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ORIGINAL RESEARCH ARTICLE

Leptin levels in patients with anorexia nervosa are reduced in the acute stage and elevated upon short-term weight restoration

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Circulating leptin concentrations are known to be low in acute anorexia nervosa (AN), which is characterized by low weight, amenorrhea and specific psychopathological features. In this study plasma leptin concentrations were determined during inpatient treatment of 23 adolescent females with AN using a sensitive radioimmunoassay (RIA) and set into relationship to leptin levels of females matched for age, body mass index (BMI; kg m^{-2}) and/or percent body fat. At referral patients had leptin concentrations well below the female controls. Weight gains led to steep increases of leptin levels which peaked at values well in excess of those observed in controls matched for BMI. In patients who reached the final treatment stage and who were followed-up after discharge, levels subsequently fluctuated and finally dropped into or below the control range. The low leptin levels at referral are likely to be involved in the pathogenesis of amenorrhea and the reduced metabolic state of acutely ill patients. Peak leptin levels reached after weight gain are possibly the cause of increased energy expenditure during this stage of the disorder.

The *obese* gene product leptin is secreted by adipocytes.[1–3] The leptin receptor gene is expressed in several tissues.[4–7] Serum leptin concentrations correlate with BMI and percent body fat,[3] suggesting that adipocytes are signaling the brain and other tissues about the size of the adipose tissue-depot. Serum leptin levels are higher in females compared to males even when leptin is corrected for differences in body composition, thus indicating a sexual dimorphic regulation.[8] Single meals do not significantly alter leptin levels.[3] Short-term fasting[9–11] and overeating[12] lead to a rapid decrease and increase, respectively, in leptin synthesis that precedes any weight alterations. Leptin concentrations decline after weight loss;[3,13] weight gain is associated with an increased leptin synthesis.[11]

Percent body fat declines to below one percent upon administration of leptin to young wild-type female mice.[2] Application of leptin to *ob/ob* mice induces weight loss via a reduction of energy intake and an increase in energy expenditure; body temperature rises and locomotor activity increases.[2,14] Leptin also corrects the reproductive defect of adult sterile female *ob/ob* mice.[5,6] In wild-type female mice exogenous application of leptin blunts the starvation-induced delay in ovulation and other metabolic consequences characteristic of semi-starvation.[15]

Anorexia nervosa is characterized by underweight, amenorrhea and specific psychopathological features consisting of intense fear of gaining weight and disturbance in the way one's body weight, size or shape is experienced.[16] This symptomatology, including the somatic consequences of semi-starvation,[17] the physiology underlying leptin synthesis and its effects on reproductive function merit determinations of circulating leptin concentrations in affected individuals, who have previously been shown to have low leptin levels during the acute stage of the disorder.[18–20]

In this study plasma leptin concentrations in 18 non-pretreated patients ranged from 0.04 to 1.69 μg L^{-1} (Figure 1) upon referral. The correlation between BMI and leptin levels in these patients was 0.48 ($P = 0.06$). Extremely low leptin concentrations (<0.1 μg L^{-1}) were observed in the two patients presenting with BMIs below 13 kg m^{-2}. Seventeen out of the 18 patients had levels below or at the 5th percentile of the reference range formed by female controls of similar age and/or

