Symposium: Adipocyte Function, Differentiation and Metabolism

Regulation of Leptin Production in Humans[1,2]

Susan K. Fried,[3] Matthew R. Ricci, Colleen D. Russell and Blandine Laferrère*

*Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ 08901 and *Obesity Research Center, St. Lukes-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, NY 10025*

ABSTRACT Serum levels of the adipocyte hormone leptin are increased in proportion to body fat stores as a result of increased production in enlarged fat cells from obese subjects. In vitro studies indicate that insulin and glucocorticoids work directly on adipose tissue to upregulate in a synergistic manner leptin mRNA levels and rates of leptin secretion in human adipose tissue over the long term. Thus, the increased leptin expression observed in obesity could result from the chronic hyperinsulinemia and increased cortisol turnover. Superimposed upon the long-term regulation, nutritional status can influence serum leptin over the short term, independent of adiposity. Fasting leads to a gradual decline in serum leptin that is probably attributable to the decline in insulin and the ability of catecholamines to decrease leptin expression, as observed in both in vivo and in vitro studies. In addition, increases in serum leptin occur ~4–7 h after meals. Increasing evidence indicates that insulin, in concert with permissive effects of cortisol, can increase serum leptin over this time frame and likely contributes to meal-induced increases in serum leptin. Further research is required to elucidate the cellular and molecular mechanisms underlying short- and long-term nutritional and hormonal regulation of leptin production and secretion. J. Nutr. 130: 3127S-3131S, 2000.

KEY WORDS: • *leptin* • *adipose tissue* • *insulin* • *glucocorticoid*

Downloaded from jn.nutrition.org by guest on May 14, 2014

It is now well established that the adipocyte hormone leptin serves as a signal that provides information about the size of energy reserves to systems regulating feeding, substrate utilization and energy balance. The function of leptin was perceived originally as a signal that prevented obesity because leptin-deficient *ob*/*ob* mice and leptin-resistant *db*/*db* mice are obese. However, it is now clear that diminished leptin levels are crucial for metabolic adaptations to starvation (Flier 1998), including the decrease in metabolic rate that allows survival for longer periods, inhibition along the reproductive and thyroid axes (Ahima et al. 1997, Kim et al. 2000) and, at least in rodents, the activity of the sympathetic nervous system (Satoh et al. 1999). Leptin is also a critical signal for the onset of puberty (Ahima et al. 1997).

To understand fully the influence of nutritional status on leptin physiology, it is necessary to understand mechanisms regulating leptin production in the fat cell. In this brief review, we will summarize knowledge of the nutritional and hormonal regulation of leptin. We will focus on available data in humans and the roles of insulin, glucocorticoids and catecholamines.

Leptin and human obesity. Leptin deficiency (Montague et al. 1997) and leptin receptor defects (Clement et al. 1998) in humans are very rare. On average, serum leptin is usually elevated in obesity and is positively correlated with body mass index, percentage of body fat and fat mass (Considine et al. 1996, Maffei et al. 1995). However, several laboratories have noted a rather wide range of plasma leptin values at any given level of body fat (Considine et al. 1996, Maffei et al. 1995). Some obese subjects exhibit notably lower or higher leptin levels than predicted from their body fat, suggesting that mechanisms other than absolute fat mass per se regulate serum leptin. This points to the possibility that a differential regulation of leptin secretion contributes to the drive to maintain a high body weight in some obese subjects.

The positive correlation between total body fat mass and serum leptin is probably explained primarily by the increased release of leptin from large compared with small fat cells (Lonnqvist et al. 1997). On average, leptin release per gram of adipose tissue is two times greater in obese than in lean subjects. Because fat cell size is usually enlarged 2–4 times in the obese, when expressed per fat cell, leptin secretion is up to 7 times higher in obese than in lean subjects. In addition, an increased number of fat cells, particularly in extreme obesity, undoubtedly contribute to increases in serum leptin. An important unanswered question is whether the increased production of leptin in enlarged fat cells from obese subjects results

[1] Presented as part of the symposium "Adipocyte Function, Differentiation and Metabolism" given at the Experimental Biology 00 meeting, San Diego, CA on April 16, 2000. This symposium was sponsored by the American Society for Nutritional Sciences and was supported by educational grants from Dupont Pharmaceuticals, Pfizer, Inc. and Zen-Bio, Inc. The proceedings of this symposium are published as a supplement to *The Journal of Nutrition*. The guest editor for the symposium publication was Naima Moustaid-Moussa, University of Tennessee, Knoxville, TN.

