The Role of Leptin in Regulating Neuroendocrine Function in Humans[1]

Susann Blüher[2] and Christos S. Mantzoros[3]

Division of Endocrinology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215

ABSTRACT Eating disorders are a group of disease states including anorexia nervosa, bulimia nervosa and binge eating on one end as well as episodic or chronic overeating resulting in obesity at the other end of the spectrum. These disorders are characterized by decreased and/or increased energy intake and are frequently associated with hormonal and metabolic disorders. The discovery of leptin, an adipocyte-secreted hormone acting in the brain to regulate energy homeostasis, and its subsequent study in human physiology have significantly advanced our understanding of normal human physiology and have provided new opportunities for understanding and possibly treating disease states, such as anorexia and bulimia nervosa. It has been recently discovered that leptin levels above a certain threshold are required to activate the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid axes in men, whereas the hypothalamic-pituitary-adrenal, renin-aldosterone, and growth hormone-IGF-1 axes may be largely independent of circulating leptin levels in humans. In this review, we summarize the latest findings related to the role of leptin in the regulation of several neuroendocrine axes, such as the hypothalamic-pituitary-gonadal and the hypothalamic-pituitary-thyroid axes in humans and discuss its potential pathophysiologic role in eating disorders. J. Nutr. 134: 2469S–2474S, 2004.

KEY WORDS: • *leptin* • *anorexia nervosa* • *bulimia nervosa* • *binge eating disorders* • *neuroendocrine axes*

Anorexia nervosa is characterized by severe restriction of food intake resulting in dramatic loss of body weight, whereas in bulimia nervosa recurring episodes of binge eating and vomiting frequently occur without significant changes in body weight (1). Episodic or chronic overeating, including the night eating syndrome which leads to obesity, are also considered to belong to the complex group of eating disorders. Although the prevalence of anorexia nervosa is increasing, dieting, occasionally excessive, in the context of obesity is the most common condition associated with food deprivation in the Western World. Malnutrition, metabolic abnormalities as well as changes in immune function, inflammation, and hormonal (mainly reproductive) changes are well known consequences of significantly decreased caloric intake (2,3).

Basic research efforts and clinical investigations focusing on the etiology of eating disorders have revealed the pathophysiological importance of several variables, including social and psychological factors, genetic predisposition, altered levels of neurotransmitters and neuropeptides, and alterations in neuroendocrine axes (4). This review focuses on the role of leptin, an adipocyte-secreted hormone regulating energy homeostasis, in normal human physiology and discusses the potential implications of these findings in eating disorders associated with energy deprivation, i.e., anorexia nervosa, bulimia nervosa, and binge eating disorders.

Leptin

Leptin is a 167-amino acid protein that is structurally related to members of the cytokine family (5,6). Leptin was discovered through positional cloning of the ob-gene (7) and is expressed primarily in white adipose tissue (8–10), but also in the stomach (11), hypothalamus (12), pituitary (13), skeletal muscle (14), placenta (15), and mammary gland (16,17). Accumulating evidence suggests that leptin is not mainly an "anti-obesity hormone," as proposed originally, but is primarily a crucial endocrine factor playing an important role in the regulation of several physiological processes activated in states of food deprivation. Leptin is mainly secreted by the white adipose tissue in direct proportion to the amount of energy stored in fat and acts by binding to specific leptin receptors. Leptin's actions in the hypothalamus and a variety of peripheral organs are mediated by the long isoform of the leptin receptor [OB-R (18,19)]. Several isoforms of this receptor, resulting from alternative splicing, are expressed in hypothalamic nuclei (20,21), the ovary, prostate, and testis, suggesting

[1] Presented at the 6th Postgraduate Course on Nutrition entitled "Nutrition and Gene Regulation" Symposium at Harvard Medical School, Boston, MA, March 13–14, 2003. This symposium was supported by Conrad Taff Nutrition Educational Fund, ConAgra Foods, GlaxoSmithKline Consumer Healthcare, McNeil Nutritionals, Nestle Nutrition Institute, The Peanut Institute, Procter & Gamble Company Nutrition Science Institute, Ross Products Division–Abbott Laboratories, and Slim Fast Foods Company. The proceedings of this symposium are published as a supplement to *The Journal of Nutrition*. Guest editors for the supplement publication were: W. Allan Walker, Harvard Medical School, George Blackburn, Harvard Medical School, Edward Giovanucci, Harvard School of Public Health, Boston, MA, and Ian Sanderson, University of London, London, UK.

[2] Current address: Children's Hospital, University of Leipzig, Germany.

[3] To whom correspondence should be addressed.
E-mail: cmantzor@bidmc.harvard.edu.

direct effects of leptin not only in energy homeostasis, but also in other physiological processes, including reproductive function (22–24).

