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FEATURE REVIEW

The role of leptin in anorexia nervosa: clinical implications

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Leptin is a hormone with pleiotropic functions affecting several tissues. Because leptin has a crucial role in the adaptation of an organism to semi-starvation, anorexia nervosa (AN) serves as a model disorder to elucidate the functional implications of hypoleptinaemia; *vice versa*, several symptoms in patients with this eating disorder are related to the low leptin levels, which are characteristic of acute AN. Weight gain in AN patients can induce relative hyperleptinaemia in comparison to controls matched for body mass index; circulating leptin concentrations in AN patients thus transverse from subnormal to supranormal levels within a few weeks. We review findings on leptin secretion in AN and focus on implications, particularly for the hypothalamus–pituitary–gonadal axis, bone mineral density and physical hyperactivity. Undoubtedly, the elucidation of leptin's function as a trigger of diverse neuroendocrine adaptations to a restricted energy intake has substantially advanced our knowledge of the pathogenesis of distinct symptoms of AN, including amenorrhoea that represents one of the four diagnostic criteria. The fact that hypoleptinaemia can induce hyperactivity in a rat model for AN has led to a series of studies in AN patients, which support the notion that application of leptin to severely hyperactive patients might prove beneficial.
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Introduction

The hormone leptin was discovered in 1994;[1] its name derived from the Greek word for thin (*leptos*) was based on the observation that exogenous application of leptin reduces fat mass in both leptin deficient *ob/ob* and wild-type mice.[2] A tremendous amount of research has since then focused on the role of leptin in obesity; in addition, the signalling pathways at the postreceptor level both within and beyond the hypothalamus have been studied extensively.[3] It has become evident that leptin by itself is not the solution for treatment of human obesity.[4]

Like other hormones, leptin is secreted in a pulsatile manner and shows a diurnal variation with an increase of about 50% during the night that might be related to an intrinsic circadian rhythm, meal timing and the sleep–wake cycle.[5,6] Leptin concentrations correlate with the amount of fat mass, with lower levels in lean individuals.[7] Females have higher leptin levels than males; this effect is not only the result of the higher per cent body fat in females but also reflects a direct influence of sex hormones on leptin secretion.[8] However, after a 36 h fast leptin levels are decreased and become similar in both sexes.[9] After a 2.5-day fast, leptin concentrations in healthy females drop by 75%.[10]

In 1996 it was first proposed that leptin's physiological role is the regulation of the neuroendocrine system during starvation.[11] Application of exogeneous leptin to prevent the starvation-induced fall in endogeneous leptin secretion was shown to substantially blunt the changes in the hypothalamic-pituary-gonadal, -adrenal and -thyroid axes in male mice; in female rodents, the starvation-induced delay in ovulation was prevented. Despite the extreme obesity, several but not all symptoms in *ob/ob* mice and leptin-deficient humans are indistinguishable from those that develop in healthy subjects upon a prolonged negative energy balance.[12–14]

In healthy humans, maintenance of a reduced body weight is accompanied by decreased energy expenditure that is mostly due to increased skeletal muscle work efficiency; the time spent in physical activity is not reduced.[14] On top of this major effect, decreased sympathetic nervous system (SNS) tone and reductions of circulating concentrations of leptin, thyroxine (T4) and triiodothyronine (T3) act coordinately to favour weight regain. Raising circulating leptin levels back to pre-weight loss levels via subcutaneous administration of leptin in subjects who had voluntarily lost 10% of their body weight resulted in pre-weight loss levels of energy expenditure, muscle work efficiency, SNS tone, T3 and T4.[14] Evidently, relative

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leptin insufficiency underlies these physiological and endocrinological alterations associated with a reduced body weight.

In light of the postulated critical role of leptin in the adaptation of an organism to semi-starvation, anorexia nervosa (AN) is a model disorder to analyse the relationship between the degree of hypoleptinaemia and both somatic and behavioural symptoms of semi-starvation. AN patients share physiological, endocrinological and psychological features with healthy subjects who have lost a substantial amount of body weight. For example, AN patients, too, have reduced basal metabolic rate, T3 and T4. Amenorrhoea, bradycardia and hypothermia are somatic symptoms of AN,[15] which also occur in healthy subjects upon substantial weight loss. Obsessive thoughts of food, prolonged and ritualistic patterns of food intake, depressed mood and increased rigidity are common to both conditions.[15,16] In spite of these similarities, the diagnostically relevant psychopathological features of AN such as intense fear of gaining weight or becoming fat and the undue influence of body weight or shape on self-evaluation clearly distinguish AN patients from weight reduced healthy subjects. In addition, total daily energy expenditure is not necessarily reduced in AN; patients can expend more energy on physical activity than controls.[17]

Because weight restoration constitutes the main initial aim in the treatment of AN, patients typically transverse from hypoleptinaemia to normal or even supra-normal levels of circulating leptin within a few weeks.[18,19] Both the initial hypoleptinaemia and the treatment-induced increments in leptin levels offer a unique opportunity to understand the physiological role of leptin. In this review, we summarize leptin-related findings in AN and analyse how this knowledge is beginning to influence our clinical perception of this eating disorder. From a clinical viewpoint, we particularly focus on the effects of hypoleptinaemia on the reproductive axis, bone mineral density and hyperactivity.

