Assessment of fracture risk in women with eating disorders

The utility of dual-energy x-ray absorptiometry (DXA)—Clinical cohort study

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Title: Assessment of fracture risk in women with eating disorders: The utility of dual-energy x-ray absorptiometry (DXA). Clinical cohort study.

Short running: DXA and fracture risk in eating disorders

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Abstract

Objective

Eating disorders (EDs) are associated with decreased bone mineral density (BMD) and increased fracture risk. The association between BMD and fracture risk in EDs is not well elucidated. We aimed to assess BMD in an ED cohort of patients with active disease and patients in remission, and to assess the predictive value of BMD on incidence of fractures.

Method

We included 344 female patients (median age 19, IQR 16; 24) referred to ED treatment. Later, patients were invited to follow-up including assessment of remission status and a DXA-scan. Information on fractures was obtained through the Danish National Registry of Patients.

Results

Patients with current AN, had significantly lower BMD compared to controls at lumbar spine (16 % lower, p<0.0001), femoral neck (18 % lower, p<0.0001) and total hip (23 % lower, p<0.0001). Recovered AN patients had higher BMD compared to those with current disease (p<0.0001 for all measures), but lower BMD compared to controls at lumbar spine (p<0.01) and hip (p<0.001). BMD did not differ between BN patients and controls. In patients with active EDNOS, BMD was lower only at the total hip (p< 0.005). We found no association between BMD and fracture risk.

Conclusion
We confirm that AN is associated with low BMD, whereas BN is not. Remission is associated with higher BMD compared to patients with active AN, but a deficit remains. We found no significant association between BMD and fracture risk, challenging the benefit of the widespread use of DXA scans in young women with ED.

**Clinical trial registration**

The study is registered in ClinicalTrials.gov, number NCT00267228.

**Keywords**

Eating disorders, anorexia nervosa, bone mineral density, fracture dual-energy x-ray absorptiometry.
Introduction

Eating disorders (ED) are multifaceted syndromes, characterized by abnormal eating habits and an excessive preoccupation with the control of body weight, and as such, patients with EDs are at risk of malnutrition, vitamin deficiencies and insufficient energy intake. Among numerous organ-systems, this deficiency state has consequences to bone, in terms of both decreased bone mass and impaired bone quality (Misra, Golden, & Katzman, 2015). As a consequence, fracture risk is increased in all anorexia nervosa (AN), bulimia nervosa (BN) and eating disorders not otherwise specified (EDNOS) patients (Vestergaard et al., 2002). Primarily investigated in AN, fracture risk increases shortly after debut of disease (Faje et al., 2014) and is sustained for many years (Lucas, Melton, Crowson, & O'Fallon, 1999; Vestergaard et al., 2002), possibly beyond disease remission.

A key component in the concept of osteoporosis and assessment of fracture risk is bone mineral density (BMD), obtained by dual-energy x-ray absorptiometry (DXA). In postmenopausal women, relative risk of suffering a major osteoporotic fracture, approximately doubles for every 1 standard deviation (SD) decrease in BMD obtained at the hip or lumbar spine (Marshall, Johnell, & Wedel, 1996), thereby providing a framework for the diagnosis of osteoporosis and everyday treatment decisions. In EDs however, there are a number of issues related to the use of DXA for assessment of BMD. First of all, the before mentioned relationship between BMD and fracture risk is derived from populations that contain the post-menopausal age span, and cannot necessarily be extrapolated to populations consisting chiefly of adolescents or young adults (Rauch et al., 2008). In accordance, the International Society for Clinical Densitometry has suggested that the diagnosis of osteoporosis in premenopausal women should not be based on BMD alone (Rauch et al., 2008). Secondly, BMD assessment by DXA relies on the assumption that the body is a three-compartment...
model, consisting of bone mineral, fat and lean body mass. As DXA only differentiates two compartments (hence, dual-energy) it is assumed that the composition of the soft tissue overlying the bone is identical to the adjacent soft tissue where the composition is measured. In AN, however, cycles of weight-loss and subsequent weight-gain alters body composition. Multiple studies have shown that rapid weight-gain in patients with AN takes place, primarily in the thighs, buttocks, and abdominal region, thereby resembling a central adiposity phenotype (El Ghoch et al., 2015; El Ghoch et al., 2014; Mayer et al., 2005; Prioletta et al., 2011). As these regions covers the most often used DXA regions of interest (hip, lumbar spine), there is an apparent risk of inaccurate estimates of true BMD (Bolotin, 2009). Nevertheless, DXA is by far the most widely available technique for assessing BMD in a clinical setting and information about how BMD reductions translate to fracture risk in ED is essential for developing evidence based practice guidelines for protecting bone health in these patients.

Another issue regarding EDs is that the trajectory of the disease includes periods of disease remission and subsequent relapse, meaning that the cumulative effect of an ED can be somewhat difficult to estimate.

