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ORIGINAL RESEARCH ARTICLE

Low leptin levels predict amenorrhea in underweight and eating disordered females

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Evidence that leptin plays an important role in reproductive function is accumulating rapidly. We hypothesized that low leptin synthesis is associated with amenorrhea. We therefore determined serum leptin levels in 43 underweight female students, who were screened for lifetime occurrence of amenorrhea. We assessed the predictive value of leptin, body mass index (BMI), fat mass and percent body fat, respectively, for lifetime occurrence of amenorrhea. Factors predicting amenorrhea were tested for their capability to predict current amenorrhea in a second cohort of 63 inpatients with anorexia nervosa (AN) or bulimia nervosa (BN). Furthermore, the relationships between serum leptin levels and of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone, respectively, were evaluated. Only leptin predicted lifetime occurrence of amenorrhea in the student cohort. The critical leptin level was in the range of 1.85 μg L^{-1}. This level served to largely separate anorectic from bulimic patients. In patients with AN mean serum $\log_{10}$ leptin levels over the first 4 weeks of inpatient treatment were correlated with mean FSH, LH and estradiol levels, respectively. Evidently, a critical leptin level is needed to maintain menstruation. In affluent populations eating disorders are likely to be a major cause of a low leptin synthesis.

The reduction of leptin synthesis upon restriction of energy intake seemingly induces the metabolic adaptation to semi-starvation.[1] Fasting leads to a rapid fall in circulating leptin levels which precedes the loss of body weight.[2–4] This reduced leptin synthesis presumably mediates other adaptive endocrine alterations[1,5–9] including the down-regulation of the hypothalamic-pituary-gonadal axis, which can set in even upon short-term fasting. The down-regulation of this axis is blunted in fasted mice, which are concomitantly treated with exogenous leptin.[1] In this context, the treatment of the infertility of *ob/ob* female mice by application of leptin[10] underscores the importance of this hormone for reproductive function. The treated *ob/ob* mice have elevated serum levels of LH, increased ovarian and uterine weights, and stimulated aspects of ovarian and uterine histology.[11] Prepubertal wild-type female mice injected with leptin reproduce earlier than controls.[12]

In the light of the aforementioned findings we hypothesized that amenorrhea in underweight females can result from a subnormal leptin synthesis. Accordingly, we measured serum leptin levels in 43 underweight female students to determine whether the occurrence of amenorrhea is related to leptin synthesis. A second cohort of 63 female inpatients with either AN or BN was used to substantiate the findings obtained in the student cohort. Patients with AN have previously been shown to have low leptin levels.[13–15]

Fifteen of 43 underweight female students whose serum leptin levels ranged from 0.47 to 13.9 μg L^{-1} (mean ± s.d.: 4.26 + 2.73 μg L^{-1}) reported at least one episode of amenorrhea lasting three months or longer. The maximal chi-square for a leptin level predicting the lifetime occurrence of amenorrhea was 9.2 ($P < 0.05$) and corresponded to leptin concentrations in the range between 1.80 and 1.87 μg L^{-1}. Seven of the nine students with leptin levels below 1.85 μg L^{-1} reported a positive history of secondary amenorrhea; one of the remaining two had BN (BMI 19.2 kg m^{-2}) and reported a past episode of oligomenorrhea, the other was a vegetarian (16.6 kg m^{-2}) who was nursing her child. Only eight of the 34 probands with leptin levels above 1.85 μg L^{-1} reported an episode of amenorrhea; additionally, two never amenorrheic females reported past episodes of oligomenorrhea. Among the six females, who fulfilled DSM-IV[16] lifetime criteria for AN ($n = 3$), BN ($n = 1$) or Eating Disorder Not Otherwise Specified ($n = 2$), four had leptin concentrations below 1.85 μg L^{-1}; only the bulimic student did not report an episode of amenorrhea.

The maximal chi-square for a BMI predicting lifetime occurrence of amenorrhea failed to reach significance ($P > 0.1$). Body fat mass and percent body fat also did not predict the occurrence of amenorrhea ($P > 0.1$).

Only two of the 30 students who were currently not using oral contraceptives reported being amenorrheic upon assessment. Among those females who had an episode of amenorrhea after assumed attainment of adult height (≥16 years of age), recalled body weights at onset of this episode of amenorrhea were similar to their present weights, thus suggesting a relatively constant leptin synthesis over time. The female reproductive system possibly adapts to low leptin levels within a certain range, thus enabling the respective

females to overcome their menstrual disorder without an increased synthesis. Alternatively, some of these underweight students might currently have had circulating leptin levels slightly above the respective levels at the time amenorrhea occurred.

