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ORIGINAL ARTICLE

Anorexia nervosa, osteoporosis and circulating leptin: the missing link

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Abstract
Summary Methods: Leptin levels were measured in 103 consecutive women with anorexia nervosa. Results: Spine BMD and Z-score values were found to be significantly lower in the low tertile compared with the highest tertile. Duration of amenorrhea and leptin level accounted for 27% of the variance in lumbar spine BMD.
Introduction The purpose of this study was to assess leptin levels and other biological variables in a population of anorexia nervosa patients.
Methods Leptin levels were measured consecutively in 103 women with anorexia nervosa (AN) with a mean age of 24.9±7.4 years. Osteodensitometry was also performed by dual energy X-ray absorptiometry (DXA).
Results Spine bone mineral density (BMD) and Z-score values were found to be significantly lower in the low tertile compared with the highest tertile. Duration of amenorrhea and leptin level accounted for 27% of the variance in lumbar spine BMD. The mean leptin level was 3.9±4.6 ng/mL (normal values, 3.5-11 ng/mL). The distribution of leptin values was not a Gaussian distribution, and a log-transformed was therefore performed. A significant correlation was found between leptin level and spinal BMD (r=0.3; p=0.002); significant correlations were observed for both femoral neck and total hip BMDs. When leptin level values were divided into tertiles, spine BMD and Z-score values were found to be significantly lower in the lower tertile (p=0.04 and p=0.02) compared with the highest tertile. For femoral neck BMDs, the T-score was slightly lower between low and high tertile, but the difference was not statistically significant (p=0.07). When multivariate analyses were performed, two independent factors which could possibly account for the variance in spinal BMDs were found. Duration of amenorrhea and leptin level accounted for 27% of the variance (p<0.0001).
Conclusion The mechanisms underlying bone loss in AN patients remain unclear and complex, involving hypoestrogenia as well as nutritional factors such as insulin-like growth factor and leptin.

Keywords Anorexia nervosa · Leptin · Osteoporosis

B. Duquesnoy passed away after the submission of this article.

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Introduction

The pathophysiological mechanisms which account for the relationship between weight, fat tissue and bone tissue are increasingly better understood. Leptin, an anorexigenic protein produced mainly by fat tissue seems to exert a controlling effect, centrally, via the sympathetic nervous system. This hypothesis is supported by experimental data suggesting that bone cell function is under neuronal control [1]. However, the data on the effect of leptin on bone tissue

are highly contradictory, and it may be that its action varies according to bone site, serum leptin level, skeletal maturation and nervous pathway [2].

Over the last few years, anorexia nervosa (AN) has become a serious public health issue in industrialised countries. The disorder carries a high rate of morbidity, with osteoporosis being one of its major complications, occurring in 20-30% of cases depending on the groups observed [3–5]. The mechanisms underlying this process of bone loss are complex, and few studies have been carried out on the role of leptin in this disorder.

Amenorrhea is a diagnostic criterion for anorexia nervosa and oestrogen deficits have been reported as a major aetiological factor for bone loss in this population. Hypoestrogenia alone cannot account for the loss in bone mass observed in anorexia patients [6, 7]. However, other factors are involved in this bone loss and nutritional factors in particular seem to play a major role. The role of the latter has been confirmed by several authors who have found significant correlations between bone mineral density (BMD) and nutritional indices such as body mass index (BMI), lean body mass, fat mass, insulin-like growth factors (IGF)-1 and leptin [5, 8, 9]. The respective roles of these biological variables were not clearly assessed, however.

The purpose of this study was to assess the level of circulating leptin in a population of anorexic patients—in whom, by definition, fat mass is low—and to examine the correlations between this biological parameter and bone loss in the study population.