Body weight in AN

Underweight is a diagnostic hallmark of AN. 'Refusal to maintain body weight at or above the minimal weight for age and height' marks the core of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV-TR weight criterion, the first out of a total of four criteria used to define this eating disorder.[20] Whereas it is unreasonable to specify a single standard for a minimally normal weight that applies to all individuals of a given age and height, 85% average body weight is provided as a guideline.[15] This relative weight is equivalent to body mass index (BMI) values, which age dependently skew between the fifth and tenth BMI centiles of different Caucasian populations.[21] Use of the 10th BMI centile as the threshold guideline takes age-dependent increments in BMI into account and implicitly entails an epidemiologically based cutoff.[22,23]

In AN, BMI only rarely drops below 10 kg/m^2;[24] upon referral for in-patient treatment, most patients have BMI in the range of 11–17; in clinical studies, mean BMI is usually in the range of 13.5–16.0. A low BMI at referral (BMI < 13 kg/m^2) predicts elevated mortality rates according to some follow-up studies in AN[24,25] and entails a lower BMI of survivors.[24] During a famine, the risk of mortality of marasmic females increases sharply upon BMI < 11 kg/m^2.[26] In contrast to marasmus, severe hypoproteinaemia and hypovitaminosis usually do not occur in AN, because the hypocaloric diets in AN patients are relatively rich in protein and vitamins.

Factors influencing circulating leptin levels in acute AN

Leptin levels in AN were first measured using an enzyme-linked immunosorbent assay in 1995;[27] in most patients, low serum levels were detected compatible with their underweight. However, due to a single severely underweight patient (BMI 11.5 kg/m^2) with a normal leptin level, the investigators speculated that AN patients might react with an upregulation of leptin production after reinstatement of an adequate caloric intake, possibly suggesting that their low body weight is physiologically defended.

Subsequent measurements of leptin levels have been based on different radioimmunoassays (RIA). Early on, hypoleptinaemia as a cardinal feature of acute AN was confirmed,[18,19,28,29] primarily reflecting the reduced fat mass. If patients are assessed at referral for in-patient treatment, their leptin levels are mostly below the fifth percentile of the reference range formed by age-matched controls;[18] this holds true for male patients, too.[30] Comparisons with younger healthy controls, whose BMI are in the same range as that of AN patients, again substantiated hypoleptinaemia as a cardinal feature of acute AN.[18] Leptin concentrations are also subnormal in the cerebrospinal fluid of AN patients; however, the cerebrospinal fluid to plasma leptin ratio is higher in patients than in controls.[19]

Apart from the type of RIA, variability in mean leptin levels reported in the literature depends on patient characteristics including point of time of blood sampling (Table 1). As in healthy individuals, BMI is correlated with leptin levels; furthermore, it appears that upon use of solid methodology for measurement of fat mass (e.g. dual-energy X-ray absorptiometry), per cent body fat explains more variation of serum leptin levels than BMI.[31] Upon adjustment for BMI levels of patients with the restricting type of AN do not differ from those of patients with the binge eating/purging type.[32]

The extent of refeeding/weight gain before blood sampling influences leptin levels in AN patients. Levels in acute AN can only be compared between studies if the patients are blood sampled in the very first days of in-patient treatment (see Table 1). Ideally, patients should also have been weight stable in the

Table 1 Synopsis of selected studies related to leptin in AN patients and other samples comprised of individuals with low fat mass (a complete overview of all studies related to leptin secretion in patients with AN is available from the authors)

Ref.	*Subjects* (n)	*Mean age in years (range)*	*Mean BMI ± s.d. (range)*	*Mean leptin levels (μg/l) (range)*	*Point in time of blood sampling*	*Correlation leptin: BMI (leptin: % body fat)*	*Detection*
[27]	AN ($n=15$; one male)	19.9 (14–32)	13.8 (11.6–17.2)	<1 ($n=10$ including $n=4$ undetectable) 1–4 ($n=5$)	During in-patient treatment	No clear relation	ELISA
[18]	AN ($n=23$)	15.8 (13.1–18.6)	14.4 (12.4–17.3)	(0.04–1.69; $n=18$)	Within the first days upon referral	$r=0.48$; $n=18$	Self-devised RIA
[118]	AN ($n=33$)	22.8 ± 6	15.7 ± 1.6	1.9 ± 1.8	Between 0800 and 0900 after an overnight fast	Not significant	Self-devised RIA
[19]	AN ($n=11$)	24 ± 6	13.5 ± 1.2	1.75 ± 0.46	After an overnight fast; at the end of a 3-week nutritional stabilization phase	Not significant	Linco Research
[28]	AN ($n=22$)	23 ± 4 (18–32)	16.3 ± 1.6	5.6 ± 3.7	After an overnight fast	$r=0.60$ ($r=0.63$)	Linco Research
[61]	AN ($n=23$)	16.2 ± 0.3* (12.2–18.8)	16.7 ± 0.2*	4.3 ± 0.6*	In the fasted state at a baseline visit	$r=0.59$ ($r=0.81$)	Linco Research
[120]	AN ($n=20$)	22.1 ± 1	13.7 ± 0.4*	1.7 ± 0.1*	While fasting	$r=0.780$ ($r=0.805$)	Linco Research
[119]	AN ($n=20$[a])	15.5 ± 1.6	17.0 ± 1.1	3.3 ± 2.1	In the fasting state at an initial visit	ND	Linco Research
	AN ($n=23$[b])	16.2 ± 1.6	16.2 ± 0.9	3.1 ± 2.4			
[39]	AN ($n=57$)	25.0 ± 7.0 (16–49)	15.2 ± 1.5	2.3 ± 1.6	At referral after an overnight fast	($r=0.57$[c,d])	Linco Research
[121]	AN ($n=18$)	23.0 ± 5.6	14.2 ± 2.3	2.5 ± 0.9	At baseline after an overnight fast	$r=0.34$; $n=13$ ($r=0.65$; $n=13$)	Linco Research
[73]	AN ($n=18$)	14.1 ± 1.2	14.4 ± 1.2	1.9 ± 1.2	After an overnight fast on day 7 of the 1st week of in-patient treatment	ND	Mediagnost
[99]	AN ($n=61$; group 1)	17.5 ± 4.6 (12.0–31.4)	14.5 ± 1.5 (10.5–17.4)	0.9 ± 1.4 (0.004–7.4)	At referral, after an overnight fast within 3 days upon admission	$r=0.484$[c] (group 1) [$r=0.691$[c] (group 1)]	Mediagnost
	AN ($n=27$; group 2)	14.5 ± 1.3 (11.5–17.4)	14.5 ± 1.3 (12.3–17.5)	1.8 ± 1.1 (0.5–4.6)			
[46]	Undernourished Gambian children ($n=472$; 251 male)	8.01 ± 0.69 (male) 8.00 ± 0.68 (female)	14.0 ± 1.01 13.89 ± 1.04	1.83 ± 0.48 (0.97–3.47) 2.36 ± 0.91 (0.86–9.31)	At the morning after an overnight fast	Leptin positively associated with BMI and body fat for age *z*-score ($P \leqslant 0.0001$)	Linco Research

