Integration of hormonal and nutrient signals that regulate leptin synthesis and secretion

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Lee M-J, Fried SK. Integration of hormonal and nutrient signals that regulate leptin synthesis and secretion. Am J Physiol Endocrinol Metab 296: E1230–E1238, 2009. First published March 24, 2009; doi:10.1152/ajpendo.90927.2008.—This review summarizes recent advances in our understanding of the pre- and posttranscriptional mechanisms that regulate leptin production and secretion in adipocytes. Basal leptin production is proportional to the status of energy stores, i.e., fat cell size, and this is mainly regulated by alterations in leptin mRNA levels. Leptin mRNA levels are regulated by hormones, including glucocorticoids and catecholamines, but little is known about the transcriptional mechanisms involved. Leptin synthesis and secretion is also acutely modulated in response to hormones such as insulin and the availability of metabolic fuels. Acute variations in leptin production over a time course of minutes to hours are mediated at the levels of both translation and secretion. Increases in amino acids and insulin after a meal activate the mammalian target of rapamycin (mTOR) pathway, leading to an increase in specific rates of leptin biosynthesis. Cross-talk among mTOR, PKA, and AMP-activated protein kinase pathways appears to integrate hormonal and nutrient signals that regulate leptin mRNA translation, at least in part through mechanisms involving its 5'- and 3'-untranslated regions. In addition, the rate of leptin secretion from preformed stores in response to hormonal cues is also regulated. Insulin stimulates, and adrenergic agonists inhibit, leptin secretion, and this likely contributes to variations in the magnitude of nutrition-related leptin excursions and oscillations. Overall, the study of leptin production has contributed to a deepening understanding of leptin biology and, more broadly, to our understanding of the cellular and molecular mechanisms by which the adipocyte integrates hormonal and nutrient signals to regulate adipokine production.

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LEPTIN IS A 16-KDA PROTEIN encoded by the obese (lep) gene that is expressed and secreted mainly by adipocytes. When energy stores are low, a fall in leptin decreases thermogenesis, promotes fatty acid (FA) over glucose oxidation, and decreases the activity of the hypothalamic pituitary adrenal and gonadal axes (1). Leptin also modulates the activities of the hematopoietic (5) and immune (52) systems, as well as angiogenesis (7).

The importance of the leptin system in regulating body fat in animals and humans is dramatically demonstrated by the extreme obesity caused by leptin deficiency (53, 93). In leptin-sensitive lean individuals, short-term increases in circulating leptin in response to feeding promote satiety. However, with chronic overfeeding and obesity, resistance to leptin action develops. Thus the ability of the adipocyte to appropriately alter leptin production to match the level of leptin resistance may be critical to limiting weight gain. Subtle alterations in the ability of the adipocyte to regulate basal and meal-induced leptin release, as well as its pulsatile pattern of secretion, may therefore establish a new “settling point”, with a defense of a body weight at an obese level. Although leptin administration may not promote weight loss in dieters, it helps maintain weight loss achieved by dieting (68). Thus unraveling the basic mechanisms regulating leptin production and secretion may point to new therapeutic approaches for treating obesity or its complications.

Chronic Changes in Nutritional Status Regulate Leptin at the Level of Gene Expression

Early studies established that the leptin mRNA expression is increased in obesity (46, 50) and that long-term alterations in food intake, e.g., starvation for 48 h or overfeeding occur in close association with variations in leptin mRNA levels, suggesting mainly transcriptional control (28, 71). Numerous descriptive studies demonstrate that the chronic hormonal milieu associated with a positive energy balance, i.e., elevated insulin and high local glucocorticoids (GC), increase leptin production by increasing leptin mRNA levels, whereas signals associated with starvation, such as catecholamines released by...
activation of the sympathetic nervous system, decrease leptin mRNA (20, 62).

