA expression of preproinsulin through a transcriptional mechanism that may involve STAT 5b [140]. Changes in insulin secretion are associated with changes in lipid metabolism within the beta cells. Both in vivo and in vitro experiments show that high concentrations of leptin induce fatty acid oxidation and inhibit lipogenesis in pancreatic islets, possibly due to increased expression of uncoupling protein 2 [141]. In leptin resistant animals, the resulting increase in triglyceride content of islets and development of lipotoxicity may contribute to their hyperinsulinemia [142]. The relevance of leptin's inhibition of insulin secretion has been illustrated by an acute hyperglycemic clamp, leptin dose–response study which showed that physiologic concentrations (serum leptin = 6 ng/ml) of leptin produced a 70% suppression of glucose-stimulated insulin secretion [143]. The inhibition of insulin secretion was apparent as early as 20 min after the start of infusion with this low dose of leptin and as early as 10 min earlier with a high dose leptin infusion (serum leptin = 261 ng/ml). The ratio of glucose infusion rate to insulin concentration did not change during the clamp, indicating that leptin did not have an insulin-like effect on glucose uptake during the 2-hour experiment [143]. The importance of leptin receptor control of beta cell function in vivo has also been illustrated by the development of hyperinsulinemia in mice in which the leptin receptor was selectively deleted from

pancreatic beta cells [144]. In these animals hyperinsulinemia leads to the development of insulin resistance [144].

3.2. Leptin and whole animal glucose metabolism

One of the first papers showing a significant effect of leptin on food intake and body weight also reported that the diabetes of leptin deficient *ob/ob* mice was corrected by a lower dose of leptin than was needed to correct energy balance [145]. The correction of hyperinsulinemia in *ob/ob* mice could not be simply explained by inhibition of insulin secretion because blood glucose was also normalized [94,145]. This effect is the opposite from that which would be expected given observations that leptin inhibits insulin-stimulated glucose metabolism in adipocytes [89]. Subsequent studies have identified tissue-specific changes in glucose metabolism in responses to leptin administration. A 5-hour icv or intravenous infusion of leptin increased whole animal glucose turnover and glycolysis, decreased liver glycogen content, but increased muscle glucose uptake and glycogen content in C57Bl/6J mice [96]. These changes in glucose metabolism were assumed to be mediated by central mechanisms because the response to leptin was exaggerated in icv infused mice compared with those receiving intravenous infusions [96]. Similarly, subcutaneous infusion of 0.5 mg leptin /kg/24 h for 8 days raised serum glucose concentration to 38 ng/ml, increased whole animal insulin-stimulated glucose uptake by peripheral tissues and exaggerated the inhibition of hepatic glucose production by insulin in rats. The inhibition of hepatic glucose output was due to a selective blockade of glycogen breakdown with no change in gluconeogenesis [146]. Glucose metabolism in adipose tissue was not measured in these studies, but because leptin reduced the size of fat depots in the leptin-infused rats [146] it can be assumed that the increase in tissue glucose uptake was limited to muscle and did not involve adipose tissue. Recent experiments with genetically engineered mice suggest that hypothalamic proopiomelanocortin (POMC) expressing neurons mediate the central effect of leptin on glucose metabolism. Selective replacement of leptin receptors in cells that express POMC in *db^{Lepr}/db^{Lepr}* mice inhibited hepatic glucose production without changing food intake or correcting body composition [147].

