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Original article

Adipokines and bone status in a cohort of anorexic patients

Isabelle Legroux-Gérot[a,b,*], Jean Vignau[c], Odile Viltart[d], Pierre Hardouin[b], Christophe Chauveau[b], Bernard Cortet[a,b]

[a] Department of rheumatology, hôpital Roger-Salengro, CHRU de Lille, 59000 Lille, France
[b] EA4490, PMOI, pathophysiology of inflammatory bone diseases, université de Lille, université Littoral Côte d'Opale, 59000 Lille, France
[c] Department of addiction medicine, hôpital Fontan, CHRU de Lille, 59000 Lille, France
[d] Inserm, UMR-S1172, Jean-Pierre-Aubert research center neurosciences and cancer (JPArc), université de Lille, CHU de Lille, 59000 Lille, France

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ABSTRACT

Introduction: Bone loss in anorexia nervosa (AN) is multifactorial; its mechanisms are not yet clearly understood and may vary depending on disease duration and severity. To determine to what extent adipokines may be involved in the bone alterations found in anorexic patients, we evaluated plasma levels for leptin, adiponectin and Pref-1 against other clinical and biological parameters in a population of anorexic patients split according to weight and bone status.

Methods: Plasma concentrations of leptin, total adiponectin, high molecular weight (HMW) adiponectin, and Pref-1 were measured. The ratio of HMW adiponectin to total adiponectin — HMW (percentage) — was calculated. We divided our population into 5 groups with different phenotypes characterizing the severity of the disease and/or the severity of bone involvement: 1 – Normal BMD and body mass index (BMI): recovery from AN; 2 – Osteopenia ($-2 < \text{Z-score} < -1$) and BMI > 17 kg/m^2; 3 – Osteopenia and BMI ≤ 17 kg/m^2; 4 – Osteoporosis (Z-score ≤ −2) and BMI > 17 kg/m^2; 5 – Osteoporosis and BMI ≤ 17 kg/m^2.

Results: The study involved 80 anorexia nervosa patients. Mean BMI was 16.8 ± 2.4 kg/m^2. No significant difference was found in total and HMW adiponectin plasma concentrations between the 5 groups. HMW (percentage) was significantly higher in group 5 compared to group 1. Leptin was significantly lower in groups 3 and 5 compared to the other groups. For the whole group femoral neck and hip BMD correlated negatively with total adiponectin and HMW adiponectin. No correlation was found between BMD (whatever the site) and plasma leptin. Multivariate analysis revealed that 2 factors — leptin and BMI — explained 10% of the variance in spine BMD. For femoral neck BMD, the 2 explanatory factors were BMI and total adiponectin which explained 14% of the variance in BMD. For total hip BMD, 27% of the variance in BMD was explained by 3 factors: leptin, BMI, and total adiponectin.

Conclusion: Bone status in anorexia nervosa is mainly determined by BMI, leptin and adiponectin.

1. Introduction

The pathophysiological mechanisms involved in bone metabolism impairment in anorexia nervosa (AN) are still largely unknown. Various mechanisms have been proposed and are probably interrelated, including oestrogen deficiency, disturbances in the growth hormone (GH)-insulin growth factor-1 (IGF1) axis, hypercorticism, and vitamin D deficiency [1–4]. More recently, several studies have highlighted the role of fat tissue — as an endocrine organ in its own right — in controlling bone metabolism via leptin — and more generally adipokines — as well as factors involved in adipocyte/osteoblast differentiation, such as preadipocyte factor-1 (Pref-1) [5–8]. Leptin is secreted primarily by adipocytes in subcutaneous fat — and to a lesser degree in visceral fat — and its serum level correlates with fat mass [9]. In addition to inhibiting appetite, leptin also regulates bone mass through a dual mechanism producing, on one hand, an inhibitory effect by stimulating the sympathetic nervous system, and on the other hand, a stimulatory effect by binding directly to receptors on osteoblasts [10,11]. Adiponectin regulates energy homeostasis through the modulation of glucose and fatty acid metabolism in peripheral tissues. It circulates in the bloodstream in the form of oligomers of different sizes. Low levels of circulating adiponectin have been reported in obesity and type 2 diabetes

* Corresponding author at
E-mail address: isabelle.legroux@chru-lille.fr (I. Legroux-Gérot).

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[12,13]. In osteoporosis, several studies have mentioned its association with low bone mass [6,14,15]. Serum adiponectin levels seem to correlate inversely with fat mass; in situations of energy deficit, bone marrow adipose tissue may be a major source of circulating adiponectin [16]. This could explain the higher serum adiponectin levels reported by some authors in anorexic patients with osteoporosis, in whom histological and MRI analyses have indicated an increase in marrow adiposity [17–20]. The increase in marrow adiposity reported in anorexic patients with osteoporosis probably plays a role in bone loss [20–22]. The physical proximity of adipocyte and osteoblast cells in bone marrow has led to the hypothesis that a dialogue exists between these cells. Further, adiponectin receptors have been reported on osteoblast cells, and serum adiponectin contributes to low bone mass by promoting osteoclast activity [23]. Moreover, as osteoblast and adipocyte cells come from the same cell line, the decrease in osteoblast differentiation would tend to induce precursors to take the adipocyte pathway.

