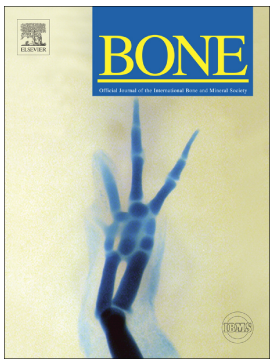

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Longitudinal Changes in Bone Parameters In Young Girls With Anorexia Nervosa

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Abbreviations

aBMD	areal bone mineral density
AN	anorexia nervosa
BA	bone area
BMAD	bone mineral apparent density
BMC	bone mineral content
BMD	bone mineral density
DXA	dual energy x-ray absorptiometry
ED	eating disorder
FM	fat mass
ISCD	International Society of Clinical Densitometry
LM	lean mass
LS	lumbar spine
ppBA	percent predicted bone area
ppBMC	percent predicted bone mineral content
SDS	standard deviation score
TB	total body
TBLH	total body less head
VFA	vertebral fracture assessment

Abstract

Background: Anorexia nervosa (AN) during childhood and adolescence has been reported to adversely affect bone health, but few studies have investigated longitudinal changes.

Method: DXA-derived bone parameters and body composition were retrospectively assessed in 111 young girls with AN with a median age of 15.4 years (10.9, 19.8). In 68 (61%) vertebral fracture assessment (VFA) was performed and in 31 (28%), a follow-up DXA was performed. Correlations with growth, changes in body composition and effects of illness duration and menstruation were examined. Size adjusted DXA standard deviation scores were calculated for total body (TB) less head bone mineral content (TBLH-BMC) and lumbar spine bone mineral apparent density (LS-BMAD)

Results: Mean (range) bone area (BA) for height centile was 27.1 (0-97), and mean lean mass for height centile was 28.8 (0-95) at baseline. Mean (range) LS BMAD was -1.0 (-2.6, 0.8) SDS at first and -1.2 (-3.0, -0.2) at second DXA (p=0.023). On follow up, lean mass for height increased from 27th centile (0, 75) to 40th centile (0, 70) (p=0.006), and fat mass for height increased from 55g/cm to 67g/cm (11.3, 124.2) (p<0.001). Duration of illness was the only negative predictor of LS BMAD (p<0.0001). Change in height SDS was the only positive predictor of change in TBLH-BMC (r=0.384, p=0.037), and change in LS BMAD (r-0.934, p<0.0001). Of 68 patients who had VFA, 4 (5.9%) had a mild vertebral fracture.

Conclusion: Bones are smaller and less dense in childhood/adolescent AN compared to healthy adolescents. Although there are significant gains in lean mass and fat mass, over time, BMAD SDS decreases slightly. Improvement in BMAD SDS is related to improvement in height SDS.

Introduction

Anorexia nervosa (AN) is an eating disorder characterised by low body weight/body mass index (BMI) secondary to a fear of weight gain and distorted body image [1]. Abnormal bone density and increased fracture risk has been reported in 85% of adult women with AN. The average annual loss of bone density in AN is approximately 2.5% [2-4].

The majority of adult bone mass is accrued during the transition from childhood to adolescence, corresponding to the period of peak incidence of AN. Optimal nutrition is an important factor in the development of a healthy skeleton, and interruptions in nutritional balance during this critical time, through development of AN, can adversely affect the attainment of peak bone mass [5-8].

AN may be further complicated by dysregulation of the growth hormone, gonadal and adrenal axes, which together may further compromise bone mass accrual through decreased bone formation[9].

Bone strength is the result of not only bone density, but also bone mass and geometry [10]. Dual energy x-ray absorptiometry (DXA) is the preferred clinical method of bone health assessment, mainly due to the low radiation dose, relatively low cost, and its ease of use. Interpretation of the two dimensional measure areal bone mineral density (aBMD) in g/cm^2 in childhood requires special consideration with results adjusted in reference to bone or body size [11-13]. The impact of chronic disease in children and adolescents, may be seen as inappropriate bone gain rather than bone loss witnessed in adulthood. A few studies have produced good quality size adjusted data in childhood/adolescent eating disorder over one year [14-15], but, to our knowledge, no studies have looked at changes in bone health over more than one year in AN, in children/adolescents relative to growth and body composition.