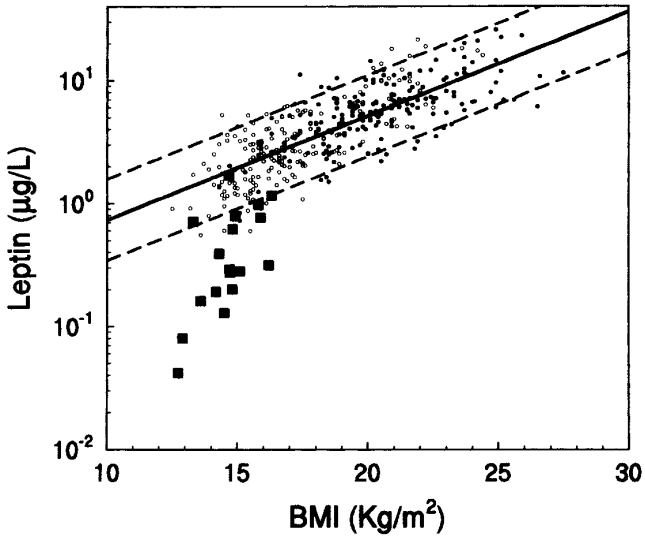

Figure 1 Plasma leptin concentrations (logarithmic scale) in 18 non-pretreated patients with anorexia nervosa *vs* body mass index (BMI; kg m^{-2}) in comparison to the reference range of healthy female controls matched for body mass index and/or age. ■ Eighteen patients at referral (age range: 13.1–18.6 years). Reference range: ○ 5.8–12.99 years; ● 13.0–18.99 years. The bold line and dashed lines represent the 50th and 5th and 95th percentiles, respectively.

BMI (Figure 1). The patients' mean (± s.d.) standard deviation score for BMI-adjusted leptin concentrations was -3.61 ± 1.85. This value was below the reference range ($P < 0.0001$).

In comparison to controls the initial leptin levels in patients were also reduced in relation to percent body fat. In the ten patients in whom BIA was performed upon referral leptin levels were at or below the fifth percentile of the reference range formed by the 108 female controls (Figure 2).

During the first treament phase weight gain consistently led to increments of leptin concentrations. The maximal leptin concentrations and the BMI-increase within the first 150 days of treatment were strongly correlated ($r = 0.73$; $P < 0.05$). However, the correlation between maximal leptin concentrations and the increase in percent body fat within the same time period was weak ($r = 0.11$; $P = 0.78$). Pooled intraclass correlations (ANOVA; 13 patients; 83 measurements) between leptin concentrations up to achievement of the maximal concentrations and BMI and percent body fat were 0.79 and 0.49, respectively.

The maximal leptin concentrations of all of the 11 patients who were treated for more than 50 days were either in the upper reference range or surmounted the 95th percentile (Figure 3). These patients' mean (± s.d.) standard deviation score was 2.40 ± 0.90 and thus above the reference range ($P < 0.0001$).

In three patients fluctuations in excess of 5.5 $\mu g\,L^{-1}$ (Figure 4) were observed in the weeks prior to and after measurement of the maximal concentrations, which were accompanied by only minimal changes of BMI. In the final treatment phase leptin levels dropped into or towards the control range. In two followed-up patients who lost weight after discharge the final leptin concentrations were again well below the reference range.

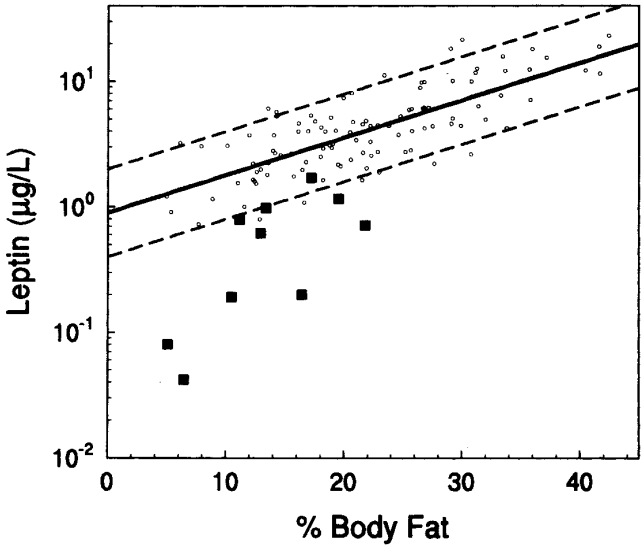

Figure 2 Plasma leptin concentrations (logarithmic scale) in 10 non-pretreated patients with anorexia nervosa *vs* percent body fat in comparison to the reference range of 108 healthy female controls aged 6.1–16.8 years; the bold line and dashed lines represent the 50th and 5th and 95th percentiles, respectively.