Thus, the value of a single DXA-obtained measurement of BMD in ED patients, in terms of fracture prediction or treatment decisions, might be affected, not only by the measured BMD at the specific time of the DXA scan, but also by the course of the disease (on-going weight loss; on-going weight gain; disease remission), and by the patients age and age at debut of disease. In this observational study of an ED cohort, combining DXA scans and register-based information on bone fracture, we therefore aim to (1) assess BMD and body composition measures in a Danish ED cohort of patients...
with active disease and patients in remission, and (2) assess the predictive value of DXA-derived Z-score on incidence of fractures.

Methods

Subjects

Patients were recruited from the Funen Anorexia Nervosa Study (FANS), a cohort comprising all patients referred to specialized ED treatment at Centre for Eating Disorders, Funen, Denmark, between January 1st 1994 and December 31st 2004. The cohort has been described in detail in previous publications (Stoving, Andries, Brixen, Bilenberg, & Horder, 2011; Winkler, Frolich, Schulpen, & Stoving, 2017). In 2008, all patients, regardless of disease course or treatment status, were invited to a follow-up visit, focussing on re-assessment of the diagnosis, weight-history, assessment of remission status and a DXA scan.

Diagnosis at first referral was obtained by a trained physician at referral, initially according to the ICD-10 classification, and the diagnosis was subsequently converted to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) coding for conformity. Height was measured on a wall-mounted stadiometer and weight was measured on a calibrated platform scale. BMI was calculated as weight divided by the square of height (kg/m\(^2\)), and % IBW was calculated using a Danish population reference defining IBW as median BMI for age and gender (Nysom, Molgaard, Hutchings, & Michaelsen, 2001). Menstrual status was self-reported, based on the six months prior to assessment, and classified into regular, irregular, and amenorrhea or as influenced by OCP use. Amenorrhea was defined as no menstrual bleedings for more than three months, irregular as some menstrual bleeds, but with no regular cycle defining it and regular as a regular cycle with periods...
ranging from 21-35 days. At follow-up, nadir weight since referral was recorded from medical charts or reported by the patient, and anthropometrics and diagnostic information was re-assessed. Since there is no consensus on how to define remission from EDs, we decided on the following criteria, incorporating two core features of EDs: (1) % ideal body weight (IBW) consistently above 85% and (2) no bulimic or purging episodes within the last six months (Stoving et al., 2012).

Inclusion criteria were a diagnosis of either AN, BN or EDNOS according to the DSM-IV and a completed DXA scan. The sole exclusion criterion was male gender, as the number of males in the cohort was too small for meaningful statistics (n=8).

All procedures performed were in accordance with the ethical standards of the 1964 Helsinki declaration. All subjects signed a written form of consent, and the study was approved by the local ethics review board (No. S-VF-20050144) and the Danish Data Protection Agency (Journal.no. 2005-41-5578).

**DXA**

DXA (Hologic, Discovery, Waltham, MA, US) was used in the assessment of body composition and BMD. Bone mineral content (BMC) and BMD measurements were obtained for the total hip, femoral neck and lumbar spine (L1-L4). From a whole body scan, body fat percentage, total fat mass and total lean body mass was obtained. Z-scores were calculated from reference data for BMD in a group of young-adult Danish women (age 20-40 years) previous reported on (Hansen, Shanbhogue, Folkestad, Nielsen, & Brixen, 2014). The coefficient of variation (CV) for the hip and spine assessment is 1.5% in our unit.
Fracture data

Information on fractures was obtained through the Danish National Registry of Patients (NRP), through the Danish Health Data Authorities. The NRP contains diagnoses from all visits to public hospitals from 1977 and forward, and from 1995, visits to outpatient clinics and emergency departments as well. The link between the patient and diagnosis in the NRP is a unique personal identification number assigned by the Danish Civil Registry System to every Danish inhabitant. The coverage of the register is secured by making reimbursements from the Danish Health Authorities depend on reporting to the NRP. In addition to a high degree of coverage, the positive predictive value of coding in the NRP is very high (>95%), making the NRP a valid and largely unbiased source for fracture data (Thygesen, Christiansen, Christensen, Lash, & Sorensen, 2011).

As the aim of study was to assess the relationship between low BMD and fracture, we chose to focus on fractures most likely due to osteoporosis. Thus, fractures likely related to major trauma (defined as more than one fracture on same referral) and fractures of the skull, sternum and ribs, fingers and toes were excluded.

Data was extracted as of July 2016. The observation period for each patient was defined from referral to data extraction, truncated by death or migration out of Denmark, in order to avoid immortal time bias. Data on residency in Denmark and death were obtained from the Danish Civil Registry System.