[2] Supported by National Institutes of Health grants RO1DK52398 (S.K.F.) and KO8 02572 (B.L.) and a grant from the American Diabetes Association (B.L.).

[3] To whom correspondence should be addressed.

0022-3166/00 $3.00 © 2000 American Society for Nutritional Sciences.

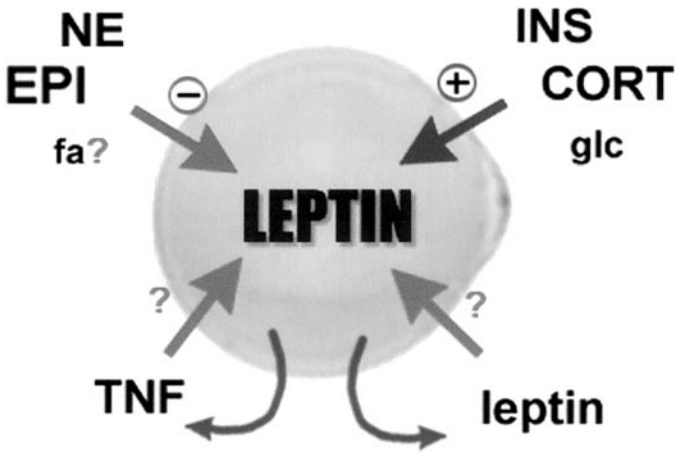

FIGURE 1 Major influences on leptin expression in human adipocytes. Leptin is upregulated by the hormones insulin (INS) and cortisol (CORT) working synergistically, and downregulated by catecholamines [norepinephrine (NE) and epinephrine (EPI)]. In addditon, tumor necrosis factor-α (TNF-α) likely serves as paracrine regulator, and may increase leptin secretion. Finally, though supported mainly by studies in rodent models, leptin likely also autoregulates its own expression and substrates such as glucose (glc) and fatty acids (fa) may also influence leptin expression.

from the chronic hormonal (i.e., hyperinsulinemia and perhaps increased cortisol turnover) and paracrine (increased cytokine production within adipose tissue) milieu associated with the obese state (**Fig. 1**). In addition, a signal arising from the physical "stretching" of the adipocyte has been proposed (Hamilton et al. 1995).

Most studies indicate that leptin levels are higher in women than men, even after correction for body fatness (Licinio et al. 1998). The mechanism involved is unclear, but one report indicates a direct effect of estrogen to increase leptin production (Casabiell et al. 1998). The sex difference is detectable even in utero and is attributable to the fetus itself (Jaquet et al. 1998).

Acute and chronic nutritional regulation of leptin. Plasma leptin levels increase with weight gain (Kolaczynski et al. 1996a, Maffei et al. 1995, Rosenbaum et al. 1997) and decrease with weight loss (Maffei et al. 1995), consistent with leptin's role as a signal of the size of adipose tissue stores. However, superimposed on the regulation of leptin secretion by level of obesity, acute nutritional and hormonal signals modulate the rate of leptin secretion with a time course encompassing effects up to ~7 h (Kolaczynski et al. 1996a, Schoeller et al. 1997). Serum leptin displays a diurnal rhythm with the highest levels between 1100 and 0100 h, after which plasma leptin declines until early afternoon (Sinha et al. 1996). The diurnal rhythm is linked to meal timing because a 6-h delay in meals produces a similar phase shift in the plasma leptin profile (Schoeller et al. 1997). Furthermore, frequent blood sampling reveals that plasma leptin levels are pulsatile, with ~30 pulses per 24 h period (Licinio et al. 1997, Sinha et al. 1996).

Plasma leptin declines gradually during 24–36 h of fasting to 40–70% of baseline in both lean and obese subjects despite little or no loss of fat mass (Boden et al. 1996, Grinspoon et al. 1997, Kolaczynski et al. 1996b). Acute massive overfeeding (120 kcal/kg) increases plasma leptin by 40% after 5 h (Kolaczynski et al. 1996a). Compared with a high fat diet (60% of calories), a low fat (20%), high carbohydrate diet produces higher peaks of leptin during the night, without affecting morning leptin levels (Havel et al. 1999). Thus, it is important to consider the excursions in plasma leptin that result from alterations in macronutrient composition or meal-timing, in addition to effects on baseline (morning leptin after an 18-h fast).

