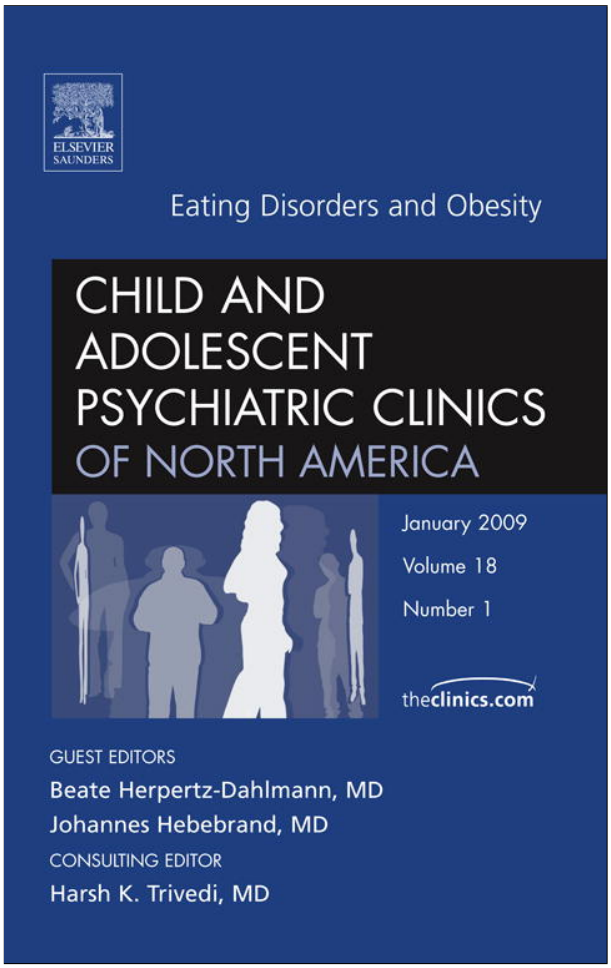

Leptin-Mediated Neuroendocrine Alterations in Anorexia Nervosa: Somatic and Behavioral Implications

Timo D. Müller[a], Manuel Föcker, MD[a], Kristian Holtkamp, MD[b], Beate Herpertz-Dahlmann, MD[b], Johannes Hebebrand, MD[a,*]

KEYWORDS

• Hyperactivity • Semi-starvation • Osteoporosis • Amenorrhea
• Hypoleptinemia • Hyperleptinema • Weight loss

In 1953, Kennedy[1] postulated that circulating signals from the periphery that change in relation to body fat stores inform the brain about changes in body fat mass and that the brain in response to these signals adjusts food intake and energy balance to achieve body weight maintenance. The first peripheral hormone implicated in the regulation of food intake was the pancreatic hormone insulin.[2] Positional cloning of the mouse *obese (ob)* gene in 1994 led to the discovery of leptin, a 16-kDa protein that is mainly secreted by white adipocytes and that, in relation to body fat mass, enters the brain through the bloodstream to activate central neuronal circuits to reduce food intake and to increase energy expenditure.[3]

Leptin is secreted into the bloodstream in a pulsatile manner and shows a diurnal variation in normal and overweight individuals, with an increase of about 50% during the night.[4] In anorectic individuals, however, this diurnal variation of leptin secretion is strikingly reduced.[5,6] Plasma levels of leptin further correlate with the amount of body fat.[7] Individuals with a low body fat mass (eg, patients with anorexia nervosa [AN]) have consistently been reported to show low to undetectable plasma levels of leptin.[8] Additionally, even after correction for body mass index (BMI) or body fat mass, plasma levels of leptin have repeatedly been shown to be higher in females than in males.[7,9,10]

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[a] Department of Child and Adolescent Psychiatry and Psychotherapy, University of Duisburg-Essen, Virchowstrasse 174, 45147 Essen, Germany
[b] Department of Child and Adolescent Psychiatry and Psychotherapy, University Clinics, RWTH Aachen, Neuenhofer Weg 21, 52074 Aachen, Germany
* Corresponding author.
E-mail address: johannes.hebebrand@uni-duisburg-essen.de (J. Hebebrand).