Table 1 Continued

Ref.	*Subjects* (n)	*Mean age in years (range)*	*Mean BMI±s.d. (range)*	*Mean leptin levels (μg/l) (range)*	*Point in time of blood sampling*	*Correlation leptin: BMI (leptin: % body fat)*	*Detection*
[48]	Ache Amerindians ($n=12$)	32.2 ± 14.0	25.2 ± 1.9	5.6 ± 3.2	After their morning meal	ND	Linco Research
[44]	AN ($n=9$)	17.2 ± 0.9	14.6 ± 0.4	Undectectable (<2)	After minimum weight gain of 10%	ND	Nichols Diagnostics
	Underweight women ($n=7$)	23.3 ± 3.1	15.7 ± 0.4	Significantly higher than in AN patients ($P>0.05$)	At different times at the menstrual cycle	ND	

Abbreviations: AN, anorexia nervosa; BMD, bone mineral density; BMI, body mass index; BN, bulimia nervosa; ELISA, enzyme-linked immunosorbent assay; ND, not determined; Ref., reference; RIA, radioimmunoassay.
*s.e.m.
[a]BMD *z*-score $\geqslant -1$.
[b]BMD *z*-score < -1.
[c]Log leptin.
[d]Fat mass.

time period before referral.[33] In healthy individuals, short-term overeating preceding any substantial weight gain induces an upregulation of leptin secretion,[34] indicating a dynamic component of leptin secretion superimposed on the well-established influence of fat mass. Leptin secretion is substantially higher from subcutaneous than visceral fat, accordingly leptin levels are dependent on body fat distribution.[35,36] In patients with acute AN, subcutaneous fat is substantially more reduced than intra-abdominal fat.[37]

Among AN patients and non-anorectic underweight females, the cognitive restraint scale of the Three Factor Eating Questionnaire was inversely correlated with leptin levels[38,39] adjusted for BMI or fat mass. An eating behaviour score was also shown to be an independent determinant of serum leptin levels in a further sample of AN patients.[40] Possibly, the scores of these scales reflect the patients' extent of current dieting. Finally, Holtkamp *et al.*[41] found a significant inverse correlation between serum leptin levels at admission and food restriction ($r=-0.73$; $P=0.01$) in 11 adolescent patients with AN as assessed by the respective scale in the Structured Interview of Anorexia and Bulimia nervosa (SIAB).[42] In healthy individuals, short-term fasting (2–3.5 days) has been shown to lead to a substantial reduction in leptin secretion without proportionate weight loss.[6,9,43]

In studies that have compared leptin levels of AN patients with those of healthy underweight females (Table 1), both per cent fat mass and leptin levels were higher in the healthy underweight, despite overlapping of BMI;[32,38,44] it appears that the higher fat mass in the healthy underweight largely explains this finding; a contributing role of acute dietary restriction in the patients appears possible.

A limited number of studies pertain to leptin secretion in individuals experiencing semi-starvation for other reasons than AN. Leptin levels were even lower in 13 juvenile female elite gymnasts with anorexia athletica than in nine patients with AN.[45] Leptin levels of both underweight and short girls (mean age 8.0 ± 0.68) suffering from moderate to moderately severe protein energy malnutrition were clearly subnormal in comparison to well-nourished controls,[46] but within the range formed by patients with acute AN. Similarly, leptin levels in children with severe protein energy malnutrition, marasmus or kwashiorkor are reduced in comparison to an age-matched control group.[47] Adult Ache Ameridians females with a mean BMI of $25.2\pm1.9\,kg/m^2$ (but nevertheless a low fat mass) had a mean leptin level of $5.6\pm3.2\,\mu g/ml$;[48] owing to a comparison with leptin levels of AN patients investigated by Grinspoon *et al.*,[28] it was concluded that the levels of the Ameridians are within the anorectic range.[48] However, leptin levels of the Grinspoon *et al.* study[28] are the highest reported in acute AN, potentially suggesting that the respective patients had already gained weight before blood sampling.

Effects of weight gain on leptin levels

During therapeutically induced weight gain leptin levels increase.[18,19,49] Presumably, both total amount and rate of weight gain are relevant for this upregulation. In patients with exceedingly low leptin levels at referral, linear leptin levels increase slowly upon initial weight gain during the first weeks of treatment to subsequently rise more rapidly (Figure 1).[18,49] However, if leptin levels are assessed logarithmically, the increase over time is linear (unpublished data). During this treatment stage, correlations between leptin levels and BMI or per cent body fat typically decline,[18,29] indicating variability of leptin secretion in response to weight gain. Potential reasons include degree of hydration and differences in relative increases in fat and fat-free mass and in subcutaneous versus visceral fat, respectively. In very underweight patients, more fat-free mass is gained initially.[50] The extent of short-term relative overfeeding could also exert an influence on leptin secretion.

