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Comparison of regional bone marrow adiposity characteristics at the hip of underweight and weight-recovered women with anorexia nervosa using magnetic resonance spectroscopy

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ABSTRACT

Bone marrow adiposity (BMA) is an underestimated tissue, with properties that may alter bone strength especially in diseases that fragilize bone such as anorexia nervosa. In the present study, we investigated the regional characteristics of BMA at the hip of 40 underweight and 36 weight-recovered anorexic women, along with 10 healthy women, using magnetic resonance spectroscopy at multiple anatomical subregions (acetabulum, femoral neck, proximal femoral diaphysis and greater trochanter) to measure bone marrow fat fraction (BMFF) and apparent lipid unsaturation levels (aLUL). Correlations between BMFF, aLUL, body fat percentage (BF), and bone mineral density (BMD) at the femoral neck and total hip, both measured using dual-energy X-ray absorptiometry, were assessed in anorexic patients. Whereas BMFF was significantly higher and aLUL significantly lower at the femoral neck of underweight and weight-recovered patients compared to controls (BMFF: 90.1 ± 6.7% and 90.3 ± 7.5% respectively versus 81.3 ± 8.1%; aLUL: 7.6 ± 1.4% and 7.3 ± 1.3% versus 9.2 ± 1.5%), BMFF and aLUL were not significantly different between the 2 subgroups of patients. Besides, three noteworthy features were observed between BMA and the other measured parameters in anorexic patients. First, synergic alterations of BMA were observed at all sites, with an inverse relationship between BMFF and aLUL ($\rho = -0.88$). Second, bone mineral compartment and BMA were associated, as a negative correlation between total hip BMD and BMFF was observed at all sites except the greater trochanter ($\rho = [-0.32;-0.29]$), as well as a positive correlation with aLUL at all sites except the proximal femoral diaphysis ($\rho = [0.25;0.37]$). Finally, we found a positive correlation between BF and BMFF at the femoral neck ($\rho = 0.35$), and a negative correlation between BF and aLUL at this same subregion ($\rho = -0.33$), which suggest a complex relationship between BMA and BF. Overall, BMA possesses regional specificities which may impair bone health, even after weight recovering.

1. Introduction

Anorexia nervosa (AN) is an eating disorder occurring predominantly in women and characterized by an intense fear of gaining weight [1]. The consequent substantial reduction of energy intake induces a hormonal imbalance responsible for multiple comorbidities, which worsen quality of life even in incomplete syndromes [2,3]. Among these many complications, bone metabolism and its mineral composition are particularly altered, with a bone loss reported in more than half of the patients, potentially fostering fragility fractures [4,5].

At the cellular level, it is important to remind that osteoblasts and marrow adipocytes have a common progenitor, the skeletal stem cell, whose fate is highly influenced by hormones. In AN as well as in osteoporosis, the final lineage allocation is in favor of adipocytes over

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osteoblasts [6], which can lead to a paradoxical "bone obesity" that impairs bone resistance to mechanical stress [7]. However, this simplistic view is complexified by the different subtypes that bone marrow adiposity (BMA) can have, the existence of regional variations corroborated by MRI or bone staining in histopathological works [8,9], and the underestimated importance of BMA composition. Indeed, several studies reported an alteration of BMA lipidic profile at the spine or on bone marrow samples obtained from iliac crest aspiration of post-menopausal women, with a decrease of lipid unsaturation levels [10–13]. Patsch et al. even observed a significant association between prevalent fragility fractures and lower unsaturation levels, but not between spinal marrow fat content and fractures [14]. Thus, while taking into consideration its spatial heterogeneity, BMA content and composition should be both assessed in order to better understand the physiopathological mechanisms that can promote region-specific skeletal complications in osteoporotic conditions.

In vivo, single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) is considered as a reference to explore BMA, as it is an accurate, reproducible and non-invasive tool that can both quantify BMA fat content and obtain a glimpse of its composition, respectively through the measurement of bone marrow fat fraction (BMFF) and apparent lipid unsaturation levels (aLUL). Regarding AN, an increased marrow adiposity at the hip and an inverse correlation between saturation levels at the femoral diaphysis and spinal BMD have been reported using this technique, although these data were based on a limited number of subjects [15–17].

Finally, only scarce literature is available on BMA in weight-recovered patients with anorexia nervosa. After short-term partial weight recovering, an abnormal body fat distribution can remain in anorexic women, and osteopenia can persist even after disease recovery [18,19]. Interestingly, even after weight normalization, anorexic women can still have an abnormal eating behavior [20]. As dietary fat intake appears to play a role in bone health, affecting both BMA composition and bone mineral density (BMD) [21,22], weight recovery may not be sufficient to restore a normal BMA. In an ancillary analysis, Fazeli et al. did not observe significant differences in BMFF at the proximal femur between healthy controls, underweight and weight-recovered anorexic patients [23]. However, for the authors, their study may suffer from insufficient statistical power to conclude this hypothesis.

Consequently, to efficiently evaluate bone health, BMA characteristics should be precisely depicted in a larger cohort of patients, through BMFF and aLUL measurements using ^{1}H-MRS at multiple sites. While focusing on multiple subregions of the hip, and with a special attention to the femoral neck, known to be a vulnerable site for fragility fractures, we aimed to compare the regional characteristics of BMA in underweight and weight-recovered anorexic women, along with age-matched healthy women as controls. The secondary objectives were to evaluate the relationship between BMA, body fat percentage and BMD at the total hip and femoral neck, both obtained using dual-energy X-ray absorptiometry (DXA).

2. Materials and methods

2.1. Ethics

This study was approved by the institutional ethics committee and written informed consent was obtained from all patients (trial registration number: 2012-A01009–34).

2.2. Study protocol

From January 2014 to June 2017, 80 women followed for anorexia nervosa in Lille University Hospital were investigated using MRI and DXA. Inclusion criteria were: (a) adult women aged between 18 and 35 years old; (b) anorexia nervosa diagnosed by a psychiatrist (V.J.), as defined by the *Diagnostic and Statistical Manual*, fourth edition [24]; (c) French health care coverage. Exclusion criteria were: (a) pregnancy; (b) any MRI contraindication; (c) MR artifacts in the hip region; (d) hip prosthesis or history of hip trauma; (e) any musculoskeletal disease which cannot be related to anorexia nervosa.

For all subjects, age, weight, height, body mass index (BMI = weight in kilograms (kg) divided by the square of the height (m^2)), smoking and alcohol were recorded. Evaluation of the physical activity was based on the European Vertebral Osteoporosis Study questionnaire: [1] light activity, mostly sedentary; [2] moderate activity involving standing and walking; [3] heavy activity with occasional carrying of heavy burdens and regular walking, and [4] very heavy physical activity involving regular heavy work or high-level sports [25]. At the time of the MR examination, patients having an abnormal BMI ($< 17.5\,kg/m^2$) were included in the "underweight anorexia nervosa" group while patients with a normal BMI ($\geq 17.5\,kg/m^2$) were in the "weight-recovered anorexia nervosa" group. Duration since weight restoration of weight-recovered anorexic patients was defined as the amount of time between the first medical report specifying a BMI above $17.5\,kg/m^2$ and the MRI examination.

Finally, 3 women with corrupted MR acquisitions and 1 patient with insufficient DXA data were excluded, resulting in a final population of 76 patients.