At referral serum leptin concentrations of 28 patients with AN and 35 patients with BN ranged from 0.06 to 3.83 μg L^{-1} and from 0.77 to 28.43 μg L^{-1}, respectively (Figure 1). Overlapping of single anorectic and bulimic patients only occurred within a narrow range of leptin concentrations that centered around the value of 1.85 μg L^{-1} leptin as calculated in the student cohort. In our previous study all 18 non-pretreated adolescents with AN also had leptin levels below 1.85 μg L^{-1} upon referral for inpatient treatment.[15]

Among the 47 eating disordered females, who were not using oral contraceptives, the leptin level of 1.85 μg L^{-1} separated those patients who were amenorrheic at referral from those who reported bleeding episodes in the 3 months prior to admission (χ^2 4.9; $P < 0.05$). Three bulimic patients were amenorrheic upon admission (respective leptin levels: 0.77, 7.13 and 16.28 μg L^{-1}). The only two patients with AN who had a bleeding episode during inpatient treatment had referral leptin levels of 1.62 and 2.71 μg L^{-1}.

Because the clinical features of AN of the binge eating/purging type overlap with those of BN, amenorrhea in females who fulfill the DSM-IV weight criterion can be the key feature that distinguishes between the two diagnoses. Our findings suggest that leptin synthesis has an influence on the diagnosis of the respective eating disorder via its impact on menstruation status. The subdivision of patients with AN according to the restricting or binge eating/purging type did not bear any systematic relationship to leptin concentrations (Figure 1).

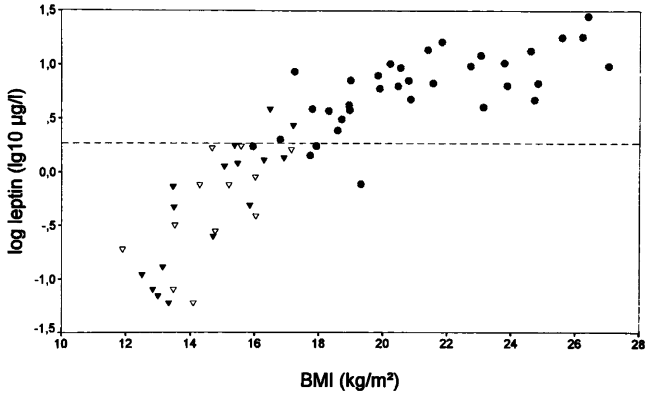

Figure 1 Relationship between transformed leptin ($\log_{10}$) concentrations at referral and body mass index upon admission of 63 female inpatients with eating disorders. Patients with bulimia nervosa (●); patients with the restricting (▽) and binge eating/purging (▼) type of anorexia nervosa. The log leptin concentrations of all 63 eating disordered patients at referral clearly correlated with their BMIs upon admission ($r = 0.81$; $P < 0.001$). The horizontal dotted line indicates the leptin level of 1.85 μg L^{-1} as calculated in the student cohort for lifetime occurrence of amenorrhea.

Low body weight, low percent body fat, weight loss, regular jogging activity, stressful life events and recent dieting have previously been identified as risk factors for amenorrhea.[7,8,9,17,18] It is important to realize that in the student cohort BMI, fat mass and percent body fat did not reliably predict lifetime occurrence of amenorrhea. The notion that fat mass or percent body fat directly plays a major role in transmitting nutritional status to the reproductive axis[19] has been rejected previously.[7] Apparently, a reduced leptin synthesis is the relevant mediator of some of the aforementioned risk factors.

In affluent countries eating disordered females might account for a considerable proportion of postmenarcheal women with leptin levels below 1.85 μg L^{-1}. Especially females with an endogenously low leptin synthesis might be prone to rapidly develop amenorrhea, if they restrict their energy intake. Some females might have more precipitous drops of leptin synthesis during fasting than others, thus predisposing them to amenorrhea. A reduced leptin synthesis possibly underlies amenorrhea in a subgroup of bulimic patients, who can have disturbances of follicular development, luteal dysfunction, reduced 24-h pulsatile gonadotropin secretion and diminished LH response to estradiol.[20,21] A diagnostic evaluation of leptin synthesis appears especially warranted in those amenorrheic or oligomenorrheic females who restrict their energy intake.