Materials and methods

The first step was to build a survey of patients with AN. For these patients, we measured several clinical and biological variables. Also, and obviously, we measured BMD since it was one of the reasons why the patients were recruited from the department of psychiatry (second step). Thereafter, we studied the relationships between these variable and bone status assessed by the measurement of markers of bone remodelling and BMD (third step). Finally, we determined (objective of the present study) the role of leptin in the assessment of bone status by using particularly simple, but also multiple regression analyses

Study population

One hundred and three anorexia nervosa patients with current anorexia nervosa were consecutively included in the study. The patients were systematically recruited from the Department of Psychiatry (JV) and were evaluated at the Rheumatology Department in Lille between March 2004 and June 2008. Mean duration of illness was 5.9±6.3 years. Mean age at the onset of the illness was 19±5.1 years. The diagnosis was made with reference to Diagnostic and Statistical Manual of Mental Disorders- fourth edition criteria. Sixty-three patients were found to have pure restrictive anorexia, while 40 patients exhibited a mixed form of the disorder (anorexia and bulimia). Mean duration of amenorrhea was 2.9±4.6 years, with a mean age at onset of 20.3±7.4 years. Three patients exhibited primary amenorrhea.

We recorded patients' weight and height and determined, by interview, their risk factors for osteoporosis, their fracture history and the types of treatment they had received, particularly oral contraceptives.

Biological data

Blood calcium and phosphate was determined (calcemia, phosphatemia, alkaline phosphatases and 24-h calciuria), as were 25-OH-D3 and parathyroid hormone (PTH) levels. Bone remodelling markers (osteocalcin and bone alkaline phosphatases, for bone formation, and serum crosslaps or C-telopeptides (CTX) and procollagen type I C terminal telopeptide (ICTP) for bone resorption) were assessed. Sera were stored at −80°C until use. Radioimmunological assays were used to measure osteocalcin (Cis-Bio International, Gif-sur-Yvette; normal values, 5.2–34.5 ng/mL) and ICTP (Orion Diagnostica, Espoo, Finland; normal values, 1.8–5.2 ng/mL) levels. Bone alkaline phosphatases were determined using a human-specific immunoradiometric method (Hybritech, Inc., Dan Diego, CA, USA; normal values, <20.5 ng/mL). Serum CTX was measured using an immunoenzymological method (ELISA; serum CrossLaps One-step, Osteometer Biotech, Herlev; normal values, 270–3,270 pmol/L). We also assessed follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, 8 AM cortisol, free urinary cortisol, TSH, T3, T4 and prolactin, as well as nutritional factors such as IGF-1, IGF BP3 and leptin (Labodia, Yens, Suisse, Radioimmunoassay). Normal values were 3.5-11 ng/mL. The limit of sensitivity for the human leptin assay was 0.5 ng/ml (100 µl sample size). Within and between assay variations were 3.4–8.3% for within percentage CV and 3.6–6.2% for between percentages CV. All samples were collected from fasting patients between 8 AM and 10 AM.

Measurement of BMD

Lumbar spine and hip bone mineral densities were determined by dual energy X-ray absorptiometry using a HOLOGIC QDR 2000 or Hologic discovery. The coefficient of variation was 1% for lumbar spine measurements and 2% for hip measurements.

Statistical analysis

Results were expressed as mean±standard deviation. The hypothesis of a normal distribution for leptin levels was assessed by using a Kolmogorov-Smirnov test. At a p level <0.20, the distribution was not Gaussian. Therefore, a logarithmic transformation was performed that permitted to obtain, in this condition, a normal distribution. Two types of statistical analyses regarding leptin levels were then performed. When raw data were taken into account due to the absence of Gaussian distribution, Kruskal Wallis or Mann–Whitney U test were used for comparing leptin levels in subpopulations of patients with AN. By contrast, for the other statistical analyses, particularly those needing adjustment (covariance analysis) and for regression analyses, we used log-transformed leptin levels since, in this condition, the distribution was a Gaussian distribution.