Leptin levels in eating disorders

Anorexia nervosa. Anorexia nervosa is associated with a significant and occasionally dramatic decrease in food intake resulting in progressive weight loss. This disease state has been defined as a complex syndrome characterized by the diagnostic criteria of a) body weight more than 15% lower than that expected for age and height, b) anxiety and fear of obesity, c) disturbed attitudes towards body weight and shape, and d) hypothalamic amenorrhea for >3 mo in postpubertal females (25). The latter is closely associated with a decrease of body weight to levels below the threshold of about 70% of the mean weight of a matched population. Interestingly, in response to this degree of weight loss, multiple endocrine axes apart from the reproductive axis are also disrupted (26).

Serum leptin concentrations are significantly reduced in the acute stage of anorexia nervosa compared to healthy, normal weight, age-matched controls (27–32), but significant positive correlations between leptin and body weight or percentage of body fat have also been described by several groups (1,28,30,31). Significantly decreased leptin levels have also been described in cerebrospinal fluid (CSF)[4] of female patients with anorexia nervosa (30) as well as in the serum of anorectic boys (33). During treatment of anorexia nervosa and recovery of body weight, serum leptin concentrations increase at a rapid rate and eventually reach higher values at a faster rate than age- and weight-matched controls (29). Thus, it appears that the lowered body fat mass is the major determinant of decreased serum leptin concentrations in anorexia nervosa (1) and that decreased serum leptin levels are transferred more efficiently into the brain and CSF. In addition to the efficiency of the leptin transporters expressed in the Blood Brain Barrier (BBB), it appears that the ratio of free to bound leptin in the circulation also plays an important role. Leptin exists in human circulation in a free form and bound to a soluble leptin receptor (sOB-R), which represents the main leptin binding activity in human serum (34) and which is the major determinant of free leptin index, the presumed biologically active form of leptin (35). Significantly higher proportions of total leptin circulate bound to a binding-protein in anorectic or lean compared to obese subjects (36), suggesting a pivotal role for leptin-binding proteins in the regulation of energy homeostasis and eating behavior. Moreover, a recent study revealed that weight gain in patients with anorexia nervosa results not only in a significant increase in serum leptin concentrations, but also in a significant reduction of sOB-R values, suggesting that elevated sOB-R levels during states of starvation might reflect a compensatory mechanism to further suppress leptin levels and action during negative energy balance (37).

Bulimia nervosa. Bulimia nervosa (BN) is characterized by episodes of binge eating, but, in contrast to anorexia nervosa, the body weight tends to remain stable over time. Serum leptin concentrations have been reported to correlate significantly and positively with BMI in bulimia nervosa (1). Similar to patients with anorexia nervosa, patients with BN have significantly lower mean serum leptin levels compared to weight- and age-matched healthy subjects (1,31,38–40); however, in general, the circulating leptin concentrations in BN are not as low as in patients with anorexia nervosa (39), and menstrual irregularities tend to occur less frequently in patients with BN than in patients with anorexia nervosa (26). Interestingly, the secretion of leptin is related to the chronic nature and severity of illness in patients with bulimia nervosa (40).

Binge-eating disorders. The group of binge-eating disorders (BED) is characterized by episodes of binge-overeating and eating-related psychopathological symptoms. In contrast to bulimia nervosa, there is no associated compensatory behavior (1). Up to 90% of obese patients present with symptoms of binge eating (41,42), in contrast to <5% of nonobese subjects (41,43).

Data about the role of leptin in these disorders is very limited, but it appears that serum leptin concentrations are significantly elevated in women with BED compared to healthy, age-matched controls (1), indicating some degree of leptin resistance. However, others reported that leptin levels are similar in obese binge- and nonbinge-eating women (44,45), and that serum leptin concentrations are positively correlated with BMI (44). Thus, the role of leptin in this group of eating disorders needs to be studied more carefully in the future. Moreover, it is important to note that mutations of the melanocortin-4 receptor, a molecule downstream of leptin, have been found in about 5% of severely obese patients with binge-eating disorders, suggesting that binge eating disorders could prove to be one of the phenotypic characteristics of subjects with a mutation in this gene (46). Mutations in genes downstream of leptin could also explain the higher leptin levels in the context of leptin resistance in these subjects.

Leptin and the regulation of neuroendocrine axes in normal subjects and subjects with eating disorders

Hypothalamic-pituitary-gonadal axis. The long isoform of the leptin receptor, OB-Rb, is highly expressed in the arcuate and ventromedial hypothalamic nuclei, areas important for the regulation of both food intake and sexual behavior (19). Moreover, it has been demonstrated that leptin receptors are expressed specifically on GnRH-secreting neurons, and that leptin accelerates GnRH-pulsatility (but not pulse amplitude) in arcuate hypothalamic neurons regulating the release of gonadotropins (47–49). Thus, it has been suggested that leptin serves as a signal to convey information to the brain about the body's fat stores and metabolic resources and acts as a permissive signal to activate the reproductive axis (24). Leptin may also facilitate GnRH secretion via indirect mechanisms, acting through altering the secretion of neuropeptides (50) and/or by releasing nitric oxide (NO) from adrenergic interneurons (47,51). It has also been suggested that leptin may exert direct effects on the pituitary level, as both leptin and the leptin receptor are expressed in pituitary cells (13,52). Finally, functional leptin receptors are also expressed in ovarian follicular cells as well as in Leydig cells (22,53,54). Moreover, leptin-mRNA is also expressed in granulosa and cumulus cells of preovulatory human follicles (55), suggesting endocrine and/or direct paracrine effects of leptin on the gonads. Based on these data, it has been proposed that leptin exerts a bimodal action on the hypothalamic-pituitary-gonadal axis depending on serum leptin levels.