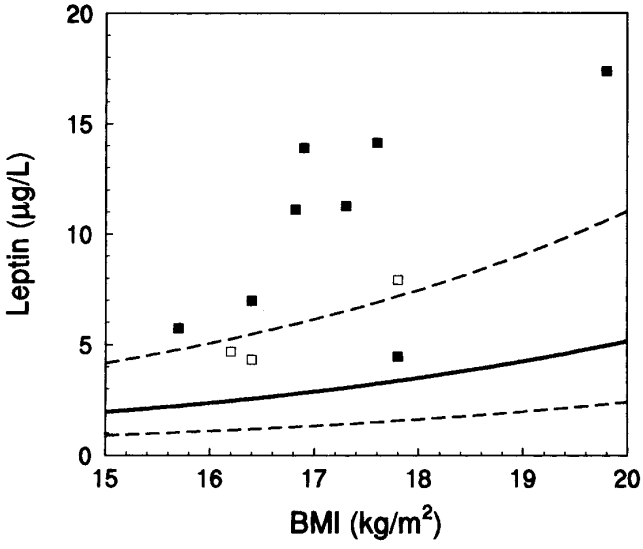

Figure 3 Maximal plasma leptin concentrations (linear scale) *vs* body mass index (BMI; kg m^{-2}) measured in 11 patients with anorexia nervosa during inpatient treatment of at least 50 days duration. ■ Eight patients who gained at least 2 kg m^{-2}; □ three patients who gained less than 2 kg m^{-2}. For specification of the reference range see Figure 1.

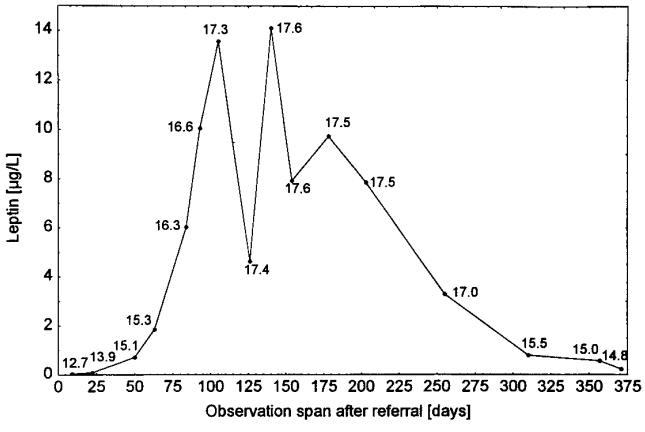

Figure 4 Plasma leptin concentrations during and after inpatient treatment (discharge occurred at day 211) of a female with anorexia nervosa aged 15.8 years upon referral. Numbers indicate body mass indexes at times when blood samplings were performed.

The variation of leptin levels over an observation span of one year is illustrated for a patient in Figure 4. This 15.8-year-old adolescent with recent onset of AN had lost 10 kg within the 2 months prior to hospitalization. Leptin synthesis increased slowly during the first 50 days of inpatient treatment (0.04–0.72 $\mu g\,L^{-1}$), in which the patient gained 6.8 kg. Thereafter leptin synthesis increased steeply up to day 100, by which time she had gained a total of 12.3 kg. The intermittent phase of fluctuating leptin levels, which lasted for approximately another 100 days and during which BMI and percent fat mass varied to a slight extent only, gave

way to a continuous drop which presently has ended at a leptin concentration of 0.23 $\mu g\,L^{-1}$ (BMI: 14.8 kg m^{-2}). After discharge renewed weight loss has totaled 6.8 kg.

This study illustrates the dynamic state of leptin synthesis in patients with AN. Single patients, who were followed up for more than a year, allowed determinations of plasma leptin levels in the acute stage of the disorder, upon short-term weight restoration achieved approximately after 100 days of inpatient treatment and finally upon renewed weight loss. The levels observed are only amenable to an interpretation under consideration of the co-investigated large number of female controls matched for age, BMI and/or percent body fat.

