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**The quantitative ultrasound method for assess low bone mass in women with anorexia
nervosa**

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ABSTRACT

Purpose: The aim of this study was to investigate the potential role of an alternative technique, quantitative ultrasound (QUS), to **assess** low bone mass in patients with anorexia nervosa (AN).

Methods: Two hundred and seven young women (134 patients with AN and 73 healthy controls) with ages ranging from 14.4 to 38.4 years participated in this observational cross-sectional study. Bone mass was concomitantly evaluated by DXA to determine areal bone mineral density (aBMD; g/cm²) at hip, lumbar spine and radius and by QUS to determine broadband ultrasound attenuation (BUA; dB/MHz) at the heel.

Results: BUA (66.5 ± 4.6 dB/MHz vs 61.0 ± 5.0 dB/MHz) and aBMD at the hip (0.916 ± 0.013 g/cm² vs 0.806 ± 0.010 g/cm²), lumbar spine (0.966 ± 0.012 g/cm² vs 0.886 ± 0.010 g/cm²) and radius (0.545 ± 0.005 g/cm² vs 0.526 ± 0.04 g/cm²) were significantly decreased (p<0.01) in patients with AN compared with controls. When patient and control data were pooled, BUA was significantly correlated with aBMD at the hip (r=0.60, p<0.001), lumbar spine (r=0.48, p < 0.001) and radius (r=0.40, p<0.001). In patients with AN, BUA and aBMD were mainly and positively correlated with weight, lean tissue mass, body mass index (BMI), and minimal BMI

life and negatively with the duration of both disease and amenorrhea. Better concordance between the two techniques was obtained when absolute BUA and aBMD values were used according to the WHO T-score classification.

Conclusion: BUA measurement at the heel by QUS appears to be a pertinent nonionizing technique to **assess** low bone mass in patients with AN.

Key terms: Quantitative ultrasound (QUS), Broadband ultrasound attenuation (BUA) bone demineralization, areal bone mineral density (aBMD), dual-X-ray absorptiometry (DXA).

Declarations

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Conflicts of interest/Competing interests: I certify that neither I (**Laurent Maimoun**) nor my co-authors (**Eric Renard, H el ena Huguet, Patrick Lefebvre, Krishna Mahadea, Vincent Boudousq, Marie Christine Picot, R emi Dor e, Pascal Philibert, Maude Seneque, Laura Gaspari, Philippe Courtet, Charles Sultan, Ariane Sultan, Didier Laux, S ebastien Guillaume, Denis Mariano-Goulart**) have a conflict of interest as described above that is relevant to the subject matter or materials included in this work.

Availability of data and material (NA)

Code availability (NA)

Mini Abstract

This study investigated the potential role of quantitative ultrasound(QUS) to assess low bone mass in anorexia nervosa patients (AN). Bone parameters from QUS and DXA were positively correlated and significantly reduced in AN compared with controls, suggesting that QUS is a pertinent technique to assess low bone mass in these patients.

1. Introduction

Anorexia nervosa (AN) is an eating disorder of multifactorial origins. Its prevalence in young females is approximately 0.4% (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition: DSM-5) per year. This disease is characterized by an intense fear of becoming fat, despite obvious thinness, and extreme behaviors for weight loss, including food restriction with or without self-induced vomiting or use of laxatives. The result is a massive weight loss and/or dramatic thinness [1]. Among the dramatic consequences of this disease, it is now well established that women with AN exhibit bone health alterations characterized by a decrease in areal bone mineral density (aBMD) at both appendicular (i.e., hip) and axial (i.e., lumbar spine) bones sites [2-6] associated with a deterioration in bone microarchitecture [3]. These bone impairments are the consequence of an uncoupling of bone formation and bone resorption [4, 7, 8] due to low levels of bone trophic hormones (i.e., insulin-like growth factor-I: IGF-I, and estrogen) and higher stress hormone levels (i.e., cortisol) [5, 6, 9]. Compared to other conditions that affect bone tissue, such as menopause, the kinetics of bone loss in patients with AN is specific and characterized by intense and very early bone demineralization [5, 10, 11]. Indeed, demineralization may be detected in adolescent girls in as little as 6 to 12 months after disease onset [10] and osteoporosis can then occur within 24 months [5, 11].

The combined alteration in bone mass and bone microarchitecture may be at the origin of the heightened fracture risk observed shortly after disease onset [12], as reported in both adults and adolescents [12-14], and it may be sustained beyond disease remission. Although weight gain and the return of menses are the most efficient ways to increase bone mass [15], their normalization still results in suboptimal bone accrual without full catch-up [15, 16].

Considering the dramatic consequences of AN on bone metabolism and fracture risk, early, systematic and adapted bone mass monitoring should be instituted for this population [5]. Dual-energy X-ray absorptiometry (DXA) is the gold standard method to diagnose osteoporosis [17] and one measure after 6 months of amenorrhea is recommended in these patients, with

monitoring every 2 years in those with low bone mass and persistent amenorrhea [18]. However, accessibility to this technique may be limited for this population as patients are often admitted to psychiatric residential treatment centers that are not authorized to use this X-ray device. In this situation, quantitative ultrasound (QUS) may be an alternative method for noninvasive assessment of skeletal status due to its nonionizing properties, portability and low cost.

Various clinical studies have demonstrated the validity of this technique (i.e., at the heel only) for routine screening of postmenopausal women to predict the risk of any type of osteoporotic fracture, independently of aBMD [19-21], and for assessing skeletal responses to mechanical loading induced by intense physical exercise [22]. Yet, despite its potential utility, few studies have used QUS to investigate the effect of AN on bone metabolism [3, 23, 24]. In addition, no clear message on the potential clinical utility of this technique for AN has been given because of the wide range of devices used and the different bone sites investigated (i.e., heel, finger, radius and tibia) and QUS parameters chosen—all of which are considered as potential confounding factors [21]—with evaluations in a limited number of patients.

Thus, the first aim of this study was to concomitantly evaluate the effect of anorexia nervosa on bone tissue by both DXA and QUS methods. The second aim was to determine whether QUS parameters are influenced by the same clinical and biological factors (i.e., anthropometric and disease-related parameters) as those evaluated by DXA. The last aim was to compare the concordance of the two measurements.