Circulating leptin levels display a pulsatile and circadian rhythm with a peak in the early morning and a nadir in the afternoon. These characteristics are similar in lean and obese subjects with the only exception being pulse amplitude, which is higher in obese subjects (56). Interestingly, the pattern of

[4] Abbreviations used: BED, binge eating disorders; BN, bulimia nervosa; CSF, cerebrospinal fluid; sOB-R, soluble leptin receptor; TSH, thyrotropin.

leptin secretion is similar to that of several other hormones which are also characterized by diurnal and circadian oscillations (**Fig. 1**). The pattern of thyrotropin (TSH) secretion, for example, is similar and appears to be synchronous to that of leptin (57). Additionally, the circadian rhythm of leptin is similar to those of prolactin, free fatty acids, and melatonin (58), but inversely related to those of ACTH and cortisol (59). Even more importantly, leptin pulsatility is synchronous to the pulsatility of serum LH and estradiol levels in normal women, especially during the night when leptin levels are relatively high, suggesting that leptin may play an important role in the regulation of physiologic levels and rhythmicity of reproductive hormones (60,61).

Hypotheses raised by these in vitro studies as well as observational studies in humans have been further corroborated by studies in mice which revealed that the delay in estrus associated with food deprivation in female mice can be reversed by leptin treatment, and that leptin rescues the starvation-induced decrease in circulating testosterone and LH concentrations in male mice (62). Moreover, the timing of puberty is accelerated in normal female mice by leptin treatment (63,64). Accordingly, delayed sexual maturation of food-restricted female rats can be accelerated by central leptin administration, despite still decreased body weight (65). Finally, sterility in obese female mice with defective leptin production has been shown to be corrected by leptin treatment (66), which also corrects the hypogonadotropic hypogonadism of mice with congenital leptin deficiency (*ob*/*ob* mice) (67). Importantly, the study of a few cases of human congenital leptin deficiency or leptin receptor mutations has demonstrated that in both conditions adults fail to undergo puberty due to a suppressed hypothalamic-pituitary-gonadal axis (68–72). However, when leptin deficient children were treated with leptin, they subsequently underwent puberty (71). Similar effects were observed in female patients with anorexia nervosa who gained weight as a result of dietary treatment—the rise in serum leptin levels was accompanied by a rise in serum LH and FSH levels (32, 73). In summary, these observations suggest that a rise in circulating leptin levels may activate the hypothalamic-pituitary-gonadal axis. Other observational studies have shown that increasing serum leptin levels during weight gain in treated patients with anorexia nervosa are significantly and positively correlated with changes in LH, FSH, testosterone, and free androgens (32,33). These data suggest that circulating leptin concentrations need to exceed a threshold for preservation of reproductive function (74) and it appears that normalization of leptin levels is an important factor required for the resumption of reproductive function in patients with anorexia nervosa.

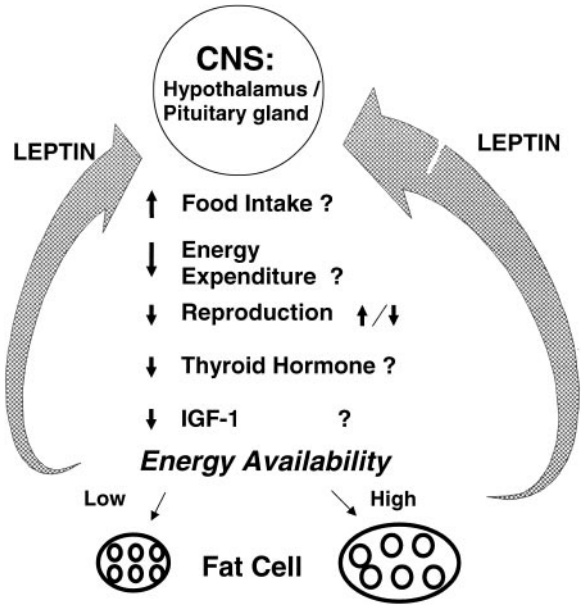

FIGURE 1 Regulation of neuroendocrine function by leptin: Undernutrition or overnutrition alter leptin secretion and serum levels, which in turn result in alterations of neuroendocrine and immune function mainly in low energy availability/low leptin states.