In female patients, who have gained an excess of approximately 2 BMI units, leptin levels can exceed the reference range for sex- and BMI-matched controls.[18] Furthermore, leptin levels in patients at target weight have been found to be disproportionately high in comparison to a healthy control group upon adjustment for BMI and % body fat.[49] Relative hyperleptinaemia as a consequence of weight gain has been observed in male patients, too.[30] The transition from initial hypoleptinaemia to relative hyperleptinaemia typically occurs within a time period of several weeks.[18,49] In underweight female patients who had regained some weight, leptin concentrations in the cerebrospinal fluid were nevertheless normal,[19] indicating that the relative hyperleptinaemia is also detectable centrally.

Hyperleptinaemia is reminiscent of a rebound phenomenon; it was speculated that the high leptin levels might represent a counter-regulation and thus predispose a patient to renewed weight loss.[18,19] It should be pointed out that relative hyperleptinaemia is not a universal finding in studies pertaining to AN patients who have gained weight or have recently reached their target weight.[51,52] Magnitude and rate of weight gain in addition to timing of blood sampling after weight gain possibly explain this discrepancy.

Despite the fact that leptin levels seemingly do not explain variability of energy expenditure in healthy individuals,[53] it is conceivable that the rapid transition from a state of hypoleptinaemia to one of relative hyperleptinaemia entails an upregulation in energy expenditure adjusted for fat-free mass,[18,19] possibly via regulation of thyroid function.[54] In accordance with this speculation, caloric intake of patients who had just achieved target weight was higher than that of weight-matched controls and of long-term recovered AN patients.[55] Preliminary evidence indeed suggests that hyperleptinaemia is associated with an elevated risk of renewed weight loss.[56] If however a patient maintains her target weight for a period of several weeks, leptin levels initially fluctuate substantially to then drop into the normal range.[18] Thus, in a patient with an initially very low leptin level, it can take more than 6 months before leptin secretion is normalized as a consequence of realimentation. Potentially, relative hypoleptinaemia (leptin concentrations adjusted for per cent body fat) persists in weight recovered patients.[51,52,57] In conclusion, AN leads to a long-term destabilization of leptin secretion, during which both relative hypoleptinaemia and hyperleptinaemia can occur.

An adequate interpretation of the clinical relevance of alterations in circulating leptin levels depends on the knowledge of the concomitant changes in splicing of leptin receptor mRNA, hypothalamic leptin receptor (long form of the leptin receptor; ObRb) density and in quantity of soluble leptin receptors (sObR or ObRe), which bind most of the circulating leptin.[58] In animal models, semi-starvation leads to an upregulation of ObRb;[59] consistent with these data, the soluble receptor is upregulated in patients with acute AN.[60–63] As a result free leptin is further reduced. Weight gain leads to a reduction of sObR.

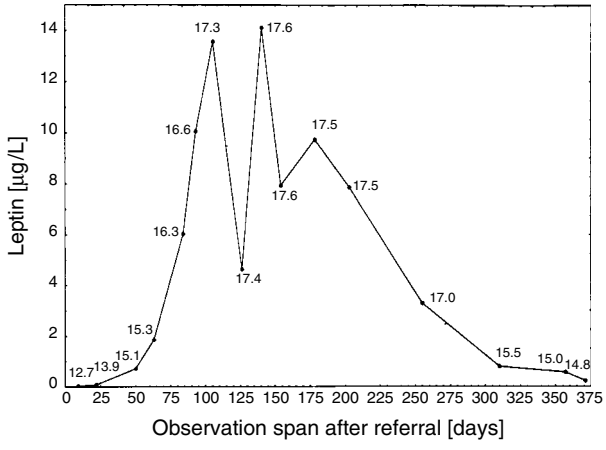

Figure 1 Plasma leptin concentrations during and after inpatient treatment (discharge occurred at day 211) of a female with AN aged 15.8 years upon referral. Numbers indicate BMIs at times when blood samplings were performed. Graphic taken from Hebebrand *et al*.[18]

Amenorrhoea and reproductive function

Primary amenorrhoea in a younger patient and secondary amenorrhoea of at least 3 months duration in a postmenarcheal female constitute the fourth DSM-IV-TR criterion for diagnosis of AN.[20] Typically, amenorrhoea sets in after the initiation of weight loss. However, onset of amenorrhoea has also been reported before substantial weight loss on an anecdotal basis;[64] possibly, such patients had already initiated a low calorie diet. Amenorrhoea also occurs in females whose energy balance is negative for a prolonged period of time for reasons other than AN.

The extensive literature on the relevance of leptin for reproductive function in energy-deprivation states

including AN has been reviewed recently.[65] Briefly, the drop in leptin levels initially induced by caloric restriction and subsequently by loss of fat mass represents the first step of a cascade, which shuts off the hypothalamic-pituitary-gonadal axis, thus entailing the development of amenorrhoea. Because in healthy females leptin regulates the minute-to-minute oscillations in the levels of luteinizing hormone (LH) and oestradiol and the nocturnal rise in leptin determines the change in nocturnal LH profile in the mid-to-late follicular phase that precedes ovulation, a reduction in circulating leptin levels could explain the disruption of hypothalamic-pituitary-ovarian function.[66] It is further assumed that a drop of leptin levels below a critical threshold potentially mediated via an upregulation of cocaine and amphetamine-regulated transcript (CART) triggers the reduction of the secretion of the hypothalamic gonadotropin-releasing hormone, which represents the initial step followed by drops in the circulating gonadotropins follicle-stimulating hormone (FSH) and LH secreted in the pituitary, which in turn entail a curtailment of ovarian oestrogen production.[32,65,67–69] Hypoleptinaemia may also affect the reproductive axis directly at the level of the pituitary and the ovary.[70,71] Leptin's relevance in reproductive function provides a physiological basis for initial observations of Frisch and Revelle,[72] who postulated a critical fat mass for the initiation of menstruation.