The critical association between leptin and normal whole animal glucose metabolism is illustrated by the severe hyperinsulinemia and hyperglycemia that are present in lipotrophic animals and humans that have very little body fat and therefore do not produce significant levels of leptin. There are also several forms of lipodystrophy in which an abnormal distribution of adipose tissue is associated with abnormally low circulating concentrations of leptin, hyperinsulinemia, insulin insensitivity and hepatic steatosis. Transgenic mouse models of lipotrophy have been developed in which transcription factors required for lipid synthesis have been either truncated [133] or inactivated [148]. Leptin replacement in lipotrophic mice that have little body fat reverses hyperglycemia and hyperinsulinemia [133,149]. In addition, transplantation of white fat from wild-type mice that express leptin will correct the hyperinsulinemia and hyperglycemia [150], whereas fat from leptin-deficient *ob/ob* mice does not [151]. The correction of insulin sensitivity is associated with restoration of insulin-induced activation of the insulin receptor and increased insulin receptor substrate 2 (IRS-2) associated PI3K in the liver [152]. Leptin replacement therapy has been used to treat patients with lipodystrophy because it improves insulin sensitivity, hepatic steatosis and reduces body fat mass [153]. In patients with lipotrophy leptin improves hyperinsulinemia, hyperlipidemia and fatty liver [154,155] within 7 days of starting treatment [155] and allows children to achieve normal puberty [156]. Many of the beneficial metabolic responses observed in lipotrophic patients are likely to be secondary to the normalization of blood glucose and reduction in muscle and liver lipid content that results from leptin suppressing appetite. In one study 3 months of leptin treatment reduced energy intake of lipotrophic patients by 50%

without producing any significant change in energy expenditure [157]. However, the early (7 days) normalization of blood glucose and triglycerides reported by Ebihara et al. [155] leaves open the possibility that leptin acts directly on insulin-responsive tissues to reverse metabolic abnormalities in lipotrophic individuals.

3.3. Insulin-like effect of leptin

Even though there is substantial evidence that leptin suppresses insulin release, it is also clear that lean animals treated with leptin remain insulin responsive and maintain normal blood glucose concentrations [158]. The apparent increase in insulin responsiveness may be attributed to the loss of fat in these animals, but there also have been a number of studies that suggest that leptin has an insulin-like effect. The most dramatic of these are demonstrations that leptin administration can normalize blood glucose in rats that are hypoinsulinemic and hyperglycemic following treatment with streptozotocin (STZ) which is toxic to pancreatic beta-cells and results in insulin-deficient diabetes. This was first reported in 1999 [159] when it was demonstrated that a continuous peripheral infusion of a high dose of leptin from a subcutaneous Alzet pump reduced food intake of the diabetic rats by 50%, blood glucose by 75%, hepatic glucose production by 70% and improved insulin sensitivity. Similar responses have been reported for rats receiving 3rd ventricle infusions [160] or daily lateral ventricle injections of leptin [161]. Leptin suppressed fasting blood glucose in both control and STZ rats to levels that were significantly lower than in vehicle injected normoglycemic animals and suppressed fasting insulin concentrations in control rats to levels that were similar to those in STZ rats. The improvement in insulin sensitivity was not due to a decrease in food intake of the leptin-treated rats because pair-fed animals remained hyperglycemic and insulin insensitive [160,161]. In addition because both central leptin injections and STZ-treatment reduced serum leptin concentrations, the changes in glucose metabolism could not be attributed to a direct effect of leptin on insulin-responsive peripheral tissues. This has recently been confirmed by a report that peripherally administered leptin corrected blood glucose and improved insulin sensitivity in STZ mice that had a liver-specific deletion of leptin receptors [162]. Systemic treatment of rats with guanethidine, which depleted peripheral tissue norepinephrine content by 60%, did not prevent the normalization of blood glucose in STZ rats receiving central injections of leptin [163], suggesting that the response was independent of activation of the sympathetic nervous system.

The long-term effect of leptin on glucose homeostasis was further investigated in a study in which insulin-deficient non-obese diabetic (NOD) mice were treated with adenovirus to over-express leptin. High circulating concentrations of leptin initially normalized blood glucose, but this effect was partially reversed after 3 weeks [164]. Leptin over-expression also was effective in correcting the diabetes in STZ rats. It was found that leptin stimulated release of IGF-1, which had insulin-like effects on muscle, but not liver tissue [164]. Hyperleptinemia also suppressed glucagon secretion and it appeared that this may be a primary mediator of the correction of diabetes in the insulin-deficient animals [164]. Recently it has been reported that knockout of the glucagon receptor prevents development of Type 1 diabetes in STZ-treated mice [165] which is consistent with evidence that leptin down-regulates glucagon in vivo in type 1 diabetic mice [164] and that physiological concentrations of leptin depolarize alpha cells and suppress glucagon secretion [166].