Direct evidence in support of this hypothesis raised from observational studies in humans comes from an interventional trial recently completed by our group. We demonstrated that leptin, given in replacement doses, fully restores the starvation induced changes of LH pulsatility and testosterone levels, thus providing conclusive evidence regarding leptin's significant role in regulating neuroendocrine processes, including reproductive function, in normal humans (75). This implies that reproductive dysfunction in states of negative energy balance, such as anorexia nervosa and the state of strenuously exercising women athletes, might be mediated by suppressed circulating leptin concentrations, and thus, reproductive function might be normalized by leptin replacement therapy. Interventional studies that have recently been completed at our institution have provided further evidence regarding the key role of leptin in the pathophysiology and hopefully treatment of reproductive dysfunction in these disorders.

Hypothalamic-pituitary-thyroid axis. It is well established that thyroid hormones are also subject to significant physiologic regulation during transition from the fed to the starving state (76). Studies in rodents have revealed that starvation rapidly suppresses both T4 and T3 concentrations (62,77), leading to central hypothyroidism; in humans the decrease of T3 levels is accompanied by the concomitant increase of reverse T3. There is currently a significant body of evidence supporting the notion that some of these changes are due to decreasing leptin levels in response to starvation (76). Exogenous leptin administration normalizes thyroid hormone concentrations in starving rodents (62). Congenital leptin deficiency is associated with impaired regulation of the hypothalamic-pituitary-thyroid axis in rodents (78) and humans (57). Mutations in the leptin receptor gene, leading to a state of leptin resistance, are also associated with hypothyroidism in humans (70), and observational studies have previously shown that leptin's circadian rhythm is associated with TSH pulsatility and circadian rhythm in both healthy men and in leptin-deficient subjects (57).

Intrigued by these experiments in rodents and observational studies in humans, we have recently completed interventional studies involving leptin administration to humans to obtain direct evidence for a role of leptin in regulating the thyroid axis in men. We showed that leptin given in replacement doses to food-deprived healthy volunteers restores TSH pulsatility-changes induced by food deprivation, increases free T4 levels, and does not significantly affect changes in T3 and rT3 levels (75). Similar data have recently been obtained by other groups studying either obese subjects dieting to achieve weight loss or rare cases of human congenital leptin deficiency (79,80).

Anorexia nervosa seems to be associated with a dysfunction of the hypothalamic-pituitary-thyroid axis (81). Mean serum levels not only of leptin, but also of TSH and thyroid hormones, are significantly altered in patients with anorexia nervosa and tend to return to levels seen in control subjects after weight recovery (81). Although as of today these observa-

tional data have not yet been supplemented by interventional studies on the regulation of the thyroid axis by leptin in eating disorders, it is expected that the role of leptin in patients with eating disorders will soon be elucidated. To this end, supportive evidence is provided by the first proof-of-concept studies recently completed at our institution.

Hypothalamic-pituitary-adrenal axis. It has also been proposed that serum cortisol concentrations are elevated in states of starvation, including anorexia nervosa (32,82–86). Elevated cortisol concentrations in the circulation have been suggested to be due not only to increased secretion of cortisol (84), but also to its increased half-life as a result of decreased metabolism (87). However, anorectic patients, despite hypercortisolism, never present with classic features of Cushing's syndrome (88), suggesting a state of "cortisol resistance" (83,89), which is probably only partial because normal sensitivity to glucocorticoid administration was observed in female patients with anorexia nervosa (82).

Although animal studies have revealed that *ob/ob* mice with congenital leptin deficiency have high circulating glucocorticoid levels (90), humans with either leptin deficiency or leptin receptor mutations have glucocorticoid concentrations within the normal range and do not present with growth retardation (69–72). In addition, leptin given in replacement doses to food-deprived healthy volunteers does not alter the starvation-induced changes in cortisol secretion and the renin-aldosterone-system (75). Thus, although direct experimental evidence provides no support for a role of leptin in regulating the hypothalamic-pituitary-adrenal axis in healthy normal subjects, interventional studies involving patients with anorexia nervosa are needed to fully elucidate the role of leptin in regulating the hypothalamic-pituitary-adrenal axis in this disorder.

Conclusions and future directions

Leptin is a hormone communicating information on the body's fat stores/energy reserves to the brain, thus maintaining normal function of several neuroendocrine axes. Several conditions including eating disorders such as anorexia nervosa and bulimia nervosa are associated with altered serum levels of leptin as well as abnormalities in neuroendocrine functions. Although most of the data available so far have been derived from animal studies or observational studies in humans, recently completed interventional studies performed in our institution have provided important insights towards the role of leptin acting as a "master hormone" in regulating neuroendocrine function in normal healthy volunteers and strenuously exercising women athletes. These studies have already provided the basis for a better understanding of the mechanisms underlying the hormonal abnormalities of subjects with eating disorders and may lead to the development of new therapeutic strategies for these conditions.

LITERATURE CITED

1. Monteleone, P., Di Lieto, A., Tortorella, A., Longobardi, N. & Maj, M. (2000) Circulating leptin in patients with anorexia nervosa, bulimia nervosa or binge-eating disorder: relationship to body weight, eating patterns, psychopathology and endocrine changes. Psychiat. Res. 94: 121–129.