Previous studies have shown conflicting results for patients with anorexia nervosa [17,18,24–26]. One explanation should be that adiponectin level is related to the severity of the disease and previous studies did include heterogeneous population. Moreover, most of the previous studies focused on total adiponectin and not on high molecular weight (HMW) adiponectin.

Also very few studies did assess both leptin and adiponectin in patients with AN and their relative role for explaining bone involvement remain controversial [27].

Pref-1 is secreted primarily by preadipocytes but also by many tissues like placenta, pituitary gland and adrenal glands. It belongs to the epithelial growth factor (EGF) family of proteins and regulates bone marrow osteoblast and adipocyte differentiation. Plasma Pref-1 was found to be high in anorexic patients, and to correlate negatively with blood leptin and whole body bone mineral density (BMD), but positively with marrow adiposity in L4 lumbar vertebrae [28].

The present study aimed:

- to explore the relationship between leptin, adiponectin, Pref-1 levels and phenotypes of patients with anorexia nervosa according to their BMI and BMD status (recovery, slight decrease in BMD and BMI, slight decrease in BMD and large decrease in BMI, large decrease in BMD and slight decrease in BMI and finally large decrease in both BMI and BMD);
- to explore the additive values of these 3 previous parameters compared to other more classical parameters who play a role in the pathophysiology of AN or in bone involvement (estrogens, IGF-1 and markers of bone remodelling);
- to assess the role of both leptin, adiponectin and Pref-1for explaining the variability of BMD in the whole cohort and particularly in women with osteoporosis.

2. Methods

2.1. Patients

The study involved 80 anorexia nervosa patients. Mean BMI was $16.8 \pm 2.4\,kg/m^2$. All patients were being followed up in the department of psychiatry (CHRU de Lille) and were referred to the department of rheumatology for a bone-health assessment. The assessment was approved by the local Ethics Committee, and all patients gave their written informed consent to participate (No. 2012 A01009 34).

We recorded patients' weight and height, and determined, by interview, their risk factors for osteoporosis as well as the types of treatment they had been receiving. According to their weight and bone status, we divided our population into 5 groups:

- group 1: normal BMD and BMI and regular menstrual cycle;
- group 2: osteopenia ($-2 <$ Z-score < -1) and BMI $> 17\,kg/m^2$;
- group 3: osteopenia and BMI $\leq 17\,kg/m^2$;
- group 4: osteoporosis (Z-score ≤ -2) and BMI $> 17\,kg/m^2$;
- group 5: osteoporosis and BMI $\leq 17\,kg/m^2$.

Patients assigned to group 1 had had one episode of anorexia nervosa but were considered "cured", having regained weight and a regular menstrual cycle. This group served as the "control" group.

For assessing the relationship between BMD (raw data and Z-scores) and adiponectin metabolism, we divided adiponectin levels (total adiponectin, HMW adiponectin and % HMW) in tertiles and analysed in the whole anorexic population, the distribution of adiponectin levels in tertiles against BMD and Z-score.

2.2. Biological data

Plasma concentrations of leptin, total adiponectin, high molecular weight (HMW) adiponectin, and Pref-1 were measured. Blood and urinary calcium and phosphate levels were also determined (calcemia, phosphatemia, alkaline phosphatases, 24-hour calciuria), and 25-hydroxyvitamin D3 — 25(OH)D3 — and parathyroid hormone (PTH), FSH, LH, estradiol, cortisol, somatomedin and bone remodelling markers.

Plasma leptin concentrations were measured by radio immunologic assay (normal values: 3.7 to 11.1 ng/mL). Plasma concentrations of total adiponectin (μg/mL), high molecular weight adiponectin (HMW adiponectin μg/mL), and Pref-1 (ng/mL) were determined by ELISA (Quantikine Immunoassay kits, R&D Systems Inc., Minneapolis, USA). The ratio of HMW adiponectin to total adiponectin — HMW (percentage) — was calculated. Bone remodelling markers — osteocalcin and bone alkaline phosphatase, for bone formation, and serum crosslaps or CTX and for bone resorption — were assessed. Osteocalcin total was measured by radioimmunoassay (Cis-Bio International, Gif-sur-Yvette; normal values: 10.4–45.6 ng/mL). Bone alkaline phosphatase were measured using a human-specific immunoradiometric method (Hybritech, Inc., Dan Diego, CA, USA; normal values: 2.9–14.5 μg/L). Serum CTX was measured by immuno-enzyme assay (ELISA) (serum crossLaps One Step, Osteometer Biotech, Herlev; normal values: 232–5115 pmol/L). IGF-I was measured by chemiluminescence (normal values: 114–451 ng/mL).