The aims of this study were : (1) to investigate factors such as body composition, duration of illness and frequency of periods and their effects on bone health; (2) to assess longitudinal changes in bone area (BA),size adjusted bone mineral content (BMC) and lumbar spine bone mineral apparent density (BMAD) in a group of young patients with AN, using the recently published UK reference data [13]; (3) to investigate changes in bone parameters in relation to changes in anthropometry such as fat mass (FM), lean mass (LM), body mass index (BMI) and height SDS, and; (4) to quantify vertebral fracture (VF) in young girls with AN.

Methods

Subjects

All females under the age of 20 years who were categorised by the referring physicians as having AN and referred for bone densitometry between January 2009 and February 2016 were included in this retrospective review. Referral for DXA was clinical, and in line with the Ionising Radiation Board recommendations. No patients were reported to be on any bone active agents, or had any concomitant condition that could affect bone health. Data were collected on duration of illness (time from diagnosis to first DXA scan), frequency of menstruation and family history of osteoporosis. This retrospective review did not require ethics approval or informed consent as it was conducted as part of healthcare evaluation of routine clinical practice and according to national guidance.

Growth and weight

Height (cm) to the nearest 0.1cm was measured on a wall-mounted stadiometer (SECA, Germany). Weight (kg) to the nearest 0.1kg was measured in light clothing on a SECA balance. Age and sex-adjusted standard deviation scores (SDS) for height, weight and BMI (kg/m^2) were calculated using the LMS method based on UK population reference data [16].

Menstruation

Frequency of periods was assessed in each female patient by the referring consultant. This was categorised as normal (if they had monthly periods), as oligomennorhoea (if the period frequency was between two and six months), or as amenorrhoea (if they had no periods for greater than 6 months). Girls who had not yet started their periods by the age of 15.9 years were categorised as normal, whereas, girls who had not started their periods aged 16.0 years and older were categorised as having amenorrhoea. The normal category was also sub-divided into premenarchal and postmenarchal.

Dual Energy X-ray absorptiometry (DXA)

Total body (TB) and lumbar spine (LS) DXA scans were obtained on 111 (girls) subjects using narrow fan beam technology on a Lunar Prodigy scanner (2009- June 2015) driven by Encore Software Version 13.0 (GE, Wisconsin, USA), or iDXA (July 2015-February 2016) driven by Encore Software Version 15.0 (GE, Wisconsin, USA). Additionally, 31 girls had a follow up DXA. We considered it justifiable to use the two different machines with two versions of software for longitudinal measures, as there are strong linear relationships between GE scanners and

software when applying the basic analysis, [13]. Furthermore, the individual equations were applied when using the UK dataset for calculating TBLH-BMC SDS and BMAD SDS for data obtained on Prodigy and iDXA models.

As outlined in previous studies, reference data were used to calculate the bone area (BA) in cm^2 and bone mineral content (BMC) in grams for age and sex [17-18]. This approach provides a percentage predicted bone area for age and a percentage predicted BMC for bone area. Moreover, by using linear interpolation, these data give predicted and percentage predicted bone area for age and sex.

Additionally, the newly published scanner-specific UK bone density dataset [13] was applied to calculate total body less head (TBLH) BMC (g) and LS bone mineral apparent density (BMAD) (gcm^3) values. Lean mass (LM) and fat mass (FM) values were derived from the TB DXA scan and expressed in g/cm height for the multiple regression analysis. The manufacturer's software also afforded centiles for BA for height, LM for height, and BMC for LM for direct comparison with a healthy Caucasian population. A bone parameter value was considered low if it was greater than 10% below the predicted mean values for the individual. The coefficient of variation (%CV) was < 1% calculated on the manufacturer's phantom, and 2.1 % and 2.5% respectively for BMC and BA calculated on 30 repeat paediatric LS scans.