2. Subjects and method

Study approval was obtained from the Regional Research Ethics Committee (Comité de Protection des Personnes Sud-Mediterranee IV, Montpellier, France; reference: 11 02 03) and permission for the clinical trials was granted by the French Medicines and Health Care Products Regulatory Agency (Agence Française de Sécurité Sanitaire des Produits de Santé; AFSSAPS).

Written informed consent was obtained from all participants and their parents when the volunteers were minors.

2.1 Participants

A total of 207 adolescents and young women with ages ranging from 14.4 to 38.5 years (mean 22.8 ± 5.5) were enrolled in this study, and 134 of them had been diagnosed with AN. Patients were consecutively recruited from the Endocrinology Department at Montpellier University Hospital (France) in 2015-2017. They fulfilled the criteria for the diagnosis of restrictive AN as defined by the DSM-5, i.e., body mass index (BMI) $<18 \text{ kg/m}^2$, fear of gaining weight and alteration in body size perception [25]. The control group (CON) was recruited in the community by advertisement and included 73 normal-weight healthy adolescent and young women with $18 < \text{BMI} < 25 \text{ kg/m}^2$. All the controls presented normal menstrual cycles and no lifetime history of eating disorders.

2.2 Methods

This study followed a case-control design. Standing height was measured with a stadiometer to the nearest 0.1 cm. Weight was determined using a weight scale with a precision of 0.1 kg. BMI was calculated as weight (kg) divided by the square of height (m).

2.2.1 Medical and menstrual histories

Each subject responded to a medical questionnaire designed to assess the general medical and menstrual history (age of menarche and menstrual function were determined for each participant. (duration of the eating disorder

2.2.2 Assays

Fasting blood samples (25 mL) were collected in the morning (08:30–10:00) in sterile chilled tubes by standard venipuncture technique. The samples were allowed to clot at room temperature and were then centrifuged at 2500 rpm for 10 min at 4°C. Serum samples were

stored at -80 °C until analysis. All samples were run in duplicate and, to reduce inter-assay variation, all the plasma samples were analyzed in a single session.

For bone turnover, plasma samples were assayed by Cobas 6000 (Roche Diagnostic, Mannheim, Germany) for osteocalcin (OC) and type I-C telopeptide breakdown products (CTX). The inter- and intra-assay CVs for the two latter parameters were lower than 7%.

For all the biological parameters analyzed, the CVs for the intra- and inter-assay variations were given by the manufacturer.

2.2.3 Bone mineral density, body fat and fat-free soft tissues

DXA (Hologic QDR-4500A, Hologic, Inc., Waltham, MA) measured the areal bone mineral density (aBMD; g/cm²) of the antero-posterior lumbar spine (L1-L4), the dominant arm radius and the proximal part of the left femur (TPF). The soft tissue body composition (fat mass [FM, kg], percentage of body fat mass [% FM] and lean tissue mass [LTM, kg]) was derived from the whole-body scan. All scanning and analyses were performed by the same operator to ensure consistency after following standard quality control procedures. Quality control for DXA was checked daily by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (DPA/QDR-1; Hologic x-caliber anthropometrical spine phantom). The coefficients of variation (CV,%) given by the manufacturer were 0.8% for the spine and radius, 1.1% at the total proximal femur, and <1% for FFST and FM. According to the ISCD (the International Society for Clinical Densitometry), we previously reported in details the least significant change calculated from 30 subjects (0.013 g/cm² at lumbar spine and 0.018 g/cm² at total hip and was [26].

In addition, all values were expressed as T-scores as absolute numbers and categorized as defined by the World Health Organization (WHO) (number of standard deviations below or above the mean bone density value for young white Caucasian women at peak bone mass) as osteopenia (DXA T-score \leq -1.0 SD and $>$ -2.5 SD) or osteoporosis (DXA T-score \leq -2.5 SD).

2.2.4 Quantitative ultrasound measurement

QUS measurements were performed with the Osteospace® bone densitometer (Medilink, Maugeio, France). The device was calibrated daily in accordance with the manufacturer's recommendations. The same operator made all measurements. The subject's foot is placed in a dry foot receptacle, and the ultrasonic transducers are coupled to the heel with a water-based coupling gel. The transducers are positioned automatically, with the aid of a laser beam. The operator positions the laser on the center of the fibular malleolus using the command key panel on the device and a mirror in the foot receptacle. Using the coordinates of the malleolus (where the laser beam is placed) and the subject's foot length, the location of the calcaneus is then determined automatically. The two probes then move automatically inward into direct contact with the foot at the determined location [27]. This procedure takes into account the anatomical differences between subjects and ensures that the same relative location will be measured for each one. Using frequential analysis of the ultrasonic transmitted signal, Osteospace® automatically measures broadband ultrasound attenuation (BUA in dB/MHz). This assumes that attenuation varies linearly with frequency, which is verified for the calcaneus in the frequency range of the experimental device (0.2-0.6 MHz) [27]. The dominant heel was measured in all subjects. The precision (reproducibility) error for BUA was previously reported in details by Njeh et al., [27]. Briefly, the heel was measured 3 times with repositioning (i.e., foot taken out of the foot receptacle and then put back) during the same visit in a subgroup of 30 women aged 25-79 years (10 women aged 23 to 30 years; 10 women aged 31 to 59 years and 10 women aged 60 to 79 years). In this condition, the coefficient of variation (CV%), the root mean squared coefficient of variation (RMSCV%) and the standardized coefficient of variation (SCV%) were 1.72%, 2.90% and 6.09%, respectively [27].

Analysis of bone turnover markers, DXA and QUS measurements were performed in the same morning.

3. Statistical Analysis

The study population is described with means and standard deviations (SD) for quantitative variables and frequencies for qualitative variables. The continuous variable distributions were tested with the Shapiro-Wilk statistic and were compared with the parametric Student's t test when the distribution was Gaussian (including all the aBMD and QUS parameters) and with the Mann-Whitney test otherwise (including OC and CTX values). For qualitative variables, groups were compared using the Chi-square test or Fisher test.

Comparisons of aBMD at various bone sites, BUA and bone turnover between patients with AN and controls, adjusted or not for age and oral contraceptives used, were performed using multiples linear regressions.

The correlations between aBMD, BUA, and the anthropometric, gynecological and disease-related parameters were measured using Pearson's or Spearman's coefficients, depending on the distribution of variables.