Depot differences. Several laboratories have reported that leptin mRNA levels and leptin secretion are higher in subcutaneous than omental adipocytes (Russell et al. 1998, Van Harmelen et al. 1998). These findings, coupled with the fact that visceral adipose tissue usually accounts for only 5–10% of total body fat, appear to explain why subcutaneous adipose tissue is the major determinant of plasma leptin in multiple regression analyses (Van Gaal et al. 1999).

Regulation of leptin production. Available evidence supports an important role for insulin, with "permissive" concentrations of cortisol, in mediating the effects of meals on serum leptin in humans. The gradual fall in leptin with fasting likely depends on the drop in insulin, in concert with increases in catecholamines. Rapid decreases in serum leptin (30–90 min) also occur upon activation of the sympathetic nervous system or administration of a β-adrenergic agonist.

Role of insulin. Early studies suggested that acute (2–3 h) hyperinsulinemia had no effect on plasma leptin levels (Dagogo-Jack et al. 1996, Pratley et al. 1996) or that increases in plasma leptin were evident only with prolonged hyperinsulinemia (several days) (Boden et al. 1997) or with supraphysiologic insulin concentrations (Utriainen et al. 1996). However, when plasma leptin levels during physiologic insulin infusion are compared with those of saline-infused controls at the same time of day, it is clear that insulin either maintains or increases plasma leptin levels, whereas levels in the control group decrease. Differences between the groups were detectable within 1 h (Malmstrom et al. 1996).

In vitro studies support a direct role for insulin in regulating leptin production. We observed that 24 h of culture of subcutaneous human adipose tissue fragments with 7 nmol/L insulin resulted in 50% higher leptin release, compared with controls cultured without insulin, without affecting leptin mRNA levels (Russell et al. 1998). However, in cultures of newly differentiated human adipocytes, insulin is critical for maintaining leptin mRNA levels and leptin release over 24 h (Wabitsch et al. 1996). Results from studies of isolated human subcutaneous adipocytes show much more delayed effects of insulin that are evident only after 72–96 h. Thus, different experimental systems appear to give discrepant results on the role of insulin in regulating leptin. It is worth noting that fragments of human fat secrete leptin at levels comparable to those calculated from arteriovenous difference studies (Klein et al. 1996), and that the direction, time course and mechanisms of hormonal effects involved appear to model in vivo results well with regard to insulin and other hormonal effects described below.

Role of glucose and glucosamine. Studies in cultured rat adipocytes seem to suggest that insulin increases leptin expression by virtue of its ability to increase adipocyte glucose utilization rather than a direct effect of insulin (Mueller et al. 1998). It is difficult to rule out this factor from available data in human adipocytes, but it is notable that the potentiation of insulin action on leptin by glucocorticoids (described below) is not associated with increased glucose utilization (Appel and Fried 1992). It has also been suggested that the flux of glucose through the glucosamine pathway serves as a "nutrient sensing" system that upregulates leptin expression during hyperinsulinemia, hypertriglyceridemia and in states of insulin resistance (Wang et al. 1998). The relative contribution of substrate and hormonal effects in regulating leptin expression

Downloaded from jn.nutrition.org by guest on May 14, 2014

in insulin-sensitive and insulin-resistant states will require further investigation.

Role of cortisol. Cortisol is generally considered a counterregulatory hormone and its secretion increases during fasting and stress. However, the development of obesity in most animal models is dependent upon moderate levels of corticosterone (the active glucocorticoid in rodents). In humans, hypercortisolemia (Cushing's syndrome) is associated with central obesity, whereas cortisol deficiency (Addison's Disease) is associated with weight loss. Cortisol turnover is usually increased in upper body obesity (Rosmond et al. 1998). Furthermore, expression of 11β-hydroxysteroid dehydrogenase, the enzyme that interconverts cortisone to cortisol, is higher in omental than subcutaneous fat, suggesting that local concentrations of cortisol may vary among depots (Ewart et al. 1999). Available data indicate that glucocorticoids potently stimulate leptin expression, but may also induce central leptin resistance (Zakrzewska et al. 1997). Thus, by affecting the balance between leptin sensitivity and production, elevated glucocorticoids may favor fat deposition and a higher set point for body fat.