Vertebral Fracture Assessment

Of the 111 cases, 68 (61%) had a lateral spine DXA for VFA as part of their bone assessment. All patients had T6- L5 acquired with standard machine protocol [19]. Lateral spine images

were analysed as previously described [20]. Lateral spine images were analysed independently by two non radiologist observers, who performed VFA in all 68 cases. Before commencing the VFA analysis, the observers defined a common protocol for point placement on each vertebral body. Each observer manually identified six landmarks corresponding to the four corners and the midpoints of the endplates, retrospectively, of each adequately visualised vertebral body starting at L4 and continuing through the thoracic spine up to T6. From these points, the software measured the anterior, middle and posterior heights, and calculated the anterior:posterior height ratio and the middle:posterior height ratio within a vertebral body. The observers also calculated the posterior:posterior height ratio when comparing the vertebrae above and below the one under examination [20]. The vertebral bodies were classified according to the extent of any height reduction as expressed by the reduction in height ratios using the scoring system developed by Genant: grade 0 (no fracture) if the reduction of any height ration was <20%, grade 1 (mild VF) if a height ratio reduction was from 20% to 25%, grade 2 (moderate VF) if the decrement was 25 to 40%, and grade 3 (severe VF) if a height ratio reduction exceeded 40% [21].

Statistics

All statistical analysis was undertaken using IBM SPSS (Version 22). All data were reported as mean with range. As data were normally distributed, one sample T Tests were used to investigate significant difference between groups. Significant difference was deemed where the value was below p=0.05. Pearson's test statistic was used to derive simple linear correlations, and multiple regression analysis was used to predict the value of variables based on the value of two or more other variables.

Results

In the cross sectional analysis, there were 111 Caucasian girls who attended for at least one DXA scan. The mean (range) age of the girls was 15.4 (10.9, 19.8), and there were 42 (37.8%) who were described as having normal gonadal function, 18 (42.8%) of whom were appropriately pre-menarchal, and 24 (57.1%) were menarchal. The remaining 69 girls had abnormal gonadal function: 11 girls (15.9%) with oligomenorrhoea, and 58 girls (84.1%) with amenorrhoea. On questionnaire survey, none of the patients reported a history of osteoporosis or non-vertebral fracture. On semiquantitative assessment [20-21], of the 68 patients, two showed a mild wedge fracture at T11, one a mild wedge fracture at T4 and one a mild biconcave fracture at T11.

3.1.1 Anthropometry/body composition

The AN group had mean height SDS -0.3 (range -3.8, 1.8) and had low weight SDS (mean -1.4, range -3.9, 1.1) and BMI SDS (mean -1.5, range -5.2, 1.6). BMI SDS was below -2.0 in 29% of the study group. The lean mass for height centile mean was 28.1 (range 0, 95), which was also skewed lower than that of the healthy population (Table 1).

3.1.2 Bone parameters

Mean (range) bone area (BA) was 1835cm^2 (1265, 2428) and 36.4cm^2 (24.4, 50.4) at the TB and LS sites in AN patients. Mean BA for height centile, as derived from the encore DXA software, was 27.1% (range 1, 97) compared to mean for healthy controls. Likewise, TB ppBA for age was low in AN patients (90%, 66, 113) as compared to healthy control data. However, at the LS site, ppBA for age (mean 96.4, range 65.1, 135.0) was normal.

The mean (range) TBLH-BMC SDS was 0.7 (-1.8, 4.8) for AN patients which did not differ significantly from the healthy population. However, at the LS site, mean BMAD SDS was -1.0 (-3.2, 2.0) SDS, which was below 90% of the predicted value.