The patients' plasma leptin levels at referral are well below those of female controls matched for age, BMI and/or percent body fat (Figures 1 and 2). In the light of the effects of fasting on leptin synthesis,[9–11] it appears that the patients' chronic restriction of energy intake induces a drop in leptin synthesis even beyond that accounted for by loss in fat mass. The low metabolic state in acute AN is possibly related to this phenomenon. In the light of leptin's effects on the reproductive system[5,6,15] the exceedingly low concentrations most likely contribute to the pathogenesis of amenorrhea. Because menstruation in patients with AN usually does not set in immediately upon weight restoration, a more prolonged normalization of leptin synthesis is required and/or additional factors are involved in this complex regulatory system. Figure 4 reveals that acute AN and its treatment lead to a long-lasting perturbation of leptin synthesis.

In patients with AN at least 50% of weight is apparently regained as fat. Especially in severely underweight patients more fat free mass is gained initially.[21] In our patients (Figure 4) leptin levels increased slowly during the first 50 days of treatment. Afterwards comparatively large leptin increments occurred despite only slight BMI-increases. Our results support a dynamic regulation of leptin synthesis, which is especially relevant, when energy intake is altered considerably. During such a dynamic period, correlations between leptin levels and BMI or percent body fat are seemingly lower than in times of stable weight regulation.

The consistent finding, that comparably small BMI increments cause marked and disproportionate rises in leptin levels beyond the reference range (Figures 3,4), suggests an abnormal sensitivity of the regulation of leptin synthesis reminiscent of a rebound phenomenon. Inadequately high leptin concentrations can theoretically result in appetite suppression and stimulation of energy expenditure. It may be hypothesized, therefore, that disturbance of the metabolic state in acute AN by realimentation is counteracted by a marked increase of leptin. Faster and/or larger weight gains possibly entail higher leptin levels than more prolonged weight gains. These findings complement those observed upon weight gain due to overfeeding in healthy subjects.[12]

Energy intake adjusted for body surface area required for maintenance of body weight in short-term weight-restored patients has been shown to be elevated above that of both long-term weight-restored patients and of healthy female controls.[22] This increased caloric requirement might contribute to poor outcome in the short-term weight-restored patients because it would be difficult for them to eat sufficient calories to maintain weight. Our results indicate that leptin might be an important mediator of this phenomenon. Interestingly, it has been suggested that a too rapid weight gain during inpatient treatment, which based on the present findings can be expected to lead to an excessive leptin synthesis, is associated with a poor prognosis.[23]

The fluctuations of leptin levels observed in some patients (Figure 4) after weight gain suggest a temporary regulatory instability during which leptin synthesis is eventually down-regulated. Our findings seem to represent the reverse of the medal when compared with leptin regulation in obese individuals upon weight loss, in whom leptin levels drop initially and rise again subsequently.[3] Maintenance of a reduced or elevated body weight is associated with compensatory changes in energy expenditure, which oppose the maintenance of a body weight that is different from the usual weight.[24] It is tempting to speculate that leptin is involved in this regulatory process. Patients with AN possibly adapt to their underweight, thus partially explaining the chronicity of this eating disorder. Because in these patients the BMI at referral is related to the weight outcome at follow-up,[25,26] it will be of interest to assess the prognostic significance of leptin levels during the acute stage of AN.

Methods

The study sample comprised 23 female adolescent inpatients with AN, of whom 18 fulfilled the DSM-IV criteria for restricting type AN and five for binge eating/purging type AN.[16] Ages upon admission ranged from 13.1 to 18.6 years (median 15.8 years). Median BMI at referral was 14.4 (range: 12.4–17.3) kg m^{-2}. Five patients had been pretreated at other hospitals immediately prior to referral. The study was approved by the ethics committees of the Universities of Giessen and Marburg; patients and controls or the parents of individuals aged <18 years gave written consent.