The mechanisms regulating long-term alterations in leptin gene expression in response to changes in the level of obesity/fat cell size or hormonal signals remain unclear. Available in vitro studies are not consistent with a major effect of insulin on leptin gene transcription. Insulin increases leptin promoter activity only after a very long treatment, 48 h, in 3T3-L1 cells (54). Furthermore, incubation of rat adipocytes with insulin for 1–2 h does not affect leptin mRNA levels, yet it increases leptin release, suggesting that insulin regulates leptin production through posttranscriptional mechanisms (8, 40, 66). In support of this interpretation, actinomycin D does not affect insulin stimulation of leptin release from isolated rat adipocytes (8). In vivo, insulin infusion into starved rats increases leptin mRNA expression in rodents after 2.5–4 h of infusion (71, 94), but this could be due to a secondary event, such as a lowering of catabolic signals including cAMP. Consistent with this idea, activation of β-adrenergic receptors (AR) decreases leptin mRNA levels over the long term (43, 64, 91).

GC increase leptin mRNA levels. In vitro incubation of rat adipocytes with a synthetic GC, dexamethasone, increases leptin mRNA level and release as early as 2 h (8). Our laboratory (58) and others (36, 56) find that, in humans, oral GCs increase leptin mRNA and serum leptin levels by approximatively twofold, 24–48 h after administration. Coadministration of a pulse of GCs with a meal or insulin increases serum leptin after 5–7 h, but it is not known whether this is because of an increase in leptin mRNA (34, 35). Long-term culture of human adipose tissue with dexamethasone for 1–7 days maintains initial leptin mRNA expression levels (39, 69). Furthermore, culture with the combination of insulin and GC increases leptin mRNA levels additively or synergistically, depending on depot (8, 39, 69, 77), suggesting that these two hormones are major regulators of leptin levels in humans.

Tumor necrosis factor-α (TNFα), a proinflammatory cytokine, decreases leptin expression over the long term (80, 82). TNFα acutely increases leptin production by adipocytes (82). In human adipose tissue, TNFα increases leptin mRNA only when added together with dexamethasone, and the mechanism involves an activation of p38 MAPK (80). Interleukin-6, another inflammatory cytokine, also increases leptin production when added in the presence of dexamethasone, but the mechanism is not yet established (81). Thus the increase in local cortisol and inflammatory cytokines in adipose tissue may contribute to higher leptin mRNA levels in obesity and contribute to higher leptin levels observed after endotoxin administration (19).

Neuropeptides may have a role in regulating leptin production. Melanin-concentrating hormone stimulates leptin release from isolated rat adipocytes (9). The mechanism appears to involve an increase in leptin promoter activity, as shown in 3T3-L1 adipocytes through MAPK and mammalian target of rapamycin (mTOR)/S6 kinase pathways (10). Neuropeptide Y (NPY) is released by sympathetic neurons that innervate adipose tissue and may also be expressed by human adipocytes (32). Kos et al. (32) reported that NPY decreases leptin release in isolated human adipocytes. This result was unexpected because NPY exerts an antilipolytic effect via an inhibition of cAMP production and is known to decrease leptin. In contrast to these results, Serradeil-Le Gal et al. (73) found that NPY increases leptin production by human adipocytes (33). Further work is needed to clarify the importance of NPY, its receptors, and the cellular signaling pathways by which they fine tune leptin expression in response to a stress (33). Taken together, available evidence indicates that the balance of nutrition-induced alterations in hormonal and neural signals regulates leptin production and mRNA levels, at least in part at the level of leptin transcription.

Very little is known about mechanisms regulating leptin gene transcription. Like other adipocyte genes that are induced during differentiation, CCAAT/enhancer-binding protein (C/EBPα) increases leptin expression by increasing promoter activity (29). The stimulatory effect of GC on leptin mRNA expression appears to occur at least in part by modulating leptin transcription. However, other than the identification of a putative GC response element in the promoter of leptin gene (25), little is known how GCs increase leptin mRNA. A recent study by Zeigerer et al. (89) showed that the addition of a peroxisome proliferator-activated receptor (PPAR)-γ agonist during differentiation of 3T3-L1 increased leptin expression; however, this may have been secondary to a general effect on differentiation. In mature adipocytes, PPAR-γ agonists actually decrease leptin promoter activity and leptin production (18, 29, 75). No PPAR response element in the leptin promoter has yet been identified; however, PPAR-γ agonists antagonize the C/EBPα-mediated activation of leptin promoter activity (29), and this mechanism may explain the effect of PPAR-γ agonists to decrease leptin expression. Identification of the sequences responsible for the hormonal regulation of leptin mRNA transcription and the cell- or tissue-specific expression of leptin remains an important gap in our knowledge of leptin biology.