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A multiple regression analysis with BMI and percent body fat as fixed variables further revealed that testosterone is negatively correlated with plasma levels of leptin in boys, but not in girls, thus indicating that higher androgen concentrations are at least partly responsible for the observed lower leptin concentrations in boys.[10] Plasma levels of leptin, however, decline on food deprivation and become similar in both sexes after a 36-hour fast.[11]

Since its discovery in 1994, a tremendous amount of research has focused on leptin-mediated signaling pathways, revealing that leptin is not only a key hormone implicated in the regulation of energy balance but it is also a pleiotropic hormone involved in various neuroendocrine and behavioral alterations associated with profound changes in energy stores. The observation that obese leptin-deficient *ob/ob* mice show endocrine abnormalities similar to those observed in semi-starved *wild-type* mice, together with the observation that prevention of the starvation-induced fall in leptin by exogenous leptin supplementation substantially blunts the changes in the hypothalamic–pituitary–adrenal (HPA), –gonadal (HPG), and –thyroid (HPT) axes, has led to the hypothesis that leptin is a key hormone mediating the neuroendocrine response to semi-starvation.[12] Indeed, hypoleptinemia induced by profound weight loss turned out to mediate various physiologic and endocrinologic alterations associated with reduced body weight, such as modulation of bone formation,[13–15] inflammation,[16,17] reproduction,[18–21] and the regulation of the HPA[12,22,23] and HPA–thyroidT axis.[24]

As leptin plays an important role in the adaptation of an organism to semi-starvation, AN can be considered as a model disorder to elucidate the relationship between hypoleptinemia induced by profound weight loss and the somatic, behavioral, and neuroendocrine alterations associated with semi-starvation. Moreover, patients with AN share many physiologic, endocrinologic, and behavioral features with healthy subjects who voluntarily or involuntarily lost a substantial amount of body weight, such as osteopenia/osteoporosis, amenorrhea, hyperactivity, hypothermia, bradycardia, reduced basal metabolic rate, and activation of the HPA axis.[8] However, even though AN shares many neuroendocrine and behavioral similarities with healthy subjects undergoing semi-starvation, the diagnostically relevant psychopathological features clearly distinguishing AN from weight reduced healthy subjects are the intense fear of gaining weight and the undue influence of body weight on self-evaluation.

In light of the postulated critical role of leptin in the adaptation of an organism to semi-starvation, the focus of this review is to summarize the pivotal role of leptin in the neuroendocrine response to profound weight loss, with special emphasis on AN. In particular, we focus on the effects of semi-starvation–induced hypoleptinemia on the HPA and HPG axis, bone formation, and the hyperactivity in patients with AN. Additionally, we highlight the consolidated findings obtained in the ''activity-based anorexia'' rat model, which serves as an animal model for AN.

LEPTIN LEVELS IN PATIENTS WITH AN

Hypoleptinemia is a cardinal feature of acute AN, and in most studies mean leptin levels only rarely exceed 2 $\mu g/L^{-1}$ on referral;[8] the low leptin levels are typically below those of healthy gender- and age-matched controls and reflect the low fat mass. The highest plasma leptin levels in patients with AN were reported by Grinspoon and colleagues[25] with 5.6 ± 3.7 $\mu g/L^{-1}$; the variation in mean leptin concentration might at least partly be explained by the time point of blood sampling, because weight regain leads to an increase in leptin secretion. This time point varies among the different studies from ''at a baseline visit''[26,27] or ''within the first days upon referral''[18,19,28–34] to ''at the end of a 3-week nutritional stabilization phase'' or ''after a minimum weight gain of

10%."[35] Other studies do not specify when during the course of treatment blood was sampled.[8] Leptin levels, however, can only be compared between studies when blood is collected at the same point in time, optimally within the first 1 to 3 days on referral, as leptin levels expeditiously increase on initiation of a refeeding therapy;[20,29,34,36,37] accordingly, leptin levels have increased from those observed on admission after 30 days of refeeding[36] or after a body weight gain of 5%.[37] However, several lines of evidence further indicate that the increase in leptin levels on refeeding is also dependent on the severity of hypoleptinemia; patients with exceedingly low plasma levels of leptin on admission show only slight linear increases in the initial phase of weight regain, which is typically followed by a subsequently more rapid increase.[20,29] Several studies have further shown that hyperleptinemia can ensue from the therapeutically induced weight gain in both female[20,29] and male[19] patients with AN, thus indicating that hyperleptinemia in these patients might contribute to difficulties in long-term weight restoration and body weight maintenance. However, not all studies have been able to confirm this finding,[34] possibly because of different rates of therapeutically induced weight gain or differences in the second time point at which patients' blood was sampled.