Correlations were sought (by simple linear regression analysis, and then by polynomial regression analysis, if necessary) between bone mineral density and log-transformed leptin level. Stepwise multiple linear regression analysis was then used when the statistical significance obtained by simple linear regression was <0.2. The purpose of these analyses was to try to determine the factors which could account for the variance in BMD. For all the other statistical analyses, $p<0.05$ was considered as statistically significant.

Results

Characteristics of patients

Patients' mean age was 24.9±7.4 years. Weight and BMI were 42.3±8 kg and 15.4±2.9 kg/m^2, respectively. Twelve patients (11.6%) had been taking or had taken some form of oral contraceptive and 24 patients (23.3%) had a previous history of bone fracture, with the most common fractures occurring in toes (seven cases), ankle (four cases), wrist (three cases) and ribs (two cases). All fractures were confirmed by visualisation of the X-ray report. One patient had had vertebral fractures (L1 and L4). Four patients had had at least two fracture events. Other risk factors for osteoporosis included the ingestion of more than 20 g of alcohol per day (five patients) and tobacco addition (42 patients). None of the patients had received long-term oral corticotherapy (Table 1).

Biology

Biological assay results are shown in Tables 1 and 2. Mean leptin was 3.9±4.6 ng/ml (normal values, 3.5-11 ng/mL).The frequency of distribution for leptin levels are reported on Fig. 1. The graph shows that leptin levels are not normally distributed since the values are skewed to the right (positively skewed). Mean values of blood and urinary calcium and phosphate assays were in normal range. Mean vitamin D was 26±15.6 ng/mL, with 44 patients displayed low vitamin D levels (<20 ng/ml). Mean bone remodelling marker levels were as follows: osteocalcin, 17.59±11.6 ng/mL; cross-laps, 6129.4±3515.1 pmol/L; ICTP, 7.4±7.5 µg/L; bone alkaline phosphatases, 12.2±7.2 µg/L. Blood estradiol was often low, with a mean of 19.7±28.7 pg/mL. FSH and LH were also low (3.4±3.4 UI/L and 2.1±3.7 UI/L, respectively). Mean blood prolactin was 11.9±10.7 ng/mL, mean IGF-1 was 0.9±0.4 UI/mL and mean IGF-BP3 was 2.9±2.4 mg/L. Thyroid function assays revealed low T3 levels in eight patients (7.8%). High 8 AM cortisol values were observed in 31

Table 1 Clinical and biological parameters of the study population (normal range)

	Anorexic
Age (years)	24.9±7.3
Weight (kg)	42.3±8
Height (cm)	164.9±6.4
BMI	15.4±2.9
Osteocalcin ng/mL (5.2–34.5)	17.5±11.6
Cross laps pmol/L (270–3,270)	6126.4±3515.1
ICTP µg/L (1.8–5.2)	7.5±5.5
bAP µg/L (<20.5)	12.2±7.2
Prolactin ng/mL (<22)	11.9±10.7
IGF-1 UI/mL (0.75–2.4)	0.9±0.4
IGF-BP3 mg/L (3.3–9.2)	2.9±2.4
Leptin ng/mL (3.5–11)	3.9±4.6

Table 2 Patients' biological parameters (normal range)

	Patients
Calcemia mg/L (85–105)	93.6±4.8
Phosphatemia mg/L (25–45)	37.1±5.6
Ionised calcium mg/L (46,8–52)	50.5±1.9
Calciuria mg/24 h (100–300)	132.8±92.9
25-OH-D3 ng/mL (20–80)	26±15.6
PTH pg/mL (12–65)	36.9±18.2
THS µUI/mL (0,4–3,6)	1.3±0.7
T3 pmol/L (3,8–5,8)	3.5±1
T4 pmol/L (8,6–15,1)	15.1±3.6
8 AM cortisol µg/100 mL (9–22)	20.1±7.6
FUC µg/diuresis (20–110)	59.4±37.4
FSH UI/L (1–9,5)	3.4±3.4
LH UI/L (0,1–77)	2.1±3.7
Estradiol pg/mL (21–649)	19.7±28.7