4. Sympathetic nervous system control of adipose tissue metabolism

4.1. Sympathetic innervation of white adipose tissue

It is now well established that white adipose tissue receives sympathetic innervation from a complex central network that includes leptin-responsive areas of the forebrain (including the hypothalamus),

midbrain and hindbrain [67]. Activation of the sympathetic efferents from the brain to white fat appears to be a primary controller of adipocyte lipolysis [167] due to the release of norepinephrine which activates β -adrenoreceptors and stimulates lipolysis [168]. In addition to its metabolic impact, sympathetic activation inhibits fat cell proliferation [125–127] and has the potential to change the size of fat depots due to a change in cell number. Central administration of leptin has been reported to increase sympathetic drive to the lumbar and renal system [169] and intrascapular brown adipose tissue (IBAT) [170], with the control of sympathetic drive to each of these tissues initiated in different areas of the brain [84,171]. A role for melanocortin receptors has been identified for the effect of leptin-induced renal and lumbar sympathetic activation [172], but not leptin-induced sympathetic activation of IBAT [173]. This is surprising given the high percentage of sites that contribute to the sympathetic outflow to brown adipose tissue that express both melanocortin 4 and leptin receptors, including the arcuate nucleus and ventromedial nucleus of the hypothalamus and the nucleus of the solitary tract and area postrema in the brain stem [57,174].

4.2. Leptin activation of sympathetic efferents to white fat

The importance of sympathetic efferents in mediating leptin's effects on white adipose tissue is less well defined. It is clear that central administration of leptin leads to a loss of fat that is maintained even when food intake is normalized [175], but experiments in which white fat depots are denervated suggest that the loss of fat is not dependent on sympathetic drive to the tissue. In experiments in which we denervated one white fat depot in rats or mice that received two week peripheral infusions of leptin that doubled circulating leptin concentrations denervation did not prevent a leptin-induced reduction in fat depot size, but did prevent the increase in adipocyte proliferation that was evident in rats with denervated fat pads [126]. In another study we tested the effects of central and peripheral leptin on norepinephrine turnover (NETO) as a neurochemical measure of sympathetic activity in various white fat depots of rats. Although it was clear that leptin could increase NETO in some, but not all, fat depots there was no correlation between norepinephrine turnover and decrease in fat pad size [176]. Similarly, Unger's group [177] has reported that adenovirus-induced hyperleptinemia reduces the size of denervated fat pads. In those rats the circulating concentrations of leptin were substantially elevated, but little of this leptin was present in cerebrospinal fluid leading to the conclusion that leptin must exert its effect on fat metabolism through mechanisms that are independent of hypothalamic activation [177].

4.3. Leptin and the sympathetic control of adipocyte metabolism

Even though the denervation studies do not show a clear association between leptin-induced increases in sympathetic drive to white fat depots and leptin-induced changes in the size of these depots, Buettner et al. [178] reported a detailed study examining the effect of infusing leptin directly into the arcuate nucleus (approximately 200 ng over 6 h) on multiple parameters of metabolism in epididymal and perirenal fat depots. Circulating glucose and insulin were clamped to isolate the effect of leptin from the indirect influence of leptin on insulin release and action. Leptin infusion caused a significant reduction in lipogenesis and fatty acid uptake, increased phosphorylation of hormone sensitive lipase and presumably increased lipolysis, but did not change adipocyte AMPK which is an index of cellular energy status. The suppression of lipogenesis was associated with inhibition of the transcription factor SREBP1 and the nuclear receptor PPAR γ , which regulate expression of lipogenesis enzymes [179,180]. Interestingly the impact of central leptin on lipogenesis was independent of hypothalamic STAT3 signaling, but required activation of PI3K. It also was shown to be dependent upon sympathetic innervation because surgical denervation or chemical ablation of catecholaminergic neurons with 6-hydroxydopamine (6OHDA)

in epididymal fat prevented the effect of leptin on lipogenesis, enzyme levels and HSL phosphorylation and facilitated an enlargement of the depot.