2.3. Healthy volunteers

Proton spectroscopic data of 10 healthy volunteer women aged between 18 and 35 years old and with a normal BMI ($> 17.5\,kg/m^2$) were acquired in the exact same conditions as the anorexic patients. Controls were recruited following the local recommendations of the ethics committee. To prevent radiation exposure, no DXA was performed. For every volunteer, age, height, weight, and BMI were recorded. All subjects were asymptomatic with regular menses, no medical or psychiatric history, no smoking habits and had not any risk factor for osteoporosis nor received any drug that could alter bone marrow adiposity. Informed consent was also obtained for all volunteers.

2.4. MR examination

MR examinations were achieved on a 3 T full-body MR scanner (Ingenia, Philips Healthcare, The Netherlands) following the same protocol as previously described [26].

Women were lying supine, with a 32-channel cardiac coil covering the proximal femoral diaphyseal shaft and the acetabular roof. Scout acquisitions consisted in T1-weighted turbo spin echo sequences in the sagittal plane (repetition time (TR)/echo time (TE) = 715/20 ms; field of view (FOV) = 210 mm; slice thickness = 3.0 mm; gap = 0.8 mm; matrix = 258 × 288 pixels; turbo factor = 5 and acquisition time = 2 min 32 s), and in the coronal and axial planes (TR/TE = 544/20 ms; FOV = 385 mm; slice thickness = 3.0 mm; gap = 0.8 mm; matrix = 427 × 528 pixels; turbo factor = 4 and acquisition time = 1 min 17 s).

Volumes of interest (VOI) were placed in 4 subregions of the right hip, considered as independent from each other for the statistical analysis: (a) the acetabulum; (b) the femoral neck; (c) the proximal femoral diaphysis and (d) the greater trochanter. The 3-plane scout acquisitions were used to guide optimal positioning of the VOI in the trabecular bone, avoiding overlap with cortical and non-cancellous bone structures. The positioning was done by MRI radiographers dedicated to imaging research protocols, under radiologist supervision, and a snapshot was saved for quality control (Fig. 1).

A single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) technique was performed using a stimulated echo acquisition mode (STEAM) with the following parameters: voxel size = $12.5 \times 12.5 \times 12.5\,mm^3$; TR/TE = 5000/20 ms; NSA 32; mixing time 16 ms; spectral bandwidth = 2500 Hz; 2048 data points

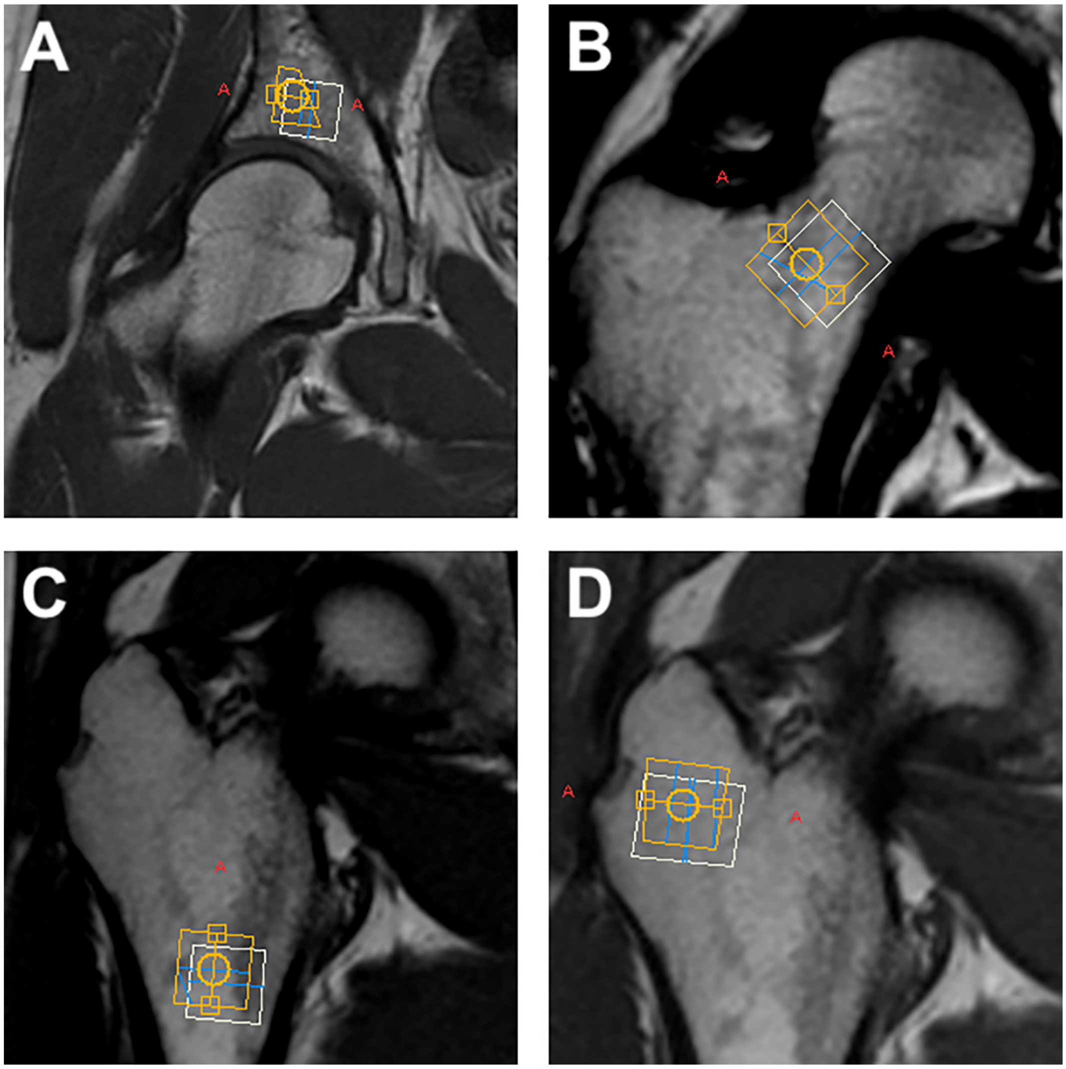

Fig. 1. – Positioning of volume of interests (VOI) on the coronal scout acquisitions, in the acetabulum (A), the femoral neck (B), the proximal femoral diaphysis (C), and the greater trochanter (D). To avoid overlap with cortical and non-cancellous structures, the positioning was also checked on axial and sagittal scout acquisitions (not shown).

Table 1

– Clinical characteristics (mean values ± standard deviation; [min–max] for BMI and duration since weight restoration) and comparisons between underweight (BMI < 17.5 kg/m^2) and weight-recovered (BMI ≥ 17.5 kg/m^2) women with anorexia nervosa, along with healthy women as controls. *: One-way ANOVA test with Tukey's post hoc comparisons showing significant differences (p-value < 0.05) between underweight anorexic women and the other groups but not between weight-recovered anorexic women and healthy women. Significant values are shown in bold (red).