3.1.3 Correlations between bone and anthropometrics/body composition

In the univariate analysis of 111 young girls with AN, there was a significant correlation between BMI SDS and bone size at TB site and LS sites as shown in Supplementary Table 1. The correlation between TB ppBA for age and BMI was r=0.44 (p<0.0001), and between LS ppBA for age and BMI was r=0.33 (p<0.001). Bone mineralisation parameters (ppBMC for BA) did not correlate with BMI SDS at TB, nor with LS BMAD, nor were there any correlations between height SDS, weight SDS, LM/height or FM/height and bone mineralisation parameters (Supplementary Table 1).

TB ppBMC for BA correlated negatively with duration of illness (r=-0.28, p=0.002), as did TBLH-BMC SDS (r=-0.24, p=0.009), and LS BMAD (r=-0.61, p<0.0001). Frequency of periods was an independent predictor of LS BMAD (r= -0.31, p=0.001). Multivariate analysis was applied, that included frequency of periods, LM, FM, duration of illness, height and BMI as predictors of TBLH-BMC, TB ppBMC for BA, and LS BMAD, (Table 3). For TBLH-BMC SDS, FM (p<0.0001), LM (p=0.001) and duration of illness (p=0.003) were negative predictors and height (p<0.0001) and BMI (p<0.0001) were positive predictors. For TB ppBMC for BA, duration of illness (p=0.005) was a negative predictors, and there were no positive predictors. Duration of illness (p<0.0001) was a negative predictor of LS BMAD. Frequency of periods was no longer a predictor of bone mineralisation in the multivariate model.

3.1.4 Follow up DXA group

Of the 111 female children/adolescents, 31 (28%) had a follow up scan. Mean time to follow up was 1.8 years (0.9 -5.7 years).

3.1.5 Anthropometrics and bone parameters at follow-up

The anthropometric characteristics and bone parameters for all patients at first and second DXA, and annual change in these parameters are reported in Table 2. Mean (range) height increased from 158.7cm (147.0, 170.7) to 161.1cm (149.2, 171.0) at first and second DXA scan respectively ($p<0.001$), however, mean height SDS was unchanged. There was a significant change in mean weight from 41.6kg (31.2, 53.6) at first DXA to 45.1 (33.8, 69.7) at second DXA ($p=0.002$), however, weight SDS remained the same (Table 2). Likewise, mean BMI changed from 16.5 (13.1, 19.6) at first DXA to 17.6 (13.6, 24.9) at second DXA ($p=0.014$), but mean BMI SDS was unchanged. There was also a significant increase in mean FM/height from 54.5g/cm (21.7, 90.7) to 66.5g/cm (11.3, 124.2) at first and second DXA respectively ($p=0.006$), but LM for height centile did not change (Table 2). Mean bone size (BA cm^2) increased at the TB site from 1799.8 cm^2 (1288, 2285) at first DXA to 1914.5 cm^2 (1510, 2320) at second DXA scan ($p<0.001$). Likewise, at the LS site, mean BA increased from 36.4cm^2 (28.1, 44.2) at first DXA to 37.9cm^2 (31.8, 47.2) at second scan ($p=0.001$). Mean bone mineral content at TB, did not change significantly over the course of the study, nor did mean TB ppBMC for BA (Table 2). However, mean BMAD reduced from -1.0 SDS at first DXA to -1.2 at second scan ($p=0.023$).

3.1.6 Correlations between bone and body composition in the follow-up group

In univariate analysis, and in terms of bone size, annual change in ppBA for age did not correlate with annual change in BMI SDS at TB ($p=0.276$), or LS sites ($p=0.443$). Likewise,

annual change in ppBA for age did not correlate with annual change in height SDS at TB (p=0.913), or LS (p=0.385), or with annual change in weight SDS at TB (p=0.190) or LS (p=0.512). Annual change in bone size (ppBA for age) did not correlate with annual change in FM/height at either TB (p=0.979) or LS (p=0.900).