2. Blüher, S. & Mantzoros, C. S. (2003) Leptin and human pubertal development. In: Leptin and Reproduction, 1st ed. (Henson, M. C. & Castracane, D., eds.). Kluwer Academic/Plenum Publishers, New York, NY. (in press).

3. Moschos, S., Chan, J. L. & Mantzoros, C. S. (2002) Leptin and reproduction: A review. Fertil. Steril. 77 (3): 433–444.

4. Hinney, A., Remschmidt, H. & Hebebrand, J. (2000) Candidate gene polymorphism in eating disorders. Eur. J. Pharmacol. 410: 147–159.

5. Madej, T., Boguski, M. S. & Bryant, S. H. (1995) Threading analysis suggests that the obese gene product may be a helical cytokine. FEBS Lett. 373: 13–18.

6. Zhang, F., Basinski, M. B., Beals, J. M., Briggs, S. L., Churgay, L. M., Clawson, D. K., DiMarchi, R. D., Furman, T. C., Hale, J. E., et al. (1997) Crystal structure of the obese protein leptin- E100. Nature 387: 206–209.

7. Zhang, Y., Proenca, M., Maffei, M., Barone, M., Leopold, L. & Friedman, J. M. (1994) Positional cloning of the obese gene and its human homologue. Nature 372: 425–432.

8. Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., et al. (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.

9. 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.

10. Leroy, P., Dessolin, S., Villageois, P., Moon, B. C., Friedman, J. M., Ailhaud, G. & Dani, C. (1996) Expression of ob gene in adipose cells. Regulation by insulin. J. Biol. Chem. 271: 2365–2368.

11. Bado, A., Levasseur, S., Attoub, S., Kermorgant, S., Laigneau, J. P., Bortoluzzi, M. N., Moizo, L., Lehy, T., Guerre-Millo, M., et al. (1998) The stomach is a source of leptin. Nature 394: 790–793.

12. Morash, B., Li, A., Murphy, P. R., Wilkinson, M. & Ur, E. (1999) Leptin gene expression in the brain and pituitary gland. Endocrinology 140: 5995–5998.

13. Jin, L., Zhang, S., Burguera, B. G., Couce, M. E., Osamura, R. Y., Kulig, E. & Lloyd, R. V. (2000) Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology 141: 333–339.

14. Wang, J., Liu, R., Hawkins, M., Barzilai, N. & Rossetti, L. (1998) A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393: 684–688.

15. Masuzaki, H., Ogawa, Y., Sagawa, N., Hosoda, K., Matsumoto, T., Mise, H., Nishimura, H., Yoshimasa, Y., Tanaka, I., et al. (1997) Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat. Med. 3: 1029–1033.

16. Smith-Kirwin, S. M., O'Connor, D. M., De Johnston, J., Lancey, E. D., Hassink, S. G. & Funanage, V. L. (1998) Leptin expression in human mammary epithelial cells and breast milk. J. Clin. Endocrinol. Metab. 83: 1810–1813.

17. Aoki, N., Kawamura, M. & Matsuda, T. (1999) Lactation-dependent down regulation of leptin production in mouse mammary gland. Biochim. Biophys. Acta 1427: 298–306.

18. Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J. & Devos, R. (1995) Identification and cloning of a leptin receptor, OB-R. Cell 83: 1263–1271.

19. Tartaglia, L. A. (1997) The leptin receptor. J. Biol. Chem. 272: 6093–6096.

20. Heretier, A., Charnay, Y. & Aubert, M. L. (1997) Regional distribution of mRNA encoding the long form of receptor in mouse brain. Neurosci. Res. Commun. 21: 113–118.

21. Huang, X. F., Lin, S. & Zhang, R. (1997) Upregulation of leptin receptor mRNA expression in obese mouse brain. Neuroreport 8: 1035–1038.

22. Cioffi, J. A., Shafer, A. W., Zupanicic, T. J., Smih-Gbur, J., Mikhail, A., Platika, D. & Snodgrass, H. R. (1996) Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nat. Med. 2: 585–589.

23. Mantzoros, C. S. (1999) The role of leptin in human obesity and disease: A review of current evidence. Ann. Intern. Med. 130: 671–680.

24. Kiess, W., Müller, G., Galler, A., Reich, A., Deutscher, J., Klammt, J. & Kratzsch, J. (2000) Body fat mass, leptin and puberty. J. Pediatr. Endocrinol. Metab. 13: 717–722.

25. American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders DSM-IV. American Psychiatric Press, Washington, DC.

26. Cantopher, T., Evans, C., Lacey, J. H. & Pearce, J. M. (1988) Menstrual and ovulatory disturbance in bulimia. BMJ 297: 836–837.

27. Hebebrand, J., van der Heyden, J., Devos, R., Kopp, W., Herpertz, S., Remschmidt, H. & Herzog, W. (1995) Plasma concentrations of obese protein in anorexia nervosa [letter]. Lancet 346: 1624–1625.