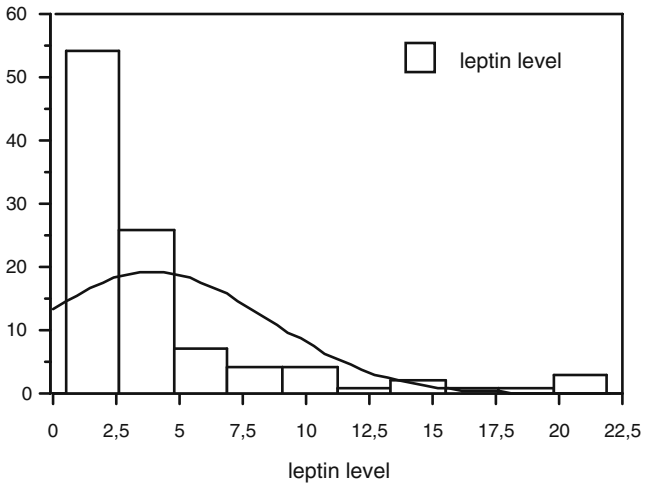

Fig. 1 Frequency distribution of leptin level with the expected frequency curve for a normal distribution. This graph shows that leptin levels are not normally distributed. The values are skewed to the right (positively skewed). The *y* axis represents the percentage of patients with a given level of leptin. The *x* axis represents the level of leptin (in ng/ml)

patients (30%) and high free urinary cortisol values in six of them (5.8%). Elevated prolactin levels were observed in eight patients (7.7%), while low somatomedin values were found in 23 patients (22.3%).

BMD measurements

Bone mineral density was normal in 30 patients (29.1%; Table 3). Osteoporosis at least at one site was observed in 23 patients (22.3%) and osteopenia in 50 patients (48.5%). Mean spine bone mineral density was 0.89±0.14, with a mean Z-score of −1.17±1.3, and a mean T-score of −1.05± 1.58. Mean hip BMD was 0.78±0.12, with a mean Z-score of −1.33±0.89, and a mean T-score of −1.19±1.08. Mean femoral neck BMD was 0.72±0.12, with a mean Z-score of −1.11±1.13, and a mean T-score of −1.31±1.19.

BMD was compared according to fracture status. BMD was not significantly different between AN patients with and without a past history of fracture. The comparison was also done after excluding the seven AN patients with a past history of toe fracture that is not considered as a fragility fracture. In this condition, we found at the femoral neck only a slight difference for BMD (p=0.049): 0.66±0.15 g/cm^2 versus 0.73±0.11 g/cm^2.

Table 3 Densitometric data for the study population

	Anorexic
Z-score spine	−1.17±1.31
T-score spine	−1.05±1.58
Spine BMD g/cm^2	0.89±0.14
T-score neck	−1.31±1.19
T-score total hip	−1.19±1.08
Neck BMD g/cm^2	0.72±0.12
Total hip BMD g/cm^2	0.78±0.12

Correlations were sought between log leptin and the various parameters. Log leptin did not correlate with age (p=0.48), age at onset of AN (p=0.35), duration of AN (p= 0.95) or age at onset of amenorrhea (p=0.06). With duration on amenorrhea, however, a correlation was observed (r= 0,21; p=0.03). Log leptin did not correlate with BMI (p= 0.09), but it did with weight (r=0.25; p=0.01). As far as the biological parameters were concerned, no correlations were found with 25 OH D3, PTH, bone remodelling markers, thyroid hormones, plasma cortisol, or free urinary cortisol and prolactin. Correlations were observed with FSH (r= 0.25; p=0.01), LH (r=0.38; p=0.001) and estradiol (r=0.24; p=0.02). However, there was no correlation with IGF=1 (p= 0.43) or IGF-BP3 (p=0.64)

Correlations were also sought between log leptin and BMD using simple linear regression analysis. A statistically significant correlation was found between log leptin and spinal BMD (r=0.3; p=0.002); significant correlations were also observed for femoral neck and total hip BMDs (r=0.23, p=0.02, and r=0.21, p=0.03, respectively)

Given the shape of the graph, polynomial (second degree) regression analysis was performed. Spinal BMD values were found to slightly better correlate with log leptin (r=0.33; p=0.005), unlike hip BMD values, for which no significant correlation was observed.