The relationships between mean $\log_{10}$ leptin levels and mean levels of LH, FSH, estradiol and progesterone, respectively, which were each based on the first 4-weekly determinations during inpatient treatment of those 47 eating disordered inpatients who were not taking oral contraceptives, are shown in Figure 2a–d. The patterns for LH, estradiol and progesterone (Figure 2a,c,d) were rather similar. Patients with mean $\log_{10}$ leptin concentrations below 1.85 μg L^{-1} clustered in the lower range of the mean concentration of the respective hormone; patients with mean $\log_{10}$ leptin levels above this value had mean hormone concentrations that showed no systematic relationship to mean $\log_{10}$ leptin levels. Overlapping of mean LH, FSH, estradiol and progesterone concentrations, respectively, occurred to a varying degree between females with mean leptin levels above and below 1.85 μg L^{-1} (Figure 2a–d). Individual mean BMIs of the same 47 patients as calculated from the first 4-weekly measurements of body weight and height during inpatient treatment were also set into relationship to mean LH, FSH, estradiol and progesterone concentrations (Figure 3a–d), respectively.

Among patients with AN the correlations between mean $\log_{10}$ leptin levels and mean LH, FSH, estradiol and progesterone levels were 0.68 ($P < 0.01$), 0.65 ($P < 0.05$), 0.48 ($P < 0.05$) and –0.14 ($P > 0.5$), respectively. The correlations between mean BMI and mean LH (0.64; $P < 0.01$), FSH (0.45; $P > 0.05$) and estradiol (0.46; $P < 0.05$) levels were slightly lower. No correlation was observed between mean BMI and mean progesterone levels ($r = 0.06$).