All biological samples were collected from fasting patients between 8 a.m. and 10 a.m. at inclusion.

2.3. Measurement of BMD, body fat and fat-free soft tissues

Lumbar spine and hipbone mineral densities were determined by dual-energy X-ray absorptiometry using a Discovery device (Hologic Inc., Bedford MA, USA). The coefficient of variation was 1% for lumbar spine measurements and 2% for hip measurements. Given the mean age of our population, results were expressed as Z-scores, and we used the WHO criterion for osteoporosis, i.e. Z-score < -2.

We also performed a total body composition analysis, measuring lean mass, fat mass, and percentage fat.

2.4. Statistical analysis

Results were expressed as mean ± standard deviation. Groups were compared using Anova or the Kruskal–Wallis test. When significant differences were found, the results were compared

Table 1
Clinical description of the population in the 5 groups.

	Group 1 ($n=15$)	Group 2 ($n=9$)	Group 3 ($n=21$)	Group 4 ($n=15$)	Group 5 ($n=20$)
Age years	25.0 ± 4.4	22.9 ± 3.9	24.7 ± 4.7	21.9 ± 3.9	23.9 ± 5.7
Age onset AN years	19.3 ± 3.4	15.3 ± 3.8[b]	19.1 ± 5.2	16.7 ± 3.2	19 ± 4.4
Age onset AMN years	21.5 ± 4.2	17.5 ± 4	20.6 ± 4.9	18 ± 3.6	19.5 ± 4.4
Duration AN years	5.8 ± 3.7	7.5 ± 5.2	5.1 ± 4.2	5.2 ± 3.1	4.9 ± 4.1
Duration AMN years	1.9 ± 2.9	3.4 ± 3.4	3.4 ± 4.7	2.4 ± 1.6	4.6 ± 4.2
Weight (kg)	51.8 ± 4.2	53.2 ± 7.2	41.3 ± 4.5[a]	50.1 ± 6.2	40.5 ± 4.1[a]
Height (cm)	168 ± 5.8	164.6 ± 6.0	164.4 ± 5.3	165.8 ± 4.8	164.7 ± 5.9
BMI kg/m^2	18.5 ± 1.7	19.5 ± 1.9	15.3 ± 1.5[a]	18.3 ± 1.6	14.9 ± 1.2[a]

AN: anorexia nervosa.
[a] BMI and weight are significantly lower in groups 3 and 5 compared to group 1 ($P<0.0001$).
[b] Mean age of disease onset is significantly lower in group 2 compared to groups 1, 3 and 5 ($P=0.003$).

2-by-2 using Student's *t*-test for independent samples, or the Mann–Whitney U test.

Lastly, stepwise multiple regression analysis was used to evaluate the factors affecting the variance in BMD. A P value < 0.20 was retained for selecting the variables included in the stepwise analysis.

2.5. Role of the funding source

This study was conducted through a grant obtained from the Nord-Pas-de-Calais District (ARCIR 2009: actions de recherches concertées d'initiative régionale).

3. Results

3.1. Clinical characteristics

Our study involved 80 female patients, aged 23.8 ± 4.7 years, diagnosed with anorexia nervosa (AN) according to DSM-IV criteria. The mean duration of anorexia was 5.5 ± 4 years. Mean age of disease onset was 18.2 ± 4.4 years. Mean duration of amenorrhea was 3.4 ± 3.8 years, with a mean age of onset of 19.6 ± 4.4 years. Of the 80 patients, 56 (70%) were found to have pure restrictive anorexia while 24 (30%) exhibited a mixed form of the disorder (bulimia and anorexia). Mean weight was 46.1 ± 7.3 kg. Mean BMI was 16.8 ± 2.4 kg/m^2.

The clinical data for the various groups are shown in Table 1. There was no significant difference in the mean age of patients across the various groups. Mean age of disease onset was significantly lower in group 2 compared to groups 1, 3 and 5. There was no difference in disease duration. BMI was obviously significantly lower in groups 3 and 5 compared to group 1, group 2 and group 4.

3.2. Biological analysis of plasma samples

The results are shown in Fig. 1.

There was no significant difference in total and HMW adiponectin plasma concentrations between the 5 groups. HMW (percentage) was significantly higher in group 5 compared to group 1. No significant difference was observed for Pref-1. Leptin was significantly lower in groups 3 and 5 compared to the other groups; in groups 3 and 5 versus 1 and 2, and in groups 3 and 5 versus 4. Plasma concentrations of IGF-I were also significantly lower in groups 3 and 5 compared to group 2 ($P=0.008$ and $P=0.01$, respectively). No difference was observed for the other parameters (serum calcium and phosphate, bone remodelling markers, thyroid function, hormone levels).