In univariate analysis, and in terms of bone mineralisation, annual change in ppBMC for BA at TB did not correlate with annual change in BMI SDS (p=0.925). There was no relationship between annual change in BMI SDS and annual change in LS BMAD (p=0.166), nor between annual change in BMI SDS and annual change in LS ppBMC for BA (p=0.261). There was a positive correlation between annual change in TBLH-BMC SDS and annual change in height SDS (r=0.384, p=0.037), but not between annual change in TB ppBMC for BA and annual change in height SDS (p=0.808). There was a strong correlation between change in BMAD and change in height SDS (r=0.934, p<0.0001). Annual change in weight SDS correlated negatively with annual change in TBLH-BMC SDS (r=-0.470, p=0.008), but did not correlate with annual change in TB ppBMC for BA (p=0.736), or with annual change in BMAD (p=0.416).

There was no relationship between change in LM and change in TB ppBMC for BA (p=0.817), or change in LS BMAD (p=0.393. Change in FM/height did not correlate with change in TB pp BMC for BA (p=0.458), or change in BMAD (p=0.197).

On multivariate analysis that included change in LM/height, change in FM/height, change in weight SDS and change in height SDS, as predictors of change in TB ppBMC for BA and change in LS BMAD (Table 4), change in height SDS was the only predictor of change in LS BMAD

(r=0.926, p<0.0001). The relationship between TBLH-BMC SDS and height SDS and between LS BMAD and height SDS is shown in Figure 1.

Discussion

Most studies in young AN patients report areal BMD (aBMD) rather than size adjusted bone parameters. However, due to the size dependency of aBMD, failure to size correct data can potentially lead to an overestimation of the prevalence of osteoporosis in patients with smaller than normal bones. Strengths of this study include the large sample size and the use of two different methods of size adjustment to calculate the DXA bone parameters. By doing this, we are able to differentiate bone size from bone mineralisation. This is the first study to our knowledge that looks at bone accrual in relation to linear growth in young people with AN.

Low BMI was reported in the present study, with 29% of girls with AN having BMI SDS less than -2.0, suggesting that almost a third of our group exhibit nutritional deficits at time of first DXA scan. A low percentage median BMI has been reported as a negative predictor of bone parameters at multiple sites [22]. We showed that the area of the skeleton was 10% smaller in AN compared to healthy children. This result is in keeping with Stone et al [14], who reported narrower bones in AN using the Møldgaard approach of size adjustment [11]. However, our height SDS was also slightly below average for AN (-0.3 SDS), which suggests bones are slightly shorter than normal. The reduced height status reported in the present

study could be due to the longer study duration, which was mean 1.8 years follow-up compared to only 12 months in previous studies [14].

Female children and adolescents with AN showed reduced LM compared to healthy controls, but appeared to have adequate TB BMC for the given LM. This result is in agreement with others [14], who reported low lean mass in patients with AN. In health, muscle and bone are very closely related, in that lean mass strongly predicts bone mineral content [23]. However, from the multiple regression analysis in the current study, there was a negative relationship between lean mass and bone, and between FM and bone, hence there appears to be a mismatch in the muscle-bone relationship in AN. One could speculate that this is most likely due to sudden loss of lean mass and fat mass in patients who are restricting their nutritional intake. Therefore, as soft tissue loss precedes bone loss, in the short term, the BMC could be high for the given lean mass (and FM). Hence, TBLH-BMC SDS, which are predicted from LM, FM, height and age, appear normal in this patient group. The current study showed in our follow up cohort, that LM/ht and FM/height increased in AN over time. Others have reported increased percent FM coupled with decreased percent LM [24].