Obviously the results of the study by Buettner [178] do not correlate with our report of a dissociation between leptin-induced changes in fat pad NETO and fat pad weight [176]. An important consideration is which fat depot was examined because epididymal was one of the fat depots that did show a leptin-induced increase in NETO and a decrease in fat pad size [176]. Perirenal fat was not examined in our study [176], but it is a white fat depot that includes a large amount of brown adipose tissue and it would not be unreasonable to assume that perirenal brown fat receives leptin-responsive sympathetic innervation similar to that in IBAT. It would be interesting to know whether hypothalamic infusion of leptin changes adipocyte metabolism in intact or denervated inguinal or retroperitoneal depots as these fat pads decreased in size with central administration of leptin, but did not show any increase in NETO [176]. If leptin-dependent central control of adipocyte metabolism is depot specific, then the translational relevance of responsiveness in epididymal fat also needs to be evaluated because humans do not have a direct equivalent of this fat depot. A point of interest in the Buettner study [178] is that central leptin increased adipocyte STAT3 phosphorylation and interleukin-6 (IL6) expression, suggesting that the effects of central leptin on adipocyte metabolism may be more complicated than increased sympathetic tone modifying levels of lipogenesis and lipolysis because IL6 interferes with insulin signaling [181], possibly by increasing SOCS3 expression [101].

Consistent with the data from Buettner's study [178], Shen et al. [182] reported a significant, 50% increase in activity of sympathetic nerves that innervate epididymal fat in rats that received an intravenous injection of 10 μ g of leptin. There was a 20-minute delay between injection of leptin and increased activity of the nerves and the response could be blocked by central anti-histamine or by making lesions of the suprachiasmatic nucleus (SCN), a site that has been shown to send sympathetic projections to white adipose tissue [183], but has not been reported to express leptin receptors [57,184]. The increase in epididymal fat sympathetic outflow was accompanied by a 60% increase in circulating concentrations of glycerol and free fatty acids that lasted for at least 2 h. The simultaneous release of fatty acids with glycerol confirms that the increase in lipolysis was not a direct effect of leptin if leptin induces a condition in which only glycerol is released from fat cells following the breakdown of triglycerides [103].

5. Leptin and adipocyte growth and development

In addition to producing direct and indirect effects on adipocyte metabolism which lead to a reduction in adipocyte size, leptin has the capability to modify adipose tissue mass by influencing the number of cells present through the inhibition of preadipocyte proliferation. Although *in vivo* studies clearly demonstrate that leptin reduces body fat mass, high concentrations of leptin stimulate preadipocyte proliferation in primary adipocyte culture. Proliferation of porcine preadipocytes was increased in the presence of 1000 ng/ml leptin [185], whereas a lower dose of 168 ng/ml (10 nM) leptin was required to increase cell proliferation in rat primary culture [186]. In the latter experiment, the cell type that responded to leptin was not determined, but proliferation was dependent on MAPK [186]. In a subsequent experiment with primary rat adipocyte culture we found a small, but significant increase in proliferation of preadipocytes, but not stromal vascular cells in the presence of 50 ng/ml leptin, whereas higher concentrations of leptin (250 ng/ml) inhibited proliferation of all cell types [187]. The difference between studies in leptin concentration that stimulated proliferation may be explained by the source of leptin or the species from which the cells were derived. The amount of leptin that stimulated proliferation of rat preadipocytes is in the range that would be found in the circulation of genetically obese rats [188] and it is possible that in conditions of extreme obesity leptin increases preadipocyte number