28. Hebebrand, J., Blum, W. F., Barth, N., Coners, H., Englaro, P., Juul, A., Ziegler, A., Warnke, A., Rascher, W. & Remschmidt, H. (1997) Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration. Mol. Psychiatry 2: 330–334.

29. Grinspoon, S., Gulick, T., Askari, H., Landt, M., Lee, K., Anderson, E., Ma, Z., Vignati, L., Bowsher R., et al. (1996) Serum leptin levels in women with anorexia nervosa. J. Clin. Endocrinol. Metab. 81: 3861–3863.

30. Mantzoros, C., Flier, J. S., Lesem, M. D., Brewerton, T. D. & Jimerson, D. C. (1997) Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J. Clin. Endocrinol. Metab. 82: 1845–1851.

31. Kopp, W., Blum, W.F., Ziegler, A., Mathiak, K., Lubbert, H., Herpertz, S., Deter, H.C. & Hebebrand, J. (1998) Serum leptin and body weight in females with anorexia and bulimia nervosa. Horm. Metab. Res. 30: 272–275.

32. Audi, L., Mantzoros, C. S., Vidal-Puig, A., Vargas, D., Gussinye, M. & Carrascosa, A (1998) Leptin in relation to resumption of menses in women with anorexia nervosa. Mol. Psychiatry 3: 544–547.

33. Wabitsch, M., Ballauff, A., Holl, R., Blum, W. F., Heinze, E., Remschidt, H. & Hebebrand, J. (2001) Serum leptin, gonadotropin, and testosterone con-

centrations in male patients with anorexia nervosa during weight gain. J. Clin. Endocrinol. Metab. 86: 2982–2988.

34. Lammert, A., Kiess, W., Böttner, A., Glasow, A. & Kratzsch, J. (2001) Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem. Biophys. Res. Comm. 283: 982–988.

35. Chan, J. L., Blüher, S., Yiannakouris, N., Suchard, M. A., Kratzsch, J. & Mantzoros, C. S. (2002) Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes 51: 2105–2112.

36. Sinha, M. K., Opentanova, I., Ohannesian, J. P., Kolaczynski, J. W., Heiman, M. L., Hale, J., Becker, G. W., Bowsher, R. R., Stephens, T. W. & Caro, J. F. (1996) Evidence of free and bound leptin in human circulation: studies in lean and obese subjects and during short-term fasting. J. Clin. Invest. 98: 1277–1282.

37. Kratzsch, J., Lammert, A., Böttner, A., Seidel, B., Mueller, G., Thiery, J., Hebebrand, J. & Kiess, W. (2002) Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence. J. Clin. Endocrinol. Metab. 87: 4587–4594.

38. Mathiak, K., Gowin, W., Hebebrand, J., Ziegler, A., Blum, W. F., Felsenberg, D., Lubbert, H. & Kropp, W. (1999) Serum leptin levels, body fat deposition, and weight in females with anorexia and bulimia nervosa. Horm. Metab. Res. 31: 274–277.

39. Jimerson, D. C., Mantzoros, C., Wolfe, B. E. & Metzger, E. D. (2000) Decreased serum leptin in bulimia nervosa. J. Clin. Endocrinol. Metabol. 85: 4511–4514.

40. Monteleone, P., Martiadis, V., Colurcio, B. & Maj, M. (2002) Leptin secretion is related to chronicity and severity of the illness in Bulimia Nervosa. Psychosom. Med. 64: 874–879.

41. Spitzer, R. L., Devlin, M. & Walsh, B. T. (1992) Binge eating disorder: a multisite field trial of the diagnostic criteria. Int. J. Eat. Disord. 11: 191–203.

42. Hsu, L.K.G., Mulliken B., McDonagh, B., Krupa, Das S., Rand., W., Fairburn, C. G., Rolls, B., McCrory, M. A., Saltzman, et al. (2002) Binge eating disorder in extreme obesity. Int. J. Obes. Relat. Metab. Disord. 26: 1398–1403.

43. Spitzer, R. L., Yanovski, S., Wadden, T., Wing, R., Marcus, M. D., Stunkard, A., Devlin, M., Mitchell, J., Hasin, D. & Horne, R. L. (1993) Binge eating disorder: its further validation in a multisite study. Int. J. Eat. Disord. 13: 137–153.

44. Adami, G., Campostano, A., Cella, F. & Ferrandes, G. (2002) Serum leptin level and restrained eating—Study with Eating Disorder Examination. Physiol. Behav. 75: 189–192.

45. Karhunen, L., Haffner, S., Lappalainen, R., Turpeinen, A., Miettinen, H. & Uusitupa, M. (1997) Serum leptin and short-term regulation of eating in obese women. Clin. Sciences 92: 573–578.

46. Branson, R., Potoczna, N., Kral, J. G., Lentes, K. U., Hoehe, M. R. & Horber, F. F. (2003) : Binge eating as a major phenotype of melanocortin 4 receptor gene mutations. N. Engl. J. Med. 348: 1096–1103.