	Women with anorexia nervosa		Healthy women	*p*-value
	Underweight	Weight-recovered		
N	40	36	10	–
Age (years)	23.3 ± 5.1	24.7 ± 5.0	25.0 ± 2.9	0.39
Body mass index (KG/M^2)	15.4 ± 1.2 [12.6–17.4]	19.8 ± 1.7 [17.7–23.4]	20.2 ± 2.6 [17.6–26.6]	**<0.0001***
Body fat percentage (%)	18.8 ± 4.9	28.4 ± 6.0	–	**<0.0001**
Spine BMD (G/CM^2)	0.83 ± 0.11	0.88 ± 0.10	–	0.11
Total hip BMD (G/CM^2)	0.75 ± 0.14	0.81 ± 0.09	–	0.06
BMD at the femoral neck (G/CM^2)	0.67 ± 0.12	0.71 ± 0.09	–	0.21
Age of onset of anorexia (years)	18.1 ± 4.6	17.0 ± 3.6	–	0.23
Duration of anorexia (years)	3.9 ± 2.7	6.3 ± 4.9	–	**0.001**
Duration since weight restoration (years)	–	1.5 ± 1.5 [0.0–7.6]	–	–
Amenorrhoeic patients	82.5% (n = 33)	61.1% (n = 22)	–	**0.04**
Age of onset of amenorrhea (years)	19.4 ± 4.8	19.6 ± 5.2	–	0.86
Duration of amenorrhea (years)	2.5 ± 2.2	1.7 ± 2.4	–	0.13
Binge-eating episodes	20.0% (n = 8)	36.1% (n = 13)	–	0.12
Smoking	15.0% (n = 6)	16.7% (n = 6)	–	0.84
Hard liquor consumption	0	0	–	–
Physical activity (EVOS score)	2.0 ± 0.6	2.4 ± 0.6	–	0.07

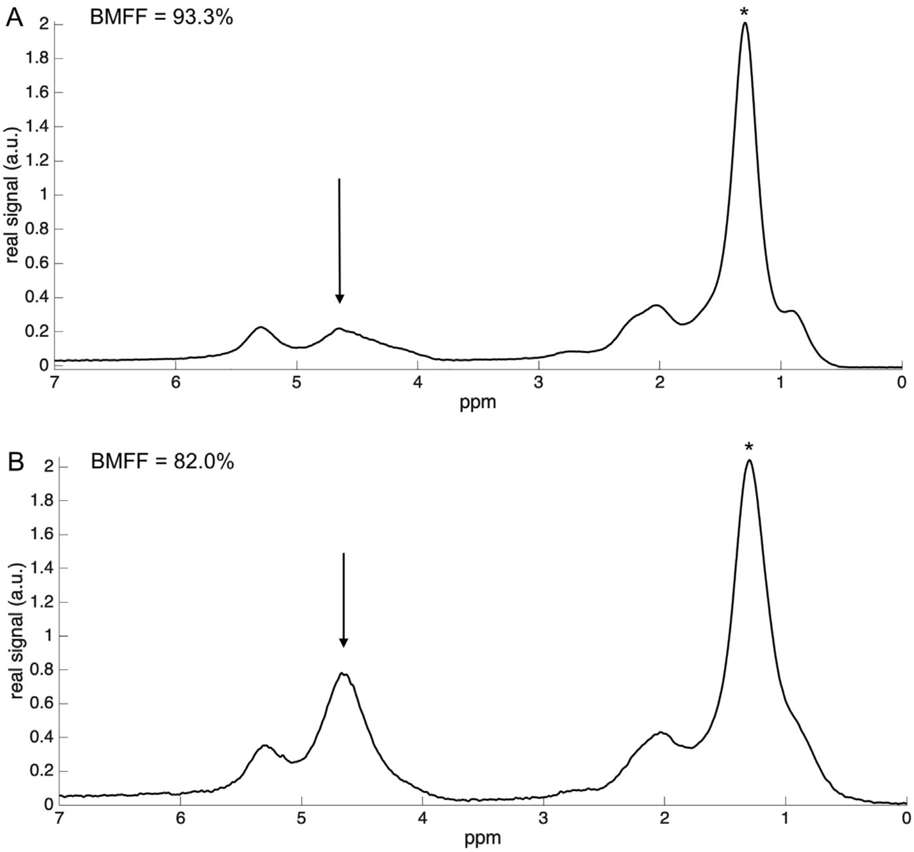

Fig. 2. – Single-voxel proton magnetic resonance spectroscopy of an underweight anorexic woman (A), showing a low water peak at 4.67 ppm (black arrow) and a high methylene peak at 1.3 ppm (asterisk), which result in a high bone marrow fat fraction (93.3%). In contrast, the water peak is higher and more visible in a healthy woman (B), resulting in a lower and normal bone marrow fat fraction (82.0%). Both spectra were acquired at the femoral neck, avoiding overlap with cortical bone and non-cancellous structures.

Table 2
– Bone marrow fat fraction (BMFF) and apparent lipid unsaturation levels (aLUL) in anorexic patients and healthy women. Values (mean values ± standard deviation) with the same letter are significantly different (p-value < 0.05). *: Kruskal-Wallis analysis with post hoc Dunn's tests highlighted significant differences between healthy women and the other groups but not between underweight and weight-recovered anorexic patients. Significant values are shown in bold (red).

		Women with anorexia nervosa		Healthy women	*p*-value
		Underweight	Weight-recovered		
BMFF (%)	Acetabulum	75.8 ± 10.1[a,b,c]	72.2 ± 10.3[d,e,f]	68.1 ± 7.4[i,j]	0.08
	Femoral neck	90.1 ± 6.7[a]	90.3 ± 7.5[d,g]	81.3 ± 8.1[k]	**<0.01***
	Proximal femoral diaphysis	89.6 ± 11.1[b]	90.5 ± 7.2[e,h]	85.1 ± 12.2[i]	0.29
	Greater trochanter	92.5 ± 10.9[c]	96.6 ± 1.5[f,g,h]	94.4 ± 3.3[j,k]	0.18
aLUL (%)	Acetabulum	10.9 ± 2.3[l,m,n]	11.1 ± 1.7[o,p,q]	12.7 ± 2.5[r,s]	0.17
	Femoral neck	7.6 ± 1.4[l]	7.3 ± 1.3[o]	9.2 ± 1.5	**<0.01***
	Proximal femoral diaphysis	7.7 ± 2.0[m]	7.3 ± 1.3[p]	8.4 ± 2.4[r]	0.46
	Greater trochanter	7.1 ± 1.6[n]	6.5 ± 0.8[q]	7.1 ± 1.4[s]	0.18

acquired; no water suppression; highest fat peak chosen to perform second order shimming and water peak manually selected to adjust frequency if necessary. Shimming procedures were employed to optimize spectral quality and maintain a mean full width at half maximum (FWHM) below 50 Hz. The global spectrum was automatically built from the acquisition of each coil channels. The acquisition time for each location was 2 min 50 s.

2.5. Bone marrow fat quantification

Spectra quality and VOI positioning were first evaluated before performing fat quantification. Acquisitions overlapping non-cancellous bone structures and/or spectra having large baseline distortions or unusual broadened peaks were discarded from the analysis to measure only signals coming from bone marrow. Based on this preliminary quality check, 20 acquisitions failed to meet the quality requirements among the 304 spectra (acetabulum: $n = 4$; femoral neck: $n = 6$; proximal femoral diaphysis: $n = 6$ and greater trochanter: $n = 4$).

Spectroscopic data were post-processed using house-built routines written in MatLab, version R2015b (MathWorks, Natick, MA, USA), optimized for BMFF and composition quantification (R.S., K.D.C.). An independent linewidth, constrained below 1.7 ppm, was assumed for the main fat peak (methylene, at 1.3 ppm), and used as a reference. The spectral location of the other fat peaks was allowed to vary by ± 0.02 ppm and the water location by ± 0.10 ppm. Using a pre-calibrated fat spectrum and these constraints, these scripts automatically fitted the acquired spectroscopic data in the frequency domain, providing reproducible measurements for each acquisition.