In most studies plasma levels of leptin were highly correlated with percent body fat[5,20,25,27,34,38] and, to a lesser extent, with BMI on referral;[5,20,25,27,29] only few studies failed to detect a clear correlation[39,40] including the first study to analyze leptin levels in AN patients using an ELISA.[39]

HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

Generally, activation of the HPA axis in response to acute stress conditions is mediated through an increased secretion of hypothalamic corticotropin-releasing hormone (CRH), which is transported through the portal system to the pituitary where CRH stimulates the production of adrenocorticotropic hormone (ACTH); increased secretion of glucocorticoids from the adrenal gland ensues.[41]

Hypercortisolism has consistently been reported in patients with acute AN.[5,14,31,41–47] This is partly provoked by an increased secretion of CRH in the hypothalamus and partly by an increased half-life of cortisol due to a decreased cortisol metabolism.[5,41] Serum levels of cortisol, however, decline on weight regain[48,49] and become similar to those observed in controls after weight reconstitution.[48]

In accordance with the findings in AN patients, activation of the HPA axis is increased in voluntarily fasted healthy subjects[50–52] as well as in rats[53] and mice[12,22] undergoing semi-starvation; corticosterone levels are increased. In 1996, Ahima and colleagues[12] first postulated that semi-starvation–induced hypoleptinemia mediates the activation of the HPA axis under severe conditions of reduced energy availability. The hypothesis was based on the observation that leptin supplementation blunts hypercorticosteronemia in semi-starved *wild-type* mice[12] and in obese leptin deficient *ob/ob* mice.[22,54] Additionally, the stress-induced increase in hypothalamic CRH release was inhibited by leptin supplementation in a dose-dependent manner.[22]

AMENORRHEA AND REPRODUCTIVE FUNCTION

Secondary amenorrhea defined as the absence of at least 3 consecutive menstrual cycles in postmenarchal females represents the fourth edition of Diagnostic and statistical manual of mental disorders diagnostic criterion for AN.[55] However, secondary amenorrhea is not confined to patients with AN; also healthy females with relatively low body fat mass, for example, underweight women,[30] women with anorexia athletica,[38] and elite women athletes[56] show an elevated incidence of amenorrhea and

disturbances in menstrual function. The observation that delayed puberty and menstrual irregularities are frequently observed in relatively thin girls has, long before the discovery of leptin, led to the hypothesis that a critical amount of body weight or body fat mass is required for the onset and maintenance of cyclical ovulation.[57]

In the state of semi-starvation the occurrence of amenorrhea reflects the physiologic neuroendocrine adaptation; adequate energy stores to sustain a pregnancy and to provide adequate nutrition to both the fetus and the mother are not available. Based on the observation that both rodents[58,59] and humans[60,61] with mutations in the leptin receptor gene or with leptin deficiency due to mutations in the leptin gene are amenorrheic and that infertility of obese leptin-deficient *ob*/*ob* mice can be restored by exogenous leptin supplementation,[58,59] it was postulated that initiation and maintenance of the human menstrual cycle requires endogenous plasma levels of leptin above a specific threshold value; accordingly amenorrhea ensues on attainment of subthreshold leptin levels. We have determined that this threshold value is around 2 μg/L.[30] Accordingly, plasma levels of leptin turned out to be a better predictor of amenorrhea than BMI, fat mass, or percent body fat,[30] thus indicating that the drop in leptin levels mediated by profound weight loss represents the first step of a neuroendocrine cascade that shuts off the HPG axis. Further studies confirmed that a drop in leptin levels below a critical threshold lowers the production of central gonadotropin-releasing hormone (GnRH), leading to decreased levels of the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn entails a curtailment of ovarian estrogen production.[18,30,62,63] Several studies further indicate that cocaine- and amphetamine-regulated transcript (CART) might be an important mediator of leptin's action on GnRH secretion[64,65] and that the drop in leptin levels below a critical threshold suppresses GnRH secretion through an upregulation of CART in the hypothalamus.[8,65]