When multivariate analyses were performed, two independent factors which could possibly account for the variance in spinal BMDs were found. Duration of amenorrhea and log leptin level accounted for 27% of the variance (p<0.0001). Using another model (not including leptin), we were able to identify three other factors, i.e., duration of amenorrhea, weight and total alkaline phosphatases. These accounted for 55.3% of the variance (p<0.0001). Duration of amenorrhea and weight accounted for 33.9% of the variance (p<0.0001) in femoral neck BMD. Duration of amenorrhea and weight also accounted for 38.6% of the variance (p<0.0001) observed in total hip BMD. Thus, duration of amenorrhea seems to be one of the major factors accounting for the variance in BMD, whether in the lumbar spine or the hip. We also found a correlation between spinal BMD and serum leptin, but were unable to find a model in which leptin accounted for the variance in BMD in the hip or femoral neck.

Also, we studied the subgroup of 63 patients with pure restrictive AN. Although this population seems to be more homogeneous, the results were not relevant. Indeed, we have done the same analyses compared with those done in the whole cohort and we did not find any satisfying model for predicting BMD values. Thus, in the first model

including log leptin and duration of amenorrhea, we explained only 13.5% in the variance of lumbar spine BMD (log leptin was not retained in this model). In the same manner, in the second model including duration of amenorrhea, weight and total alkaline phosphatases we explained only 12.7% in the variance of lumbr spine BMD (only weight was considered in this model to be relevant). Finally, in this subpopulation, no other model was robust enough to be retained. Also patients with restrictive AN were not different from other AN patients in terms of BMD, BMI, fracture status and biological status (data not shown).

We then sought to compare leptin level according to bone density. Although there is no clear definition of osteoporosis in this population of patients with AN, arbitrarily, we chose the dual energy X-ray absorptiometry (DXA) definition of osteoporosis according to the World Health Organisation classification that is only validated for post-menopausal women. Therefore, we divided the patients into three groups: normal BMD, osteopenic and osteoporotic. Patients with normal BMDs had the highest leptin levels, osteoporotic patients had the lowest and osteopenic patients had intermediary levels (Fig. 2). A significant difference was observed between the first two groups alone: the patients with normal BMDs were found to have significantly higher log leptin levels than the osteoporotic patients (p=0.04). However, when adjustments were made for weight and duration of amenorrhea, the difference was no longer significant, although it did remain significant when an adjustment was made for duration of AN.

We also divided leptin levels into tertiles, with the first tertile ranging from 0.5-7.5 ng/ml, the second from 7.5-14.6 ng/ml and the third for values in excess of 14.6 ng/ml. Spine BMDs and Z-scores were significantly different according to the tertile of leptin (p=0.01 for both comparison). Also, spine BMDs and Z-scores were significantly lower in the lower tertile (p=0.04 and p=0.02, respectively; Fig. 3) than in the higher tertile. Moreover, the difference remained significant after adjustments were made for age (p=0.005), weight (p=0.04), BMI (p=0.02), duration of anorexia nervosa (p=0.03), age of onset of anorexia nervosa (p=0.009) and duration of amenorrhea (p=0.03), but not after being adjusted for age of onset of amenorrhea (p=0.06). After making several adjustments (for age, BMI and duration of AN), first tertile values remained significantly lower than third tertile values (p=0.01).

There was no significant difference in total hip BMDs across the three groups. After adjusting for age, the difference between the first and third tertiles (log leptin) was found to be significant (p=0.04). When the results were expressed as Z-scores, there was no significant difference between the three groups (Fig. 4).