In vivo effects of glucocorticoid administration on leptin. Numerous reports in the literature indicate that glucocorticoid increases plasma leptin and leptin mRNA in vivo. In humans, dexamethasone (a synthetic glucocorticoid) increased plasma leptin (Larsson and Ahren 1996) after as little as 9 h (Miell et al. 1996) and subcutaneous adipose tissue leptin mRNA after 2 d (Kolaczynski et al. 1997). The increase in plasma leptin in response to glucocorticoid appears to be greater in obese vs. lean humans (Dagogo-Jack et al. 1997). Glucocorticoids induce insulin resistance and hyperinsulinemia (Larsson and Ahren 1996, Papaspyrou-Rao et al. 1997), thus raising the possibility that the in vivo effects of glucocorticoid on leptin are indirect. However, Larsson and Ahren (1996) found that the increases in plasma leptin with dexamethasone were not related to changes in plasma insulin or insulin sensitivity.

In vitro effects of glucocorticoids on leptin. Insulin and cortisol act synergistically over the long term in both subcutaneous and omental fat to maintain leptin expression. We have also noted depot differences in the regulation of leptin expression in human fat in response to culture with dexamethasone in the absence of insulin (Russell et al. 1998). After 24–48 h of culture, dexamethasone increased leptin mRNA in both omental and subcutaneous fat, but increased leptin release into the medium only in omental fat.

Interactions between insulin and glucocorticoid in leptin regulation

In adipose tissue of obese subjects, the combination of insulin and dexamethasone (but neither hormone alone) maintains initial rates of leptin release and expression of leptin mRNA for up to 7 d of culture. Leptin secretion is merely maintained over 7 d in the obese, whereas in adipose tissue of lean subjects, culture with maximal concentration of insulin and dexamethasone actually increases leptin release into the medium between d 1 and 7 of culture (unpublished observation). These data imply that a chronically hyperinsulinemic state, with at least permissive levels of glucocorticoid, may explain in part the elevated leptin expression observed in obesity.

As in our results in cultured fragments of human fat, synergism between insulin and cortisol in promoting leptin expression has been noted in cultures of newly differentiated human fat cells (Wabitsch et al. 1996). Contrasting these results, however, are reports that dexamethasone actually decreases insulin-stimulated leptin release into the medium from cultured isolated human adipocytes (Considine et al. 1997, Halleux et al. 1998). The reason for this discrepancy is not known, but may relate to the inclusion of serum in the culture medium.

Glucocorticoids, added with or without insulin, appear to increase leptin by a transcriptional mechanism, as judged by inhibitor studies (Bradley and Cheatham 1999, Russell et al. 1998). A glucocorticoid response element has been identified in the promoter region of the human leptin gene, but there are no reports on its functionality (Gong et al. 1996).

Importance of cortisol in the regulation of leptin. Given findings that cortisol potentiates insulin effects on leptin expression, it may appear paradoxical that leptin and cortisol exhibit reciprocal diurnal rhythms (Havel et al. 1999, Licinio et al. 1997). This inconsistency may arise from the failure to consider variability in serum insulin levels. According to results from several in vitro models, glucocorticoids increase leptin expression most strongly in the presence of insulin, with a time delay of 3–7 h. Similarly, in vivo, in subjects who are fed (i.e., high insulin), administration of a bolus of dexamethasone induces higher serum leptin levels compared with those administered saline after 4–5 h, yet dexamethasone had no effect in subjects who had fasted (Laferrère et al. 1998). Our recently reported preliminary data indicate that insulin also promotes a dexamethasone-induced rise in serum leptin (Laferrère et al. 1999), suggesting that the meal effect is due at least in part to the rise in serum insulin. In addition, recent data from rat studies indicate a potentially important role for gut peptides (Attoub et al. 1999).

The sympathetic nervous system and β-adrenergic agonists. There is ample evidence to suggest that short- or long-term stimulation of adipose tissue β-adrenergic receptors (β-AR) inhibits leptin in rodents and adipose cell lines (Li et al. 1997, Trayhurn et al. 1995). Increases in cyclic AMP production may directly affect leptin mRNA transcription because a cyclic AMP-responsive element has been identified in the promoter region of the leptin gene (Gong et al. 1996). In human adipose tissue, we reported that isoproterenol has both short- and long-term effects on leptin expression. Isoproterenol decreases leptin release after 1.5–3 h of incubation of adipose tissue from obese (Ricci and Fried 1999) or lean subjects (unpublished). Over the longer term, culture of human omental or subcutaneous adipose tissue with isoproterenol for 24 h decreased leptin accumulation in the medium in parallel with a decrease in its mRNA level (Ricci and Fried 1999). This occurs even when insulin or insulin and/or dexamethasone are present in the incubation medium (Halleux et al. 1998, Ricci and Fried 1999).