In patients with AN the increase in leptin levels due to a refeeding therapy leads to increments of FSH and LH secretion when leptin levels exceed 1.2 μg/L (FSH)[20] and 1.85 μg/L (LH),[18] respectively. Accordingly, treatment of amenorrheic women with recombinant leptin increased mean LH levels and LH pulse frequency after 2 weeks and increased maximal follicular diameter, the number of dominant follicles, ovarian volume, and estradiol levels over a period of 3 months;[66] these females did not fulfill criteria for AN at the time of the study. However, in patients with acute AN, increments of leptin levels and subsequently of the gonadotropins are not able to rapidly restore regular menstruation; 85% of adolescents with AN resumed menses within 6 months after restoration of 90% of standard weight.[67] Obviously, it takes time for the reproduction axis to normalize, including the renewed growth of the ovaries.

BONE FORMATION

A persisting decrease in bone mineral density (BMD) is one of the most profound long-term consequences of prolonged AN,[8,68,69] and the association of BMD deficiency with AN has consistently been shown in both female[68–72] and male patients with AN.[73] The peak physiologic calcium accretion and bone mass accrual is between age 11 and 16 years[74–76] and this time coincides with the highest incidence for the development of AN.[77] Sufficient energy availability is crucial for bone mass development during this period, and prolonged semi-starvation at this age is an important risk factor for the development and persistence of osteopenia and osteoporosis later in life.[8] Indeed, the decrease in BMD is not immediately restored on weight regain[69,70,78–80] and 1 study even reported persisting BMD deficiency more than 10 years after the diagnosis of AN.[78] Accordingly, osteopenia and osteoporosis have consistently been

described in 50% to 92% and 13% to 38% of patients with AN, respectively.[25,81] In light of these long-term complications, it is not surprising that anorectic patients show a 7-fold increased incidence of spontaneous fractures[82] and 1 study even reported a 57% cumulative incidence of fractures at the hip, radius, and spine 40 years after the diagnosis of AN.[83]

Semi-starvation–induced hypercortisolism is implicated in the development of BMD deficiency, and thus osteopenia and osteoporosis, as high serum levels of cortisol inhibit bone formation, increase bone resorption, impair calcium absorption from the gut, and affect the secretion of several hormones implicated in bone metabolism.[13,14,84,85] Leptin, however, is indirectly involved in the frequently observed BMD deficiency in AN, because semi-starvation–induced hypoleptinemia entails hypercortisolism due to activation of the HPA axis. Several lines of evidence further indicate that leptin also directly influences bone metabolism; leptin is known to stimulate the expression and secretion of central CART,[86] which inhibits bone loss through inhibition of bone resorption.[87] Thus, semi-starvation–induced hypoleptinemia in combination with an autonomic dysfunction might at least partly explain the consistently observed impairment of bone metabolism in patients with AN.[8]

HYPERACTIVITY IN PATIENTS WITH AN AND SEMI-STARVED RATS

Elevated levels of physical activity are commonly observed in patients with AN[8,28,33,88–93] and some investigators view this phenomenon as a core clinical symptom of AN.[8,88,91,94] In light of the lack of a consistent operational definition of ''hyperactivity,'' excessive exercise is described in 31% to 80% of patients with AN.[91] In some studies, patients with AN were reported to have an addictive, obsessive-compulsive drive to exercise.[90,91,95] The hypothesis that physiologic mechanisms due to semi-starvation underlie this phenomenon was supported by the observation that rats increase their running wheel activity up to 300% to 500% within a few days, when food is either supplied for only a limited period per day (eg, 1–2 h/d)[96–99] or when food is restricted to 60% of the ad libitum food intake.[32] In the most extreme cases, in which food was only supplied for 1 h/d, rats even ran themselves to death within approximately 21 days.[100] This phenomenon called ''activity based anorexia'' (ABA)[101] or ''semi-starvation–induced hyperactivity'' (SIH) is considered as an animal model of AN,[8,32,91,98] which shows several symptoms of AN, such as weight loss, low adiposity, hypothermia, low leptin levels, high levels of physical activity, and, in females amenorrhea.[91]