In order to derive relevant fracture rates for the general population a control group was randomly drawn from the Danish Civil Registry system, based on year and month of birth and gender. Each patient was matched 1:5.
Statistics

Standard descriptive statistics was applied. Distribution of data was evaluated visually by normal probability plots. Non-normally distributed parameters were further evaluated with histograms to determine distribution. Data are presented as mean +/- SD for normally distributed data or median and interquartile range (IQR) for non-normally distributed data. Differences in DXA measurements between diagnostic groups and healthy control were assessed using one-way ANOVA or Kruskal-Wallis, as appropriate. If the overall p-value of the test was considered significant, differences between individual groups were assessed by Tukey-Kramer post hoc test for parametric data and Dunns test with Bonferroni correction, for non-parametric data.

Poisson regression was used to assess association between absolute BMD, BMD Z-scores and fracture risk. Fracture rates were calculated by dividing the number of fractures with total person-years of observation time, and presented as number of fractures per 1000 patient-years. In patients with more than one hospital contact for fractures at the same anatomical location, we only included coding events that were at least 180 days apart in order to avoid counting a follow-up visit as a new fracture event. Incidence rate ratio were calculated based on a cut-off of two standard deviations below mean for sex and age (Z-score <-2), as proposed by the International Society for Clinical Densitometry (Rauch et al., 2008), in order to assess whether this proposed cut-off had any discriminative power in terms of fracture prediction.

A p-value of 0.05 was considered significant. Stata (Version 14.0, StataCorp, TX, US) was used for all statistical analysis.
Bias:

Non-response bias was assessed by comparing patients attending follow-up to those who either declined or did not reply to invitation, by pairwise t-tests. We expected a proportion of missing data from the medical charts at referral. Missing data was regarded as missing completely at random and analyses were carried out only on complete cases; hence there was no imputation into the dataset.

Results

Subjects

From the underlying study population, a total of 344 patients (35%) fulfilled the criteria for entering the study (Figure 1). Median age at referral was 17 years (range 14–41) in patients with AN, 21 years (range 13-42) in BN and 20 years (range 14-39) in EDNOS. Those who did complete both DXA and clinical examination at follow-up did not differ from those who either declined or did not respond to the invitation, in terms of age at debut of disease, baseline BMI and % IBW, nadir BMI and % IBW or number of ED-related admissions (supplemental Table 1). The fraction of patients in remission within two years of debut of disease was comparable as well. Characteristics at follow-up are displayed in Table 1. Apart from obvious differences in body weight and BMI, patients with current AN differed from those in remission, in terms of lower nadir % IBW (p<0.0001), lower height (p<0.05) and longer duration of disease (p<0.0005). There was no difference in the use of OCPs between the two groups. In both BN and EDNOS, the only descriptive parameter, which differed between patients with current disease and those in remission, was duration of disease.
The amount of missing data was low (<5%), except for nadir BMI/% IBW (< 7% in AN; < 30% in BN and < 28% in EDNOS) (supplemental Table 2).

**DXA**

As expected, patients with current AN had lower fat %, fat mass and lean body mass, compared to their recovered counterparts (p<0.0001 for all measures). In both BN and EDNOS, no body composition measures differed between those with current disease and those who had recovered. Patients with current AN, had significantly lower BMD compared to healthy controls at lumbar spine (16 % lower, p<0.0001), femoral neck (18 % lower, p<0.0001) and at the total hip (23 % lower, p<0.0001). The prevalence of low bone density, defined as Z-score < -2.0 at femoral neck, total hip or lumbar spine, was 50.0 % in the current AN group vs. 9.2 % in the remission group. Even though BMD at lumbar spine, femoral neck and total hip were higher in the group of recovered AN patients compared to those with current disease (p<0.0001 for all measures), mean BMD in the recovered group were still lower compared to the healthy control group in lumbar spine (p<0.01) and total hip (p<0.001). In patients with current BN, BMD values did not differ between patients and controls at neither site, although the prevalence of low bone mass in current BN was higher than predicted from the normally distributed reference population (12.5 %). There was no difference in mean BMD between patients with current BN, and those who had recovered, although the prevalence of low bone mass differed (12.5 % vs. 0 %). In patients with active EDNOS, mean BMD was lower only at the total hip (p< 0.005), and no difference was observed between patients with current disease, and those who have recovered.
Fractures

Fractures are shown in table 3. As both BMD and fracture risk did not differ between patients with BN and EDNOS and controls, further analysis were only carried out for AN patients. Poisson regression showed no significant association between fracture risk and any of lumbar BMD ($r^2 = 0.012; p = 0.15$), total hip BMD ($r^2 = 0.009; p = 0.21$) or femoral neck BMD ($r^2 = 0.021; p = 0.09$). Equal non-significant associations were found for lumbar spine BMD Z-score ($r^2 = 0.009, p = 0.21$), total hip BMD Z-score ($r^2 = 0.009, p = 0.22$) and femoral neck BMD Z-score ($r^2 = 0.005, p = 0.33$). Adjustment for age, duration of disease and remission status, did not improve either association (data not shown). Incidence rates and incidence rate ratios, stratified by Z-score of minus two are shown in table 4. Although fracture incidence rates were higher in the group of AN patients with Z-score below minus two, the difference in IRRs were far from significant at any region. In the BN and EDNOS groups, the number of patients with a Z-score below minus two and a fracture, consisted of only one and three patients, respectively, providing far too little power for further statistical analysis (data not shown).