In this pilot study 13 patients were assessed upon referral and during inpatient treatment. Six of these cases were followed-up after discharge (total observation spans: $n = 4 \leq 100$ days; $n = 3$ between 100 and 200 days; $n = 3$ between 200 and 300 days; $n = 3$ between 300 and 416 days). An additional ten patients were assessed upon referral only, because they had either been admitted recently, declined further investigations or discharged themselves with parental consent during the initial phase of inpatient treatment.

Body height and weight were measured upon referral. The first blood sampling for determination of plasma leptin levels was performed within the first days upon referral at 8 am, thus excluding an influence of the circadian rhythm of leptin synthesis.[27] Nine of

the 13 patients observed during inpatient treatment consented to biweekly blood samplings; the remaining four consented to less frequent samplings. Weight measurements were performed concomitantly with the blood samplings after an overnight fast at 8 am. The six followed-up patients were reassessed at different time intervals for up to four times after discharge.

Ten patients consented to determinations of body composition upon referral and during inpatient treatment on the biweekly basis using bioelectric impedance analyses (BIA). Body fat mass was calculated from measured resistance according to a gender and fatness-specific equation (<30% body fat) for predicting lean body mass, which in contrast to other equations allowed determinations of fat mass in even the most cachectic patients.[28] During inpatient treatment weight gain was achieved using individualized behavior therapy. After an initial phase patients received between 2100 and 2900 kilocalories daily.

The reference range of serum leptin levels in relationship to age was determined in 187 healthy females aged 13.0–18.99 years (mean BMI ± s.d.; $20.57 \pm 2.53\ kg\ m^{-2}$). To additionally match for BMI and leptin concentrations, younger ($n = 205$; age range 5.8–12.99 years; mean BMI ± s.d.: $17.23 \pm 2.52\ kg\ m^{-2}$) females also served as controls. All controls were ascertained in school classes. Body composition was additionally determined by BIA in a total of 108 females (age range: 6.1–16.8 years; mean 11.3 years). Individuals who acknowledged having an acute or chronic somatic illness were excluded from the study. Data pertaining to the control sample including developmental stage, estradiol and testosterone levels will be published elsewhere (Blum *et al*, unpublished data).

The reference range for leptin concentrations based on the controls was used to adjust the patients' leptin levels for BMI by calculating standard deviation scores (Z-scores) for patients' initial and maximal leptin concentrations. Repeated measurement ANOVA was used to calculate pooled intraclass correlations between leptin concentrations up to achievement of the maximal leptin concentrations and BMI and percent body fat, respectively.

Leptin in patients and controls was measured with a sensitive RIA. Recombinant human leptin was used for raising a polyclonal antibody in rabbits (final dilution 1 : 30 000), for production of tracer by the chloramine T method[29] (20 000 cpm per tube) and for preparation of standards (geometrical dilution between 12.5 and $0.049\ \mu g\ L^{-1}$). All components including the serum samples were appropriately diluted in assay buffer ($0.05\ mol\ L^{-1}$ sodium phosphate, pH 7.4, $0.1\ mol\ L^{-1}$ NaCl, 0.1% (v:v) gelatine from teleost fish (Sigma, München, Germany), 0.1% triton X-100 (Serva, Heidelberg, Germany), 0.05% (w:v) NaN_3). The assay mixture was composed of 100 μl each of standard/diluted sample, tracer, and 1st antibody and was incubated overnight at room temperature. Separation of bound and free tracer was achieved by adding 500 μl of cold 4% (v:v) polyethylene glycol 6000 containing goat-anti-rabbit IgG (DSL, Houston, TX, USA). After centrifugation, the supernatant was decanted and the precipitate was counted in a gamma counter. Half-maximal binding of tracer occurred at $0.9\ \mu g\ L^{-1}$. Sensitivity of undiluted samples was $0.03\ \mu g\ L^{-1}$. Inter- and intra-assay coefficients of variation were 8.5 and 0.8%, respectively.

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