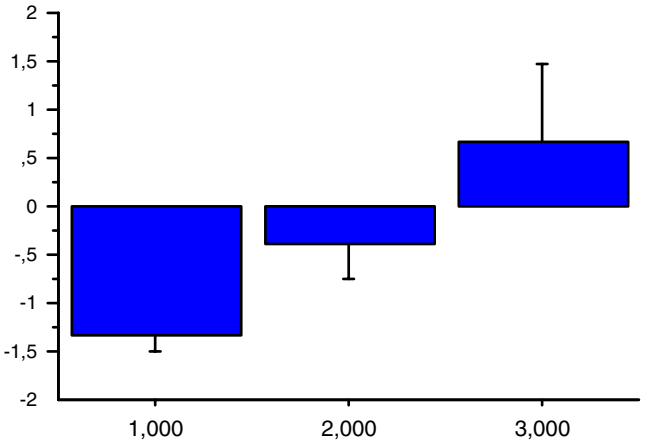

Fig. 3 Lumbar spine Z-scores (y axis) according to leptin levels (distributed in tertiles, x axis): p for trend=0.007

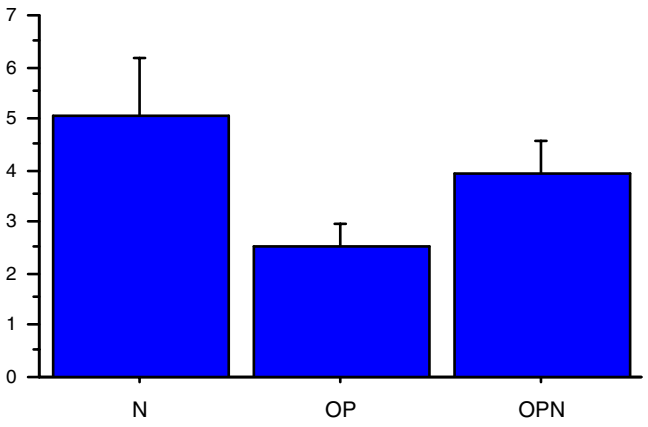

Fig. 2 Leptin level (y axis in ng/ml) according to densitometric status (N, OPN, OP). N vs OP: p=0.04. *N* normal women, *OP* osteoporotic women, *OPN* osteopenic women

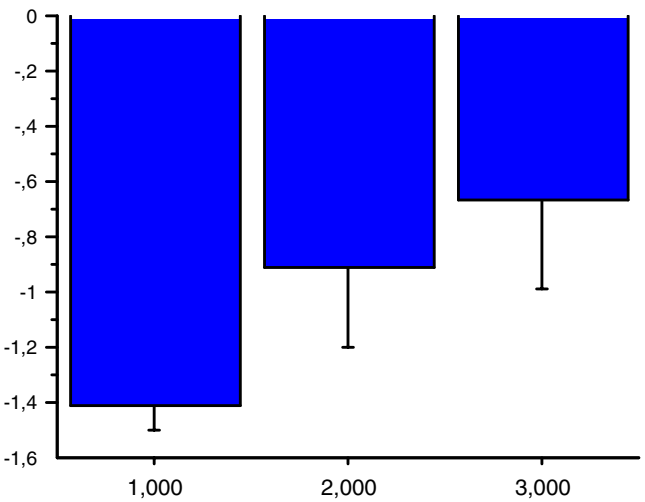

Fig. 4 Total hip Z-scores (y axis) according to leptin levels (distributed in tertiles, x axis): p for trend=0.07