Discussion

In one the largest published cohorts of ED patients, we confirm that AN is associated with a high prevalence of low bone mass, defined as Z-score below minus two. Furthermore, we show that disease remission is associated with higher BMD compared to patients with active AN, but a deficit remains compared to the general population. Although BMD is decreased, a proposed cut-off of Z-score below minus two, did not predict increased fracture risk.
Due to the design of the study, all patients with active disease at follow-up had a minimum duration of disease of four years, and were offered treatment including efforts towards nutritional rehabilitation, if deemed necessary. The effect of the latter is likely reflected in the quite large difference between nadir % IBW and current % IBW (68.0 ± 9.9 % vs. 80.6 ± 7.1 %) in patients with active AN. A proportion of patients with active disease and a % IBW > 85 %, was due to our definition of remission, integrating both weight and purging behaviour. In terms of the weight criterion for remission, the DSM-IV outlines the diagnostic cut-off for AN as 85 % of expected body weight, leaving some room for interpretation of what to be expected (Thomas, Roberto, & Brownell, 2009). Some studies have used a cut-off BMI 17.5 kg/m² as in the ICD-10 diagnostic system, and others have applied formulas based on regional height-weight charts (Pai & Paloucek, 2000). By applying % median IBW, we allowed for tracking during growth from adolescents to adulthood and integrating a local reference material, hereby increasing the external validity.

Standing height in the group of patients with current AN, was lower compared to both former AN patients and healthy subjects in the DXA control group (height 167.4 ± 6.3 cm, p < 0.05 compared to patients with active disease). That malnutrition results in deceleration of linear growth is well known, and accordingly, patients with adolescent-onset AN, have been shown to have a lower final height than healthy controls (Modan-Moses et al., 2012). Even though weight restoration accelerates growth, catch-up is often not fully attained, indicating that the growth spurt period is very sensitive to malnutrition.

We report decreased BMD in all of femoral neck, total hip and lumbar spine in patients with current AN compared to a group of age-matched healthy controls. Both the absolute BMD-deficit and prevalence of low z-score (below minus two) in hip and lumbar spine, are similar to the findings in
others cohorts comparable on age and duration of disease (Bachmann et al., 2014; Grinspoon et al., 2000; Hofman, Landewe-Cleuren, Wojciechowski, & Kruseman, 2009; Winston, Alwazeer, & Bankart, 2008). Furthermore, patients with similar age at debut of disease and BMI at baseline, but with shorter duration of disease, are reported to have higher BMD (DiVasta et al., 2016; Levy-Shraga et al., 2016; Misra et al., 2011). Thus, our findings support the notion that the adverse effect of malnutrition on bone, progresses with increasing duration of disease. Of greater interest is our finding of only partly restored BMD in the group of patients with a history of AN. In spite of complete disease remission, and consequently normal body weight for a median period of up to 6.9 years, BMD were persistently lower at both lumbar spine and total hip, compared to healthy controls.

Some of the apparent increase in BMD upon disease remission, might be explained by an increase in extra-osseous fat depots after weight-gain (Bolotin, 2009), but the BMD deficits compared to normal-weight controls, suggest this does not explain the entire difference. With a mean age of 28.5 years, the group of recovered AN patients were just about the expected accrual of peak bone mass (Bonjour, Chevalley, Ferrari, & Rizzoli, 2009). From a theoretical point of view, a 10% decrease in peak bone mass (approximately similar to the deficit in the AN remission group) would advance the development of osteoporosis with 10 years (Hernandez, Beaupre, & Carter, 2003), implying an increased fracture risk beyond the follow-up period of our study.

Despite no difference in mean BMD, the prevalence of Z-score below minus two was higher in patients with current BN compared to both patients in remission and healthy controls. Possible explanations for decreased BMD in BN includes menstrual disturbances (in-spite of normal weight)
and a history of AN, none of which were evident in the total of nine patients with low BMD in our population. We did not, however, have information on smoking or biochemistry (hypercortisolemia, thyroid, calcium and vitamin D), factors that could contribute to low BMD. In one of the few studies assessing BMD in patients with BN, Neassén et al reports low spinal BMD in 77 patients with current BN. In their study, spinal BMD was positively associated to levels of estrogen and testosterone and negatively associated with history of amenorrhea and levels of cortisol (Naessen, Carlstrom, Glant, Jacobsson, & Hirschberg, 2006). Together with similar findings from other studies (Baker, Roberts, & Towell, 2000; Morris et al., 2004), it seems likely that decreased BMD in BN is associated to AN-like traits, more than to factors or behaviour exclusively seen in BN.