Progress in our understanding of the regulation of leptin expression has been slowed by the lack of cell culture lines that express high levels of leptin and respond robustly to hormonal signals. The classic 3T3-L1 adipocyte cell line expresses very low levels of leptin mRNA. Although an alternate method of differentiation can increase leptin mRNA expression and permit analysis of the regulation of leptin secretion (76, 89), the levels are still far below those observed in adipose tissue in vivo. After transplantation of 3T3-F442A preadipocyte into mice, leptin mRNA levels achieve normal in vivo levels, suggesting the importance of factors that are missing ex vivo (51). Additionally, although insulin and GC are known to increase leptin levels in intact fragments of adipose tissue additively or synergistically (8, 39, 69, 77), the fact that this is not observed in 3T3-L1 (63) or other cell culture models represents a significant obstacle to mechanistic studies of relevance to the human adipocyte.

**Posttranscriptional Mechanisms Dominate the Short-Term Nutritional Regulation of Leptin Production**

Studies from several laboratories demonstrate that insulin does not affect leptin mRNA levels yet increases leptin release twofold after 1–2 h of incubation (8, 38, 40, 66). Our studies in rat adipose tissue used biosynthetic labeling to directly demonstrate the translational control of leptin by feeding, starvation, insulin, and adrenergic agonists (40, 65). Short-term starvation (14 h) decreases relative rates of leptin biosynthesis despite little or no effect on mRNA levels. The decline in leptin translation is more marked in younger/leaner compared with...
older/obese rats. In vitro, insulin increases rates of leptin biosynthesis by approximately two- to threefold in adipose tissue of younger rats (6–7 wk old) without affecting leptin mRNA levels and has a smaller effect (up to 1.5-fold) in older/more obese rats (12–14 wk old). The magnitude of the insulin effect on leptin mRNA translation is also greater in starved compared with fed rats (40, 65), most likely attributable to the lower baseline. Consistent with the importance of the translational control in the nutritional regulation of leptin expression, more leptin mRNA is associated with polysome fractions in adipose tissue from fed compared with starved rats. Furthermore, in vitro insulin treatment increases leptin mRNA association with translationally active polysome fractions (40).

Activation of the sympathetic nervous system may antagonize the insulin stimulation of leptin translation. β-AR agonists decrease leptin synthesis and secretion within hours without affecting leptin mRNA levels (17, 65). Addition of isoproterenol, a β-AR agonist, to incubations of rat adipose tissue acutely blocks the insulin stimulation of relative rates of leptin biosynthesis without affecting leptin mRNA levels (65). Thus the counterregulation of leptin biosynthesis by insulin and catecholamines has the potential to fine tune the rate of leptin production over a short time course, 1 h or less.

Mechanisms Regulating Leptin Translation

Alterations in translational initiation or efficiency could mediate changes in leptin mRNA translation with feeding or insulin. Leptin mRNA of both human and mouse includes a short 5′-untranslated region (UTR) that lacks a 5′-terminal oligopyrimidine but is predicted to have conserved secondary structures that may affect translation (66). Using reporter constructs transfected into 3T3-L1 adipocytes, we found that the 5′-UTR of leptin mRNA increases translation of luciferase by two- to threefold without affecting its mRNA level. Consistent with this result, Kandror’s group also found that the 5′-UTR of human leptin mRNA increases translation of luciferase in constructs that include the inhibitory 5′-UTR (40). Thus available evidence suggests that insulin derepresses leptin translation through a mechanism that involves an interaction of the 5′- and 3′-UTRs. Chakrabarti et al. (14) also showed that the mouse 5′-UTR is not sufficient for the stimulation of leptin translation by insulin or nutrients present in DMEM (i.e., glucose and amino acids). They also demonstrated that insulin provokes a small stimulation of the translation of the bicistronic reporter construct in HEK-293 cells regardless of the presence of the leptin 5′-UTR. In our experiments using 3T3-L1 adipocytes, insulin caused a small but consistent increase in both control and 5′-UTR reporter constructs that is likely explained by its well-known effect on general protein synthesis. Unraveling the novel mechanisms by which 5′- and 3′-UTRs of leptin specifically regulate insulin-stimulated leptin synthesis, over and beyond its effects on general protein synthesis, is a key question for future research. Furthermore, central to understanding shifts in leptin production with fasting and refeeding, it will be important to investigate the mechanisms by which activation of the cAMP pathway antagonizes insulin-induced luciferase activity (P. Brauner and S. Fried, unpublished observations).