When we divided the adiponectin levels in tertiles and analysed the distribution against BMD and Z-score, we found significant results with total adiponectin and HMW adiponectin but not with % HMW. The total adiponectin levels were higher in the lower tertile for spine ($P=0.01$) and femoral neck BMD ($P=0.002$). The results were similar, when we expressed the results as Z-scores for all the sites: spine ($P=0.02$), hip ($P=0.01$), femoral neck ($P=0.03$) and whole body ($P=0.01$). The HMW adiponectin levels were higher in the lower tertile for spine ($P=0.01$), femoral neck ($P=0.001$) and whole body BMD ($P=0.02$). The results were similar for Z-scores for all the sites: spine ($P=0.01$), hip ($P=0.003$), femoral neck ($P=0.01$) and whole body ($P=0.02$) (Fig. 2). The same analyses were done with % HMW but the results were not significant.

3.3. Bone mineral density and body composition

In our population of 80 patients, 35 (43.75%) had osteoporosis at least at one site and 30 (37.5%) had osteopenia (Z-scores between −1 and −2). BMD was found to be normal in 15 patients (18.75%).

Table 2
Densitometric description and total body analysis results of the population in the 5 groups.

	Group 1 ($n=15$)	Group 2 ($n=9$)	Group 3 ($n=21$)	Group 4 ($n=15$)	Group 5 ($n=20$)
Spine BMD	0.95 ± 0.24	0.85 ± 0.05	0.89 ± 0.06	0.78 ± 0.06[a]	0.73 ± 0.08[a]
Spine Z-score	−0.05 ± 1.01	−1.54 ± 0.49	−1.11 ± 0.6	−2.67 ± 0.68[a]	−2.68 ± 0.81[a]
Hip BMD	0.93 ± 0.09	0.8 ± 0.06	0.82 ± 0.07	0.78 ± 0.09[a]	0.66 ± 0.09[a]
Hip Z-score	−0.16 ± 0.77	−1.21 ± 0.49	−0.99 ± 0.59	−1.46 ± 0.77[a]	−2.3 ± 0.64[a]
Neck BMD	0.82 ± 0.1	0.69 ± 0.04	0.72 ± 0.07	0.66 ± 0.08[a]	0.6 ± 0.09[a]
Neck Z-score	−0.35 ± 0.99	−1.48 ± 0.38	−1.17 ± 0.56	−1.87 ± 0.68[a]	−2.25 ± 0.73[a]
% fat	26.8 ± 5.3	29.5 ± 7.8	19.2 ± 3.8[b]	27.2 ± 6.8	19.5 ± 4.5[b]
Fat mass (g)	13,899 ± 3587	16,205 ± 6810	7927 ± 1924[b]	13,610 ± 4308	7831 ± 2274[b]
Lean mass (g)	35,163 ± 3299	34,796 ± 3026	31,119 ± 3934[b]	34,104 ± 4733	30,245 ± 2211[b]

BMD: bone mineral density.
[a] BMD significantly lower groups 4 and 5 vs. groups 1, 2 and 3.
[b] Values significantly lower groups 3 and 5 vs. groups 1, 2 and 4.