In children and adolescents, bone is modelling rather than remodelling, hence, one would not expect to see bone loss in young patients, but rather inappropriate gain compared to size and gender matched controls. This can be demonstrated by a reduction in the SDS rather than change in absolute values. Such deficit in bone mineral accrual was significant at the LS, as could be seen from the reduced BMAD SDS in the follow up of AN girls. The decline in BMAD SDS over time seen in the current study did not correlate with a change in BMI, LM or FM but

did correlate with change in height SDS. Deficits in LS BMAD have been associated with reduced frequency of menstruation, which has been attributed to oestrogen deficiency [25]. However, an association between frequency of menses and bone mineralisation was not observed in the current study. Restoration of menses has been shown to significantly improve BMD [24], but full recovery may not be attained [26]. Weight gain has been shown to lead to recovery of bone mineral deficits in some [5, 7, 26-29], but not all adolescent studies [14, 30-33]. These studies are reviewed extensively elsewhere [34].

Due to size dependency limitation [35], areal BMD as measured by DXA incorporates both bone size and bone mineralisation. It is a two dimensional scan of a three dimensional object, therefore underestimates BMD in smaller children with smaller bones (short and/or slender). In the multiple regression model, BMI predicted bone mineralisation at TB, but not the LS site. We also showed from the univariate analysis that BMI influences bone size to greater extent than bone mineralisation.

We report a vertebral fracture rate of 5.9% in childhood/adolescent ED in a population of children adolescents referred for bone density assessment. This is comparable with 3.8% vertebral fractures reported by others [36], in a cross sectional analysis of 13-26 year old girls and young adults with AN (n=80). According to DiVasta et al [36], the incidence of vertebral fracture is not predicted by BMI or BMD Z scores. Although the current study did not have a large enough fracture group for reliable statistical analysis, our results showed, with size adjusted BMAD, that all four patients did have bone density scores of <-1.0 SDS.

There are several limitations to this study. The TBLH-BMC SDS in the present study is calculated from an algorithm that includes LM, FM, height and age. Hence, for patients with abnormally low LM and FM, these data should be interpreted with caution. The use of DXA has also been criticised for its sensitivity and inability to differentiate between trabecular and cortical bone. Due to the retrospective nature of this study, we were unable to determine which patients had been continually affected by their eating disorder, or which patients had been in periods of remission. However, we were able to assess nutritional status with BMI SDS and change in BMI SDS status over time. Additionally, we did not have access to blood samples in the present to determine bone biomarkers, or growth factors. Furthermore, children/adolescents who were identified as having vertebral fractures did not undergo conventional x-ray for confirmation. We recently reported low incidence of VF in young people with AN in comparison to other disease groups [37]. When considering asymptomatic vertebral fracture assessment, there are limited data on fracture incidence in healthy children/adolescents. Therefore, we were unable to compare our VF data to the norm.

In conclusion, bones are smaller in size and less dense in childhood/adolescent AN compared to healthy adolescents. Deficits in bone mineralisation are apparent at the LS in AN, but not obviously at the TB. As expected, bone size for age is related to BMI SDS. On follow up, although there are significant gains in lean mass and fat mass, BMAD SDS decreases slightly. Improvement in BMAD SDS is related to improvement in height SDS, which suggests that the drive is for linear growth and bone accrual rather than an increase in body composition where calorific intake is limited.

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Table 1. Characteristics of patients with anorexia nervosa

	Anorexia Nervosa
Girls n (%)	111 (100%)
Duration of illness (years)	1.7 (0.4, 6.0)
Age (years)	15.4 (10.9, 19.8)
Height (cm)	159.7 (136.3, 174.0)
Height SDS	-0.3 (-3.8, 1.8)
Weight (kg)	43.7 (29.4, 66.1)
Weight SDS	-1.5 (-3.9, 1.1)*
BMI (wt/ht^2)	17.1 (12.8, 24.9)*
BMI SDS	-1.5 (-5.2, 1.6)*
FM/height (g/cm)	59.8 (9.7, 116.1)
TB BA (cm)	1835 (1265, 2428)
TB ppBA for Age (%)	90.0 (66, 113)*
TB pp BMC for BA (%)	98.5 (88, 116)
TB BA for Ht (centile)	27.1 (1, 97)*
TB LM for Ht (centile)	28.8 (0, 95)*
TB BMC for LM (centile)	52.3 (1, 99)
TBLH BMC SDS	0.7 (-1.8, 4.8)
LS BA (cm)	36.4 (24.4, 50.4)
LS ppBA for Age (%)	96.4 (65.1, 135.0)
LS BMAD SDS	-1.0 (-3.2, 2.0)*