The pre-calibrated fat spectrum contained the following peaks: $-(CH_2)_n-CH_3-$ (methyl: 0.90 ppm; L_1), $-(CH_2)_n-$ (methylene: 1.30 ppm; L_2), $-CH_2-CH_2-CO-$ (β-carboxyl methylene: 1.59 ppm; L_3), $-CH_2-CH=CH-$ (α-olefinic methylene: 2.00 ppm; L_4), $-CO-CH_2-CH_2-$ (α-carboxyl methylene: 2.25 ppm; L_5), $-CH=CH-CH_2-CH=CH-$ (diallyl-methylene: 2.77 ppm; L_6), $-CH_2-O-CO-$ (glycerol: 4.20 ppm; L_7),

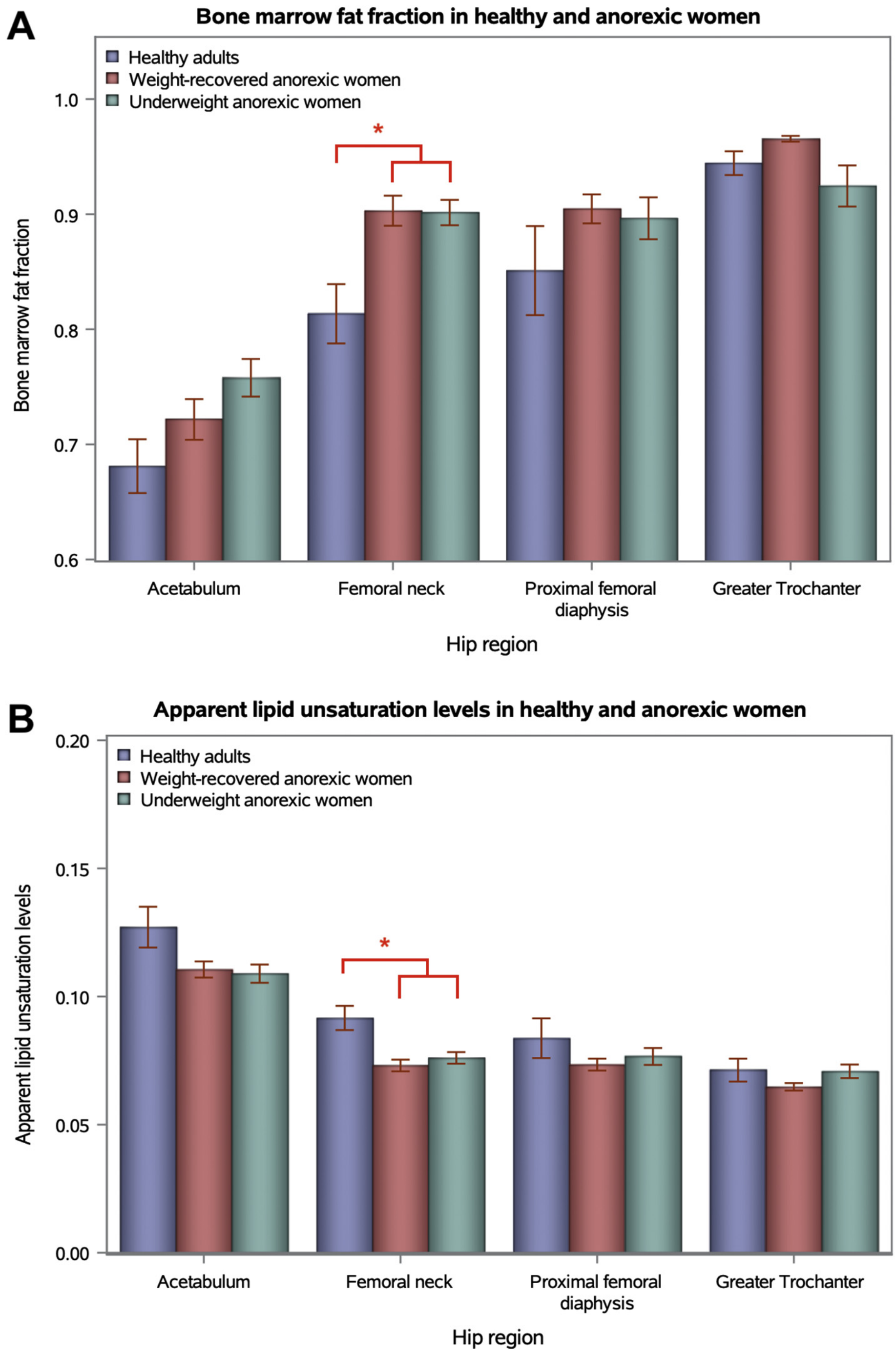

Fig. 3. – Mean bone marrow fat fractions (BMFF) (A) and apparent lipid unsaturation levels (aLUL) (B) at 4 subregions of the hip of anorexic and healthy women. Error bars indicate standard error. Asterisks show significant differences regarding BMFF or aLUL between anorexic and healthy women.

–CH = CH– (olefinic double bond 5.3 ppm; UL). Water peak was defined at 4.67 ppm. BMFF was calculated as the percentage of fat signal relative to total signal intensity (fat + water). The apparent lipid unsaturation level (aLUL) was calculated as follows, using the olefinic peak (UL) as the most representative unsaturated lipid: aLUL (%) = UL/(UL + ΣL_{1-7}).

2.6. Evaluation of bone mineral density and body composition

BMD of the spine, total left hip, left femoral neck and body fat percentage were assessed using DXA (Discovery W, Hologic). Except for 3 patients who already had a DXA scanner within the year (22, 50 and 84 days between the scan and the MR acquisition), all examinations (MR and DXA) were performed the same day.

2.7. Statistical analysis

Statistical analysis was completed with SAS University Edition, version 9.04 (SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was used to check for normality. Demographic characteristics were evaluated using one-way ANOVA with Tukey's post hoc tests and Student *t*-tests for quantitative variables, and chi-squared tests for categorical data. Comparisons of BMD, BMFF, and aLUL between sites and groups were performed using non-parametric tests (Mann-Whitney rank-sum

Table 3
– Spearman's rank-order correlation coefficients at 4 subregions of the hip of all women with anorexia nervosa (underweight and weight-recovered anorexic patients), between bone marrow fat fraction (BMFF), apparent lipid unsaturation levels, total hip bone mineral density (BMD), BMD at the femoral neck and body fat percentage, adjusted for body mass index and amenorrhoeic status. Significant values are shown in bold (red).

	Acetabulum		Femoral neck		Proximal femoral diaphysis		Greater trochanter	
	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value
A: BMFF								
Total hip BMD	−0.30	**0.01**	−0.32	**0.01**	−0.29	**0.02**	−0.22	0.08
BMD at the femoral neck	–	–	−0.20	0.12	–	–	–	–
Apparent lipid unsaturation levels	−0.89	**<0.0001**	−0.80	**<0.0001**	−0.74	**<0.0001**	−0.79	**<0.0001**
Body fat percentage	0.22	0.07	0.35	**<0.01**	0.19	0.12	0.06	0.61
B: Apparent lipid unsaturation levels								
Total hip BMD	0.37	**<0.01**	0.33	**<0.01**	0.22	0.08	0.25	**0.04**
BMD at the femoral neck	–	–	0.20	0.12	–	–	–	–
Body fat percentage	−0.23	0.06	−0.33	**<0.01**	−0.18	0.15	−0.12	0.32

or Kruskal-Wallis analyses followed by post hoc Dunn's tests [27]). Because a non-linear relationship was presumed between BMFF, aLUL, body fat percentage and BMD at the femoral neck and total hip, Spearman's rank-order method was used to calculate correlation coefficients (ρ), adjusted for BMI and the amenorrhoeic status. Unless specified otherwise, all data were written in the form mean ± standard deviation (*p*-value). A *p*-value below 0.05 was considered to be statistically significant.