The agreement between the DXA and QUS continuous measurements were evaluated with Gwet's agreement coefficient 1 (Gwet's AC1) for measurements according to the WHO classification (normal/osteopenia/osteoporosis) and with Bland and Altman's method for absolute T-scores. It should be noted that the WHO criteria (T-score) are only applicable to postmenopausal women or, at the minimum, women who have reached their peak bone mass. In the current study, however, we used the same T-score to examine the concordance between the two techniques even though some of the subjects had not reached their optimal BMD values. The Z-score is recommended to compare young patients with an age-, sex-, and race-matched reference range [28], but unfortunately, no reference curve is available for Osteospace® QUS in young population.

The sensitivity and specificity of the BUA were also estimated and, using the method of Blake et al [29], we determined the grey zone of the BUA, an area of values where the discriminatory performance is 'insufficient', in the sense that a value in this zone does not allow

the target disease to be scored as either present or absent [30]. The upper triage threshold was defined as the T-score below which 90% of the osteoporotic patients lie. The lower threshold was defined as the T-score above which 90% of the non-osteoporotic patients lie. The 95% confidence intervals (CI) on the triage thresholds were estimated according to the binomial distribution. The percentage of patients who met the criteria for referral for a DXA examination was calculated with the 95% CI.

To identify factors influencing aBMD at various bone sites and BUA, multiple linear regressions adjusted for age and other potential confounding factors, were performed. Candidate variables were stepped into the model with a stepwise selection. To determine entry and removal from the model, significance levels of 0.20 and 0.10 were used, respectively. These analyses were performed in each group (AN and CON) to take into account the characteristics of AN.

The statistical significance was set at 0.05 and the analyses were performed using Statistical Analysis Systems Enterprise Guide version 4.3 (SAS Institute, Cary, NC, USA).

4. Results

The anthropometric characteristics and the gynecological data of the 134 patients with AN and the 73 CON are summarized in **Table 1**. The age distribution ranged from 14.4 to 38.6 years and subjects with AN were slightly older than the control group (23.1 ± 3.1 vs. 22.8 ± 6.5 yr, respectively; $p=0.02$). There were no significant differences between the two groups with regard to height, whereas based on the underlying disease of interest, weight, BMI, body LTM, and body FM (kg and %) were significantly lower in AN compared with CON ($p<0.01$). Concerning the characteristics of AN, the mean age of onset was 17.9 ± 4.0 yr (range: 12.0 to 33.0 yr) and the mean duration was 4.9 ± 5.3 yr (range: 0.3 to 24.6 yr).

Concerning the gynecological profile (**Table 1**), the age of menarche was not different between groups (13.0 ± 1.4 ys for CON vs 13.0 ± 1.6 ys for AN). Hormonal contraceptive use was significantly lower in patients than controls (35% vs 67%, respectively, $p<0.01$). The mean duration of amenorrhea for those not taking hormonal contraceptives was 23.1 ± 43.7 months. Menstrual disorders were more frequent in patients with AN than in CON (85% vs 21%, $p<0.01$). Controls with menstrual disorders had only minor variations in cycle duration (~28 days), but no cases of secondary amenorrhea were encountered. None of the subjects presented primary amenorrhea.

Bone characteristics

Areal bone mineral density

Table 2 presents the aBMD for the two groups at various bone sites. Compared with CON, patients with AN presented significantly lower values ($p<0.01$) at the hip, lumbar spine and radius. The differences between controls and patients ranged from -3.7% for the radius and -12.2% for the hip.

Quantitative ultrasound (QUS)

BUA measured at the heel was significantly lower in AN patients compared with controls (-8.3%, $p < 0.001$) (**Table 2**).

Bone biochemical markers

Markers of bone turnover are described in **Table 2**. The marker of bone formation (OC) was significantly lower in patients with AN (-17.3%; $p < 0.01$), whereas the marker of bone resorption was significantly higher than in controls (+50.0%, $p < 0.01$; CTX).

Adjustment on age and hormonal contraceptive use did not modify the difference in the degree of significance between patients with AN and CON for any aBMD, QUS or bone turnover markers.

Classification of participants according to WHO criteria

Supplementary Table 1 showed the repartition of the participants based on their T-score at lumbar spine and hip, according to the WHO definition. At lumbar spine, 44 (60%) healthy controls presented normal values, 28 (38%) were osteopenic and one (2%) was osteoporotic. At this same bone site, 57 (42%) patients with AN presented normal values, 61 (46%) were osteopenic and 16 (12%) were osteoporotic. At hip, 56 (77%) healthy controls presented normal values, 17 (23%) presented osteopenia and none presented osteoporosis. For the same bone site, 51 (38%) patients with AN presented normal values, 66 (50%) presented osteopenia and 16 (12%) presented osteoporosis.

When the two localizations (lumbar spine and hip) were combined, one healthy control (1%) and 22 (16%) patients with AN had osteoporosis in either or both localizations.

For BUA measured at the heel, the repartition of healthy control and patients with AN according to normal, osteopenic and osteoporotic status was 62 (85%) vs. 56 (42%), 11 (15%) vs. 67 (50%), and 0 (0) vs 11 (8%), respectively.

Correlation between DXA and QUS measurements

For the whole population, BUA was positively correlated ($p < 0.001$) with aBMD measured at all bone sites (**Figure 1 A to C**). The coefficients of correlation ranged from 0.399 at radius to 0.604 at hip. When subgroup analyses were performed, the correlations between BUA and aBMD were better for patients than for controls at lumbar spine ($r = 0.45$, $p < 0.001$; $r = 0.20$, $p = 0.08$, respectively), hip ($r = 0.55$, $p < 0.001$; $r = 0.38$, $p < 0.001$, respectively), and radius ($r = 0.35$, $p < 0.001$; $r = 0.31$, $p < 0.05$, respectively).

Concordance between DXA and QUS measurements

The agreement between T-scores evaluated by DXA at the lumbar spine and hip and QUS according to the WHO classification (normal/osteopenia/osteoporosis) in the whole population, patients with AN and CON is shown in **Table 3**. Gwet's AC1 evaluation showed agreement percentages of 0.43 and 0.48 for lumbar spine and hip in the whole population, 0.38 and 0.34 for patients with AN, and 0.56 and 0.68 for CON.