Consistent with the fairly rapid inhibitory effects of β-AR stimulation on leptin release, two studies have demonstrated that infusion of isoproterenol or epinephrine (Carulli et al. 1999) decreases plasma leptin by 19–27% over 120 min in lean men and women (Donahoo et al. 1997). A 3-h infusion of epinephrine (at levels similar to that seen in strenuous exercise) decreased plasma leptin by 22% and abdominal subcutaneous adipose tissue leptin mRNA relative abundance by 47% in obese men and women (Carulli et al. 1999). We also found a rapid decrease in serum leptin when sympathetic nervous system activity was stimulated by placing women in a cold chamber at 4°C (Ricci et al. 2000). Using open-flow microperfusion in subcutaneous human adipose tissue in vivo, Orban et al. (1999) noted a transient decrease in interstitial fluid leptin concentration within 90 min of addition of isoproterenol. It is unlikely that fatty acids per se explain the decrease in leptin with isoproterenol (Stumvoll et al. 2000).

Downloaded from jn.nutrition.org by guest on May 14, 2014

Given the relatively rapid effects of β-adrenergic activation on leptin release, we speculate that the pulsitility of leptin secretion in vivo may be mediated in part by sympathetic innervation of adipose tissue. There is precedent for this notion in the observation of Bergman that fatty acid release from dog omental adipose tissue is pulsitile and blocked by propanolol (Getty et al. 2000)

Regulation of leptin secretion. Virtually nothing is known about the cellular pathway(s) involved in leptin secretion, i.e., whether leptin secretion per se is regulated (increases/decreases in response to a secretagogue) or is merely constitutively secreted at a rate proportional to its synthesis. The fairly rapid effects of β-adrenergic stimulation on leptin release from adipose tissue in vivo and in vitro suggest the possibility that leptin secretion per se is regulated by cAMP. A recent report suggests that cholecystokinin may regulate leptin secretion directly (Attoub et al. 1999). Additionally, tumor necrosis factor, potentially an important paracrine regulator of leptin, transiently increases release of leptin from a preformed pool in 3T3-L1 adipocytes (Kirchgessner et al. 1997).

A number of lines of evidence, derived mainly from studies of rodent models, suggest that insulin directly influences leptin secretion. Barr et al. (1997) found that insulin stimulated leptin release from rat adipocytes and that this efflux was associated with an initial decrease in cellular leptin content. Using confocal microscopy, Barr et al. (1997) showed that in the absence of insulin, intracellular leptin was localized to the endoplasmic reticulum, whereas after insulin treatment, leptin was found at the plasma membrane, consistent with the hypothesis that insulin directly increased leptin secretion. Similarly, Bradley and Cheatham (1999) found that incubation of rat adipocytes with insulin increases leptin release during a 2-h incubation without affecting leptin mRNA. Insulin increases the relative rate of release of a number of adipocyte secretory proteins, independently of alterations in protein synthesis (Scherer et al. 1995). In the future, it will be important to determine whether insulin also directly influences the intracellular trafficking of leptin and the importance of such mechanisms to short-term changes (minutes to hours) in serum leptin in response to physiologic and nutritional signals.

Summary and conclusions

We have reviewed the evidence that effects of insulin and glucocorticoid underlie the chronic upregulation of leptin expression in obesity. Catecholamines chronically decrease leptin expression over the long term and may also decrease leptin secretion; in combination with declines in plasma insulin, these mechanisms probably contribute to declines in leptin during fasting. In addition, leptin levels are regulated over a time course of minutes (pulsitility) to hours (diurnal rhythm linked to meal timing). There is strong evidence that insulin, with a permissive role for glucocorticoids, plays a critical role in nutritionally induced variations in serum leptin levels.

LITERATURE CITED

Ahima, R. S., Dushay, J., Flier, S. N., Prabakaran, D. & Flier, J. S. (1997) Leptin accelerates the onset of puberty in normal female mice. J. Clin. Investig. 99: 391–395.

Appel, B. & Fried, S. K. (1992) Effects of insulin and dexamethasone on lipoprotein lipase in human adipose tissue. Am. J. Physiol. 262: E695–E699.