3. Results

3.1. Population demography

Slightly more than half of the patients (52.6%, $n = 40$) had underweight anorexia nervosa, while 36 women had a BMI above 17.5 kg/m^2 at the moment of the MR examination and were considered as weight-recovered anorexic women. The mean age was respectively 23.3 ± 5.1 and 24.7 ± 5.0 years in both groups and was not significantly different from controls (25.0 ± 2.9 years, $p = 0.39$). Healthy and weight-recovered anorexic women had a significantly higher BMI than underweight anorexic women (respectively 20.2 ± 2.6 and 19.8 ± 1.7 kg/m^2 versus 15.4 ± 1.2 kg/m^2, $p < 0.0001$).

Among all anorexic patients, 21 women (27.6%) reported binge-eating and purge episodes. Mean age of onset of anorexia nervosa was 17.6 ± 4.2 years, with a mean disease duration of 5.0 ± 4.1 years and age of onset of amenorrhea beginning later, at 19.5 ± 4.9 years. Habits comprised of mild physical activity (mean EVOS score of 2.2 ± 0.7) and 12 smokers (15.8%). No regular hard liquor consumption was declared by the participants.

Weight-recovered women with anorexia nervosa had a significantly higher body fat percentage and longer duration of anorexia nervosa compared to underweight patients (body fat percentage: 28.4% ± 6.0 versus 18.8% ± 4.9, $p < 0.0001$; duration of anorexia nervosa: 6.3 ± 4.9 versus 3.9 ± 2.7 years, $p = 0.001$). There were significantly less amenorrhoeic women in the weight-recovered group than in the underweight anorexia nervosa group (61.1% versus 82.5% respectively, $p = 0.04$). However, there was no significant difference regarding the duration of the amenorrhea (1.7 ± 2.4 years in the weight-recovered group and 2.5 ± 2.2 years in the underweight group, $p = 0.13$). The mean duration since weight restoration was 1.5 ± 1.5 [0.0–7.6] years.

Total hip BMD was close to the significance threshold between patient groups, slightly higher in the weight-recovered group (0.81 ± 0.09 g/cm^2 versus 0.75 ± 0.14 g/cm^2, $p = 0.06$), whereas there were no significant differences concerning spinal BMD between underweight and weight-recovered women (respectively 0.83 ± 0.11 g/cm^2 versus 0.88 ± 0.10 g/cm^2, $p = 0.11$). The other demographic characteristics were not significantly different between the 2 patient groups. Detailed characteristics are summarized in Table 1.

3.2. BMFF and aLUL values between groups

A marked decrease in water peak amplitude could be visible on MR spectra in underweight anorexic women compared to healthy women (Fig. 2). Mean BMFF values were significantly higher at the femoral neck of underweight or weight-recovered anorexic women compared to healthy women (respectively 90.1 ± 6.7% or 90.3 ± 7.5% versus 81.3 ± 8.1%, $p < 0.01$; Table 2 and Fig. 3A).

Conversely, mean aLUL values were significantly lower at the femoral neck of underweight or weight-recovered anorexic women compared to healthy women (respectively 7.6 ± 1.4% or 7.3 ± 1.3% versus 9.2 ± 1.5%, $p < 0.01$; Table 2 and Fig. 3B).

3.3. BMFF and aLUL values between subregions

Mean BMFF and aLUL values were significantly different between subregions (Kruskal-Wallis analysis, $p < 0.0001$) but with patterns depending on the patient group.

In underweight anorexic women, BMFF at the acetabulum was significantly lower than BMFF at the other subregions (75.8 ± 10.1% versus 90.1 ± 6.7% or 89.6 ± 11.1% or 92.5 ± 10.9% for the femoral neck, proximal femoral diaphysis, and greater trochanter respectively). In weight-recovered anorexic women, BMFF was significantly different between all site-pairs, except between the proximal femoral diaphysis and femoral neck. Finally, in healthy women, BMFF at the acetabulum was significantly lower than BMFF at the greater trochanter or proximal femoral diaphysis (68.1 ± 7.4% versus 94.4 ± 3.3% or 85.1 ± 12.2% respectively), and the greater trochanter had higher values than BMFF at the femoral neck (81.3 ± 12.2%) (Table 2 and Fig. 3A).

Regarding aLUL patterns, underweight and weight-recovered patients had significantly higher mean values at the acetabulum compared to the other subregions. In contrast, healthy women also had significantly higher mean aLUL values at the acetabulum compared with the greater trochanter or proximal femoral diaphysis (12.7 ± 2.5% versus 7.1 ± 1.4% or 8.4 ± 2.4% respectively), but not with the femoral neck (9.2 ± 1.5%) (Table 2 and Fig. 3B).

3.4. Relation between BMFF, aLUL, and BMD in anorexic women

A strong negative correlation between aLUL and BMFF was observed, with a Spearman's rank-order coefficient of −0.88 when pooling all subregions, and coefficients comprised between −0.89 and − 0.74 when considering individually each region ($p < 0.0001$; Table 3 and Fig. 4A). This correlation remained strong when analyzing independently each subgroup ($p < 0.0001$; Tables 4 and 5). In

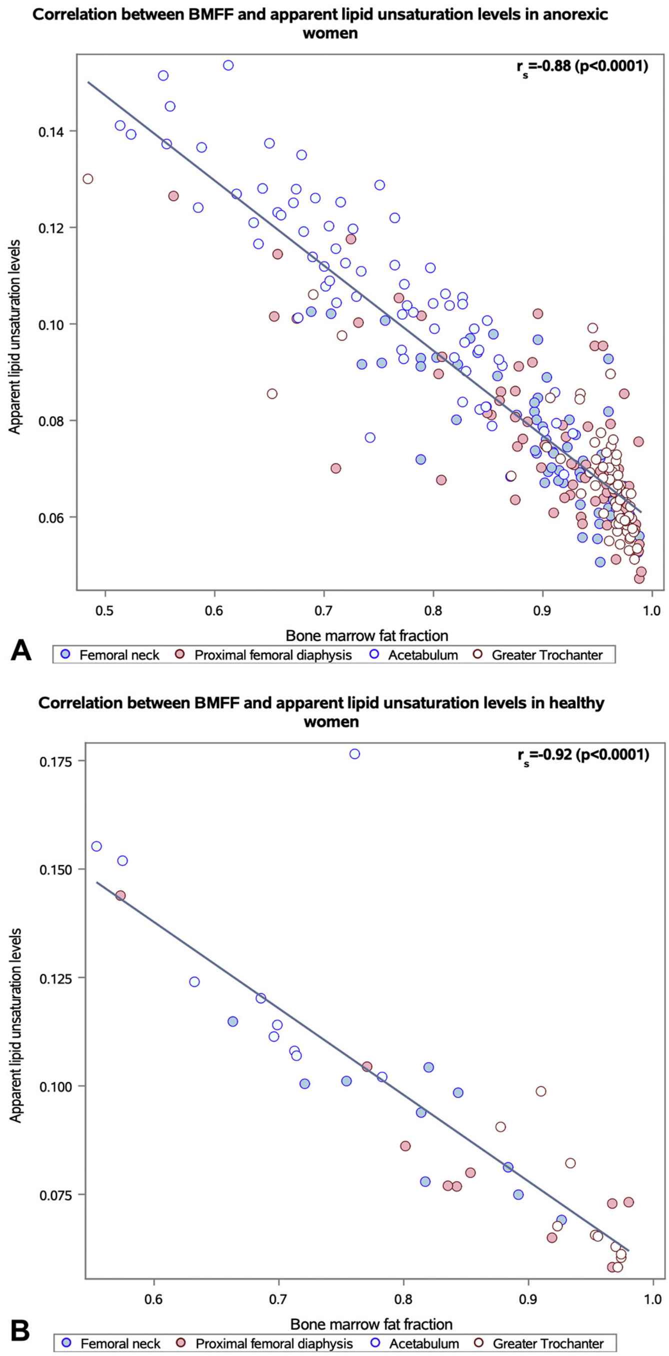

Fig. 4. – Strong negative correlation between bone marrow fat fraction and apparent lipid unsaturation levels in the hip of anorexic women (A) and healthy women (B) suggesting a close relationship between bone marrow adiposity unsaturation profile and its quantitative importance. r_s: global Spearman's rank-order coefficient (pooling all subregions), adjusted for BMI and the amenorrhoeic status.

comparison, a strong negative correlation between aLUL and BMFF was also observed in healthy women ($\rho = -0.92$, $p < 0.0001$; Fig. 4B).