The agreement between the absolute T-score evaluated by DXA and QUS at lumbar spine and hip in the whole population (A and B), patients with AN (C and D) and healthy controls (E and F) was assessed with Bland and Altman's method (**Figure 2**). There was no proportional bias (the slope of the regression line did not significantly differ from 0) for any analysis. In contrast to the whole population and healthy controls, no fixed bias was found in patients with AN for lumbar spine and hip (mean value of the difference differs significantly from 0; $p = 0.0026$ for lumbar spine and $p = 0.02$ for hip in whole population, $p < 0.0001$ and $p = 0.02$ in control group, respectively).

Table 4 presented the upper (90% sensibility) and lower (90% specificity) T-score thresholds calculated from the method of Blake et al., [29] to determine osteoporotic and nonosteoporotic patients with the Osteospace® QUS device. The lower triage threshold was a T-score of -1.5 [-1.89; -1.22] and the upper threshold was a T-score of -0.79 [-1.22; -0.66]. The percentage of patients who met the criteria for referral for an DXA examination was 23% (95%

CI: 16-32%). When these new cut-offs were applied, 17 patients were considered as osteoporotic.

Relationships of aBMD and BUA with the anthropometric, gynecological and disease-related parameters

When the data of all subjects were pooled (data not shown), BUA and aBMD were significantly and positively correlated with weight, BMI, FM, LTM (all, $p < 0.001$) and negatively correlated with CTX (radius excepted).

When subgroup analysis was performed in patients with AN, BUA and aBMD were positively correlated with weight (radius excepted), LTM, BMI (radius excepted), minimal BMI life, FM (only for BUA), and age of disease onset (only for radius), whereas negative correlations were observed with disease duration (radius excepted), amenorrhea duration (radius excepted) and OC levels (only for lumbar spine and radius). In controls, BUA and aBMD were positively correlated with weight, LTM and BMI (radius excepted).

Multiple regression analysis

Multiple regression analysis was performed to determine which independent factors might have influenced BUA and aBMD measured at the various bone sites in patients with AN (**Supplementary Table 2**) and CON (**Supplementary Table 3**). Our final models explain between 12% and 36% of the aBMD variance, depending on the bone site analyzed. In the AN group, the independent variables for L1-L4, hip and radius aBMD were principally duration of amenorrhea, minimal BMI life and osteocalcin. For controls, the independent variables for BMD were principally WB LTM. For BUA, the independent factors were age, minimal BMI life, WB LTM and CTX for patients and only LTM for controls.

5. Discussion

This study confirmed that AN induces a dramatic alteration in bone metabolism characterized by alterations in aBMD and bone turnover. Interestingly, BUA obtained from QUS at the heel was also significantly decreased in these patients to the same extent, suggesting the potential clinical utility of this technique for this disease.

Numerous studies have reported that AN induces a dramatic alteration in bone mass [2-6]. Our data confirmed that, aBMD was decreased at all bone sites in these patients, but particularly the hip and lumbar spine, whereas the radius seemed to be less affected. An uncoupling of bone turnover determined by bone biochemical markers was also observed characterized by a decrease in bone formation and an increase in bone resorption.

Conversely to DXA [2-6] or pQCT analysis [3], there are few data based on QUS parameters measured at the heel available in this specific population [3, 31]. In our study, the BUA values were significantly reduced in patients with AN compared with controls. Our data confirmed those of previous studies [3, 31]. Nevertheless, the difference in BUA values reported in these studies between patients and healthy controls appeared more marked (ranging from ~20% to ~30%) [3, 31] compared with our results (~8%). It is interesting to note, however, that whatever the study [3, 31], the amount of BUA difference was of the same order of magnitude as for the aBMD difference. This difference is somewhat unexpected because the patients with AN investigated here and in the other two studies [3, 31] were relatively comparable in terms of age, BMI and factors related to the disease (age of onset, duration of AN and duration of last menses), but differed for the contraceptive used [3]. It should therefore be kept in mind that the bone status of these patients is not only related to the clinical characteristics at the time of investigation, but also to the history of the disease. Thus, the minimum lifetime BMI, which represents the severity of underweight since the onset of AN, was higher in our study compared with Milos' patients (14.8 vs 13.2 kg/m²) [3], suggesting that the disease histories of our patients were less severe. This assumption was reinforced by the lower prevalence of patients with

osteoporosis in our study (16.4%) compared with other reports (21 to 40%) [3, 11, 32]. The apparent low severity of disease in our patients is probably due to the recruitment process in our clinical department, which began managing the patients early after disease onset.

In addition to BUA, the QUS method provides other physical parameters characteristic of bone mineral and structure. Thus, in adolescent patients with AN (n=14), Oswiecimska et al. [24] reported a lack of increase in the absolute amplitude-dependent speed of sound (Ad-SOS) values and an obvious decrease in the Z-scores of this parameter during medical care (19.4 months), suggesting that the difference with the reference values for the normal healthy young population increased. In this previous study, the absence of bone gain contrasted with an increase in weight. However, the lack of a control group and the difference in the bone site evaluated by QUS (i.e., proximal phalanges of fingers), which may be differently impacted by bone loss, makes any comparison with our results difficult. Hand phalanges are mostly constituted of cortical bone, whereas the heel is constituted more than 90% of trabecular bone [33]. Nevertheless, the SOS parameter, which was not directly obtained by the QUS device used in our study (i.e., Osteospace[®]), seems less discriminant than BUA to differentiate patients from controls [3]. In another study, unexpectedly higher SOS values measured at the distal radius and the mid-shaft of the tibia were reported in adolescents with AN compared with controls, while at same time, aBMD at the lumbar spine and hip was lower [34]. Moreover, SOS was negatively correlated with BMI and no significant correlations between SOS and aBMD were observed [34]. These unexpectedly higher SOS values were also observed by Kutilek et al. [35] in adolescent patients. These results were attributed to the minimal soft tissue that may violate underlying operating assumptions of this QUS methodology [21, 34]. Moreover, it is also probable that for SOS estimation, the bone morphology has to be taken into account because the ultrasonic propagation can lead to guided modes for which the value of SOS cannot be directly interpreted.