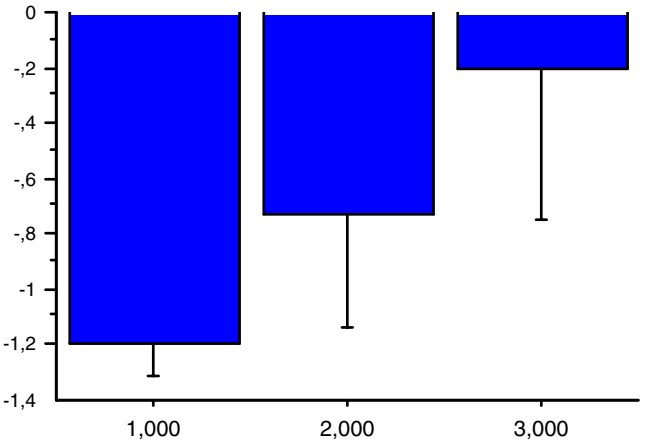

Fig. 5 Femoral neck Z-scores (*y* axis) according to leptin levels (*x* axis, distributed in tertiles): *p* for trend=0.09

For femoral neck BMDs, the T-score was slightly lower between group 1 (first tertile) and 3 (third tertile), but the difference was not statistically significant (p=0.07). There was no significant difference between the three groups when the results were expressed as BMD (p=0.08) or as Z-scores (p=0.13; Fig. 5). After adjustments were made, the differences were never significant.

Discussion

Leptin is a hormonal polypeptide secreted by adipocytes. Over the last few years, several authors have demonstrated the relationship between bone tissue, weight and sex hormones. Leptin receptors have been identified in the hypothalamus. Leptin-deficient mice (ob/ob) or leptin-receptor deficient mice (db/db) have been found to be obese and hypogonadal [1]. However, while one would have expected these mice to be osteoporotic, on account of their hypogonadism, they exhibited a high bone mass phenotype, reflecting the link between bone mass, weight and sex hormones. Leptin seems to act via the sympathetic nervous system, and neuroreceptors have been observed on osteoblasts [10]. Leptin seems to have a bimodal effect on bone tissue, with low serum levels having a somewhat positive effect, and high levels having a negative effect.

In the literature, a few authors have measured leptin levels in anorexia nervosa patients. The leptin levels were generally low [11–13], especially during the active phase of the disease. A loss of the daily cycle, and particularly the night time peak have also been reported [14, 15]. In our study, we also found low serum leptin levels in our patients. Uzum et al., on the other hand, reported a high leptin-to-fat mass ratio in their AN patients [16]. Misra et al. observed low values in their population, but fat mass was determined by impedance measurement in the first study and by DXA in the second [11].

We found no correlation between leptin and BMI, but we did find a correlation between leptin and weight. In another study, Gati et al. compared leptin levels in 56 patients with eating disorders with a control group (22 patients), and observed low leptin values only in the anorexia nervosa group, with a correlation to BMI. In the control group, and in patients with bulimia, no correlation was found between leptin level and BMI [17].

However, a positive correlation was observed between leptin level and sex hormones in our patients, highlighting the link between fat mass and sex steroids. Several authors have reported a parallel increase in LH and leptin during weight recovery in anorexic patients [18]. The normalisation of leptin levels is indispensable for menstrual cycle recovery in these patients. This explains why, when serum leptin remain low, amenorrhea persists, despite weight recovery. In a previous study, eight patients [19] exhibiting hypothalamic amenorrhea caused by intense physical exercise or weight loss, were treated with leptin for 3 months. Their initial leptin levels were low (mean: 3 ng/ml) but had risen by the end of the treatment. The patients had lost weight, mainly by loss of fat mass. Three of the patients recovered their menstrual cycles, exhibiting a significant increase in LH and estradiol levels at 3 months. Their IGF-1 and osteocalcin levels were also significantly higher in the third month. These observations illustrate leptin's ability to stimulate bone formation and sex steroids.

We failed to find a correlation between leptin and IGF-1, bone markers or thyroid function in our study.