Because patients with acute AN and healthy underweight postmenarcheal females typically have a leptin level of below and above approximately 2 μg/l, respectively, a leptin level below this concentration has been invoked to represent the critical threshold value for amenorrhoea.[32,67] Interestingly, currently eumenorrhoeic lean females with a leptin level below 2 μg/l mostly had been amenorrhoeic in the past.[32] Possibly, the reproductive axis is able to adjust to leptin levels minimally below the initial threshold value.

Obviously, inter-individual differences in the critical leptin level required to maintain reproductive function exist, so that it seems more appropriate to speak of a threshold range. Factors that might influence the leptin level, which triggers amenorrhoea on an individual basis potentially include age, the dynamics of the drop in circulating leptin over time, the ratio of leptin to serum leptin-binding proteins and the premorbid leptin level. It is conceivable that in premorbidly lean patients, the restriction of food intake, which has not yet led to substantial weight loss, entails a drop in circulating leptin sufficient to initiate the cascade leading to amenorrhoea; in healthy females, a 3-day fast induces a 70–80% reduction of baseline leptin levels.[6]

Realimentation induced increments in leptin levels of postmenarcheal AN patients presenting with very low leptin levels (<0.1 μg/l) and undetectable FSH and LH levels are followed within a few weeks by increments of FSH and subsequently of LH.[68] In those patients who do not present with severe hypoleptinaemia, FSH is already detectable upon admission. The threshold leptin level for normalization of LH levels is in the range of 1.85 μg/l.[68] In a further study, the leptin threshold value for FSH was found to be in the range of 1.2 μg/l.[73] It appears possible that the threshold ranges for various leptin-induced adaptations of the hypothalamic-pituitary-gonadal axis (and other systems) to energy depletion differ.

Obviously, the mere increments of leptin levels and subsequently of the gonadotropins are insufficient to rapidly induce menstruation. In patients with acute AN, it can take several months before ovarian function normalizes. Thus, ovaries of severely emaciated patients are small. When the initial increment in FSH before the rise of LH (LH:FSH ratio <1) ensues, multifollicular ovaries appear. The emergence of a dominant follicle upon attainment of premorbid weight is accompanied by an increase in uterine area and associated with increased levels of LH and oestradiol and an LH:FSH ratio greater than 2. Visualization of a dominant follicle upon pelvic ultrasound predicts onset of menstruation in a substantial proportion of patients within 1 month.[74]

Inhibin B, an early marker of gonadal activity, is not detectable in severely underweight AN patients; Inhibin B serum levels increase during weight gain, again signalling gradual awakening of the reproductive function. Inhibin B values are highly correlated with leptin and BMI.[51] Because leptin receptors have been detected in ovaries, leptin may act at the level of the ovary[71,75,76] in addition to the action of leptin at the pituitary[70] and the hypothalamic levels. Because leptin is itself produced in the ovaries, the occurrence of full-length leptin receptors in ovarian follicles, corpus luteum and medulla may indicate a leptin-dependent autocrine and paracrine loop for steroid production and for any other as yet unknown peripheral leptin function.[75]

Achievement of a weight of approximately 90% of standard body weight has been shown to lead to resumption of menses in 85% of adolescent AN patients within 6 months;[77] amenorrhoeic in comparison to eumenorrhoeic patients of a similar BMI and per cent fat mass are characterized by lower levels of leptin, gonadotropins and oestradiol, despite similar BMI.[67,77,78] A direct comparison of 74 patients fulfilling all diagnostic criteria for AN with 42 eating disorderd eumenorrhoeic females who only fulfilled the first three DSM-IV criteria for AN, but who nevertheless had a similar BMI and severity of eating disorder symptomatology, revealed higher fat mass and per cent fat mass in the eumenorrhoeic in comparison to the amenorrhoeic females; as expected levels for oestradiol, insulin growth factor-1 (IGF-1) and leptin (3.7 ± 0.3 vs 2.8 ± 0.2 μg/l; $P=0.04$) were lower in the amenorrhoeic patients.[79]

In 17 adult AN patients who were not menstruating after 6–12 months of treatment during which mean BMI and mean leptin levels increased from 14.9 ± 0.5 to 19.3 ± 0.4 and 2.2 ± 0.1 to 6.4 ± 1.4 μg/l, respec-

tively,[52] clomiphene treatment led to an on average five- and 50-fold increase in LH and oestradiol levels. Serum leptin levels were not different in those nine patients who initiated menstruation from those who did not; the mean leptin level of all 17 patients was lower than in healthy controls. The normal response to clomiphene in these patients indicated that the hypothalamic-pituitary axis was intact. The authors argue that persistence of hypoleptinaemia in weight-recovered patients possibly contributes to persisting hypothalamic amenorrhoea.

To our knowledge, the relationship between leptin secretion and the reproductive axis has been investigated in only three male AN patients.[30] At referral, leptin levels were all below 0.25 μg/l; the single patient with a leptin level above the fifth age centile dropped well below this centile during early in-patient treatment.[30] Testosterone levels were initially low and increased concomitantly to leptin during realimentation.