Due to the vague definition of the EDNOS diagnosis, not much can be drawn from the diagnosis by itself in terms of the course of the disease and outcome. Accordingly, patients with current EDNOS shared some features familiar with AN (low nadir % IBW), others with BN (current % IBW and body composition) and yet others in between (BMD values). Although we should be very cautious drawing conclusions, EDNOS does not seem to be associated with decreased BMD, at least in our sample of the population.

One of the major aims of the study was to assess the relationship between BMD and fracture risk. In both BN and EDNOS, the absolute number of fractures was low. Taken together with BMD values similar (or close) to the background population, it came as no surprise, that we could not show a relationship between BMD and fracture risk. In AN our fracture rates was higher than rates reported from the general population (Vestergaard et al., 2002), and thus consistent with a number of other studies (Faje et al., 2014; Nagata, Golden, Leonard, Copelovitch, & Denburg, 2016; Vestergaard et
We were not, however, able to show a significant association between BMD and fracture risk. Nor could we show that a cut-off of Z-score below minus two stratifies populations of different fracture risk, unsurprising given the result of the Poisson regression. One problem regarding the interpretation of the data is without doubt the relative low number of osteoporotic fractures in our cohort. In the AN group, 145 patients suffered 41 fractures, providing too little statistical power to reject a possibly association between BMD and the risk of fracture. In regression analyses, $r^2$ values between 0.005 and 0.021 (depending of DXA region) suggest that even though a larger sample size may result in a statistical significant association between BMD and fracture risk, the clinical significance is very limited. We were able to follow 145 patients with AN for a mean follow-up time of 18.6 years after diagnosis, and we are confident that the NRP captures the vast majority of the fractures. Thus, even though the relative fracture rate is significantly increased in AN, the absolute number of fractures, is still quite low. In spite of the long mean follow-up time, the mean patient age at the end of the study was only 36 years. What would be really interesting is to show whether a BMD deficit in early adulthood transforms into a marked increase in fracture risk when patients enter their fifth and sixth decade. Unfortunately, our data cannot answer this question, yet.

Overall, our data does not support the use of a single DXA scan as a fracture prediction tool in adolescent onset AN. A similar conclusion has been reached by Faje et al, based on DXA scans and self-reported fracture data on 310 patients with AN (Faje et al., 2014). In our study, BMD is lower compared to age-matched controls, even after disease remission, but the significant BMD deficit, does not seem to directly translate into significant fracture risk, at least not short-term.

Possible explanations for the weak association between BMD and fracture risk might be changes in extra-osseous tissue composition and bone marrow adiposity, but probably more importantly,
differences in physical activity. As a part of the treatment standard care, patients were asked to refrain from excessive physical activity, but due to the design of the study, we were not able measure compliance. It is very likely that a high level of strenuous exercise increases fracture risk, regardless of BMD, thus explaining some of the variation.

We suggest that efforts should be made towards establishing a DXA independent risk assessment, including disease characteristics such as nadir body weight, age at onset, and disease burden (expressed as years of disease multiplied with the degree of underweight) combined with bone imaging modalities, which minimizes the impact of changes in extra-osseous fat distribution and bone marrow, and allow for assessment of bone microarchitecture (e.g. HR-pQCT)

**Strengths and limitations**

The present study is one of few prospective studies of ED combining basic clinical information, BMD, and fractures. One of the major strengths is the longitudinal design, enabling us to characterise the course of the disease, in terms of both diagnosis and weight. It is well known that there is substantial diagnostic cross-over between eating disorder diagnoses, meaning that proportion of patients with an intake diagnosis of AN will move to BN over time (and to a lesser degree, vice versa) (Eddy et al., 2008). Due to the design of the study, we cannot account for diagnostic crossover or remission/relapse of disease from follow-up and DXA scan, to the retrieval of fracture information. Still, our definition of remission ensures at least six months of normal weight and absence of purging behaviour, prior to assessment. Furthermore, we were able to evaluate BN and EDNOS in the light of a history of AN or not.
We chose not to include menstrual status in the assessment of remission. This could be viewed as potential limitation, as resumption of menses is an established predictor for an increase in BMD during rehabilitation in AN (Miller et al., 2006). The challenge is, however, that the clinical course and time-span from complete or partial weight-recovery to resumption of menses is highly unpredictable (El Ghoch, Calugi, Chignola, Bazzani, & Dalle Grave, 2016; Tinahones et al., 2005; Winkler et al., 2017). If we applied resumption of menses as a strict remission criterion, we would likely classify a number of subjects with persistent normal-weight and no other risk factors for bone loss (hence, normalised cortisol and growth hormone secretion) as patients with current disease. This could threaten the external validity of the fracture risk estimates, as the population of low weight patients with current disease, would be diluted. The uncertainties related to the assessment of menstrual function (also including active or previous use of oral contraceptives), has led to the omission of amenorrhea as a diagnostic criteria for AN in DSM-5, (Association, 2013) thereby justifying our definition of remission.

An obvious limitation is the absence of follow-up DXA scans. If we have had the opportunity to compare baseline DXA scans with repeated scans after remission or prolonged disease, we could have associated changes in BMD to fracture risk. Unfortunately, this was not the case.