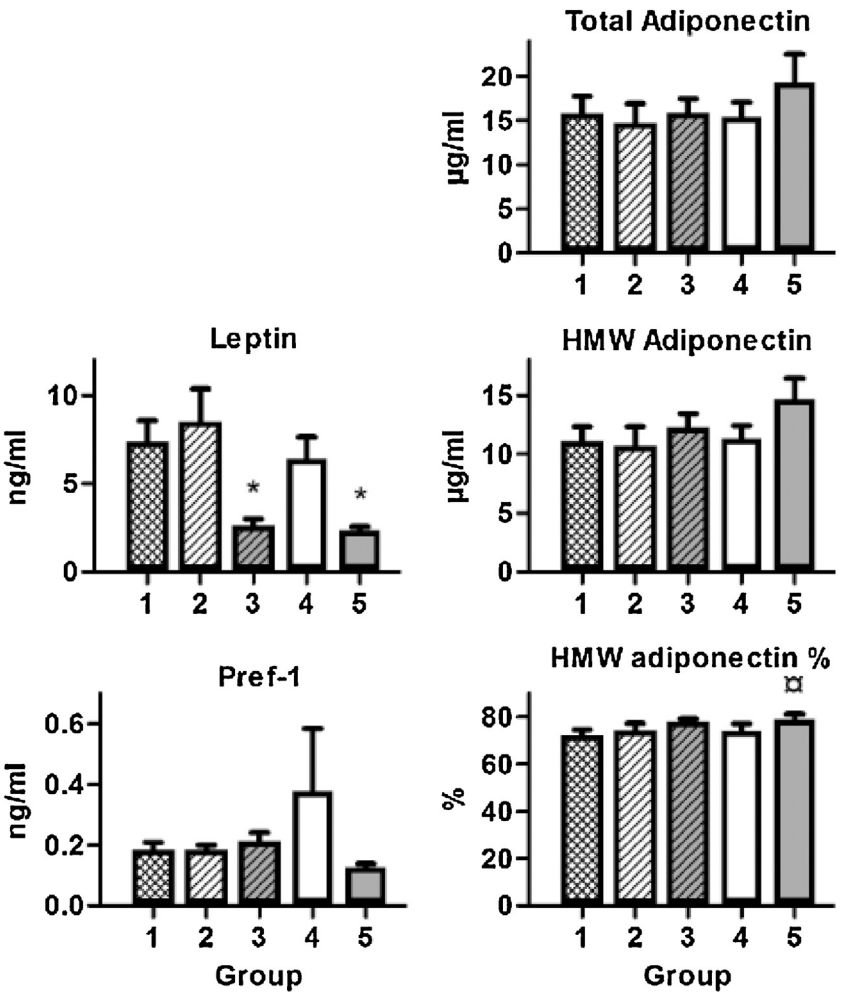

Fig. 1. Biological description of the population in the 5 groups. * Groups 3 and 5 vs. groups 1 and 2, $P = 0.0002$; groups 3 and 5 vs. group 4, $P = 0.003$. ¤ Group 5 vs. group 1, $P = 0.04$.

The results for the various groups are shown in Table 2. As expected, BMD at all 3 sites was significantly lower in the osteoporosis groups — groups 4 and 5 — than in groups 1, 2 and 3. Regarding body composition analysis results, percentage fat and fat mass were found to be significantly lower in groups 3 and 5 compared to groups 1, 2 and 4 ($P < 0.0001$). A significant drop in lean mass was also observed in group 5 compared to groups 1, 2 and 4 ($P = 0.002$), and in group 3 compared to group 1 ($P = 0.001$) and groups 2 and 4 ($P = 0.01$).

3.4. Linear regression analysis

We sought correlations between adiponectin levels and the other biological parameters and BMD results in the entire study population (Table 3), in groups 4 and 5 — patients with osteoporosis — in groups 3 and 5 (patients with BMI $\leq 17\ kg/m^2$) (Table 4).

3.4.1. In the entire study population

No correlation was found between adipokines and BMI. Negative correlations were found between weight and HMW adiponectin and weight and HMW (percentage). Total adiponectin correlated negatively with fat mass and IGF-I. HMW adiponectin was correlated negatively with percentage fat, fat mass, lean mass and IGF-I. HMW (percentage) correlated positively with PTH and blood estradiol. No correlation was found between the various adipokine forms and insulin. Pref-1 correlated positively with HMW (percentage) ($r = 0.24$; $P < 0.05$), but not with total adiponectin or HMW adiponectin.

Positive correlations were found between fat mass and BMI ($r = 0.81$; $P < 0.0001$), IGF-1 ($r = 0.46$; $P < 0.0001$), insulin ($r = 0.58$; $P < 0.0001$) and leptin. Additionally, percentage fat correlated positively with BMI ($r = 0.75$; $P < 0.0001$), IGF-1 ($r = 0.46$; $P < 0.0001$), insulin ($r = 0.54$; $P < 0.0001$), and leptin. There was a positive correlation between lean mass and BMI ($r = 0.64$; $P < 0.0001$) and leptin, but not with IGF-1 or insulin.

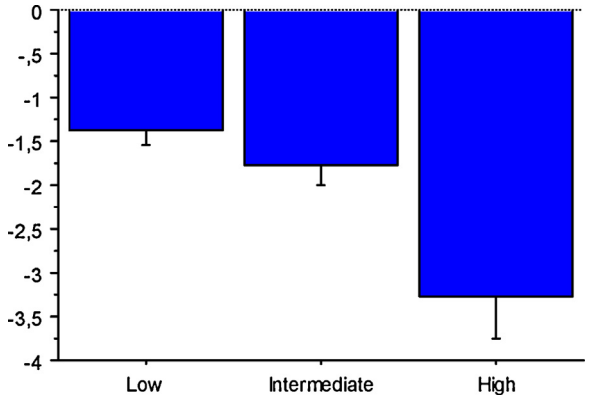

Fig. 2. Lumbar spine Z-score according to tertiles of high molecular weight (HMW) adiponectin. Error bars are SEM ($P = 0.01$).

Table 3
Correlations between adipokines and other parameters in the entire study population* BMD.