Our study revealed that in the context of AN, QUS may be of some interest. This is based on the observation that, not only were BUA and aBMD concomitantly lower in patients compared with healthy controls, but the difference also varied by the same magnitude of about 8–12%. Moreover, BUA and aBMD appeared positively and significantly correlated, even though these associations were less significant than those observed for aBMD measured at both lumbar spine and hip, for example. This result is not surprising because BUA is not fully explained by aBMD, but also to a lesser extent by microarchitectural parameters [21, 36, 37]. As suggested by Resch et al. [31], the low BUA in AN patients may therefore also be caused by the structural alteration in bone. In addition, BUA and aBMD are influenced by the same anthropometric and disease-related factors. Thus, clinical factors in these patients, such as weight, LTM, BMI and minimal BMI life, are positively correlated with both these parameters, whereas disease and amenorrhea duration exert negative influences and may be considered to have deleterious effects. These observations were previously observed in this population of patients for aBMD [3-6, 9, 31, 32, 38, 39], but only partially or not reported for BUA [3, 31, 35]. Moreover, we observed that the major independent factors associated with reduced aBMD or BUA were relatively comparable and included such crucial parameters as minimal BMI life.

Given that QUS methods are free of ionizing radiation, portable and relatively cost-effective for bone status evaluation, their use may increase accessibility to better detection and the assessment of low bone mass for patients outside of hospitals or institutions adapted to dispose of X-ray technologies. Nevertheless, several ambiguous points should be clarified before they can be used in clinical routine for this specific population. For example, it is well known that malnutrition is associated with abnormalities of sodium-potassium pumps, inducing an increase in sodium and water retention [40]. The occurrence of truncal and leg edema in patients with AN has been well described particularly during refeeding therapy [40], and calcaneal edema has been demonstrated to reduce BUA [41]. In this context, the ISCD recommended that patients with severe edema should be excluded from heel measurements [21]. Moreover, despite our

finding that BUA values was positively correlated with the aBMD values measured at all bone sites, particularly at the hip, we observed that the concordance between the two techniques to correctly classify patients as osteoporotic was limited. This results was somewhat expected because the WHO's classification of normal, osteopenic and osteoporotic status is established from aBMD values using DXA technologies at specified skeletal sites [42]. The better concordance between BUA and aBMD observed for absolute T-score values, particularly in patients, may reinforce the the ISCD's caution that the WHO criteria should not be applied to other technologies and other skeletal sites [21]. In our study, the determination of triage thresholds (i.e., T-score \leq -1.5 for osteoporotic and T-score \geq -0.79 for nonosteoporotic patients) seems to support the ISCD's recommendations on this point and may help to determine more adapted T-scores for QUS devices, particularly Osteospace®. **In addition, T-score <-1.5 improves the identification of patients with anorexia with osteoporosis.** Moreover, as the derivation of T-scores is heavily dependent on the reference database [43], better concordance between these two techniques may be obtained by using the same reference population concomitantly.

We are aware that our study presents some limitations. Although the mean age of patients and controls was relatively close (22.8 ± 6.5 years vs 23.1 ± 3.1 years), the difference was at the limit of significance. Moreover, the variances were not homogenous in the two groups. Nevertheless, these differences at the margin did not influence our results, as the adjustment for age did not modify the degree of significance. In addition, the cross-sectional design and the single measurement of bone parameters did not allow us to determine whether the kinetics of loss or regain of bone mass due to weight and menstruation recovery would be demonstrated in the same way by the two techniques. Future longitudinally designed studies would be useful to determine the sensitivity of these two techniques to monitor bone mass variation and determine fracture risk. Applying the WHO's osteoporosis definition (i.e., T-score $<$ -2.5 SD) to young women might be questionable as this classification was originally meant to be applied to

postmenopausal women only. However, Z-scores for a young population were not available for the QUS device used in this study, and this was the only way at our disposal to try to classify our patients according to the level of demineralization with both techniques. Last, the lack of information on two points—the patients' vitamin D status and the relationship between altered DXA and QUS bone parameters in the presence of fragility fractures in AN women—was also a limitation of this study.

BUA measurement at the heel by QUS appears to be a pertinent nonionizing technique to assess low bone mass in patients with AN. However, before BUA can be used in clinical routine, the concordance between DXA and QUS techniques in terms of the WHO T-score classification will need to be improved. Moreover, it seems important to determine whether QUS is sufficiently sensitive to monitor bone mass variations during the phases of the disease, such as weight loss or regain, which was unfortunately not evaluated in the current study.

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TABLES AND LEGENDS

Table 1. Characteristics of adolescent and young adult women with anorexia nervosa and controls.

Legend: Values are presented as mean \pm SD. BMI: body mass index. FM: fat mass; LTM: lean tissue mass; AN: anorexia nervosa. * Controls presented only minor alterations in the duration of menstrual cycles (~28 days). Menstrual disorders and duration of amenorrhea were only reported in patients with anorexia without oral contraceptive use.

Table 2. Comparisons of areal bone mineral density at various bone sites, ultrasound parameters and markers of bone turnover between patients with anorexia nervosa and controls adjusted or not by age and oral contraceptive use.

Legend: Values are presented as mean \pm SD. *Difference (%) = ((mean AN-mean CONT)/meanCONT)*100. aBMD: areal bone mineral density; BUA: broadband ultrasound attenuation, OC: osteocalcin; CTX: type I-C telopeptide breakdown products.

Table 3: Agreement between aBMD and BUA T-scores categorized according to the WHO classification in the whole population, patients with anorexia nervosa and controls.

Legend: Data are presented as number (percentage). Gwet's AC1 are agreement coefficients between aBMD and BUA T-scores. AN: anorexia nervosa; BUA: broadband ultrasound attenuation. Classification according to WHO criteria: normal (DXA T-score $> -1SD$), osteopenia (DXA T-score $\leq -1.0 SD$ and $> -2.5 SD$) and osteoporosis (DXA T-score $\leq -2.5 SD$). 95 % CI: 95 % confidence interval.

Table 4: Lower and upper triage thresholds for the determination of osteoporotic and nonosteoporotic patients from Osteospace® QUS device.

Legend: Patients with T-score ≤ -1.5 were classified as osteoporotic.

Patients with T-score ≥ -0.79 were classified as nonosteoporotic.

Patients with T-score ranging from $]-1.50 ; -0.79[$ (23%) require referral for an DXA examination.