47. Yu, W. H., Kimura, M., Walczewska, A., Karanth, S. & McCann, S. M. (1997) Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci. U.S.A. 94: 1023–1028.

48. Cunningham, M. J., Clifton, D. K. & Steiner, R. A. (1999) Leptin's actions on the reproductive axis: Perspectives and mechanisms. Biol. Reprod. 60: 216–222.

49. Lebrethon, M. C., Vandersmissen, E., Gerard, A., Parent, A. S., Junien, J. L. & Bourguignon, J. P. (2000) In vitro stimulation of the pre-pubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. Endocrinology 141: 1464–1469.

50. Terasawa, E. (1998) Cellular mechanism of pulsatile LHRH release. Gen. Comp. Endocrinol. 112: 283–295.

51. Yu, W. H., Walczewska, A., Karanth, S. & McCann, S. M. (1997) Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. Endocrinology 138: 5055–5058.

52. Iqbal, J., Pompolo, S., Considine, R. V. & Clarke, I. J. (2000) Localization of leptin receptor-like immunoreactivity in the corticotropes, somatotropes, and gonadotropes in the ovine anterior pituitary. Endocrinology 141: 1515–1520.

53. Karlsson, C., Lindell, K., Svensson, E., Bergh, C., Lind, P., Billig, H., Carlsson, L. M. & Carlsson, B. (1997) Expression of functional leptin receptors in the human ovary. J. Clin. Endocrinol. Metab. 82: 4144–4148.

54. Caprio, M., Isidori, A. M., Carta, A. R., Moretti, C., Dufau, M. L. & Fabbri, A. (1999) Expression of functional leptin receptors in rodent Leydig cells. Endocrinology 140: 4939–4947.

55. Cioffi, J. A., Van Blerkom, J., Antczak, M., Shafer, A., Wittmer, S. & Snodgrass, H. R. (1997) The expression of leptin and its receptors in pre-ovulatory human follicles. Mol. Hum. Reprod. 3: 467–472.

56. 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.

57. Mantzoros, C. S., Ozata, M., Negrao, A. B., Suchard, M. A., Ziotopoulou, M., Caglayan, S., Elashoff, R. N., Cogswell, R. J., Negro, P., et al. (2001) Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: Evidence for possible partial TSH regulation by leptin in humans. J. Clin. Endocrinol. Metab. 86: 3284–3291.

58. Matkovic, V., Ilich, J. Z., Badenhop, N. E., Skugor, M., Clairmont, A., Klisovic, D. & Landoll, J. D. (1997) Gain in body fat is inversely related to the nocturnal rise in serum leptin levels in young females. J. Clin. Endocrinol. Metab. 82: 1368–1372.

59. Licinio, J., Mantzoros, C., Negrao, A. B., Cizza, G., Wong, M. L., Bongiorno, P. B., Chrousos, G. P., Karp, B., Allen, C., et al. (1997) Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat. Med. 3: 575–579.

60. Licinio, J., Negrao, A. B., Mantzoros, C., Kaklamani, V., Wong, M. L., Bongiorno, P. B., Mulla, A., Cearnal, L., Veldhuis, J. D., et al. (1998) Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc. Natl. Acad. Sci. U.S.A. 95: 2541–2546.

61. Licinio, J., Negrao, A. B., Mantzoros, C., Kaklamani, V., Wong, M. L., Bongiorno, P. B., Negro, P. P., Mulla, A., Veldhuis, J. D., et al. (1998) Sex differences in circulating human leptin pulse amplitude: Clinical implications. J. Clin. Endocrinol. Metab. 83: 4140–4147.

62. Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E. & Flier, J. S. (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382: 250–252.

63. Chebab, F. F., Mounzih, K., Lu, R. & Lim, M. E. (1996) Early onset of reproductive function in normal mice treated with leptin. Science 275: 88–90.

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

65. Gruaz, N. M., Lalaoui, M., Pierroz, D. D., Englaro, P., Sizonenko, P. C., Blum, W. F. & Aubert, M. L. (1998) Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food-restricted female rats. J. Neuroendocrinol. 10: 627–633.

66. Chebab, F., Lim, M. & Lu, R. (1996) Correction of the sterility defect in homozygous obese female mice by treatment with human recombinant leptin. Nat. Genet. 12: 318–320.

67. Swerdloff, R. S., Batt, R. A. & Bray, G. A. (1976) Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology 98: 1359–1364.

68. Strobel, A., Issad, T., Camoin, L., Ozata, M. & Strosberg, A. D. (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. Nat. Genet. 18: 213–215.

69. Ozata, M., Ozdemir, I. C. & Licinio, J. (1999) Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J. Clin. Endocrinol. Metab. 84: 3686–3695.

70. Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., et al. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392: 398–401.

71. Farooqi, I. S., Jebb, S. A., Langmack, G., Lawrence, E., Cheetham, C. H., Prentice, A. M., Hughes, I. A., McCamish, M. A. & O'Rahilly, S. (1999) Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N. Engl. J. Med. 341: 879–884.