The possibility that leptin mRNA stability is regulated has not been addressed. Leptin, like other cytokine mRNAs, includes several adenosine-uridine-rich elements that are known to regulate their half life (90). We may not have seen any variations in our leptin UTR reporter mRNA expression levels in our previous studies because they were not designed to address this issue, i.e., we used a reporter construct that was driven by a strong promoter (SV 40 promoter) (40).

Nutrients May Signal Alterations in Leptin Production

In addition to alterations in insulin, changes in circulating levels of glucose, amino acids, and lipids may also contribute to increases in leptin production in response to meals. Conversely, a decline in energy or nutrient availability, via a decrease in mTOR and increase in AMP-activated protein kinase (AMPK) signaling may contribute to the decreased leptin levels with fasting.

Increases in circulating branch chain amino acids (BCAAs) likely contribute to meal-induced increases in leptin. Administration of BCAAs, particularly leucine, elicits a rise in leptin after 3 h, as shown by Lynch et al. (47). This group also showed that norleucine, which does not increase serum insulin, also increases leptin, providing evidence that BCAA increase leptin translation via activation of mTOR (47). Whether amino acids and insulin effects on leptin translation are additive and which factor dominates in vivo will require further research.

Glucose also has been suggested to mediate nutritionally induced leptin levels since glucose infusion increases leptin mRNA levels in adipose tissue and plasma leptin levels (42). Furthermore, it has been suggested that the insulin effect on
leptin depends on insulin-stimulated glucose uptake and metabolism in rat adipocytes (42, 55). Mueller et al. (55) concluded that insulin’s effect on glucose metabolism explains its ability to stimulate leptin gene expression via a SP-1 site and thus its increased release. In their study, however, insulin stimulates leptin secretion only after 48–96 h. Thus it is unlikely that this mechanism contributes to the insulin stimulation of leptin release over a time course of hours (3, 8, 38). Using isolated rat adipocytes, Cammisotto et al. (13) showed that 5 mM compared with 0 mM glucose increased leptin release from rat adipocytes, but increasing glucose up to 25 mM did not cause a further increase. We obtained similar results using rat adipose tissue (M.-J. Lee and S. Fried, unpublished observations). Other metabolizable hexoses or lactate also stimulate leptin release (13, 41, 55), suggesting that glucose metabolism into trioses or generation of ATP, rather than glucose itself, plays a role in inducing leptin production or secretion. It therefore seems likely that the inhibition of leptin release from cultured adipocyte-treated inhibitors of glucose uptake (55) were secondary to a nonspecific effect on energy status that resulted in an activation of AMPK and subsequent inhibition of general protein synthesis. Alternatively, because leptin secretion requires energy, the decline in ATP with inhibition of glucose uptake may inhibit the process of leptin secretion per se. Studies are needed to assess the short- and long-term effect of glucose and other substrates on relative rates of leptin biosynthesis and secretion.

FA exert short- and long-term effects on leptin expression. In short-term experiments (2 h), FAs antagonize insulin-stimulated leptin secretion from rat adipocytes without affecting basal levels (12). Blocking triacylglycerol synthesis with triacsin C, which raised intracellular FA availability, also inhibits leptin release after 2 h (74). The effect of FAs on leptin release is independent of mitochondrial FA oxidation (12), suggesting that their effect over the short term is mediated by an inhibition of insulin signaling. Consistent with this idea, infusion of intralipid/heparin to humans for several hours to raise FAs does not affect baseline leptin levels (59) but blocks the stimulatory effect of insulin (22). Furthermore, decreased FA uptake secondary to CD36 deficiency is associated with elevated serum leptin despite lower adiposity (27). The higher leptin levels were attributed to the absence of FA antagonism of the stimulatory effect of a glucose load on serum leptin in the absence of CD36. On the other hand, the perilipin-null mouse exhibits high basal lipolysis, which should raise intracellular FA availability, yet it also has high leptin (78). This observation seems inconsistent with studies showing a suppressive role of FAs on leptin, pointing out the need for further analysis of the mechanisms by which endogenous and exogenous FAs affect leptin production.