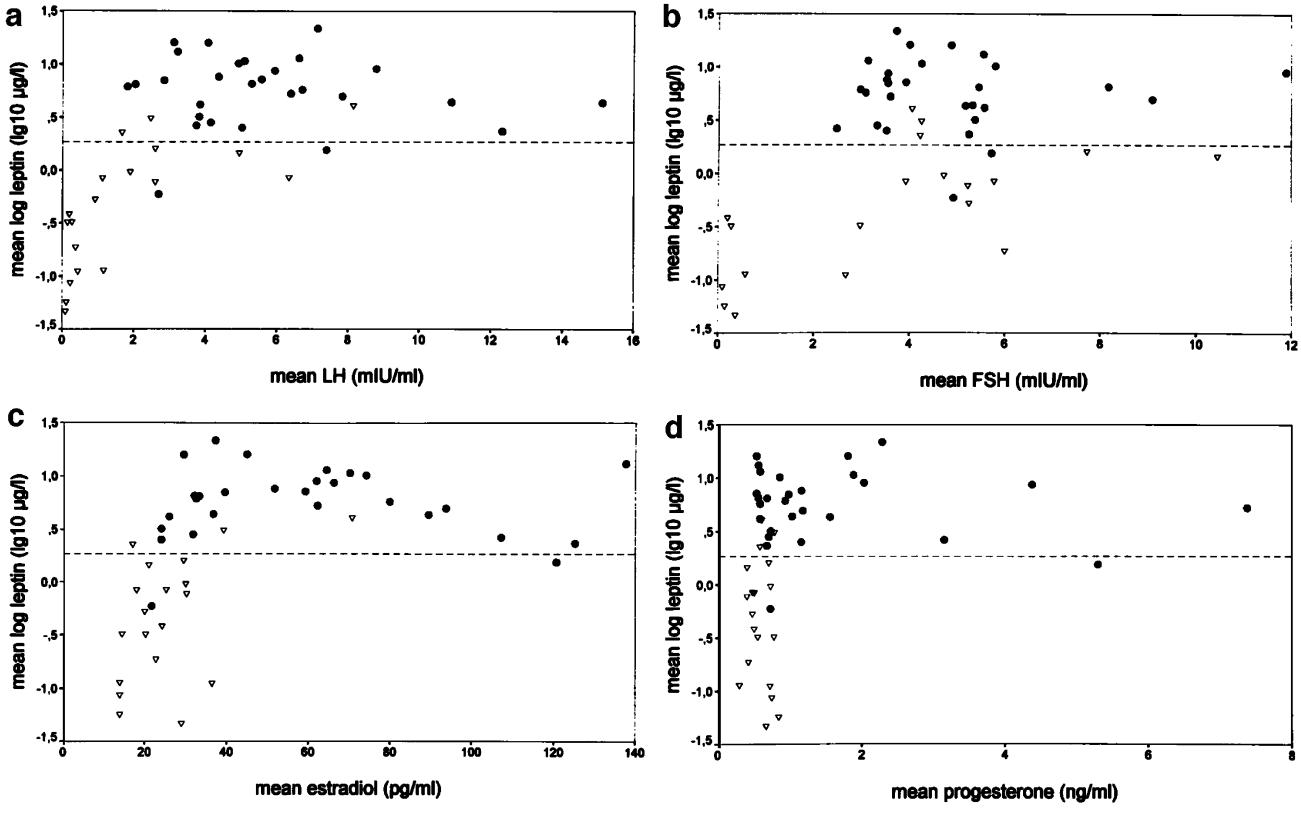

Figure 2 Relationships between mean transformed leptin levels (log_{10}) and mean concentrations of luteinizing hormone (a), follicle stimulating hormone (b), estradiol (c) and progesterone (d) in 28 patients with bulimia nervosa (●) and 19 patients with anorexia nervosa (▽). For every patient the mean concentration for each hormone was calculated from the respective first 4-weekly determinations during inpatient treatment. The horizontal dotted line indicates the leptin level of 1.85 $\mu g\ L^{-1}$ as calculated in the student cohort for lifetime occurrence of amenorrhea.

Because systematic relationships between mean log_{10} leptin levels above 1.85 $\mu g\ L^{-1}$ and mean concentrations of the pituary and gonadal hormones were not apparent (Figure 2a–d) in the eating disordered patients, leptin appears to function as a switch, which turns off the reproductive axis, if circulating leptin drops below a critical level. Based on our RIA this critical level is in the range of 1.85 $\mu g\ L^{-1}$. Because some of the students with slightly lower leptin levels were currently menstruating, the value of 1.85 $\mu g\ L^{-1}$ represents the upper limit of this range. Obviously, amenorrhea due to other pathogenetic mechanisms can occur in females with leptin concentrations well above 1.85 $\mu g\ L^{-1}$. In the light of the presence of leptin receptors in both hypothalamus and ovaries,[22,23] it is unclear how leptin might function as the switch regulating menstrual function. The assumed hypothalamic effect of leptin on secretion of gonadotropin releasing hormone and thus of FSH and LH is a prerequisite, but is not sufficient for normalization of ovarian function.

Theoretically, exogenous application of leptin should have beneficial effects on the disturbance of the reproductive axis in females with a reduced leptin synthesis. However, possible metabolic and psychological consequences of exogenous leptin application need to be evaluated before such a therapeutic trial can be attempted. Because leptin levels rise considerably above 1.85 $\mu g\ L^{-1}$ during weight gain in patients with AN without leading to rapid onset of menstruation, the physiological normalization obviously takes time and possibly involves additional factors.[15]

Materials and methods

Study cohort I As described previously,[24] a BMI equivalent to or below the 10th age-centile,[25] absence of somatic disease and a cigarette consumption below 10 cigarettes per day formed the inclusion criteria for the 43 underweight female students. They were reimbursed for their voluntary participation. Written informed consent was obtained and the study was approved by the ethics committee of the University of Marburg.

The students were blood sampled at 8 am after an overnight fast. Body height and weight were measured in light clothing. Bioelectrical impedance analyses were performed in all students. Using a semi-structured interview the students were probed as to the lifetime occurrence of amenorrhea of at least 3 months duration. Frequency of amenorrheic episodes were recorded. Episodes of oligomenorrhea lasting 6 or more months were additionally noted. The students were asked to recall their body weight at the time(s) these menstrual disorders set in. Students were also ques-