Further limitations include absence of dietary and exercise data, and absence of lateral spine scans to assess possible vertebral fractures. Finally, the relatively low number of fractures limits the statistical power, but as discussed, we still consider the findings on fracture risk clinical relevant.

The fraction of missing data was high for reported nadir BMI (and thus IBW) in both BN and EDNOS. The likely explanation is that the emphasis on weight is much less pronounced in BN and EDNOS compared to AN, thus the reporting into the medical charts was less comprehensive. This
is of course a limitation in the interpretation of the potential effect of nadir weight on fracture risk in both BN and EDNOS, although our BMD data suggests otherwise.

Conclusion

In conclusion, we report decreased BMD in AN patients compared to normal-weight controls both in remitted and in active disease whereas patients with BN or EDNOS had normal BMD values. We show that BMD was higher in a group of AN patients in disease remission, but a deficit compared to controls, remained. Though the relative risk of fractures was doubled in AN compared with healthy control subjects, the absolute fracture risk remains relatively low, in accordance with the generally low fracture rates in young adults. Interestingly, the relative risk of fractures was increased in AN even in patients whose BMD is not substantially reduced, a phenomenon reminiscent of the fracture risk seen in diabetes and glucocorticoid exposure (Buckley et al., 2017; Napoli et al., 2017).

In this clinical cohort with 377 DXA scans, there was no significant association between BMD and fracture risk. A cut-off of BMD Z-score minus two, corresponding to the definition of low bone mass in premenopausal women, had no predictive power in terms of fracture prediction. We suggest that fracture prediction efforts in EDs should not be made on the basis of routine DXA scans. Instead, efforts should be made to establish risk factors based on the course and burden of the specific disease.
Tables