72. 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., et al. (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 26/387: 903–908.

73. Ballauff, A., Ziegler, A., Emons, G., Sturm, G., Blum, W. F., Remschidt, H. & Hebebrand, J. (1999) Serum leptin and gonadotropin levels in patients with anorexia nervosa during weight gain. Mol. Psychiatry 4: 71–75.

74. Di Carlo, T., Tommaselli, G. A., De Filippo, E., Pisano, G., Nasti, A., Bifulco, G., Contaldo, F. & Nappi, C. (2002) Menstrual status and serum leptin levels in anorectic and menstruating women with low body mass index. Fertil. Steril. 78: 376–382.

75. Chan, J., Heist, K., DePaoli, A. M., Veldhuis, J. D. & Mantzoros, C. S. (2003) The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J. Clin. Invest. 111: 1409–1421.

76. Flier, J. S., Harris, M. & Hollenberg, A. N. (2000) Leptin, nutrition, and the thyroid; the why, the wherefore, and the wiring. J. Clin. Invest. 105: 859–661.

77. Blake, N. G., Eckland, D. J., Foster, O. J. & Lightman, S. L. (1991) Inhibition of hypothalamic thyrotropin-releasing hormone messenger ribonucleic acid during food deprivation. Endocrinology 129: 2714–2718.

78. Othake, M., Bray, G. A. & Azukizawa, M. (1977) Thermogenic defect in pre-obese ob/ob mice. Nature 266: 60–62.

79. Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., et al. (2002) Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest. 110: 1093–103.

80. Rosenbaum, M., Murphy, E. M., Heymsfield, S. B., Matthews, D. E. & Leibel, R. L. (2002) Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. J. Clin. Endocrinol. Metab. 87: 2391–2394.

81. Nedvidkova, J., Papezova, H., Haluzik, M. & Schreiber, V. (2000) Interaction between serum leptin levels and hypothalamo-hypophyseal-thyroid axis in patients with anorexia nervosa. Endocr. Res. 26: 219–230.

82. Gordon, C. M., Emans, S. J., DuRant, R. H., Mantzoros, C., Grace, E., Harper, G. P. & Majzoub, J. A. (2000) Endocrinologic and psychological effects

of short-term dexamethasone in anorexia nervosa. Eat Weight Disord. 5: 175–182.

83. Walsh, B. T., Roose, S. P., Katz, J. L., Dyrenfurth, I., Wright, L., Vande Wiele, R. & Glassman, A. H. (1987) Hypothalamic pituitary-adrenal-cortical activity in anorexia nervosa. Am. J. Obstet. Gynecol. 117: 435–449.

84. Baranowska, B. (1990) Are disturbances in opioid and adrenergic systems involved in the hormonal dysfunction of anorexia nervosa? Psychoneuroendocrinology 15: 371–379.

85. Herpertz, S., Wagner, R., Albers, N., Blum, W. F., Pelz, B., Langkafel, M., Kopp, W., Henning, A., Oberste-Berghaus, C., et al. (1998) Circadian plasma leptin levels in patients with anorexia nervosa: relation to insulin and cortisol. Horm. Res. 50: 197–204.

86. Herpertz, S., Albers, N., Wagner, R., Pelz, B., Kopp, W., Mann, K., Blum, W. F., Senf, W. & Hebebrand, J. (2000) Longitudinal changes of circadian leptin, insulin and cortisol plasma levels and their correlation during refeeding in patients with anorexia nervosa. Eur. J. Endocrinol. 142: 373–379.

87. Boyar, R. M., Hellman, L. D., Roffwarg, H., Katz, J., Zumoff, B., O'Connor, J., Bradlow, H. L. & Fukushima, D. K. (1977) Cortisol secretion and metabolism in anorexia nervosa. N. Engl. J. Med. 296: 190–193.

88. Støving, R. K., Hangaard, J., Hansen-Nord, M., and Hagen, C. (1999) A review of endocrine changes in anorexia nervosa. J. Psychiatr. Res. 33: 139–152.

89. Hotta, M., Shibasaki, T., Masuda, A., Imaki, T., Demura, H., Ling, N. & Shizume, K. (1996) The response of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone CRH; and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. J. Clin. Endocrinol. Metab. 62: 319–324.

90. Dubuc, P. U. (1977) Basal corticosterone levels of young ob/ob mice. Horm. Metab. Res. 9: 95–97.

91. Howard, J. K., Lord, G. M., Matarese, G., Vendetti, S., Ghatei, M. A., Ritter, M. A., Lechler, R. I. & Bloom, S. R. (1999) Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. J. Clin. Invest. 104: 1051–1059.

92. Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R. & Lechler, R. I. (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394: 897–901.

93. Martin-Romero, C., Santos-Alvarez, J., Goberna, R. & Sanchez-Margalet, V. (2000) Human leptin enhances activation and proliferation of human circulating T lymphocytes. Cell Immunol. 199: 15–24.