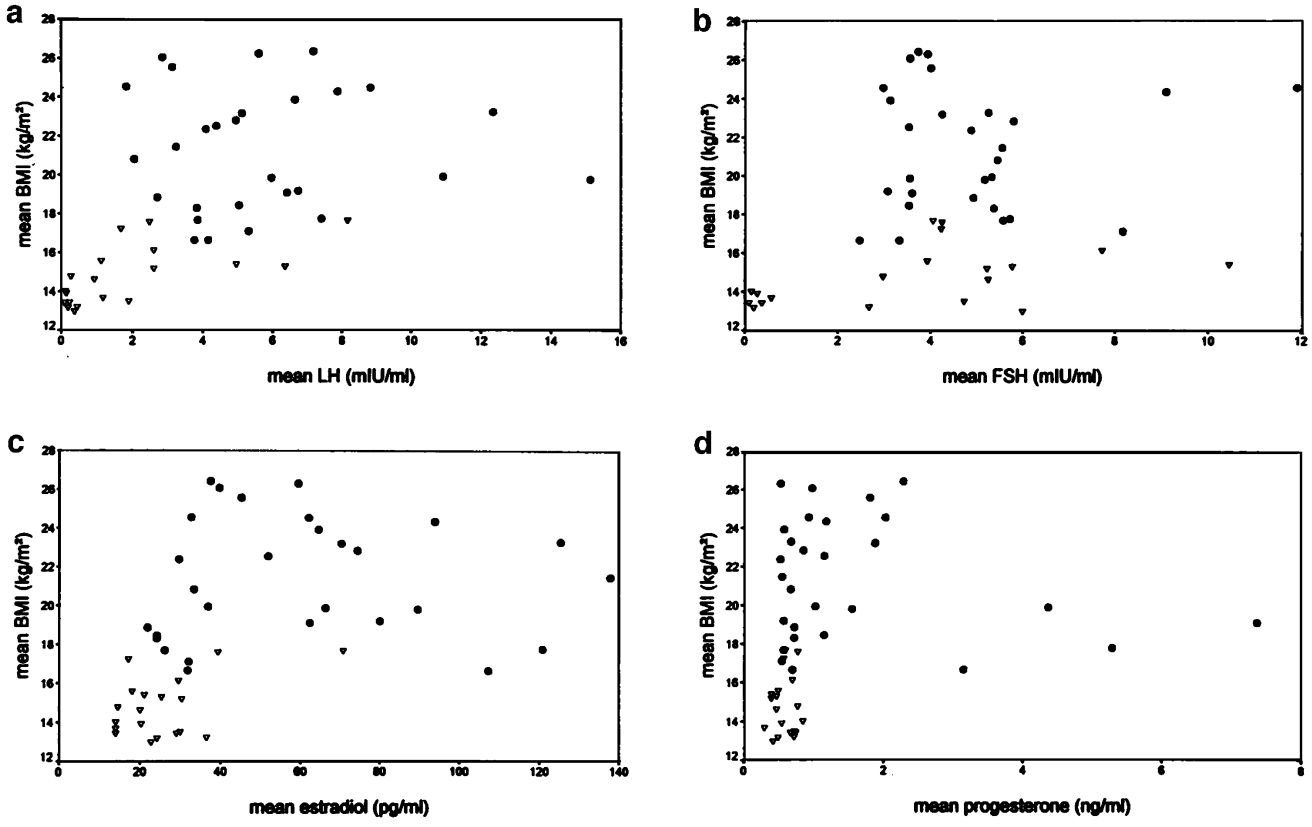

Figure 3 Relationships between mean body mass indexes (kg m^{-2}) and mean concentrations of luteinizing hormone (a), follicle stimulating hormone (b), estradiol (c) and progesterone (d) in 28 patients with bulimia nervosa (●) and 19 patients with anorexia nervosa (▽). For each patient the mean concentration for each hormone was calculated from the respective first 4-weekly determinations during inpatient treatment. Similarly, the mean body mass index was calculated from body weights measured during the first 4 weeks of inpatient treatment and height at referral.

tioned as to the present use of oral contraceptives. All students were screened with the eating disorders module of the updated version (kindly provided by Professor Wittchen, Munich) of the Composite International Diagnostic Interview,[26] thus allowing diagnoses of AN and BN and Eating Disorders Not Otherwise Specified according to the DSM-IV criteria.[16]

The mean age (± s.d.) of the students was 24.3 ± 3.8 years (range 18.7–34.6 years). The mean BMI (± s.d.) was 17.6 ± 0.7 kg m^{-2} (range 14.6–19.0). Body fat mass and percent body fat were calculated from measured resistance (BIA 2000-5; Data Input GmbH, Frankfurt, Germany) according to a gender-specific equation for underweight individuals.[27] Mean fat mass and percent body fat (± s.d.) were equivalent to 9.5 ± 1.6 kg (range 4.6–14.3) and 18.8 ± 3.1% (range 12.1–27.5), respectively.