In 2000, Exner and colleagues[32] first postulated that semi-starvation–induced hypoleptinemia triggers SIH. The hypothesis was confirmed experimentally by way of subcutaneous implantation of mini-pumps containing leptin or vehicle in rats undergoing semi-starvation; vehicle treated rats increased their activity up to 300% within 7 days, whereas the activity of the leptin treated rats remained at baseline.[32] A second experiment showed that leptin normalized activity levels even after SIH had set in.[32] The suppression of SIH by exogenous application of leptin was subsequently confirmed by an independent group.[98]

The SIH triggered by hypoleptinemia in rats suggests that the same mechanism might underlie the hyperactivity in patients with AN. Indeed, Exner and colleagues[32] reported the highest levels of restlessness (assessed by self-ratings) in patients with AN when leptin levels and body weight were at their lowest. Additionally, activity levels decreased when leptin levels and body mass increased owing to inpatient treatment.[32] Consistently, serum leptin levels were negatively correlated with motor restlessness (assessed by expert ratings using visual analog scales) in 61 females with

AN.[33] The result was independently confirmed in 27 adolescents with AN, in whom motor restlessness was assessed by expert ratings using the Structured Inventory for Anorectic and Bulimic Syndromes (SIAB).[33] To corroborate these findings, various qualities of physical activity (excessive exercise, motor and inner restlessness) were assessed by accelerometry and self- and expert ratings in an independent sample of 26 inpatients with AN; leptin levels were significantly associated with all qualities of activity and restlessness.[28]

Semi-starvation–induced hyperactivity is seemingly not confined to patients with AN. Thus, survivors of captivity after World War II reported an increase in activity, productivity, and creativity in the initial phase of reduced food availability.[91,102,103] Additionally, voluntary periods of severe food restriction (“fasting cures”) are practiced frequently in the European population as part of a traditional self-care health-promoting activity, and these voluntary fasting cures have been shown to be associated with mood enhancement, leptin depletion, and activation of the HPA axis.[50,51]

The increase in activity levels in times of reduced energy availability has been interpreted as increased foraging behavior. Thus, in evolutionary terms, starvation-induced increase in activity levels might be a phylogenetically old pathway to increase the odds for survival.[91] The decrease in activity due to application of leptin as a satiety signal supports this hypothesis.

The molecular mechanisms underlying the induction of SIH by hypoleptinemia remain unclear. Several leptin-mediated first- and second-order systems in the brain and the periphery might be associated with the effect of leptin on SIH. These could act in concert; alternatively, a single system underlies SIH. The hypothalamic melanocortinergic (MC) system[86] might be involved in the leptin-mediated effect on SIH, because MC receptor expression is increased in the ventromedial hypothalamus of SIH rats, suggesting that a hyperactive MC system underlies the SIH phenotype.[104] However, central infusion of alpha-melanocyte—stimulating hormone[105] or agouti-related protein[99] had no effect on total daily running activity. Other possible pathways downstream of the leptin receptor that might be implicated in SIH are the serotonergic/dopaminergic system or the HPA axis. The increase in glucocorticoids mediated by semi-starvation–induced activation of the HPA axis might play an important role in the development of SIH, because SIH was completely suppressed in adrenalectomized rats and restored after corticosterone treatment.[106,107] Additionally, application of corticosterone increases locomotor activity in ad libitum-fed rats[108] and mice.[109] In accordance with the animal models, urinary free cortisol has recently been shown to correlate with activity levels (measured by accelerometry) in 36 women with acute AN.[110]

NEURONAL FUNCTIONS

Recently, several studies have demonstrated that the actions of leptin are not restricted to the hypothalamus and the hypothalamus–pituitary axis. Instead, many other brain areas including brain stem, cerebellum, amygdala, substantia nigra, and especially the hippocampus are highly enriched with leptin receptors. New findings indicate an enhancement of *N*-methyl-D-aspartic acid receptor function and hippocampal long-term potentiation by leptin and thus underline the importance of this hormone in hippocampal learning and memory function.[111] Accordingly, memory impairments are observed in leptin-insensitive rodents, whereas leptin administration improves memory processes. Interestingly, severely underweight anorectic patients also display memory deficits, especially concerning working memory, which often alleviate with weight gain.[112]