Adipokines	Clinical data				Biological data			Bone			
	Weight	Fat mass	% fat	Lean mass	IGF-1	PTH	Blood estradiol	Whole body BMD	Spine BMD	Femoral neck BMD	Total hip BMD
Leptin	ns	$r=0.77^{***}$	$r=0.73^{***}$	$r=0.28^{**}$	ns	ns	ns	ns	ns	ns	ns
Total adiponectin	ns	$r=-0.24^{*}$	ns	ns	$r=-0.26^{*}$	ns	ns	$r=-0.22^{*}$	ns	$r=-0.23^{*}$	$r=-0.31^{**}$
HMW adiponectin	$r=-0.29^{**}$	$r=-0.28^{**}$	$r=-0.23^{*}$	$r=-0.28^{**}$	$r=-0.25^{*}$	ns	ns	ns	ns	$r=-0.21^{*}$	$r=-0.29^{**}$
HMW (percentage) adiponectin	$r=-0.23^{*}$	ns	ns	ns	ns	$r=0.38^{**}$	$r=0.23^{*}$	ns	ns	ns	ns
Pref-1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

BMD: bone mineral density; IGF1: insulin growth factor-1; PTH: parathyroid hormone; Pref-1: preadipocyte factor-1; ns: non significant.
* $P<0.05$.
** $P\leq 0.01$.
*** $P<0.0001$.

Table 4
Correlations between adipokines and bone parameters in osteoporosis or BMI < 17.

Patients with osteoporosis	Osteoporosis groups (4 and 5)				Groups with BMI < 17.5 (3 and 5)			
Adipokines	Whole body BMD	Spine BMD	Femoral neck BMD	Total hip BMD	Whole body BMD	Spine BMD	Femoral neck BMD	Total hip BMD
Leptin	ns	ns	ns	ns	ns	ns	ns	ns
Total adiponectin	$r=-0.41^{**}$	$r=-0.41^{**}$	$r=-0.39^{*}$	$r=-0.48^{**}$	$r=-0.41^{**}$	$r=-0.44^{**}$	$r=-0.33^{*}$	$r=-0.40^{**}$
HMW adiponectin	$r=-0.35^{*}$	$r=-0.36^{*}$	ns	$r=-0.44^{**}$	$r=-0.36^{*}$	$r=-0.43^{**}$	ns	$r=-0.34^{*}$
HMW (percentage) adiponectin	ns	ns	ns	ns	ns	ns	ns	ns
Pref-1	ns	ns	ns	ns	ns	$r=0.32^{*}$	ns	ns

BMD: bone mineral density; HMW: high molecular weight; Pref-1: preadipocyte factor-1; ns: non significant.
* $P<0.05$.
** $P\leq 0.01$.

No correlation was found between spine BMD and the biological parameters. Femoral neck BMD correlated negatively with duration of amenorrhea ($r=-0.37$; $P<0.01$), total adiponectin, and HMW adiponectin, but positively with BMI ($r=0.32$; $P<0.01$). Total hip BMD correlated negatively with duration of amenorrhea ($r=-0.35$; $P<0.01$), total adiponectin ($r=-0.31$; $P<0.01$), and HMW adiponectin, but positively with BMI ($r=0.38$; $P<0.001$). Whole body BMD also correlated negatively with total adiponectin. No correlation was found between BMD at any of the sites and Pref-1 or leptin. Markers of bone remodelling were not correlated with both BMD and other biological variables.

3.4.2. *In groups 4 and 5 (patients with osteoporosis)*

Spine BMD correlated positively with weight ($r=0.35$; $P<0.05$), and BMI ($r=0.35$; $P<0.05$), but negatively with total adiponectin and HMW adiponectin. Femoral neck BMD also correlated positively with weight ($r=0.43$; $P=0.01$) and with fat mass ($r=0.35$; $P<0.05$), and negatively with total adiponectin. Total hip BMD correlated positively with weight ($r=0.64$; $P<0.0001$), BMI ($r=0.54$; $P=0.001$), percentage fat ($r=0.41$; $P=0.01$), fat mass ($r=0.51$; $P=0.01$), and lean mass ($r=0.54$; $P<0.001$), and negatively with total adiponectin and HMW adiponectin. Whole body BMD correlated positively with weight ($r=0.37$; $P<0.05$), BMI ($r=0.33$; $P<0.05$), and lean mass ($r=0.38$; $P<0.05$), and negatively with total adiponectin and HMW adiponectin. Markers of bone remodelling were not correlated with both BMD and other biological variables.

3.4.3. *In groups 3 and 5 (patients with BMI ≤ 17 kg/m²)*

Spine BMD correlated negatively with total adiponectin, HMW adiponectin, and Pref-1. Femoral neck BMD correlated positively with weight ($r=0.32$; $P<0.05$) and BMI ($r=0.34$; $P<0.05$), and negatively with total adiponectin. Total hip BMD correlated positively with weight ($r=0.35$; $P<0.05$) and BMI ($r=0.34$; $P<0.05$), and negatively with total adiponectin and HMW adiponectin. Markers of bone remodelling were not correlated with both BMD and other biological variables.

Multivariate analysis performed on the entire study population revealed that 2 factors — leptin and BMI (adjusted R-squared = 0.1, $P=0.008$) — explained the variance in spine BMD. For femoral neck BMD, the 2 explanatory factors were BMI and total adiponectin (adjusted R-squared = 0.14, $P=0.002$). For total hip, the variance in BMD was explained by 3 factors: leptin, BMI, and total adiponectin (adjusted R-squared = 0.27, $P<0.0001$).