FAs may also exert long-term effects on basal leptin expression in vitro (27, 74). This effect may be mediated, at least in part, via a stimulation of PPAR activity, since FAs are PPAR-γ agonists and PPAR-γ agonists are known to decrease leptin gene expression and circulating leptin in vivo (Ref. 79 and our unpublished observation in vitro in human adipose tissue). Furthermore, the higher baseline leptin mRNA levels in CD36-null mice may be secondary to lower FA uptake with consequent lower activation of PPARRs (27). It will therefore be of interest to compare the effects of different types of FAs that differentially affect PPAR activation on leptin.

Signaling Pathways Involved in Insulin and Amino Acid-stimulated Leptin Translation: Integration at the Level of mTOR

Present knowledge of the pathways that mediate the short-term control of leptin translation by hormones, nutrients, and energy status is summarized in Fig. 1. Studies using inhibitors have shown that insulin increases leptin production in adipocytes through phosphoinositide (PI) 3-kinase/PKB/mTOR. Bradley and Cheatham (8) showed that inhibitors of PI 3-kinase and MEK1/MEK2, as well as mTOR, blocked insulin-stimulated leptin release from isolated rat adipocytes without affecting leptin mRNA expression levels. Furthermore, expression of constitutively active AKT induces a more than 20-fold increase in leptin protein levels without affecting leptin mRNA levels (4). We further demonstrated that inhibitors of PI 3-kinase, AKT, or mTOR block insulin-stimulated leptin biosynthesis (40). Recent studies by Chakrabarti et al. (14) identified activation of mTOR complex 1 (mTORC1) as the critical downstream event mediating the insulin stimulation of leptin translation (14). Increasing mTORC1 activity by overexpression of Rheb or dominant negative AMPK increases leptin translation, and this does not require the presence of leptin 5′-UTR (14). In the physiological context, independent of their metabolism into glycolytic substrate or TCA cycle intermediates, amino acids can increase leptin within 2–4 h by acting as signaling molecules activating mTOR (47, 48, 66). However, the downstream events through which activation of mTOR leads to a specific increase in relative rates of leptin biosynthesis, over and above a small increase in general protein synthesis, remain to be determined.

It is notable that decreasing mTOR activity by adipose specific knockout of raptor (a component of the mTORC1) (60) results in lower leptin levels, whereas knockout of mTOR downstream translational repressor 4E-BP1 and 2 results in higher circulating leptin levels (37). However, these changes were appropriate for the concomitant changes in fat mass. The nutritional status of the mice when the leptin measurements were made was not stated, thus it is not possible to discern whether manipulating mTOR signaling specifically mediates the response to nutrient (amino acid) and hormonal (insulin) signals. In vitro studies of cultured adipocytes derived from these genetically altered mice may help define the role of the mTOR pathway in the regulation of leptin translation.

Recent studies point to the interplay between mTOR (mTORC1 complex) and AMPK signaling pathways, providing a mechanism to couple leptin synthesis with energy availability (30, 85). Kandror’s group (14) demonstrated that removing the negative effect of AMPK by overexpressing dominant negative AMPK increases mTORC1 and leptin translation. This observation raises the possibility that AMPK tonically inhibits leptin translation in cultured cells and that the activation of AMPK by low cellular energy status such as fasting may inhibit leptin translation through mTOR pathway. It is not known whether this mechanism also modulates leptin synthesis in primary adipocytes. Thus the ability of insulin and/or nutrients (glucose and amino acids such as leucine) to stimulate leptin translation may result, at least in part, through relieving the inhibitory effect of AMPK. Thus cross-talk between nutrient and energy availability signaling through PI 3-kinase/PKB and AMPK may link nutritional status to the regulation of
leptin production. Studies are needed to delineate the effects of AMPK activation on general protein synthesis from any possible specific effect on leptin translation.

Activation of PKA pathway may also regulate leptin release during the starved state. Increases in cAMP are likely to mediate the β-adrenergic inhibition of leptin release. Cong et al. (15) showed that insulin antagonizes the β-AR suppression of leptin release from rat primary adipocytes during 24-h cultures through PI 3-kinase-dependent activation of PDE3-decreasing cAMP. We have also found that dbcAMP can mimic the effect of β-AR to inhibit leptin translation (P. Brauner and S. Fried, unpublished observation). Thus exercise, cold stress, and fasting may decrease leptin production via cAMP signaling. Increasing intracellular cAMP has been shown to decrease mTOR kinase activity and phosphorylation of mTOR downstream targets, 4E-BP1 and S6K, in several cell types including adipocytes (26, 45, 72). Thus cAMP may decrease overall or specific rates of leptin translation through cross-talk with the mTOR pathway.