to facilitate the expansion of white fat mass. In contrast to the response of cells exposed directly to leptin, proliferation of both preadipocytes and stromal vascular cells was inhibited by serum from rats that had been infused with leptin for 5 days [187]. Leptin concentration in the serum of the leptin-infused rats was increased from 1.2 to 3.2 ng/ml and serum was included in the incubation media at 0.25% by volume, excluding a direct effect of leptin on proliferation. These data suggest that leptin induces release of a circulating factor that has a potent inhibitory effect on proliferation of all cell types present in adipose tissue. Therefore, *in vivo* it is likely that increased circulating concentrations of leptin inhibit enlargement of fat depot size in part by preventing an increase in cell number.

Fat depot size is determined not only by the number of cells present, but also by the number of preadipocytes that differentiate into lipid filled adipocytes. In two of the cell culture studies described above leptin had no effect on preadipocyte differentiation [185,187], but in one experiment leptin was reported to produce a 30% increase in differentiation [186] that was associated with increased expression of lipoprotein lipase and peroxisome proliferator-activated receptor gamma (PPAR γ 2), a primary regulator of preadipocyte differentiation into mature adipocytes [189]. In contrast to the results from primary culture leptin had no effect on proliferation or differentiation of 3T3-L1 cells, a cell line derived from murine fibroblasts that can differentiate and fill with lipid under appropriate conditions [190], but did reduce the amount of triglyceride that accumulated in the cells, consistent with the expected effect of leptin on mature adipocytes [117]. The previous experiments suggest that leptin does not influence the differentiation of preadipocytes to adipocytes, whereas data from an *in vivo* experiment suggests that hyperleptinemia can lead to dedifferentiation of adipocytes [191]. Adenovirus-induced over-expression of leptin caused a 20-fold increase in circulating leptin, resulted in a loss of visible fat in rats and was associated with an inhibition of expression of PPAR γ and aP2, both of which are markers of differentiated adipocytes. At the same time Pref1 expression, a marker of preadipocytes, increased without any change in total DNA content of the fat depot. These data imply not only that the adipocytes had lost their lipid stores, but also had stopped expressing the proteins that are essential for lipid filling and reverted to the phenotype of preadipocytes [191]. Thus, in experimental conditions high concentrations of leptin can potentially directly and/or indirectly de-differentiate adipocytes. It is, however, unlikely that this would occur naturally because production of endogenous leptin should decrease in parallel with the reduction in fat mass in a leptin-responsive animal.

6. Summary

There is ample evidence that administration of exogenous leptin to leptin-responsive animals inhibits food intake and leads to a reduction in body fat mass. Although the negative energy balance of the animals causes secondary changes in metabolism to increase lipid mobilization, there also is a role for leptin to specifically modify adipocyte metabolism to inhibit lipid accumulation and promote lipid mobilization. Because adipocytes express both long and short leptin receptors [60], there is the opportunity for the hormone to act directly on the cells to reduce cell size. Evidence from *in vitro* [110,90] and *in vivo* studies [120] suggests that the direct effects of leptin on adipocyte metabolism make a small contribution to the control of fat mass *in vivo*. By contrast, leptin-induced changes in insulin secretion contribute to the normalization of circulating concentrations of insulin and peripheral tissue insulin sensitivity [145]. Although leptin stimulates whole animal glucose uptake [96], this effect is limited to muscle because prolonged exposure of adipocytes to leptin results in a loss of insulin sensitivity and an inhibition of insulin stimulated lipogenesis and reversal of insulin inhibition of lipolysis [89]. Central control of adipose metabolism by leptin is mediated by the sympathetic nervous system and many of the brain regions that are involved in the control of sympathetic outflow to

white fat also express leptin receptors [57,174]. Leptin-induced sympathetic control of adipocytes metabolism may be depot specific [176], but there is the potential for neural control of lipolysis, lipogenesis, and cell proliferation [178]. In addition leptin may modify whole body fat mass simply by inhibiting cell proliferation through the release of circulating inhibitors of proliferation [187].

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