This article is protected by copyright. All rights reserved.
<table>
<thead>
<tr>
<th></th>
<th>AN (n=49)</th>
<th>AN - remission (n=96)</th>
<th>p-value</th>
<th>BN (n=86)</th>
<th>BN - remission (35)</th>
<th>p-value</th>
<th>EDNOS (n=44)</th>
<th>EDNOS - remission (n=34)</th>
<th>p-value</th>
<th>Controls (n=128)</th>
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</thead>
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<tr>
<td>Age (y)</td>
<td>29.9 ± 8.4</td>
<td>28.5 ± 7.0</td>
<td>0.28</td>
<td>31.9 (27.5; 36.6)</td>
<td>31.0 (27.7; 33.5)</td>
<td>0.22</td>
<td>29.7 (23.9; 35.8)</td>
<td>26.4 (23.7; 31.8)</td>
<td>0.11</td>
<td>29.2 ± 6.2</td>
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<td>Age at debut of disease (y)</td>
<td>16.3 ± 4.6</td>
<td>16.1 ± 5.0</td>
<td>0.85</td>
<td>17 (15; 20)</td>
<td>16 (15; 18)</td>
<td>0.56</td>
<td>17 (14; 20)</td>
<td>15 (13; 17)</td>
<td>0.41</td>
<td>N/A</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>45.6 ± 5.5</td>
<td>61.8 ± 8.4</td>
<td>&lt;0.0001</td>
<td>64.3 (58.9; 74.4)</td>
<td>62.1 (58.5; 72.7)</td>
<td>0.54</td>
<td>60.6 (53.9; 71.8)</td>
<td>61.4 (56.4; 70.8)</td>
<td>0.76</td>
<td>62.7 ± 7.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.07</td>
<td>1.68 ± 0.06</td>
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<td>1.66 ± 0.07</td>
<td>1.68 ± 0.05</td>
<td>0.18</td>
<td>1.68 ± 0.06</td>
<td>1.66 ± 0.07</td>
<td>0.26</td>
<td>1.68 ± 0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.0 ± 1.5</td>
<td>21.9 ± 2.5</td>
<td>&lt;0.0001</td>
<td>23.4 (21.4; 26.6)</td>
<td>22.7 (20.5; 25.6)</td>
<td>0.26</td>
<td>21.4 (19.5; 25.1)</td>
<td>22.0 (19.9; 26.3)</td>
<td>0.36</td>
<td>22.1 ± 1.9</td>
</tr>
<tr>
<td>% IBW</td>
<td>80.6 ± 7.1</td>
<td>104.5 ± 12.0</td>
<td>&lt;0.0001</td>
<td>117.1 (101.7; 126.9)</td>
<td>112.4 (97.8; 122)</td>
<td>0.26</td>
<td>113.0 (93.9; 120.5)</td>
<td>116.1 (94.6; 125.0)</td>
<td>0.58</td>
<td>109.1 ± 9.1</td>
</tr>
<tr>
<td>BMI at referral (kg/m²)</td>
<td>15.3 ± 2.1</td>
<td>16.7 ± 2.0</td>
<td>&lt;0.0001</td>
<td>21.5 (19.2; 23.5)</td>
<td>20.2 (18.6; 23.0)</td>
<td>0.33</td>
<td>19.8 (17.0; 21.4)</td>
<td>18.7 (17.4; 21.0)</td>
<td>0.42</td>
<td>N/A</td>
</tr>
<tr>
<td>Nadir BMI (kg/m²)</td>
<td>13.8 ± 1.9</td>
<td>15.5 ± 2.5</td>
<td>&lt;0.0005</td>
<td>19.0 (17.4; 21.3)</td>
<td>18.8 (18.0; 20.4)</td>
<td>0.83</td>
<td>17.0 (16.0; 19.9)</td>
<td>17.6 (15.7; 19.0)</td>
<td>0.71</td>
<td>N/A</td>
</tr>
<tr>
<td>Nadir % IBW</td>
<td>68 ± 9.9</td>
<td>77.4 ± 12.6</td>
<td>&lt;0.0001</td>
<td>94 (85; 104)</td>
<td>92 (87; 102)</td>
<td>0.98</td>
<td>83 (78; 97)</td>
<td>89 (80; 92)</td>
<td>0.70</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of disease (y)</td>
<td>11 (6; 18)</td>
<td>6 (3; 11)</td>
<td>&lt;0.0005</td>
<td>15 (11; 19)</td>
<td>5 (3; 7)</td>
<td>&lt;0.0001</td>
<td>12 (9; 16)</td>
<td>4 (3; 8)</td>
<td>&lt;0.0001</td>
<td>N/A</td>
</tr>
<tr>
<td>Time since remission (y)</td>
<td>N/A</td>
<td>6.9 (4.2; 9.5)</td>
<td>-</td>
<td>8.3 (6.4; 10.5)</td>
<td>-</td>
<td>N/A</td>
<td>5.6 (4.1; 7.1)</td>
<td>-</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Menstrual status</td>
<td></td>
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</tr>
<tr>
<td>Amenorrhrea (n, %)</td>
<td>29 (56)</td>
<td>12 (13)</td>
<td>&lt;0.001</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.48</td>
<td>4 (10)</td>
<td>2 (6)</td>
<td>0.49</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Irregular (n, %)</td>
<td>6 (12)</td>
<td>18 (19)</td>
<td>0.28</td>
<td>4 (5)</td>
<td>2 (6)</td>
<td>0.97</td>
<td>4 (10)</td>
<td>5 (16)</td>
<td>0.56</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Regular (n, %)</td>
<td>12 (23)</td>
<td>51 (52)</td>
<td>&lt;0.005</td>
<td>57 (77)</td>
<td>28 (88)</td>
<td>0.81</td>
<td>23 (57)</td>
<td>20 (65)</td>
<td>0.99</td>
<td>128 (100)</td>
</tr>
<tr>
<td>Use of OCP (n, %)</td>
<td>5 (9)</td>
<td>15 (16)</td>
<td>0.33</td>
<td>13 (17)</td>
<td>2 (6)</td>
<td>0.13</td>
<td>9 (23)</td>
<td>4 (13)</td>
<td>0.30</td>
<td>*</td>
</tr>
</tbody>
</table>
Table 1: Patient characteristics at the time of follow-up, stratified on remission status. P-values < 0.05 are considered significant. Data are reported as mean +/- 1 SD or median and IQR, according to distribution. % IBW was calculated using a Danish population reference defining IBW as median BMI for age and gender (Nysom, Molgaard, Hutchings, & Michaelsen, 2001). * No available data on OCP use in the control group. IBW%: % ideal body weight; OCP: oral contraception pill; N/A: not applicable