Attoub, S., Levasseur, S., Buyse, M., Goiot, H., Laigneau, J. P., Moizo, L., Hervatin, F., Le Marchand-Brustel, Y., Lewin, J. M. & Bado, A. (1999) Physiological role of cholecystokinin B/gastrin receptor in leptin secretion. Endocrinology 140: 4406–4410.

Barr, V. A., Malide, D., Zarnowski, M. J., Taylor, S. I. & Cushman, S. W. (1997) Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinology 138: 4463–4472.

Boden, G., Chen, X., Kolaczynski, J. W. & Polansky, M. (1997) Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J. Clin. Investig. 100: 1107–1113.

Boden, G., Chen, X., Mozzoli, M. & Ryan, I. (1996) Effect of fasting on serum leptin in normal human subjects. J. Clin. Endocrinol. Metab. 81: 3419–3423.

Bradley, R. L. & Cheatham, B. (1999) Regulation of *ob* gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes 48: 272–278.

Carulli, L., Ferrari, S., Bertolini, M., Tagliafico, E. & Del Rio, G. (1999) Regulation of *ob* gene expression: evidence for epinephrine-induced suppression in human obesity. J. Clin. Endocrinol. Metab. 84: 3309–3312.

Casabiell, X., Pineiro, V., Peino, R., Lage, M., Camina, J., Gallego, R., Vallejo, L. G., Dieguez, C. & Casanueva, F. F. (1998) Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. J. Clin. Endocrinol. Metab. 83: 2149–2155.

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P. & Guy-Grand, B. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature (Lond.) 392: 398–401.

Considine, R. V., Nyce, M. R., Kolaczynski, J. W., Zhang, P. L., Ohannesian, J. P., Moore, J.H.J., Fox, J. W. & Caro, J. F. (1997) Dexamethasone stimulates leptin release from human adipocytes: unexpected inhibition by insulin. J. Cell Biochem. 65: 254–258.

Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J. & Bauer, T. L. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334: 292–295.

Dagogo-Jack, S., Fanelli, C., Paramore, D., Brothers, J. & Landt, M. (1996) Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 45: 695–698.

Dagogo-Jack, S., Selke, G., Melson, A. K. & Newcomer, J. W. (1997) Robust leptin secretory responses to dexamethasone in obese subjects. J. Clin. Endocrinol. Metab. 82: 3230–3233.

Donahoo, W. T., Jensen, D. R., Yost, T. J. & Eckel, R. H. (1997) Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. J. Clin. Endocrinol. Metab. 82: 4139–4143.

Ewart, H. S., Carroll, R. & Severson, D. L. (1999) Lipoprotein lipase activity is stimulated by insulin and dexamethasone in cardiomyocytes from diabetic rats. Can. J. Physiol. Pharmacol. 77: 571–578.

Flier, J. S. (1998) Clinical review 94: what's in a name? In search of leptin's physiologic role. J. Clin. Endocrinol. Metab. 83: 1407–1413.

Getty, L., Panteleon, A. E., Mittelman, S. D., Dea, M. K. & Bergman, R. N. (2000) Rapid ascillations of free fatty acids in normal and fat-fed dogs are driven by the central nervous system, not by insulin. Obes. Res. 7: 33s (abs.).

Gong, D. W., Bi, S., Pratley, R. E. & Weintraub, B. D. (1996) Genomic structure and promoter analysis of the human obese gene. J. Biol. Chem. 271: 3971–3974.

Grinspoon, S. K., Askari, H., Landt, M. L., Nathan, D. M., Schoenfeld, D. A., Hayden, D. L., Laposata, M., Hubbard, J. & Klibanski, A. (1997) Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. Am. J. Clin. Nutr. 66: 1352–1356.

Halleux, C. M., Servais, I., Reul, B. A., Detry, R. & Brichard, S. M. (1998) Multihormonal control of *ob* gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. J. Clin. Endocrinol. Metab. 83: 902–910.

Hamilton, B. S., Paglia, D., Kwan, A. Y. & Deitel, M. (1995) Increased obese mRNA expression in omental fat cells from massively obese humans. Nat. Med. 1: 953–956.

Havel, P. J., Townsend, R., Chaump, L. & Teff, K. (1999) High-fat meals reduce 24-h circulating leptin concentrations in women. Diabetes 48: 334–341.