The postulated critical role of leptin in the regulation of the hypothalamic-pituitary-gonadal axis has directly been proven via leptin treatment of females with hypothalamic amenorrhoea. In this proof-of-principle study eight females (BMI range: 18.8–24.4 kg/m^2; fat mass 12.5 $\pm$ 2.8 kg) with hypothalamic amenorrhoea (mean duration: 5.1 $\pm$ 4.0 years) due to strenuous exercise or low weight received recombinant human leptin (r-metHumLeptin; 0.08 mg/kg body weight; 40% of daily dose at 0800 hours, 60% at 2000 hours) for up to 3 months; six other females (BMI range: 18.5–22.1 kg/m^2) with hypothalamic amenorrhoea (mean duration: 5.6 $\pm$ 4.4 years) served as controls. None of the cases or controls fulfilled criteria for an active eating disorder. Leptin levels ranged from 1.4 to 6.9 and 1.4 to 7.2 μg/l, respectively. Three cases but none of the controls had an ovulatory menstrual cycle during leptin treatment. Another two cases had a preovulatory follicle, but did not ovulate. Leptin treatment significantly increased the maximal follicular diameter, number of dominant follicles, ovarian volume (during the follicular phase) and endometrial thickness. LH-pulse patterns improved or normalized in six cases. Levels of LH, oestradiol, IGF-1, baseline T3 and T4 levels increased during leptin treatment. Cases reported a qualitative decrease in appetite during the third month of treatment. Among the five patients treated for 3 months, body weight declined by approximately 2 kg, which was accounted for by a loss of fat mass.[80]

Welt and co-workers thus proved that leptin treatment of patients with hypothalamic amenorrhoea can restore reproductive function. The elevation of circulating leptin levels triggered the hormonal changes leading to ovulation; most cases had a leptin level >2 μg/l at baseline. It was not reported whether cases had a history of weight loss or an eating disorder before onset of amenorrhoea; they had however been weight stable for at least 3 months before the initiation of leptin treatment. It is also unknown if their leptin levels had previously dipped into the range of levels observed in acute AN (<2 μg/l). Conceivably, some of the cases might have had considerably higher leptin levels before onset of amenorrhoea. Again, the long-term functional implications of a perturbation of leptin secretion entailing levels within or slightly above the postulated threshold for AN are underscored.

Bone mineral density

One of the most important long-term somatic complications of AN is a decrease in bone mineral density.[81] Anorectic patients have a sevenfold increased incidence of spontaneous fractures, which occur at multiple sites.[82] In 85% of weight recovered ($\pm$10% ideal body weight), AN patients whose menses had resumed a bone mass density deficiency persisted 11 years after diagnosis.[83]

Peak bone mass is largely built during adolescence;[84] the age range of peak calcium accretion is 10.5–14.6 years in girls and 12.0–15.9 years in boys.[85] During this period, sufficient nutrient supply and physical exercise play a very important role in the build-up of peak bone mass. Thus, adolescent AN-induced semi-starvation is an important factor in the development of osteopenia and osteoporosis in later life.[86]

Starvation is accompanied by high serum cortisol, which enhances bone resorption, and by low serum concentrations of oestradiol, an important hormone for bone mineralization. Accordingly, hypoleptinaemia has an indirect effect on bone density via its relevance for the hypothalamic-pituitary-gonadal axis and hypothalamic-pituitary-adrenal axis.[11] Bone mineral density in fully recovered AN patients has been shown to be inversely related to the duration of amenorrhoea and directly related to the duration of postmenarcheal menses before onset of AN-induced amenorrhoea.[87] Oestrogen use (e.g. in contraceptives) by itself does not have a significant effect on bone formation in AN patients. Weight gain, however, is a strong predictor of increasing bone mass in AN.[88–90]

The reason why oestrogens can increase bone mass in healthy postmenopausal females but not in adolescents with AN can potentially be explained by the action of leptin, which is by itself a powerful regulator of bone mass. Bone continuously renews itself by remodelling, which comprises two phases, namely resorption by osteoclasts and formation by osteoblasts. Although several animal studies demonstrated that leptin has a strong antiosteogenic effect,[91] leptin's effects on bone metabolism are not fully understood. For example, an inverse relationship between the cross-linked carboxyterminal telopeptide of type 1 collagen (CTX), a marker of bone resorption, and leptin concentrations in foetal blood has been detected.[92] Furthermore, a positive correlation between bone mass and serum leptin levels was observed in non-obese women after adjustment for body weight and fat mass.[93] On the other hand, leptin-deficient *ob/ob* mice have a high bone mass.

Substitution of leptin in these animals is followed by bone mass reduction.

Recent findings indicate that leptin induces bone loss via the central nervous system (CNS) and by regulating input from the SNS. Thus, knockout mice lacking the β2-adrenergic receptor are resistant to leptin's central bone-reducing effects. However, leptin-deficient *ob/ob* mice demonstrate an increase in bone resorption, despite a reduction in β-adrenergic signalling that should result in diminished bone resorption.[94]

An important component in the leptin–CNS–bone interplay seems to be the neuropeptide CART that might explain the contradiction.[95] Current observations in CART knockout mice suggest that CART seems to inhibit bone loss. CART neurons are widely distributed in the brain and spinal cord and are found also in regions rich in neurons that express high concentrations of sex steroid receptors. Hypothalamic cell groups potentially integrate several signals,[94] including that of leptin and sex steroids to coordinate the CNS regulation of bone mass, probably mediated by sympathetic neurons. However, in AN it has long been known that starvation reduces nor-epinephrine turnover in the hypothalamus.[96] Hypoleptinaemia in combination with a dysfunction of the autonomic system may be one possible explanation for an impairment of bone metabolism in AN.