All values mean (range). * > 10% lower than healthy

Table 2. Characteristics of patients with anorexia nervosa at first and second DXA scan

	Initial DXA n= 31	**Follow up DXA n= 31**	**Δ/year**	**P**
Age (years)	14.4 (11.1, 17.1)	16.2 (12.1, 19.8)		
Height (cm)	158.7 (147.0, 170.7)	161.1 (149.2, 171.0)	1.65 (0.0, 9.29)	<0.001
Height SDS	-0.1 (-2.2, 1.2)	-0.2 (-1.7, 1.3)	-0.16 (-1.56, 0.91)	0.079
Weight (kg)	41.6 (31.2, 53.6)	45.1 (33.8, 69.7)	2.18 (-4.55, 12.08	0.002
Weight SDS	-1.4 (-3.8, 0.2)*	-1.5 (-3.7, 1.3)*	0.75 (-2.7, 5.7)	0.182
BMI (wt/ht^2)	16.5 (13.1, 19.6)	17.6 (13.6, 24.9)	0.05 (-1.8, 1.54)	0.014
BMI SDS	-1.6 (-4.2, -0.4)*	-1.5 (-4.5, 1.6) *	0.1 (-1.7, 2.0)	0.360
LM for height centile	27.29 (0, 75)*	39.58 (0, 70)*	4.3 (-23.6, 44.2)	<0.001
FM/height (g)	54.5 (21.7, 90.7)	66.5 (11.3, 124.2)	7.16 (-22.73, 35.24)	0.006
BA at TB (cm^2)	1799.8 (1288, 2285)	1914.5 (1510, 2321)	61.2 (-112.5, 222)	<0.001
TB ppBA for Age (%)	92.3 (72, 124)	91.7 (75, 106)	-1.2 (-10.7, 8)	0.335
TB pp BMC for BA (%)	97.2 (88, 108)	95.2 (84.9, 111)	-1.0 (-9, 8)	0.027
TB BA for Ht (centile)	27.3 (2, 78)*	39.6 (2 , 97)*	6.2 (-47.8, 58)	0.009
TB BMC for LM (centile)	53.0 (6, 96)	49.8 (9, 99)	-2.1 (-34.6, 28.3)	0.242
TBLH BMC SDS	0.7 (-2.0, 3.8)	0.6 (-1.4, 3.0)	-0.15 (-2, 1.43)	0.208
BA at LS (cm^2)	36.4 (28.1, 44.2)	37.9 (31.8, 47.2)	0.95 (-1.3, 5.1)	0.001
LS BMAD SDS	-1.0 (-2.6, 0.8)*	-1.2 (-3.0, -0.2)*	-0.18 (-1.6, 0.5)	0.023

All values mean (range). P value ≤ 0.05 deemed significant, * > 10% lower than healthy

Table 3. Association between bone parameters and anthropometrics in patients with anorexia nervosa (n=111): results of multivariable linear regression analysis