Table 1. Characteristics of adolescent and young adult women with anorexia nervosa and controls.

Parameters	Controls (n=73)	Patients with AN (n=134)	p-value
Age (years)	23.1 ± 3.1	22.8 ± 6.5	0.02
Anthropometric data			
Weight (kg)	58.8 ± 6.8	44.1 ± 5.2	<0.01
Height (cm)	165.6 ± 5.6	165.4 ± 6.0	0.740
BMI (kg.m ⁻²)	21.4 ± 1.9	16.1 ± 1.5	<0.01
Minimal BMI life (kg/m ²)	19.1 ± 1.7	14.8 ± 1.6	<0.01
Whole-body FM (%)	28.2 ± 4.7	16.7 ± 5.6	<0.01
Whole-body FM (kg)	16.7 ± 3.9	7.6 ± 2.9	<0.01
Whole-body LTM (kg)	40.1 ± 4.5	35.6 ± 4.2	<0.01
Characteristics of AN			
Age of AN onset (yr)	-	17.9 ± 4.0	-
Duration of AN (yr)	-	4.9 ± 5.3	-
Hyperactivities, n(%)	-	37 (29)	-
Gynecological data			
Age of menarche (yr)	13.0 ± 1.4	13.0 ± 1.6	0.770
Menstrual disorders, (n and %)*	5 (21)	70 (85)	<0.01
Duration of amenorrhea (months)*	-	23.1 ± 43.7	-
Oral contraceptives, (n and %)	49 (67)	45 (35)	<0.01

Legend: Values are presented as mean ± SD. BMI: body mass index. FM: fat mass; LTM: lean tissue mass; AN: anorexia nervosa. * Controls presented only minor alterations in duration of menstrual cycles (~28 days). Menstrual disorders and duration of amenorrhea were only reported in patients with anorexia without oral contraceptive use.

Table 2. Comparisons of areal bone mineral density at various bone sites, ultrasound parameters and markers of bone turnover between patients with anorexia nervosa and controls adjusted or not by age and oral contraceptive use.

Dependent variables	Controls (n=73)	Patients with AN (n=134)	Difference (%)*	p-value
Areal BMD				
Lumbar spine (g/cm ²)	0.968 ± 0.093	0.884 ± 0.108	-8.7	<0.01
Lumbar spine T-score (SD)	-0.58 ± 0.83	-1.15 ± 1.05	-	<0.01
Hip	0.918 ± 0.098	0.806 ± 0.118	-12.2	<0.01
Hip T-score (SD)	-0.33 ± 0.78	-1.18 ± 0.98	-	<0.01
Radius	0.546 ± 0.035	0.526 ± 0.044	-3.7	<0.01
Radius T-score (SD)	-0.11 ± 0.73	-0.34 ± 1.08	-	0.13
Adjusted areal BMD				
Lumbar spine (g/cm ²)	0.966 ± 0.107	0.886 ± 0.110	-8.3	<0.001
Lumbar spine T-score (SD)	-0.58 ± 1.00	-1.16 ± 1.02	-	<0.001
Hip	0.916 ± 0.112	0.806 ± 0.116	-12.0	<0.001
Hip T-score (SD)	-0.33 ± 0.90	-1.19 ± 0.92	-	<0.001
Radius	0.545 ± 0.041	0.526 ± 0.043	-3.5	<0.01
Radius T-score (SD)	-0.11 ± 1.0	-0.40 ± 1.0	-	0.11
Ultrasonic parameters				
BUA (dB/MHz)	66.5 ± 4.6	61.0 ± 5.0	-8.3	<0.01
BUA T-score (SD)	-0.06 ± 0.84	-1.09 ± 0.91	-	<0.01
Adjusted Ultrasonic parameters				
BUA (dB/MHz)	66.6 ± 4.9	61.0 ± 5.1	-8.4	<0.001
BUA T-score (SD)	-0.04 ± 0.89	-1.09 ± 0.92	-	<0.001
Markers of bone turnover				
OC (ng/ml)	28.3 ± 9.4	23.4 ± 11.2	-17.3	<0.01
CTX (ng/ml)	0.50 ± 0.22	0.75 ± 0.34	+50.0	<0.01
Adjusted markers of bone turnover				
OC (ng/ml)	29.4 ± 10.1	22.4 ± 10.2	-23.8	<0.001
CTX (ng/ml)	0.54 ± 0.28	0.72 ± 0.30	+33.3	<0.001

Legend: Values are presented as mean ± SD. *Difference (%) = ((mean AN-mean CONT)/meanCONT)*100. aBMD: areal bone mineral density; BUA: broadband ultrasound attenuation, OC: osteocalcin; CTX: type I-C telopeptide breakdown products.

Table 3: Agreement between aBMD and BUA T-scores categorized according to the WHO classification in the whole population, patients with anorexia nervosa and controls.

		BUA					Gwet's AC1 [95% CI]
		N(%)					
			Normal	Osteopenia	Osteoporosis	Total	
		aBMD	Whole population	Normal	77 (37)	24 (11)	0 (0)
Osteopenia	39 (19)			42 (20)	8 (4)	89 (43)	
Osteoporosis	2 (1)			12 (6)	3 (1)	17 (8)	
Lumbar spine	Normal		79 (38)	28 (14)	0 (0)	107 (52)	0.48 [0.38;0.57]
	Osteopenia		37 (18)	42 (20)	4 (2)	83 (40)	
	Osteoporosis		1 (1)	8 (4)	7 (3)	16 (8)	
Patients with AN	Lumbar spine	Normal	37 (28)	20 (15)	0 (0)	57 (43)	0.38 [0.26;0.50]
		Osteopenia	18 (13)	35 (26)	8 (6)	61 (45)	
		Osteoporosis	1 (1)	12 (9)	3 (2)	16 (12)	
	Hip	Normal	28 (21)	23 (17)	0 (0)	51 (38)	0.34 [0.22;0.47]
		Osteopenia	26 (20)	36 (37)	4 (3)	66 (50)	
		Osteoporosis	1 (1)	8 (6)	7 (5)	16 (12)	
Controls	Lumbar spine	Normal	40 (55)	4 (5)	0 (0)	44 (60)	0.56 [0.41;0.71]
		Osteopenia	21 (29)	7 (9)	0 (0)	28 (38)	
		Osteoporosis	1 (1)	0 (0)	0 (0)	1 (1)	
	Hip	Normal	51 (70)	5 (7)	0 (0)	56 (77)	0.68 [0.52;0.85]
		Osteopenia	11 (15)	6 (8)	0 (0)	17 (23)	
		Osteoporosis	0 (0)	0 (0)	0 (0)	0 (0)	

Data are presented as number (percentage). Gwet's AC1 are agreement coefficients between aBMD and BUA T-scores. AN: anorexia nervosa; BUA: broadband ultrasound attenuation. Classification according to WHO criteria: normal (DXA T-score > -1SD), osteopenia (DXA T-score ≤-1.0 SD and >-2.5 SD) and osteoporosis (DXA T-score ≤-2.5 SD). 95 %CI: 95 % confidence interval.