Jaquet, D., Leger, J., Levy-Marchal, C., Oury, J. F. & Czernichow, P. (1998) Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J. Clin. Endocrinol. Metab. 83: 1243–1246.

Kim, M. S., Small, C. J., Stanley, S. A., Morgan, D. G., Seal, L. J., Kong, W. M., Edwards, C. M., Abusnana, S., Sunter, D., Ghatei, M. A. & Bloom, S. R. (2000) The central melanocortin system affects the hypothalamo-pituitary thyroid axis and may mediate the effect of leptin. J. Clin. Investig. 105: 1005–1011.

Kirchgessner, T. G., Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. (1997) Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. J. Clin. Investig. 100: 2777–2782.

Klein, S., Coppack, S. W., Mohamed-Ali, V. & Landt, M. (1996) Adipose tissue leptin production and plasma leptin kinetics in humans. Diabetes 45: 984–987.

Kolaczynski, J. W., Considine, R. V., Ohannesian, J., Marco, C., Opentanova, I., Nyce, M. R., Myint, M. & Caro, J. F. (1996b) Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes 45: 1511–1515.

Kolaczynski, J. W., Goldstein, B. J. & Considine, R. V. (1997) Dexamethasone,

JN THE JOURNAL OF NUTRITION

Downloaded from jn.nutrition.org by guest on May 14, 2014

OB gene, and leptin in humans; effect of exogenous hyperinsulinemia. J. Clin. Endocrinol. Metab. 82: 3895–3897.
Kolaczynski, J. W., Ohannesian, J. P., Considine, R. V., Marco, C. C. & Caro, J. F. (1996a) Response of leptin to short-term and prolonged overfeeding in humans. J. Clin. Endocrinol. Metab. 81: 4162–4165.
Laferrère, B., Fried, S. K., Osborne, T., Uy, N., Kovera, A., Albu, J. & Pi-Sunyer, F. X. (1999) Synergistic effects in in vivo insulin and dexamethasone on serum leptin in humans. Obes. Res. 7: 65s (Abst.).
Laferrère, B., Fried, S. K., Hough, K., Campbell, S. A., Thornton, J. & Pi-Sunyer, F. X. (1998) Synergistic effects of feeding and dexamethasone on serum leptin levels. J. Clin. Endocrinol. Metab. 83: 3742–3745.
Larsson, H. & Ahren, B. (1996) Short-term dexamethasone treatment increases plasma leptin independently of changes in insulin sensitivity in healthy women. J. Clin. Endocrinol. Metab. 81: 4428–4432.
Li, H., Matheny, M. & Scarpace, P. J. (1997) β3-Adrenergic-mediated suppression of leptin gene expression in rats. Am. J. Physiol. 272: E1031–E1036.
Licinio, J., Mantzoros, C., Negrao, A. B., Cizza, G., Wong, M. L., Bongiorno, P. B., Chrousos, G. P., Karp, B., Allen, C., Flier, J. S. & Gold, P. W. (1997) Human leptin levels are pulsatile and inversely related to pituitary-adrenal. Nat. Med. 3: 575–579.
Licinio, J., Negrao, A. B., Mantzoros, C., Kaklamani, V., Wong, M. L., Bongiorno, P. B., Negro, P. P., Mulla, A., Veldhuis, J. D., Cearnal, L., Flier, J. S. & Gold, P. W. (1998) Sex differences in circulating human leptin pulse amplitude: clinical implications. J. Clin. Endocrinol. Metab. 83: 4140–4147.
Lonnqvist, F., Nordfors, L., Jansson, M., Thorne, A., Schalling, M. & Arner, P. (1997) Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. J. Clin. Investig. 99: 2398–2404.
Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R. & Ranganathan, S. (1995) Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. Nat. Med. 1: 1155–1161.
Malmstrom, R., Taskinen, M. R., Karonen, S. L. & Yki-Jarvinen, H. (1996) Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. Diabetologia 39: 993–996.
Miell, J. P., Englaro, P. & Blum, W. F. (1996) Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Horm. Metab. Res. 28: 704–707.
Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., Sewter, C. P., Digby, J. E., Mohammed, S. N., Hurst, J. A., Cheetham, C. H., Earley, A. R., Barnett, A. H., Prins, J. B. & O'Rahilly, S. (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature (Lond.) 387: 903–908.