Among all patients with anorexia nervosa, and after controlling for BMI and amenorrhoeic status, significant negative correlations between BMFF and total hip BMD were observed in the acetabulum, femoral neck (Fig. 5) and proximal femoral diaphysis (Spearman's coefficients of −0.30, −0.32 and − 0.29 respectively, $p < 0.05$; Table 3). However, in the subgroup analysis, a significant negative correlation between BMFF and total hip BMD was observed only in the proximal femoral diaphysis of underweight patients ($\rho = -0.36$, $p = 0.04$; Table 4) and at the acetabulum and femoral neck of weight-recovered patients (respectively $\rho = -0.42$ and $\rho = -0.41$, $p < 0.05$; Table 5). A significant positive correlation was also observed between aLUL and total hip BMD at the acetabulum, femoral neck and greater trochanter (respectively $\rho = 0.37$, $\rho = 0.33$ and $\rho = 0.25$, $p < 0.05$) (Table 3). In underweight anorexic patients, a positive correlation was statistically significant only in the proximal femoral diaphysis and the greater trochanter (respectively $\rho = 0.41$ and $\rho = 0.44$, $p < 0.05$; Table 4), whereas in weight-recovered patients, only measurements at the femoral neck remained significant ($\rho = 0.41$, $p = 0.03$; Table 5).

Finally, no statistically significant association was highlighted between BMD at the femoral neck and BMFF or aLUL at this location (Table 3 and Fig. 5).

3.5. Relation between BMA and body fat percentage in anorexic women

Regarding BMFF, a moderate positive correlation with body fat percentage was only found at the femoral neck when pooling all anorexic patients ($\rho = 0.35$, $p < 0.01$) (Table 3 and Fig. 5), whereas it was close to significance in weight-recovered women for the same location ($\rho = 0.34$, $p = 0.06$; Table 5). In underweight anorexic women, a significant positive correlation was observed at the femoral neck and the acetabulum (respectively $\rho = 0.41$ and $\rho = 0.45$, $p = 0.01$; Table 4).

Similarly, with respect to aLUL, a negative correlation with body fat percentage was observed at the femoral neck when pooling all anorexic patients or considering only weight-recovered anorexic women (respectively $\rho = -0.33$ and $\rho = -0.37$, $p < 0.05$; Fig. 5) (Tables 3 and 5). In underweight patients, a significant negative correlation was only observed at the acetabulum ($\rho = -0.42$, $p = 0.01$; Table 4).

4. Discussion

BMA has features that can be imaged and subsequently become interesting imaging biomarkers. To understand better this underestimated tissue, we investigated the regional characteristics of BMA at the hip of anorexic patients, assessing both its quantitative importance and its unsaturation levels, respectively through BMFF and aLUL measurements using ^{1}H-MRS. Interestingly, whereas we observed significantly higher BMFF values and lower aLUL at the femoral neck of anorexic patients compared to healthy adults, we did not find any significant differences between underweight and weight-recovered anorexic patients, regardless of the subregion. The strong inverse relationship we found between BMFF and aLUL, along with the discrete different regional patterns of BMA and close relationship between BMA and BMD, also suggest bone alterations that may foster fragility fractures. These last observations are in agreement with previous works focused on the hip or the spine of anorexic women [15,26].

4.1. Regional characteristics of bone marrow adiposity at the hip

As defined in the *Diagnostic and Statistical Manual*, altered nutritional intake is a key characteristic of anorexia nervosa [24]. The extreme depletion of body fat which occurs in these patients is associated with an increase in fracture risk and profound changes in bone composition and microarchitecture, such as in weight-bearing bones like the tibia where an enlargement of trabecular spacing has been reported [28,29]. In a nationwide register study, Vestergaard et al. reported a significant increased fracture risk of the femoral neck of women with anorexia nervosa, with an incidence rate ratio of 7.17 (95% confidence limits = 3.36–15.3) compared to 3.49 (1.50–8.11) in the spine [5]. An increase in BMFF was observed after sleeve gastrectomy surgery, a bariatric intervention which may have negative effects on skeletal health and promote fractures [30,31]. These results suggest that the mineral and non-mineral bone compartments must reach an equilibrium to maintain bone strength. Subsequently, the understanding of the regional variations of BMFF is of prime importance to dissect the

Table 4
– Spearman's rank-order correlation coefficients at 4 subregions of the hip of underweight anorexic women, between bone marrow fat fraction (BMFF), apparent lipid unsaturation levels, total hip bone mineral density (BMD), BMD at the femoral neck and body fat percentage, adjusted for body mass index and amenorrhoeic status. Significant values are shown in bold (red).

	Acetabulum		Femoral neck		Proximal femoral diaphysis		Greater trochanter	
	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value
A: BMFF								
Total hip BMD	−0.24	0.15	−0.24	0.17	−0.36	**0.04**	−0.23	0.18
BMD at the femoral neck	–	–	−0.08	0.65	–	–	–	–
Apparent lipid unsaturation levels	−0.88	**<0.0001**	−0.81	**<0.0001**	−0.75	**<0.0001**	−0.87	**<0.0001**
Body fat percentage	0.45	**0.01**	0.41	**0.01**	0.25	0.15	0.07	0.69
B: Apparent lipid unsaturation levels								
Total hip BMD	0.31	0.07	0.25	0.14	0.41	**0.02**	0.44	**0.01**
BMD at the femoral neck	–	–	0.10	0.57	–	–	–	–
Body fat percentage	−0.42	**0.01**	−0.29	0.09	−0.27	0.12	−0.17	0.32

Table 5
– Spearman's rank-order correlation coefficients at 4 subregions of the hip of weight-recovered anorexic women, between bone marrow fat fraction (BMFF), apparent lipid unsaturation levels, total hip bone mineral density (BMD), BMD at the femoral neck and body fat percentage, adjusted for body mass index and amenorrhoeic status. Significant values are shown in bold (red).

	Acetabulum		Femoral neck		Proximal femoral diaphysis		Greater trochanter	
	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value
A: BMFF								
Total hip BMD	−0.42	**0.02**	−0.41	**0.03**	−0.18	0.34	−0.17	0.35
BMD at the femoral neck	–	–	−0.35	0.07	–	–	–	–
Apparent lipid unsaturation levels	−0.87	**<0.0001**	−0.80	**<0.0001**	−0.72	**<0.0001**	−0.72	**<0.0001**
Body fat percentage	0.06	0.76	0.34	0.06	0.11	0.58	0.24	0.19
B: Apparent lipid unsaturation levels								
Total hip BMD	0.28	0.15	0.41	**0.03**	−0.05	0.79	−0.13	0.50
BMD at the femoral neck	–	–	0.34	0.08	–	–	–	–
Body fat percentage	−0.04	0.85	−0.37	**0.04**	−0.03	0.89	−0.14	0.47

pathological mechanisms that precede skeletal complications in energy-intake restriction conditions and those that may limit skeletal recovery after weight-restoration.