Table 4: Lower and upper triage thresholds for the determination of osteoporotic and nonosteoporotic patients with the Osteospace® QUS device.

	Lower triage T-score (95%CI)	Upper triage T-score (95%CI)	Percentage referred for DXA (95%CI)
	-1.50 [-1.89 ; -1.22]	-0.79 [-1.22 ; -0.66]	23% [16 ; 32]
Patients with anorexia nervosa (n)	17	36	
Controls (n)	0	38	
Whole population (n)	17	74	

Legend: Patients with T-score \leq -1.5 were classified as osteoporotic.

Patients with T-score \geq -0.79 were classified as nonosteoporotic.

Patients with T-score within a range of]-1.50; -0.79[(23%) required a referral for DXA examination.

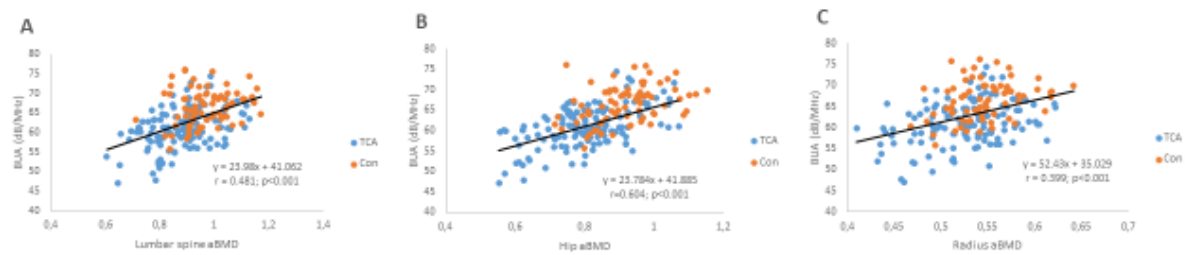


Figure 1. Correlation analysis of aBMD measured at various sites and BUA measured at the heel

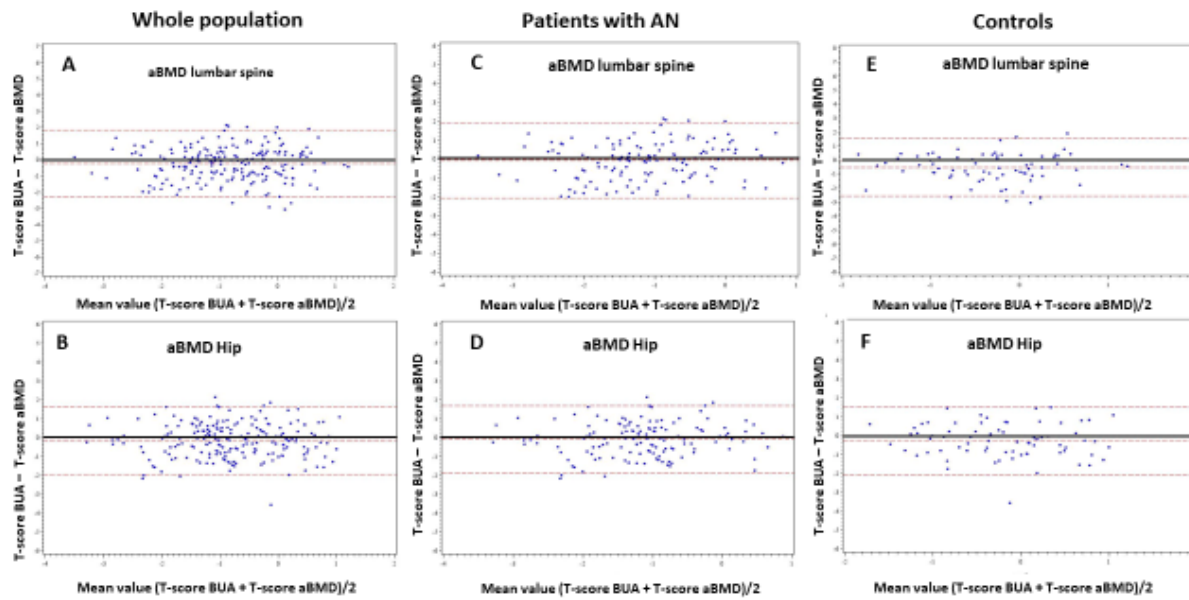


Figure 2. Bland and Altman approach assessed the agreement between absolute T-score evaluated by QUS and DXA, which served as gold standard, at lumbar spine and hip in whole population (A and B), in patients with AN (C and D) and healthy controls (E and F). y-axis shows the difference (T-score), and x-axis shows the mean (T-score) of the two measurements compared. Horizontal lines are added at value for zero (black continuous line) and at the mean (red dotted lines) of the difference and at 95% of confidence interval (CI) (red dotted lines). BUA: broadband ultrasound attenuation, aBMD, areal bone mineral density.

Supplementary material

Supplementary Table 1. Repartition of healthy controls and patients with anorexia nervosa based on the WHO criteria and according to the different bone sites.

Legend: Data are presented as number (percentage). BUA: broadband ultrasound attenuation. Classification according to the WHO criteria: normal (DXA T-score $> -1SD$), osteopenia (DXA T-score $\leq -1.0 SD$ and $> -2.5 SD$) and osteoporosis (DXA T-score $\leq -2.5 SD$).

Supplementary Table 2. Univariate and multivariate regression analysis of factors influencing aBMD at various bone sites and BUA in patients with anorexia nervosa.