Mueller, W. M., Gregoire, F. M., Stanhope, K. L., Mobbs, C. V., Mizuno, T. M., Warden, C. H., Stern, J. S. & Havel, P. J. (1998) Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 139: 551–558.
Orban, Z., Remaley, A. T., Sampson, M., Trajanoski, Z. & Chrousos, G. P. (1999) The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. J. Clin. Endocrinol. Metab. 84: 2126–2133.
Papaspyrou-Rao, S., Schneider, S. H., Petersen, R. N. & Fried, S. K. (1997) Dexamethasone increases leptin expression in humans in vivo. J. Clin. Endocrinol. Metab. 82: 1635–1637.
Pratley, R. E., Nicolson, M., Bogardus, C. & Ravussin, E. (1996) Effects of acute hyperinsulinemia on plasma leptin concentrations in insulin-sensitive and insulin-resistant Pima Indians. J. Clin. Endocrinol. Metab. 81: 4418–4421.
Ricci, M. R. & Fried, S. K. (1999) Isoproterenol decreases leptin expression in adipose tissue of obese humans. Obes. Res. 7: 233–240.
Ricci, M. R., Fried, S. K. & Mittleman, K. D. (2000) Acute cold exposure decreases plasma leptin in women. Metabolism 49: 421–423.
Rosenbaum, M., Nicolson, M., Hirsch, J., Murphy, E., Chu, F. & Leibel, R. L. (1997) Effects of weight change on plasma leptin concentrations and energy expenditure. J. Clin. Endocrinol. Metab. 82: 3647–3654.
Rosmond, R., Dallman, M. F. & Bjorntorp, P. (1998) Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J. Clin. Endocrinol. Metab. 83: 1853–1859.
Russell, C. D., Petersen, R. N., Rao, S. P., Ricci, M. R., Prasad, A., Zhang, Y., Brolin, R. E. & Fried, S. K. (1998) Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am. J. Physiol. 275: E507–E515.
Satoh, N., Ogawa, Y., Katsuura, G., Numata, Y., Tsuji, T., Hayase, M., Ebihara, K., Masuzaki, H., Hosoda, K., Yoshimasa, Y. & Nakao, K. (1999) Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. Diabetes 48: 1787–1793.
Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem 270: 26746–26749.
Schoeller, D. A., Cella, L. K., Sinha, M. K. & Caro, J. F. (1997) Entrainment of the diurnal rhythm of plasma leptin to meal timing. J. Clin. Investig. 100: 1882–1887.
Sinha, M. K., Sturis, J., Ohannesian, J., Magosin, S., Stephens, T., Heiman, M. L., Polonsky, K. S. & Caro, J. F. (1996) Ultradian oscillations of leptin secretion in humans. Biochem. Biophys. Res. Commun. 228: 733–738.
Stumvoll, M., Fritsche, A., Tschritter, O., Lehmann, R., Wahl, H. G., Renn, W. & Haring, H. (2000) Leptin levels in humans are acutely suppressed by isoproterenol despite acipimox-induced inhibition of lipolysis, but not by free fatty acids. Metabolism 49: 335–339.
Trayhurn, P., Duncan, J. S. & Rayner, D. V. (1995) Acute cold-induced suppression of *ob* (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic system. Biochem. J. 311: 729–733.
Utriainen, T., Malmstrom, R., Makimattila, S. & Yki-Jarvinen, H. (1996) Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. Diabetes 45: 1364–1366.
Van Gaal, L. F., Wauters, M. A., Mertens, I. L., Considine, R. V. & De Leeuw, I. H. (1999) Clinical endocrinology of human leptin. Int. J. Obes. Relat. Metab. Disord. 23 (suppl. 1): 29–36.
Van Harmelen, V., Reynisdottir, S., Eriksson, P., Thorne, A., Hoffstedt, J., Lonnqvist, F. & Arner, P. (1998) Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 47: 913–917.
Wabitsch, M., Jensen, P. B., Blum, W. F., Christoffersen, C. T., Englaro, P., Heinze, E., Rascher, W., Teller, W., Tornqvist, H. & Hauner, H. (1996) Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45: 1435–1438.
Wang, J., Liu, R., Hawkins, M., Barzilai, N. & Rossetti, L. (1998) A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature (Lond.) 393: 684–688.
Zakrzewska, K. E., Cusin, I., Sainsbury, A., Rohner-Jeanrenaud, F. & Jeanrenaud, B. (1997) Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. Diabetes 46: 717–719.

Downloaded from jn.nutrition.org by guest on May 14, 2014