Additionally, BMA is a complex entity, functionally distinct from other adipose tissues. In rodents, two skeletal adipocyte subpopulations from proximal and distal tibia were identified, with different cellular properties [8]. Histologically, regions with low hematopoiesis and high lipid unsaturation (usually known as “yellow marrow”) contrast with areas with active hematopoiesis and low lipid unsaturation (“red marrow”) that may be more prone to physiopathological changes, age and hormonal fluctuations [8,32,33]. Although differences can be visible on T1-weighted MR sequences, slight variations in BMA composition may go undetected. As a result, we used ^{1}H-MRS to obtain a more accurate snapshot of BMA content and composition at multiple anatomical subregions of the hip. VOI positioning was standardized to obtain reproducible and comparable measurements while covering a relatively large volume (2 mL) of the spongy bone.

In our study, we demonstrated regional increasing values of BMFF as follows: acetabulum < proximal femoral diaphysis ≃ femoral neck < greater trochanter, with discrete regional variations depending on the disease status and weight recovering. Even though the highest amount of BMA was observed in the greater trochanter, this region of the hip is the least prone to loading forces. On the contrary, the femoral neck is more exposed to axial compression. In anorexic patients, we reported higher BMFF values in this subregion compared to controls, in addition to an inverse relationship between BMFF and total hip BMD. Although the exact physiopathological mechanisms cannot be determined, as ^{1}H-MRS cannot discriminate intra from extracellular lipids, our results might explain in part the greater occurrence of fractures at this location [5].

Following weight recovery in patients with AN, BMD in the hip progressively returns to a normal value despite a persistently elevated fracture risk remaining up to 10 years after diagnosis [4,5,29,34]. Fazeli et al. did not find any difference regarding BMFF at the hip of healthy subjects, underweight and weight-recovered anorexic women [23]. In contrast, we observed an altered BMA profile at the femoral neck of anorexic women compared to controls, but not between underweight and weight-recovered patients. This finding is of importance as it raises two points that might contribute to the enduring fracture risk. First, weight recovery may not be sufficient to restore a normal BMA. In our study, weight-recovered patients, who restored only recently their BMI (with an average of 1.5 years since weight normalization) still had an abnormal eating behavior, and 61.1% of them were amenorrhoeic, two parameters which also affect bone metabolism. Moreover, as DHEA + estrogen/progestin administration can stop disease-related changes in marrow composition in women with anorexia nervosa, persistence of an abnormal BMA may be indicative of a remaining hormonal dysfunction that lasts even after weight recovery [35]. Second, BMA appeared to have regional differences that may foster region-specific skeletal complications. We reported significant changes in BMA at the femoral neck of anorexic women compared to healthy subjects, but not in the other anatomical sites. As a result, BMA could recover differently between the different subregions of the hip. In particular, these differential variations might evolve at the expense of the femoral neck, where we reported an abnormally remaining accumulation of marrow adiposity in weight-recovered women compared to healthy subjects. Vajapeyam et al. also reported site-specific variations of BMA characteristics in the distal femoral diaphysis of adolescent girls with anorexia nervosa, following administration of DHEA + estrogen/progestin medication or placebo [35]. These data bolster the existence of region-specific patterns of BMA, and the need of taking into consideration the exact location assessed, especially in longitudinal studies.

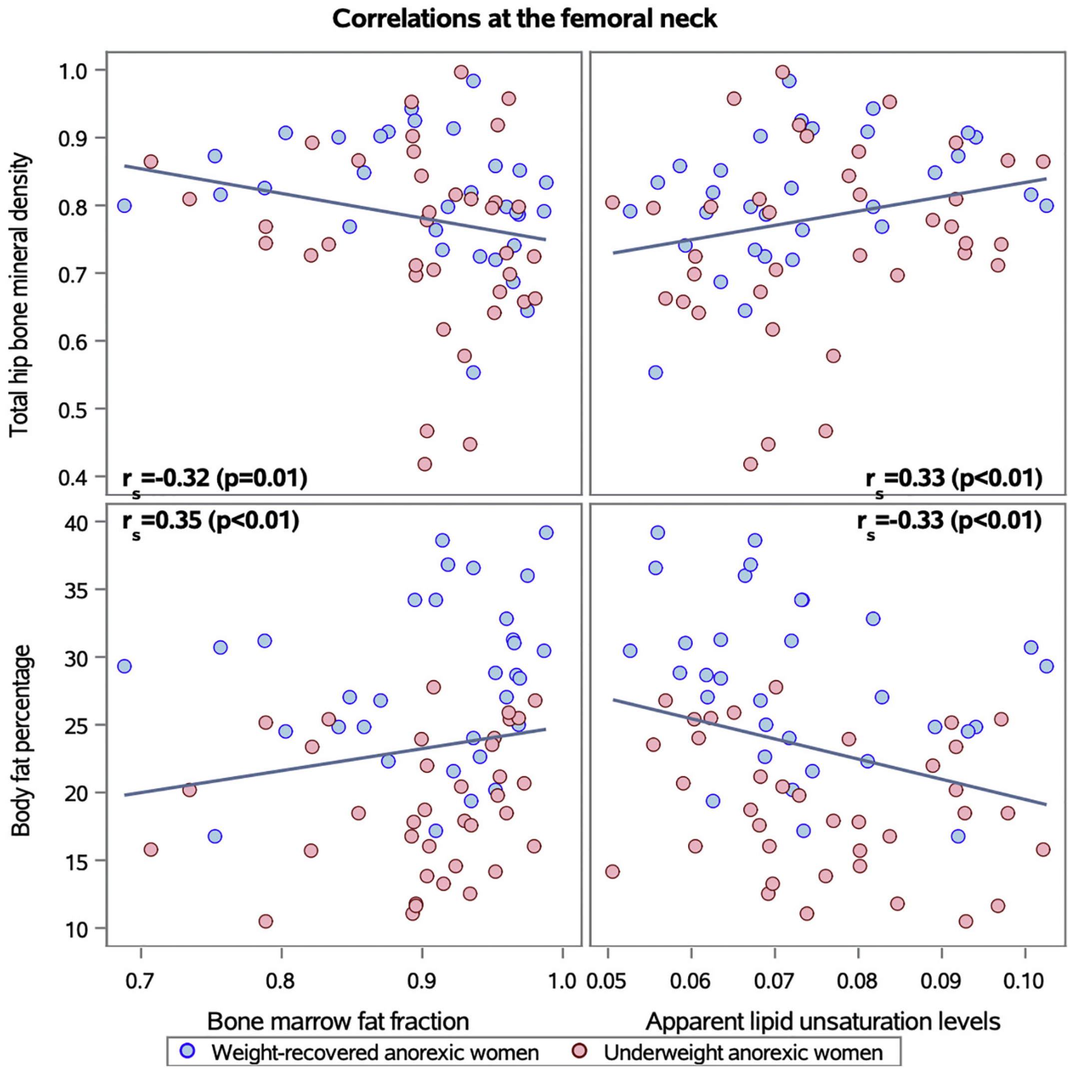

Fig. 5. – Correlations between bone marrow fat fraction (BMFF) or apparent lipid unsaturation levels (aLUL), and total hip bone mineral density (BMD) or body fat percentage, at the femoral neck of women with anorexia nervosa. Negative correlations were found between BMFF and total hip BMD as well as aLUL and body fat percentage, whereas a significant positive correlation and a positive trend were found between BMFF and body fat percentage, and aLUL and total hip BMD. Spearman's rank-order correlation coefficients (r_s) and significance (*p*-value) are indicated, as well as linear regression curves to show trends.