Legend: aBMD: areal bone mineral density; L1-L4: lumbar spine; BUA: broadband ultrasound attenuation, BMI: body mass index; WB: whole body; FM: fat mass; LTM: lean tissue mass; Menstrual disorders (Yes vs. No); Oral contraceptives (Yes vs. No); CTX: type I-C telopeptide breakdown products. * Variables not introduced into the multivariate model for reasons of colinearity.

Supplementary Table 3. Univariate and multivariate regression analysis of factors influencing aBMD at various bone sites and BUA in controls.

Legend: aBMD: areal bone mineral density; L1-L4: lumbar spine; BUA: broadband ultrasound attenuation, BMI: body mass index; WB: whole body; FM: fat mass; LTM: lean tissue mass; Menstrual disorders (Yes vs. No); Oral contraceptives (Yes vs. No); CTX: type I-C telopeptide breakdown products. * Variables not introduced into the multivariate model for reasons of colinearity.

Supplementary Table 1. Repartition of healthy controls and patients with anorexia nervosa based on the WHO criteria and according to the different bone sites.

	All (n=207)	Controls (n=73)	Patients with AN (n=134)
Lumbar spine			
Normal	101 (49)	44 (60)	57 (42)
Osteopenia	89 (43)	28 (38)	61 (46)
Osteoporosis	17 (8)	1 (2)	16 (12)
Hip			
Normal	107 (52)	56 (77)	51 (38)
Osteopenia	83 (40)	17 (23)	66 (50)
Osteoporosis	16 (8)	0 (0)	16 (12)
Osteoporosis at lumbar spine or hip	23 (11)	1 (1)	22 (16)
BUA			
Normal	118 (57)	62 (85)	56 (42)
Osteopenia	78 (38)	11 (15)	67 (50)
Osteoporosis	11 (5)	0 (0)	11 (8)

Data are presented as number (percentage). BUA: broadband ultrasound attenuation. Classification according to the WHO criteria: normal (DXA T-score > -1SD), osteopenia (DXA T-score \leq -1.0 SD and >-2.5 SD) and osteoporosis (DXA T-score \leq -2.5 SD).

Supplementary Table 2. Univariate and multivariate regression analysis of factors influencing aBMD at various bone sites and BUA in patients with anorexia nervosa.

		P-values			
Groups	Parameters	L1-L4 aBMD	Hip aBMD	mid-radius aBMD	BUA
Univariate analysis	- Age	p=0.60	p=0.01	p=0.01	p<0.01
	- Weight	p<0.001*	p<0.001*	p=0.06*	p<0.001*
	- Height	p<0.01*	p<0.001*	p=0.33*	p=0.03*
	- BMI	p<0.01	p=0.01	p=0.11	p<0.001
	- Minimal BMI life	p<0.001	p<0.001	p<0.01	p<0.001
	- WB FM	p=0.10	p=0.16	p=0.18	p<0.01
	- WB LTM	p<0.001	p<0.001	p=0.01	p<0.001
	- Age of AN onset	p=0.40	p=0.86	p<0.001	p=0.78
	- Duration of AN	p=0.09	p<0.001	p=0.91	p<0.001
	- Amenorrhea duration	p=0.03	p<0.001	p=0.05	p<0.01
	- Age of menarche	p=0.25	p=0.92	p=0.23	p=0.85
	- Menstrual disorders	p=0.19	p=0.33	p=0.44	p=0.89
	- Oral contraceptives	p=0.50	p=0.372	p=0.26	p=0.51
	- Osteocalcin	p=0.02	p=0.04	p<0.001	p=0.30
	- CTX	p=0.94	p=0.79	p=0.19	p=0.10
Multivariate analysis	- Age	p=0.98	p=0.23	p<0.01	p<0.01
	- Minimal BMI life	p<0.001	p<0.001	p=0.01	p=0.01
	- WB FM				p=0.10
	- WB LTM				p=0.02
	- Amenorrhea duration		p=0.01	p=0.06	
	- Osteocalcin	p=0.15	p=0.01	p<0.001	
	- CTX				p=0.04
	Final model	R ² =0.23	R ² =0.36	R ² =0.26	R ² =0.35

aBMD: areal bone mineral density; L1-L4: lumbar spine; BUA: broadband ultrasound attenuation, BMI: body mass index; WB: whole body; FM: fat mass; LTM: lean tissue mass; Menstrual disorders (Yes vs. No); Oral contraceptives (Yes vs. No); CTX: type I-C telopeptide breakdown products. * Variables not introduced into the multivariate model for reasons of colinearity.

Supplementary Table 3. Univariate and multivariate regression analysis of factors influencing aBMD at various bone sites and BUA in controls.

Groups	Parameters	P-values			
		L1-L4 aBMD	Hip aBMD	mid- radius aBMD	BUA
Univariate analysis	- Age	p=0.38	p=0.86	p=0.14	p=0.75
	- Weight	p<0.01*	p<0.001*	p=0.01*	p=0.01*
	- Height	p=0.08*	p<0.001*	p=0.13*	p=0.09*
	- BMI	p=0.01	p=0.01	p=0.04	p=0.04
	- WB FM	p=0.29	p=0.15	p=0.58	p=0.11
	- WB LTM	p<0.001	p<0.001	p<0.01	p=0.03
	- Age of menarche	p=0.29 p=0.49	p=0.59 p=0.99	p=0.19 p=0.80	p=0.90 p=0.05
	- Oral contraceptives	p=0.38 p=0.37	p=0.97 p=0.38	p=0.24 p=0.07	p=0.20 p=0.10
	- Osteocalcin -CTx				
	Multivariate analysis	- Age	p=0.32	p=0.78	p<0.001
- WB LTM		p<0.001	p<0.001	p<0.01	p=0.03
- Age of menarche				p=0.01	p=0.06
- Oral contraceptives				p<0.01	
- CTX		R ² =0.18	R ² =0.29	R ² =0.35	R ² =0.12
Final model					

aBMD: areal bone mineral density; L1-L4: lumbar spine; BUA: broadband ultrasound attenuation, BMI: body mass index; WB: whole body; FM: fat mass; LTM: lean tissue mass; Menstrual disorders (Yes vs. No); Oral contraceptives (Yes vs. No); CTX: type I-C telopeptide breakdown products. * Variables not introduced into the multivariate model for reasons of colinearity.