In the patients with osteoporosis — groups 4 and 5 — the factors explaining the variance in spine BMD were leptin, BMI and total adiponectin (adjusted R-squared = 0.35, $P=0.001$). For femoral neck, the factors explaining the variance in BMD were BMI and total adiponectin (adjusted R-squared = 0.2, $P=0.01$), and for total hip the 3 explanatory factors were leptin, BMI, and total adiponectin, which explained a significant part of the variance at this site (adjusted R-squared = 0.47, $P<0.0001$). For whole body BMD, leptin, BMI, and total adiponectin (adjusted R-squared = 0.33, $P=0.002$) were also found to be explanatory factors.

In patients with BMI ≤ 17 kg/m², the factors explaining the variance in spine BMD were BMI and total adiponectin (adjusted R-squared = 0.21, $P=0.004$). This was also the case for femoral neck and total hip BMDs (adjusted R-squared = 0.22, $P=0.003$ and 0.27, $P=0.001$, respectively). For whole body BMD, the factors explaining the variance in BMD were BMI and total adiponectin (adjusted R-squared = 0.21, $P=0.004$).

4. Discussion

Our study involved a sizeable cohort of anorexia nervosa patients, allowing us to form sub-groups based on BMI and bone status. Group results were compared with group 1, which comprised patients who had a history of anorexia nervosa but could be considered “cured”.

No difference was found in total serum adiponectin, but a significant increase in HMW (percentage) adiponectin was observed in group 5 — the group of patients with osteoporosis and BMI ≤ 17 kg/m² — compared to group 1. Moreover, the total

adiponectin levels and HMW adiponectin were higher in the lower tertile for spine and femoral neck BMD. The results were similar, when we expressed the results as Z-score for all the sites: spine, hip, femoral neck and whole body. In anorexia nervosa patients, adiponectin results are contradictory. Whereas earlier studies [24,25] reported lower or unchanged adiponectin levels in anorexia nervosa patients compared to controls, a more recent study conducted by Terra et al. [26] compared 28 patients to 33 controls and reported significantly higher concentrations of circulating total and HMW adiponectin in patients. The patients were older, with a mean age of 27.4 ± 1.4 years. In a study involving 11 anorexia nervosa patients (mean age 16.4 years) compared to 26 controls, Pannacciulli et al. [18] also found significantly higher adiponectin levels in their patient group. Similarly, in a study conducted by Delporte et al. [17] involving 26 anorexia nervosa patients versus 24 controls, adiponectin levels were found to be 30% higher in the patient group while leptin levels were lower. They also reported negative correlations between adiponectin and BMI and adiponectin and fat mass. The most recent data seem to argue in favour of an increase in HMW adiponectin — and indeed total adiponectin — in anorexia nervosa patients with osteoporosis. In our study, we found no difference in total adiponectin, but in the absence of a real control group, comparisons with data from the literature are difficult.

We sought correlations between adipokines and other metabolic parameters and found that total adiponectin correlated negatively with fat mass. HMW adiponectin was also found to correlate negatively with percentage fat, fat mass and lean mass. In the study by Terra et al. [26], the authors also reported a negative correlation between total adiponectin and percentage fat, but also BMI and disease duration after adjusting for age. Several authors have also mentioned negative correlations between adiponectin and BMI [24,26], contrary to our findings. These data tend to confirm a negative correlation between total adiponectin and fat mass.

Thus, anorexia nervosa patients with low BMIs were able to produce adiponectin despite having little peripheral fat, which suggests that the adiponectin is not being secreted by subcutaneous or visceral adipocytes.

Several authors have reported an increase in circulating adiponectin levels during caloric restrictions in animals and man [18,25,29], despite the loss of visceral and subcutaneous fat.

A strong relationship between resting energy expenditure and adipokines was found in a recent study, which explored both leptin and adiponectin in AN patients [16]. The authors demonstrated a positive correlation with resting energy expenditure and markers of bone formation, leptin and a negative correlation with bone resorption markers but no correlation with BMD. In the present study, we did not find relationships between the level of bone remodelling markers and both biological variables (including adipokines) and BMD whereas we found a significant negative correlation between adiponectin levels and hip BMD as Maïmoun et al. found [27]. Also Maïmoun et al. [27] measured only total adiponectin whereas we measured different type of adiponectin according to their molecular weight. Finally, they did not perform multivariate analysis perhaps owing to the sample size, which was smaller compared with our study (50 AN patients against 80 in our own study). Cawthorn et al. [16] compared adiponectin secretion in peripheral fat and marrow fat in mice and rabbits. They found that marrowfat secreted more adiponectin than peripheral fat, and that mice with no marrowfat did exhibit an increase in serum adiponectin levels in response to caloric restrictions but this increase is blunted compared to wild-type control mice. These results clearly demonstrate that the increase in marrow fat observed during caloric restrictions contributes to increasing serum adiponectin levels, and several recent studies have reported an increase in marrow fat, evaluated by MRI, in anorexia nervosa patients with osteoporosis [21,30]. However, the role of adiponectin in bone metabolism remains unclear, and other animal studies failed to confirm these results [31]. Lastly, data from studies in man are lacking or insufficient. Unlike some studies, we did not find a correlation between adiponectin and spine BMD in our population. In a study comparing 17 anorexia nervosa patients to 19 controls, Misra et al. [32] found an inverse correlation between spine BMD, as well as femoral neck and whole body BMD, and adiponectin. In our study we also found an inverse correlation between femoral neck, total hip BMD and total and HMW adiponectin; however spine BMD correlated inversely with total and HMW adiponectin in only anorexia nervosa patients with osteoporosis. These correlations were also found in patients with BMI $\leq 17\,kg/m^2$.