Interestingly, in our study, we did not observe a statistically significant association between BMFF at the femoral neck and BMD at this same location, contrasting with data in the literature [9]. Two reasons might partly explain the absence of statistical significance. First, the femoral neck is more prone to variability related to patient positioning and has a significantly higher precision error than the total hip. Therefore, inherent biases might affect statistical comparisons. Secondly, we observed a negative correlation between these two parameters in the subgroup analysis regarding weight-recovered anorexic women ($\rho = -0.35$, $p = 0.07$), and an almost null Spearman's rank-order correlation coefficient in the underweight group ($\rho = -0.08$, $p = 0.65$). Consequently, the evolution of bone marrow adiposity and bone mineral density probably depends on multiple factors including disease recovery and might reach a plateau in low weight anorexic women.

Whereas these region-specific characteristics reflect the variable quantitative importance of BMA, its lipidic profile, assessed through the aLUL, showed less variability at the hip. Mean aLUL values were higher at the acetabulum than the greater trochanter, femoral neck and proximal femoral diaphysis in anorexic women. In contrast, aLUL at the femoral neck of healthy women remained significantly lower compared to anorexic women. Yeung et al. suggested that changes in BMA composition may impair bone strength, after observing a comparable reduction in unsaturated lipids in the lumbar spine of osteoporotic patients [10].

Finally, we found a negative correlation between BMFF and aLUL in all subregions. As it was observed in both anorexic and healthy women, the phenomenon appears not to be disease-specific. However, the strength of this correlation has not a straightforward meaning, as methodological and physiological explanations may mingle together. First, T_2 value of bone marrow water is known to be inversely correlated with bone marrow proton-density fat fraction (PDFF) [36] and T_2 changes of bone marrow lipids can also emerge with an increasing BMI [35]. As we measured T_2-weighted fat fractions, the increasing overestimation of observed BMFF might therefore comprise a T_2 effect. Nevertheless, decreased unsaturated and increased saturated lipid levels have also been described in bone conditions where fat accumulation was reported [10,37]. Because high content of saturated fatty acids may induce a secretion of pro-inflammatory cytokines (such as IL-6), which have also been observed in adults with AN [37,38], a synergic effect of an increased adiposity and altered BMA composition might thus arise above a certain threshold and play an active role in the impairment of bone health, weakening bone strength and fostering fragility fractures in underweight and weight-recovered anorexic women.

4.2. Relation between marrow adiposity and body composition

DXA easily provides body composition parameters, such as body fat percentage. In our study, we observed a significant and positive but moderate correlation between this last parameter and BMFF at the femoral neck of anorexic patients. However, this result contrasts with other studies, which reported a negative correlation between BMA and other fat depots [15]. The explanation remains unclear, but two critical elements may intervene in these discrepancies.

First, the technique used to assess body composition may potentially lead to different results. Although DXA is acceptable and well correlated with body composition in AN, CT and MRI have a higher level of precision to assess separately subcutaneous and visceral adipose tissues, even if it comes at a greater cost [39]. Fazeli et al. reported a positive correlation between BMA and body fat percentage in the femoral epiphysis using DXA [23]. On the other hand, Bredella et al. found a significant negative correlation between marrow fat content in the femoral metaphysis and diaphysis based on an MR-approach to evaluate body composition, although no statistical significance was found in the femoral epiphysis [15]. Second, the underweight and weight-recovered anorexic patients were seeking nutritional management help at our institution, which may have had an impact on BMA.

Nonetheless, regarding BMA composition, we found that aLUL at the femoral neck was significantly and negatively correlated with body fat percentage in our study. Previous studies also reported negative correlations between marrow composition and body fat compartments, such as the subcutaneous and visceral adipose tissues assessed by MRI [16].

Consequently, correlations between BMA and body composition are partially conflicting with the literature, underlying the importance of a standardized methodology and reinforcing the idea that the relation between these two entities is probably multifactorial and might evolve with different rates following disease recovery.

4.3. Limitations

Our study has four main limitations. First, the cross-sectional design does not allow to make any assumptions about causality. Moreover, as our inclusion criteria included anorexic women followed in our institution, patients belonged to a treatment-seeking population. To limit these confounding factors, correlations were adjusted for BMI and the amenorrhoeic status, known to be a predictive factor of change in BMD during follow-up [40].

Second, there is only a limited number of controls, as the main goal of our study was to assess bone marrow adiposity in several regions at the hip of anorexic patients. However, this number is similar to other studies [15,17], and we selected gender- and age-matched volunteers to improve comparison accuracy. Moreover, although there was neither personal or familial medical history, nor clinical evidence of osteopenia, we did not assess hip BMD in the control group.

Third, ^{1}H-MRS is an advanced technique which is sensitive to artifacts. Approximately 7% of our acquisitions did not pass quality control. Besides, by using single-TE ^{1}H-MRS sequences, no T2 correction was applied [41] and the reported BMFF is affected by T_2-weighting effects, especially since BMI had been proved to be an effect modifier of T_2 relaxation times of water and lipids [35,36,42]. The same applies to the apparent lipid unsaturation level measurements, whose effect may also be driven by J-modulations [42]. However, all examinations were performed in the same MR scanner and in the exact same conditions, which allowed comparison between groups. Regarding DXA, body composition was not adjusted for body water, which can also be responsible for variations in anorexic women [39].

Finally, even if ^{1}H-MRS is considered as the gold standard to assess BMA in vivo, this imaging modality cannot discriminate abnormal fatty deposition from red to yellow bone marrow reconversion. Further studies are required to better depict the pathological mechanisms underlying BMA accumulation in patients with anorexia nervosa. Parametric maps provided by sequences like quantitative chemical-shift sequences can also be a way to improve the analysis of bone marrow adiposity in diseases such as AN, allowing the evaluation of regional variations.

5. Conclusion

Our study highlighted alterations in BMA at the femoral neck of young women with AN compared to healthy subjects, which could also be observed in weight-restored anorexic patients. By investigating the regional characteristics of BMA in these women, we could also depict discrete regional patterns and significant correlations between BMFF, aLUL, total hip BMD, and BF. Our data suggest a slow-evolving marrow fat profile, with regional specificities, and alterations that may last even after weight recovery, impair bone health and potentially contribute to hip fractures, especially at the femoral neck. To conclude, the assessment of BMA characteristics at this peculiar location could be useful in examining the long-term fracture risk for such patients.

CRediT authorship contribution statement

Sammy Badr: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Isabelle Legroux-Gérot**: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft. **Jean Vignau:** Conceptualization, Investigation, Resources, Writing - review & editing. **Christophe Chauveau:** Conceptualization, Writing - review & editing. **Stefan Ruschke:** Methodology, Software, Writing - review & editing. **Dimitrios C. Karampinos:** Methodology, Software, Writing - review & editing. **Jean-François Budzik:** Conceptualization, Formal analysis, Writing - original draft, Supervision. **Bernard Cortet:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Supervision. **Anne Cotten:** Conceptualization, Methodology, Writing - original draft, Supervision, Conceptualization, Methodology, Writing - original draft, Supervision.

Declaration of Competing Interest

DC Karampinos receives grant support from Philips Healthcare.

The other authors declare no conflict of interest in link with the present study.

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