However, we did find a significant correlation between spinal BMD and leptin level (r=0.3; p=0.002). The correlation was also significant for femoral-neck and total-hip BMDs (r=0.22, p=0.02 and r=0.21, p=0.03, respectively). Some authors have studied the relationship between circulating leptin and BMD in patients with anorexia nervosa, but their findings are sometimes contradictory and their study populations small. There seems to be a significant positive correlation between serum leptin and BMD in adult menopausal women [20] and to a lesser extent in men [21]. However, only serum leptin, and not fat mass leptin, was significantly lower in women with fractures as opposed to women without fractures. Where anorexia nervosa is concerned, other authors have found, as we have in our study, a correlation between BMD—particularly spinal BMD—and leptin, but also insulin, FT [3] and cortisol [22].

Multivariate analysis was performed to identify factors which could account for the variance in BMD. At the spine, two independent factors (duration of amenorrhea and leptin level) accounted for 28% of the variance in BMD (p< 0.0001). However, three other factors, i.e., duration of

amenorrhea, weight and total alkaline phosphatases, were found to account for 55.3% of the variance ($p<0.0001$). At the hip, on the other hand, leptin did not seem to account for the variance in BMD, and at the femoral neck, duration of amenorrhea and weight accounted for 33.9% of the variance ($p<0.0001$). Duration of amenorrhea and weight also accounted for 38.6% ($p<0.0001$) of the variance in total hip BMD. Thus, while duration of amenorrhea remains a major factor in accounting for the variance in BMD, whether at the spine or hip, leptin also seems to be a predictive factor of spinal BMD. In a study involving 17 anorexia nervosa patients, Misra et al. [23] measured several nutritional factors (leptin, adiponectins, GH, IGF-1, cortisol, ghrelin and YY peptides), as well as blood estradiol, and studied their correlations with BMD. The results were compared with 19 controls. Leptin correlated with BMD at the three sites. They also found an inverse correlation between leptin and OPG. When multiple regression analysis was performed, the variance in BMD correlated negatively with adiponectins in both the AN and control groups. In the AN group, leptin, lean body mass and adiponectins were independent variables which could account for the variance in BMD throughout the entire body ($r^2=0.74$). The authors also reported another correlation between lean body mass, YYP, leptin, ghrelin and oestrogens, and hip BMD ($r^2=0.84$). However, in a previous study [11], involving 23 anorexic patients and 21 controls, the same authors studied the predictive factors of BMD (ghrelin, IGF-1, cortisol, oestrogens, leptin). While ghrelin seemed to be an important factor in both groups, leptin was found to be a predictive factor of spinal and hip BMD only in healthy patients, as was the case with cortisol. It is difficult to draw definitive conclusions at these two studies owing to the low number of patients evaluated compared with our own study.

In a previous study, we had already highlighted the fact that duration of amenorrhea played a major role in accounting for the variance in BMD in both spine and hip, but IGF-1 also seemed to play a role in determining bone mineral density in the hip, while blood estradiol played a greater role in the spine [5]. In our study, we observed a significant correlation between leptin and blood estradiol. Thus, these two nutritional factors (leptin and IGF-1) could have preferential bone-tissue targets for action: the hip for IGF-1, and the spine for leptin.

This study has its limitations. There were no control groups, and we only assessed leptin levels and not those of digestive hormones–ghrelin and YY peptides in particular, which had already been studied in previous works, and which also seem to be involved in changes in bone mass. It also has several strengths: the high number of patients evaluated and thorough biological evaluation. Moreover, AN is a heterogeneous disease. Therefore, we also analysed the subpopulation of AN patients with a pure restrictive disease. However, overall in this subpopulation, results were poorer compared with the whole population.

In conclusion, we were able to demonstrate that leptin is a predictive factor of spinal BMD in anorexia nervosa patients. A significant correlation was also found between leptin and estradiol, confirming the relationship existing between bone tissue, fat tissue and sex hormones. Further work would be necessary to gain a better understanding of the mechanisms underlying the interaction between these various organs and the numerous mediators which have an effect on bone mass, with a view to improving the treatment of anorexia nervosa patients.

Conflicts of interest None.

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