Interplay between cAMP and AMPK signaling in adipocytes may also contribute to the β-AR-mediated decrease in leptin release (23, 31, 87). Koh et al. (31) showed that β-AR agonists increase the AMP/ATP ratio and activate AMPK activity in rat adipocytes. A recent paper by Gauthier et al. (23) showed that β-AR-stimulated lipolysis increases use of ATP for FA activation and that this activates AMPK activity. Thus it seems possible that the decline in serum leptin associated with activation of the sympathetic nervous system with fasting, cold, or exercise involves the cross-talk among the PKA, AMPK, and mTOR pathways, as illustrated in Fig. 1.

In studies of rat adipocytes or adipose tissue, stimulation of β-AR did not affect basal leptin secretion but antagonized the insulin stimulation of leptin secretion after as little as 30 min of treatment (24, 65). In human adipose tissue, however, β-AR stimulation decreased basal leptin release without affecting leptin biosynthesis (65). Thus there may be significant species differences in the adrenergic regulation of leptin.
Regulation of Leptin Release at Posttranslational Steps

In addition to alterations in its synthesis, the posttranslational processing of leptin in the short term (i.e., within minutes to an hour) may also be regulated at the level of secretion per se (3, 38). Newly synthesized leptin can be stored, degraded intracellularly before being secreted, or secreted. Secreted leptin may also be subject to reuptake and secretion (38). Nutritional status (chronically or acutely) and hormonal stimuli, i.e., insulin or adrenergic agonists, could affect any of these processes to regulate leptin release.

We demonstrated that a substantial quantity of leptin protein resides in a detergent-sensitive compartment of human adipose tissue, sufficient to account for leptin release from adipose tissue of humans for over 3 h (70) and rats for 1 h (38). Moreover, the amount of leptin in human adipose tissue is highly correlated with fat cell size and obesity (40, 70). The size of the preformed leptin pool also varies as a function of nutritional status and obesity. Leptin content is higher in adipose tissue from older/obese compared with younger/lean rats, and in fed compared with starved mice (Ref. 39; M. J. Lee and S. Fried, unpublished observation; see Fig. 2). Thus we proposed that a preformed leptin pool may provide a reservoir that can be rapidly released and contribute to changes in plasma leptin that occur hours after meals, as illustrated in Fig. 2. This mechanism may permit more rapid changes in circulating leptin than would be possible with alterations in de novo synthesis. For example, variations in the release of stored leptin may contribute to pulsatile variations (~30 min) in plasma leptin of humans (44) and fed conditions (2).

With pulse-chase experiments, we directly demonstrated that, independent of protein synthesis, insulin increases (38) while isoproterenol decreases (P. Brauner and S. Fried, unpublished results) secretion of newly synthesized leptin from rat and human adipose tissue over a time course of 30–60 min, showing that insulin or catecholamines act on the leptin secretion step. Nutritional status affects insulin induction of leptin secretion. Insulin increases leptin secretion in adipose tissue from fed but not overnight fasted rats (38). This may be due to the fact that starvation depletes a specific pool of preformed leptin that is subject to regulated secretion. Repletion of this pool in fed rats may allow insulin to promptly increase serum leptin, helping to maintain energy balance (Fig. 2).

Little is known about the molecular mechanisms controlling leptin secretion. Leptin exists in different compartments from other adipocyte secretory products, namely lipoprotein lipase, adiponectin, or glucose transporter 4 (3, 6, 67). Inhibition of vesicular trafficking with Brefeldin A (49) or monensin (42) blocks leptin release into medium and leads to its intracellular accumulation, indicating that leptin secretion is regulated by the classical secretory pathway. However, leptin does not appear to colocalize within Golgi (3), and Roh et al. (67) suggested that, at least in adipocytes from young rats, separate leptin storage vesicles may exist. Like adiponectin (84), the endoplasmic reticulum (ER) may be a critical site for regulating leptin folding (it has one disulfide bond) and secretion. Future studies in which the expression or binding activity of potential chaperones is manipulated might help to explain the cellular trafficking of leptin with variations in nutritional or physiological status.