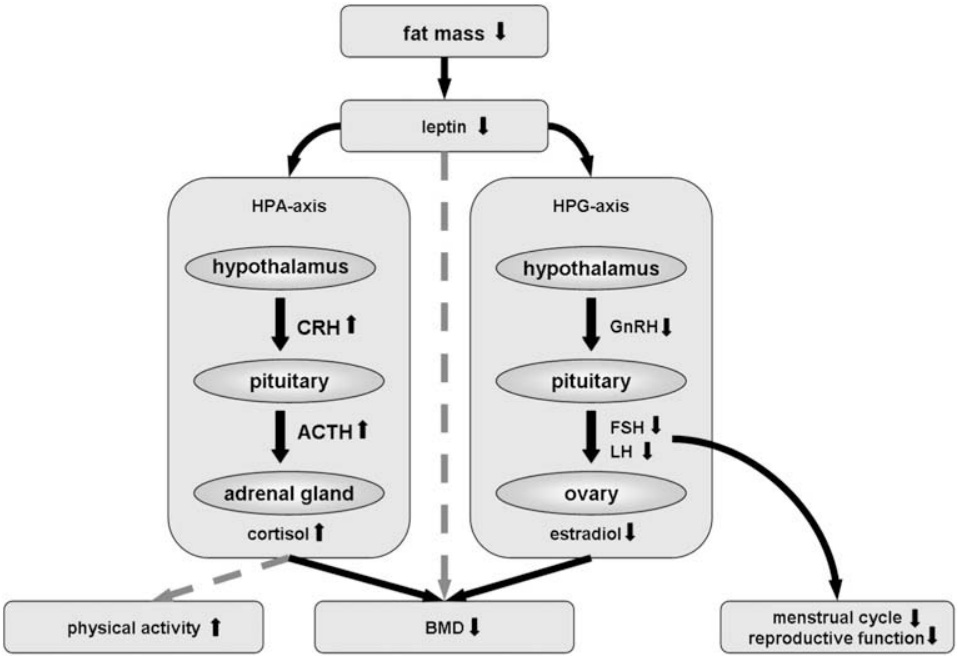

Fig. 1. Semi-starvation–induced alterations of the hypothalamus–pituitary–adrenal (HPA) and –gonadal (HPG) axis in patients with anorexia nervosa. ACTH, adrenocorticotropic hormone; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; BMD, bone mineral density; ↑, increased; ↓, decreased; dashed line, indirect influence; continuous line, direct influence.

SUMMARY

The crucial role of leptin in the neuroendocrine alterations associated with AN is indisputable (**Fig. 1**). Plasma levels of leptin are strikingly reduced during the acute phase of the disorder and increase on weight regain. Whereas hypercortisolism rapidly normalizes on weight restoration, disturbances of ovarian function and decreased BMD can persist for several months up to years after body weight has been restored to normal values. The degree of hypoleptinemia in the acute phase of AN can be considered as an indicator of the severity of the disorder; the lower the leptin level, the more semi-starvation has progressed, and the less adipose tissue exerts its pleiotropic function on other tissues. Accordingly, secretion of gonadotropins (FSH, LH) decreases when leptin levels drop below specific thresholds, thus entailing the development of amenorrhea. Hypoleptinemia-induced hyperactivity reminds us of the difficulty to distinguish between primary and secondary (semi-starvation induced) psychopathological symptoms in AN.

In light of the pivotal role of leptin in mediating the neuroendocrine alterations associated with semi-starvation, the determination of leptin levels should become part of the routine clinical evaluation at referral.[8] It was previously also discussed to include hypoleptinemia as a diagnostic criterion of AN.[94] A prerequisite of that recommendation would be the definition of both the normal reference range and the range of leptin levels observed in patients with AN. As leptin levels potentially differ from those on admission after initiation of weight restoration, the time point of blood sampling needs to be considered when comparing different studies. Measurement of leptin levels may potentially help to detect AN early on during childhood and to assess the severity of the disorder.

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