<table>
<thead>
<tr>
<th></th>
<th>AN (n=49)</th>
<th>AN - remission (n=96)</th>
<th>p-value</th>
<th>BN (n=86)</th>
<th>BN - remission (35)</th>
<th>p-value</th>
<th>EDNOS (n=44)</th>
<th>EDNOS - remission (n=34)</th>
<th>p-value</th>
<th>Controls (n=128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density</td>
<td></td>
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<tr>
<td>Total body (g/cm²)</td>
<td>1.020 ± 0.091</td>
<td>1.084 ± 0.067</td>
<td>&lt;0.0001</td>
<td>1.118 ± 0.088</td>
<td>1.125 ± 0.601</td>
<td>0.96</td>
<td>1.094 ± 0.079</td>
<td>1.091 ± 0.077</td>
<td>0.85</td>
<td>0.828 ± 0.113</td>
</tr>
<tr>
<td>Femoral neck (g/cm²)</td>
<td>0.682 ± 0.121***</td>
<td>0.802 ± 0.090</td>
<td>&lt;0.0001</td>
<td>0.802 ± 0.114</td>
<td>0.815 ± 0.094</td>
<td>0.59</td>
<td>0.791 ± 0.133</td>
<td>0.809 ± 0.117</td>
<td>0.60</td>
<td>0.941 ± 0.109</td>
</tr>
<tr>
<td>Total hip (g/cm²)</td>
<td>0.722 ± 0.136***</td>
<td>0.875 ± 0.092**</td>
<td>&lt;0.0001</td>
<td>0.915 ± 0.146</td>
<td>0.941 ± 0.109</td>
<td>0.32</td>
<td>0.875 ± 0.141*</td>
<td>0.908 ± 0.127</td>
<td>0.28</td>
<td>1.005 ± 0.101</td>
</tr>
<tr>
<td>Lumbar spine (g/cm²)</td>
<td>0.844 ± 0.129***</td>
<td>0.967 ± 0.102*</td>
<td>&lt;0.0001</td>
<td>1.013 ± 0.111</td>
<td>1.034 ± 0.140</td>
<td>0.28</td>
<td>1.009 ± 0.129</td>
<td>1.005 ± 0.129</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>BMD Z-score</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.9 (-1.7; 0.2)</td>
<td>-0.4 (-1.0; 0.2)</td>
<td>&lt;0.0001</td>
<td>-0.2 (-0.7; 0.5)</td>
<td>0.0 (-0.6; 0.3)</td>
<td>0.84</td>
<td>-0.1 (-0.8; 0.7)</td>
<td>-0.2 (-0.8; 0.2)</td>
<td>0.66</td>
<td>-0.3 (-0.8; 0.3)</td>
</tr>
<tr>
<td>Total hip</td>
<td>-1.5 (-2.3; -0.6)</td>
<td>-0.9 (-1.4; -0.3)</td>
<td>&lt;0.0001</td>
<td>-0.4 (-1.1; 0.3)</td>
<td>-0.2 (-0.8; 0.3)</td>
<td>0.75</td>
<td>-0.5 (-1.3; 0.2)</td>
<td>-0.6 (-1.2; 0.0)</td>
<td>0.69</td>
<td>-0.2 (-0.7; 0.3)</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>-1.4 (-2.5; -0.4)</td>
<td>-1.0 (-1.6; -0.4)</td>
<td>&lt;0.0001</td>
<td>-0.4 (-1.2; 0.4)</td>
<td>-0.3 (-0.1; 0.1)</td>
<td>0.68</td>
<td>-0.3 (-1.0; 0.7)</td>
<td>-0.4 (-1.2; 0.0)</td>
<td>0.26</td>
<td>0.0 (-0.2; 0.3)</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Table 2: DXA-values. Low bone mass defined as z score < -2. P-value < 0.05 was considered significant. *p-value <0.01 compared to controls; **p-value<0.001 compared to controls; ***p-value<0.0001 compared to controls.
Table 3: Distribution of fractures and incidence rates according to diagnosis and fracture site. Incidence rates marked in bold are significantly increased compared to control. Rates are omitted for regions with three fractures or less. IR: Incidence rate

<table>
<thead>
<tr>
<th>Fracture Site</th>
<th>All fractures (n)</th>
<th>IR/1000 patient years</th>
<th>IRR compared to controls</th>
<th>p-value on discriminative power</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>35</td>
<td>12.9 (9.3-18.1)</td>
<td>1.9 (1.3-2.8)</td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-score &gt; -2.0</td>
<td>28</td>
<td>12.7 (8.8-18.4)</td>
<td>1.9 (1.2-2.8)</td>
<td></td>
</tr>
<tr>
<td>z-score &lt; -2.0</td>
<td>7</td>
<td>14.2 (6.8-29.8)</td>
<td>2.1 (0.9-4.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Femoral neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-score &gt; -2.0</td>
<td>30</td>
<td>12.2 (8.5-7.5)</td>
<td>1.8 (1.2-2.7)</td>
<td></td>
</tr>
<tr>
<td>z-score &lt; -2.0</td>
<td>5</td>
<td>22.2 (9.2-53.3)</td>
<td>3.3 (1.1-7.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-score &gt; -2.0</td>
<td>26</td>
<td>12.4 (8.4-18.2)</td>
<td>1.9 (1.2-2.8)</td>
<td></td>
</tr>
<tr>
<td>z-score &lt; -2.0</td>
<td>7</td>
<td>13.8 (6.6-29.0)</td>
<td>2.1 (0.8-4.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4: Fracture rates according to the presence of low bone mass in patients with AN. Stratified by the presence of Z-score – 2 at lumbar spine, femoral neck or total hip. Fracture incidence rates for controls is not shown. p-values on the difference between low bone mass and normal-for-age bone mass. A p-value < 0.05 are considered significant. IR: Incidence rate. IRR: Incidence rate ratio. NS: not significant.
Figure legends

Figure 1: Inclusion flow
References


FANS cohort n=1064

Death n=12

Out-of-county referrals n= 66

Study population n=986

Invited to follow-up n=986

Declined or did not reply to invitation n=615

Completed DXA n=371

Incomplete diagnostic information (n=1)
Insufficient remission status (n=18)
Male (n=8)

Fracture data n=344