Turnover of Newly Synthesized Leptin: Insulin Prevents Intracellular Degradation of Leptin by Increasing Secretion

Another potential site of regulatory control of leptin production is degradation. In pulse-chase experiments, only a fraction, up to 50%, of the labeled leptin that disappeared from cells could be recovered in the medium, suggesting that some of the newly synthesized leptin is degraded within adipose tissue (38). Insulin prevented this intracellular leptin degradation by moving it out of the cells, increasing the half-life of newly secreted leptin by 10.2 ± 0.33. Little is known about the molecular mechanisms controlling leptin secretion. Leptin exists in different compartments from other adipocyte secretory products, namely lipoprotein lipase, adiponectin, or glucose transporter 4 (3, 6, 67). Inhibition of vesicular trafficking with Brefeldin A (49) or monensin (42) blocks leptin release into medium and leads to its intracellular accumulation, indicating that leptin secretion is regulated by the classical secretory pathway. However, leptin does not appear to colocalize within Golgi (3), and Roh et al. (67) suggested that, at least in adipocytes from young rats, separate leptin storage vesicles may exist. Like adiponectin (84), the endoplasmic reticulum (ER) may be a critical site for regulating leptin folding (it has one disulfide bond) and secretion. Future studies in which the expression or binding activity of potential chaperones is manipulated might help to explain the cellular trafficking of leptin with variations in nutritional or physiological status.
synthesized leptin from 50 min to 150 min (38). Although several studies (61, 88) have suggested leptin is degraded through proteasomal pathway, our results with inhibitors indicate that leptin is also degraded by another pathway. Lysosomal degradation of leptin seems likely since exogenously added 125I-leptin is degraded (38). Thus secreted leptin may be subject to reuptake and degradation, providing another level of control of net leptin secretion. Further analysis of the mechanisms regulating leptin degradation in different compartments may reveal novel mechanisms that fine tune leptin secretion as a function of nutritional state.

Conclusion

In states of chronic nutrient excess or restriction, serum leptin concentrations are regulated at the mRNA expression level through both transcriptional and posttranscriptional mechanisms. In response to meal ingestion and consequent variations in hormone levels and nutrient availability, regulation at posttranscriptional steps, including translation, storage, secretion, or degradation, adjusts the rate of leptin production and release.

Future Directions

As we understand more about how leptin is regulated at pre- and posttranscriptional levels, we will get a step closer to the development of methods to manipulating diurnal excursions and oscillations in serum leptin levels and thereby could modulate its downstream effectiveness. Additionally, it will be important to dissect how the adipocyte senses its size to adjust basal leptin production to the level of body fat and the degree of central leptin resistance. Interestingly, recent research points to the potential for cross-talk between the leptin action in the hypothalamus and leptin production in the periphery. Leptin signaling through mTOR activity in the hypothalamus regulates leptin action on food intake, and this pathway becomes resistant with high-fat-diet-induced obesity (16). The extent to which the adipocyte mTOR pathway also becomes resistant to insulin or nutrient signals may be a critical determinant of the ability of the adipocyte to upregulate leptin production and close the feedback loop. In addition, hypothalamic leptin signaling through PI 3-kinase can activate sympathetic nerves to regulate adipocyte lipogenesis and endocannabinoid production in adipose tissue (11). This mechanism may also regulate leptin transcription and translation, providing direct central control of leptin production and an additional feedback loop. Another layer of regulation involves the autocrine feedback of leptin production at the level of the adipocyte (83, 92). Although some of the nutrients (glucose, amino acids, and fatty acids) and endocrine or neural signals (insulin, GCs, catecholamines, NPY, and melanin-concentration hormone) have been identified, their relative importance and mechanism of action remain unclear. Finally, fat cells produce many other adipokines, including adiponectin, RBP 4, and serum amyloid A. Some mechanisms for regulating the production of these adipokines may be common with leptin, but others are likely to be unique. The elucidation of how the adipocyte integrates signals and functions as an endocrine cell that modulates appetite, fuel metabolism, inflammation, angiogenesis, and reproduction may point to novel therapeutic approaches to preventing obesity and its metabolic consequences.

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