Study cohort II Eighty-two inpatients with eating disorders were consecutively ascertained at the Department of Psychosomatics of the Medical Center Benjamin Franklin, Free University of Berlin, between June 1991 and September 1995. Exclusion criteria were diabetes mellitus, discharge prior to 4 weeks of inpatient treatment and lack of consent as required by the ethics committee of the University. Based on the respective clinical charts the remaining 63 patients were retrospectively classified according to the DSM-IV criteria prior to measurement of leptin levels. Twenty-eight patients had AN (12 with the restricting and 16 with the binge eating/purging type, respectively). All 35 patients with BN had the purging type.

The mean ages (± s.d.) of patients with AN and BN were 22.5 ± 4.8 and 24.5 ± 6.4 years, respectively. Mean referral BMIs (± s.d.) of the anorectic and bulimic patients were 14.7 ± 1.5 and 21.1 ± 3.0 kg m^{-2}, respectively. A total of 16 patients were using oral contraceptives at referral (nine with AN and seven with BN). By definition all patients with AN had been amenorrheic for at least 3 months prior to referral. Three patients with BN had not had a bleeding episode during the 3 months prior to referral (BMIs at referral between 19.0 and 21.8 kg m^{-2}). None of the three bulimic patients with BMIs below 17.5 kg m^{-2} was amenorrheic upon referral.

Patients were blood sampled at 8 am after an overnight fast on a weekly basis during inpatient treatment. Serum samples were frozen at –80°C prior to determination of hormone levels. Estradiol, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by fluoroimmunoassays (Delfia®, Wallac, Turku, Finland). Sensitivity was 13 pg

ml^{-1}, 0.25 ng ml^{-1}, 0.05 mIU ml^{-1} and 0.05 mIU ml^{-1}, respectively. Inter- and intraassay coefficients of variation were all below 10%. Leptin levels were measured using a sensitive radioimmunoassay as described previously.[28] Sensitivity of undiluted samples was 0.03 μg L^{-1}. Inter- and intra-assay coefficients of variation were 8.5 and 0.8%, respectively. The serum leptin levels of all 63 eating disordered patients at referral were set into relationship to the corresponding BMIs (Figure 1).

Due to the fact that in this retrospective part of our study blood sampling during treatment had been performed on a weekly basis only, serum levels of leptin, estradiol, progesterone, FSH and LH could only be determined accordingly. In order to assess the relationship between serum leptin levels and the serum levels of the gonadal and pituary hormones (Figures 2 and 3), the mean level of each hormone was calculated for the first 4 weeks of inpatient treatment, thus covering the length of a normal menstrual cycle. Only those 47 females (19 AN; 28 BN) who were not using oral contraceptives were included in these analyses.

Because serum levels of estradiol, progesterone, FSH and LH are subject to considerable variation depending on the phase of the menstrual cycle, it would obviously have been advantageous to base calculations of mean levels for each hormone on more frequent determinations within the 4-week period. This consideration only applies to those 25 females with bulimia nervosa, who were not amenorrheic upon admission and who were not using oral contraceptives. We refrained from evaluating hormone levels at later time periods of the 12-week treatment program, because leptin levels rose considerably in some patients with AN who concomitantly gained weight. In both anorectic and bulimic patients the BMI differences between values at referral and at week 4 were negligible (–0.16 and 0.08 kg m^{-2}, respectively). The corresponding differences of leptin levels were –0.14 and 0.65 μg L^{-1}, respectively.

The predictive value of leptin, BMI and percent body fat, respectively, for lifetime amenorrhea in cohort I was evaluated using a maximum χ^2 approach with $\epsilon = \frac{1}{4}$.[29] This approach takes multiple testing of simple 2×2 contingency tables into account. The usual χ^2 test for 2×2 contingency tables was applied to current amenorrhea in cohort II in order to test the accuracy of the predictors found in cohort I. Due to the retrospective character of this part of our study we were not able to incorporate information pertaining to past episodes of amenorrhea in the eating disordered patients (cohort II).

In all scatterplots a logarithmic transformation ($\log_{10}$) was performed for leptin levels, in order to obtain a resolution of the extremely low values observed in AN. This transformation has also been used by other investigators. However, the other variables analyzed in this study are not customarily transformed. Accordingly, non-transformed values for these variables were used.

For comparisons of mean values the two sample two-sided *t*-test was applied. Pearson correlation coefficients were calculated and the corresponding tests were performed.

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