Hyperactivity

Elevated levels of physical activity have consistently been reported in patients with AN;[97–100] some investigators view this phenomenon as a core clinical symptom of AN.[23,97] Different terms have been invoked for the elevated activity, including excessive or compulsive exercise, intense athleticism, an exaggerated need for physical activity, paradoxical liveliness, hyperactivity, overactivity, motor restlessness or diffuse restlessness.[99] The various descriptions of 'hyperactivity' reflect different aspects or qualities of one or more related behaviours or psychopathologic features. The connotations underlying these terms refer to different aetiologic mechanisms including conscious efforts of the patient (to work off calories), compulsive behaviour, an altered state of mind and a neurobiological phenomenon. In light of the lack of a consistent operational definition of 'hyperactivity', it is not surprising that it has been observed in 31–80% of AN patients.[101–103]

'Anorexia-based activity' or 'semi-starvation-induced hyperactivity (SIH)' is viewed as an animal model of AN.[99] In essence, rats with access to a running wheel develop hyperactivity upon food restriction. Running wheel activity levels increase by 300–500% within a few days after the onset of caloric restriction; if the experiment is not terminated, rats virtually run themselves to death within 7–10 days. SIH is more pronounced in female and young rats.[99] The hypothesis that hypoleptinaemia triggers SIH was confirmed experimentally via subcutaneous implantation of minipumps containing leptin or vehicle in rats undergoing food restriction:[98] Vehicle-treated rats showed a fourfold increased activity after 7 days. In contrast, the activity levels of leptin-treated animals remained at baseline (Figure 2). A second experiment proved that leptin was able to 'rescue' rats that had already developed SIH; the

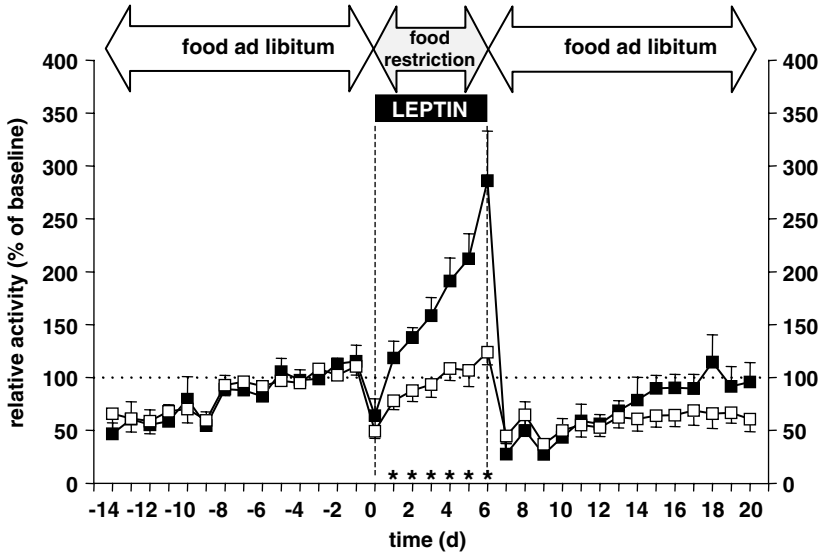

Figure 2 Suppression of SIH by leptin. Running wheel activity was recorded in rats, which were restricted to 60% of their *ad libitum* food intake for 1 week ($n=7$ in each treatment group). Rats were treated with leptin (open symbols) or vehicle (closed symbols) via implanted minipumps beginning on the first day of food restriction. The duration of leptin application is indicated by the black bars and the period of food restriction is highlighted by the shaded arrow. The recording of running wheel activity started 2 weeks before treatment. Activity is presented as a percentage $\pm$ s.e.m. of mean activity during the second pre-treatment week, to adjust for individual variation in baseline activity. Asterisks denote a significant effect of leptin treatment on activity ($P<0.05$). Graphic taken from Exner *et al*.[98]

elevated activity levels dropped to baseline. This key role of leptin in SIH has recently been confirmed in an independent study.[104] Accordingly, hypoleptinaemia can trigger a strong behavioural response, which can be corrected via increments of circulating leptin levels.

Subsequent studies have aimed to elucidate the mechanism by which hypoleptinaemia induces hyperactivity. Central infusion of agouti related peptide (AgRP), an orexigenic neuropeptide co-expressed with neuropeptide Y (NPY), has been reported to counteract hyperactivity in food-restricted rats;[105] this finding is surprising, because both NPY and AgRP are upregulated as a consequence of a drop in circulating leptin levels.[106] However, because only locomotor activity, but not running wheel activity, was attenuated by AgRP, this finding is not inevitably in contrast to the leptin-mediated effect on SIH. Another study of the same group likewise revealed no significant effect of centrally infused AgRP on running wheel activity.[107] Nevertheless, it has been suggested that a hyperactive melanocortinergic system underlies the SIH phenotype.[104,105,107] Other possible effectors downstream of the leptin receptor that might be implicated in SIH are the serotonergic and/or dopaminergic system as well as the hypothalamic-pituitary adrenal axis.

The SIH triggered by hypoleptinaemia suggests that the same mechanism might underlie the hyperactivity in patients with acute AN. Indeed, patients, who ranked their motor restlessness on a visual analogue scale during therapeutically induced weight gain, reported highest levels at referral, when leptin levels were lowest. As leptin levels increased, subjective experiences of motor restlessness decreased.[98] In two independent patient samples, leptin levels at referral for in-patient treatment were inversely correlated with activity levels as assessed by the respective therapists.[100] A closer look revealed that activity levels were lower in severely emaciated patients with almost non-detectable leptin levels than in patients who had somewhat higher levels, suggesting that the relationship between leptin and activity levels follows an inverted U.[108] Presumably, the postulated effect of hypoleptinaemia on activity levels declines when patients are close to starvation and thus critically ill. In rats, SIH similarly ceases close to death.[99]

Attempts to dissect the core phenotype underlying hyperactivity in acute AN have not been successful. Thus, leptin levels not only contributed to the variance of physical activity level as assessed by an objective measure (accelerometry) but also to patient self-reported motor and 'inner' restlessness described as being jittery, anxious and restless.[108] Theoretically, hypoleptinaemia could affect activity levels directly and/or indirectly via specific psychopathological features. For example, independent effects of anxiety on activity levels need to be considered.[41]