Contrary to other authors, we found no significant difference in serum Pref-1 level between the various groups in our study, but we did not have a group of healthy controls without a history of anorexia nervosa. We found no correlation between serum Pref-1 levels and fat mass, percentage fat and lean mass results from BMD examinations. For the various adiponectin forms, we only found a positive correlation between Pref-1 and HMW (percentage, $r = 0.24$; $P = 0.03$). However, when the two groups of patients with BMI $\leq 17\,kg/m^2$ were considered together, a moderate correlation was observed between spine BMD and Pref-1 ($r = 0.32$; $P = 0.04$). Pref-1 seems to be an interesting candidate since it regulates marrow osteoblast and adipocyte differentiation. Aronis et al. [8] reported an increase in serum Pref-1 in 18 anorexia nervosa patients compared with healthy subjects. They also reported negative correlations between Pref-1 and BMD, and positive correlations with marrow fat, in both anorexia nervosa patients and healthy subjects. Other authors, such as Fazeli et al. [33], also reported an increase in Pref-1 levels in anorexia nervosa patients compared to healthy subjects, as well as an inverse correlation between Pref-1 and percentage fat. Pref-1 is one of many factors involved in adipocyte differentiation and its role may vary depending on the nutritional status of the patient. The role of serum Pref-1 in peripheral fat deposits is unknown and further studies will be needed to determine its effect on marrowfat compared to visceral and subcutaneous fat.

We also found that serum leptin levels were significantly lower in groups 3 and 5 (with BMI $\leq 17\,kg/m^2$) compared to the other groups. In the literature, several authors reported low leptin levels in anorexia nervosa patients [34–36]. We previously evaluated serum leptin levels in 103 anorexia nervosa patients grouped according to bone status and found low levels in patients with osteoporosis [5]. In the previous study when multivariate analyses were performed, the independent factors that could best explain the variance in spine BMD were found to be duration of amenorrhea and leptin level, which accounted for 28% of the variance. On the other hand, in the previous study, leptin did not explain the variance in hip BMD. Similarly in the present study, leptin and BMI were found to explain 10% of the variance in spine BMD. However, when patients with osteoporosis were considered alone, 35% of the variance in spine BMD was explained by 3 factors: leptin, BMI and total adiponectin. For femoral neck BMD, 14% of the variance was explained by BMI and total adiponectin. In anorexia nervosa patients with osteoporosis, the figure increased to 20% with the same factors. For total hip, 27% of the variance in BMD was explained by 3 factors — leptin, BMI, and total adiponectin, and in anorexia nervosa patients with osteoporosis, the figure increased to 47% with the same factors. These findings suggest that adipokines seem to particularly influence BMD for patients with the most severe disease (low BMI and or low BMD).

Moreover, apart from IGF-I level, other parameters measured in this present study (estradiol, FSH, LH, cortisol, markers of bone turn over) did not seem to play a role for explaining phenotypes of

anorexia nervosa patients according to BMD and BMI. In the same manner, these parameters did not explain the variability of BMD.

There are certain limitations to our study. We did not have a real control group, but we compared our patients with those in group 1, who were anorexia nervosa patients who were considered "cured" and who had normal BMIs and BMDs. Therefore, this group 1 could be considered as a surrogate for a control group. We divided our population into several groups according to bone status and BMI to reflect different disease profiles. While this approach may seem logical, it necessarily results in fewer subjects in each group. Lastly, since our study was a cross-sectional study, no longitudinal data is available. However, compared to data in the literature, our study group was sizeable, and many parameters were analysed in detail.

This study argues in favour of adipokine involvement as an explanatory factor of bone status impairment in anorexia nervosa. The variance in BMD in anorexia nervosa patients with osteoporosis was explained predominantly by 3 factors: BMI, leptin, and adiponectin. To the extent that these 3 factors are, by definition, independent — which is the principle underlying stepwise linear regression analysis — our hypothesis is that BMI is a reflection of lean mass, leptin a reflection of subcutaneous and visceral fat, and adiponectin a possible reflection of marrow fat.

Disclosure of interest

The authors declare that they have no competing interest.

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