	Regression standardised β coefficient (95% confidence interval) and p value associated with each predictor		
	TBLH-BMC SDS (R^2 =0.311)	TB pp BMC for BA (R^2 =0.174)	LS BMAD (R^2 =0.389)
Period frequency	0.038 (-0.198, 0.301) p=0.683	0.040 (-0.967, 1.450) p=0.693	-0.146 (-0.297, 0.025) p=0.096
Fat mass	-2.153 (0.001, 0.000) p<0.0001	-0.488 (-0.002, 0.001) p=0.339	-0.220 (0.000 , 0.000) p=0.615
Lean mass	-2.411 (0.001, 0.000) p=0.001	-0.140 (-0.002, 0.001) p=0.827	-0.370 (0.000, 0.000) p=0.503
Duration of illness	-0.274 (-0.503 , -0.105) p=0.003	-0.255 (-2.217 , -0.289) p=0.011	-0.581 (-0.571, -0.314) p<0.0001
Height	2.386 (0.230 , 0.568) p<0.0001	0.319 (-0.585, 1.057) p=0.569	0.388 (-0.065, 0.154) p=0.422
BMI	2.790 (0.983, 2.489) p<0.0001	0.516 (-2.231, 5.075) p=0.442	0.409 (-0.312, 0.661) p=0.478

Correlations deemed significant where p<0.05

Table 4. Association between Δ bone parameters and Δ anthropometrics in patients with anorexia nervosa (n=31): results of multivariable linear regression analysis

	Regression standardised β coefficient (95% confidence intervals) and p value associated with each predictor	
Annual change in parameter	Δ TB pp BMC for BA (R^2 =0.062)	Δ LS BMAD (R^2 =0.864)
Δ Fat mass/height	-0.245 (-0.211 , 0.078) p=0.402	-0.148 (-0.011, 0.002) p=0.159
Δ Lean mass/height	0.449 (-1.733 , 5.225) p=0.312	0.020 (-0.145, 0.165)p=0.899
Δ Weight SDS	0-.269 (-6.276 , 3.443) p=0.554	0.084 (-0.160 , 0.271) p=0.600
Δ Height SDS	0.074 (-2.297, 3.316) p= 0.712	0.926 (0.669, 0.918) p<0.0001

Correlations deemed significant where p<0.05

Highlights:

1. Lumbar spine bone mineral apparent density reduces in girls with anorexia nervosa girls with illness greater than one year
2. Changes in lumbar spine bone mineral apparent density are dependent on linear growth
3. Vertebral fractures can occur in anorexic girls/adolescents referred for bone density scans
4. It is important to take bone size into account in analysis of paediatric bone mineral density

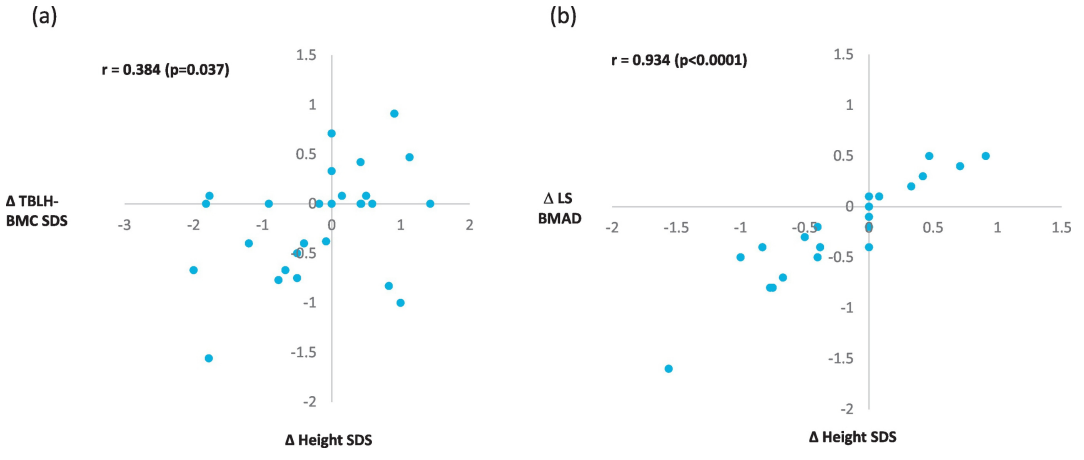
(a)
r = 0.384 (p=0.037)
Δ TBLH-BMC SDS
Δ Height SDS
(b)
r = 0.934 (p<0.0001)
Δ LS BMAD
Δ Height SDS

Figure 1