It is unclear if and to what extent hyperactivity is a behavioural phenotype specific to patients with acute AN. Thus, there are a number of models of low leptin levels in laboratory animals and in humans who are not evidently associated with increased levels of physical activity.[99] Nevertheless, increased nervousness, anxiety and motor activity have anecdotally been reported in semi-starved humans. Age, gender, diet and activity levels before onset of food restriction need to be taken into account. For example, AN patients who had been physically active before the onset of their eating disorder are potentially more prone to develop hyperactivity in acute AN.[102] It has been proposed that the hyperactivity in AN patients reflects the activation of a phylogenetically old system in thus predisposed individuals.[99,109]

Conclusions and outlook

There is no doubt that leptin plays an important role in AN, both with respect to clinical symptomatology and course. Leptin secretion is profoundly perturbed in this eating disorder. Normalization of secretion after weight recovery requires time, the recovery of leptin-dependent somatic and behavioural symptoms may take even longer. The degree of hypoleptinaemia in acute AN is an indicator of the severity of the disorder; thus, pronounced hypoleptinaemia is not only indicative of an exceedingly low fat mass but also reveals that the neuroendocrine adaptation to semi-starvation has maximally progressed in such critically ill patients.

In this context, both threshold values for specific functions and quantitative relationships need to be considered. A threshold value implies that leptin acts as a switch; thus amenorrhoea inevitably ensues if leptin concentrations fall below a specific minimal value; obviously, the situation is more complex at the endocrinological level because potentially different threshold values exist for specific sub-functions such as regulation of FSH and LH. For more complex functions such as ovulation and regular menses a supra-threshold concentration of leptin is necessary but not sufficient. In other situations, leptin levels may be correlated with quantitative variables; the degree of hyperactivity in the SIH animal model could, for instance, theoretically depend on the circulating leptin concentration. Finally, both a threshold and a quantitative relationship could apply to a single phenotype; SIH could potentially only arise if leptin levels fall below a specific concentration, the degree of hypoleptinaemia then influencing the severity of the hyperactivity. Studies of short-term fasted females revealed that changes of leptin levels within the physiologic range have no major physiologic effects in leptin-replete humans,[110] substantiating the critical role of sub-threshold leptin concentrations for the initiation of the physiologic adaptation to a semi-starvation.

We argue that determination of leptin levels in patients with acute AN should thus become part of the routine clinical evaluation at referral; a prerequisite for this recommendation is that both the normal

reference range and the range of leptin levels observed in AN patients are known to the clinician. For this purpose, additional efforts to assess precisely the range of leptin levels in larger numbers of non-pretreated patients with acute AN are required for the diverse RIAs on the market. Ideally, future research in AN should include descriptions of the mean and range of leptin concentrations of non-pretreated patients at referral; in our own studies, leptin levels have ranged from <0.1 to $1.8\,\mu$g/l. Based on such knowledge, both the clinician and the researcher would be able to assess the severity of the disorder in terms of the extent of semi-starvation: The lower the leptin level of an individual patient, (a) the more semi-starvation has progressed, (b) the less fat mass remains and (c) the less the adipose tissue exerts its leptinergic function on other tissues. The leptin level in acute AN allows inference as to the residual function of the hypothalamic-pituitary-gonadal axis and potentially to the extent that hyperactivity has an endocrinological basis. The consideration of the leptin level in the diagnostic assessment procedure thus adds considerably to the mere determination of a patient's BMI. Leptin threshold ranges for specific functions warrant further research as do possible quantitative causal relationships. We have previously discussed that future classification systems could include hypoleptinaemia as one of the diagnostic symptoms of AN.[23] Leptin levels could potentially help to detect early AN and/or a restricting pattern of eating behaviour in underweight individuals.

Somatic, psychopathological and cognitive symptoms, and imaging and endocrinological results could conceivably differ according to the degree of the patients' hypoleptinaemia. Tracking of leptin levels over time could contribute to generate hypotheses as to the influence of leptin concentrations on psychopathology. In rats, application of leptin decreases depression-like activity in the forced swim test[111] and treatment of *ob/ob* mice with leptin attenuates anxiety-related behaviour.[112] In AN patients, depression and anxiety are common and frequently recede during weight gain. Leptin has been postulated to play a role in cognition[113] and could accordingly also have implications for AN patients, among whom cognitive functions are particularly reduced in severely emaciated patients. AN patients show an increase of wakefulness after sleep onset, a higher number of arousals and a reduction of slow-wave sleep and slow-wave activity;[114] in *ob/ob* mice sleep regulation is altered.[115] Exogeneous application of leptin to leptin-deficient human adults was shown to have sustained effects on tissue composition in the human brain.[116] In patients with acute AN, pseudoatrophy cerebri is a frequent finding, which is usually reversible during weight gain;[117] knowledge of leptins's effects on the brains of leptin-deficient individuals could potentially account for some of the imaging findings in AN patients.

The aforementioned findings merely illustrate some of the findings that can now be pursued in an attempt to delineate further implications of hypoleptinaemia in AN-related research. *Vice versa*, research into the functions of leptin on the human organism will benefit by including specific studies in AN patients. It is likely that the discovery of leptin and its functions will turn out to be the major endocrinological finding in AN to explain symptoms related to semi-starvation.

As has been pointed out previously,[18,19,98] leptin can be considered as a potential therapeutic agent to reverse some of the symptoms of acute AN. Obviously, particular precautions need to be taken in initial studies to ensure that patients do not lose weight or suffer metabolic complications. If such studies become possible, the effects of increasing circulating leptin concentrations on the semi-starved human organism could be assessed.

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