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Skeletal status and laboratory investigations in adolescent girls with anorexia nervosa

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Abstract

To our knowledge anorexia nervosa (AN) adversely influences bone density, but whether qualitative characteristics of bone are also affected is not known. For this reason we investigated prospectively the changes in skeletal status in a population of 18 adolescent girls with AN aged 11.5–18.1 years (mean 15.9±1.9 years) using both dual-photon X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) measurements, bone turnover markers (osteocalcin, bone alkaline phosphatase – bALP, carboxy-terminal cross-linked telopeptide of type I collagen – ICTP), and laboratory investigations (serum total and ionised calcium, serum phosphate, urine calcium/creatinine ratio, luteinizing hormone – LH, follicle-stimulating hormone – FSH, estradiol). Measurements of bone mineral density at the spine (s-BMD) and total body (TB-BMD) and amplitude-dependent speed of sound (Ad-SOS) of the hand phalanges were performed at baseline, 7.8±2.4 and 19.4±5.6 months of follow-up. The mean values of TB-BMD, s-BMD and Ad-SOS measurements did not change during the period of observation. The mean *Z*-scores for TB-BMD and Ad-SOS were significantly lower after 19.4 months of observation vs. baseline (−1.06±1.00 vs. −0.67±0.98 vs. and −0.50±0.88 vs. 0.26±1.75, respectively). *Z*-scores for s-BMD decreased non-significantly ($p=0.08$). Among bone turnover markers, we observed a significant increase in bALP and a non-significant increase in osteocalcin serum concentrations which were below normal ranges for age, sex and Tanner stage at baseline. High baseline serum ICTP concentration decreased non-significantly, reaching normal ranges during the observation. We conclude that anorexia nervosa seriously affects skeletal status in adolescent girls. Bone turnover markers analysed together with densitometric parameters suggest that AN influences both bone formation and resorption processes. QUS measurements at hand phalanges may be an appropriate method in the evaluation of skeletal status in patients with AN.

Keywords: Anorexia nervosa; Adolescents; Bone mineral density; Bone quantitative ultrasound; Bone turnover markers

Introduction

Anorexia nervosa (AN) is a psychiatric disorder characterized by abnormal eating behaviours that result in weight loss and has potentially serious medical consequences. Long-term studies with up to 10 years of follow-up demonstrated that only 42–48% of patients recover to normal weight and attain regular menstrual function [1]. AN is chronic and severe eating disorder and many complications may occur during its course. One of the most important medical consequences of AN is a decrease in bone mineral density, leading to 7-fold increased risk of spontaneous fractures [2]. Recent studies have demonstrated that the prevalence of osteopenia at lumbar spine in adolescent patients (42–44%) is similar as in adult ones [3–5]. Rapid bone loss that occurs within 6 months of disease onset and persists despite disease recovery is well documented in adult women with AN [6–8]. Only a limited number of studies have focused on recovery of osteopenia in adolescents and whether the deficit in bone mineral density in these patients persists or is latter corrected is uncertain. Because this period is a particularly critical time for bone mineral accumulation, deficits incurred

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Table 1
Clinical characteristics of subjects studied at baseline, at second and third measurement

	Baseline	Second measurement	Third measurement	Δ per month
Age (years)	15.90±1.86	16.59±1.88 *	17.51±1.80 *	
Weight (kg)	41.05±7.62	48.10±10.11 **	50.29±10.22 **	1.13±1.48
Height (m)	1.61±0.07	1.62±0.06	1.63±0.06	0.002±0.006
BMI (kg/m^2)	15.79±2.09	18.27±2.76 **	18.90±2.91 **	0.40±0.57

Data expressed as mean±SD.
* Significant versus baseline value, $p<0.05$.
** Significant versus baseline value, $p<0.005$.

during this time may be permanent and contribute to increased spontaneous fracture risk for the rest of life.

Although many studies have shown, that AN is associated with low BMD, it remains unknown whether it adversely affects other bone characteristics, which may be assessed by QUS (quantitative ultrasound). QUS of hand phalanges is a recently developed, free of ionizing radiation, portable and relatively cost-effective method of bone status evaluation. QUS provides information not only about bone mass but also determines qualitative features of bone tissue such as elasticity and architecture and is proven to discriminate between healthy and osteoporotic individuals [9,10]. In several recent studies, phalangeal QUS measurements were used in order to assess skeletal status in female children and adolescents [11–15].

To our knowledge no prospective studies on skeletal status in girls with AN using both dual photon X-ray absorptiometry (DXA) and QUS have been published previously.

Bone metabolism changes dramatically during adolescence, but there are relatively few and contradictory data concerning the effect of AN and recovering from the disease on bone turnover in this population [6,16–19]. Some of the available studies lack the simultaneous measurement of BMD, qualitative assessment of bone, bone formation and bone resorption markers [16–18]. Therefore the mechanisms that trigger bone loss in adolescents with AN are not fully understood. The aim of this study was to investigate prospectively the changes in bone mineral density, qualitative characteristics of bone and laboratory investigations (including bone formation and resorption markers) in a population of adolescent girls with AN.

Materials and methods

Subjects

In this study we examined 18 girls from the Upper Silesia industrial region hospitalized for anorexia nervosa at the age of 11.5–18.1 years at baseline (mean age 15.9±1.9 years). Mean duration of the disease prior to hospitalization was 14.9±13.6 months (range 3–56 months). All examined patients were at IV–V Tanner stage [20]. All patients were in the acute phase of disease and met DSM-IV (Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition) diagnostic criteria [21] for AN (refusal to maintain body weight more than 85% of that expected, intensive fear of gaining weight or becoming fat and absence of at least 3 consecutive menstrual cycles in postmenarchal females). Sixteen patients had restricting type and two patients had bulimic type of AN. At baseline amenorrhea was present in all 18 patients. Some patients had secondary amenorrhea ($n=15$) or were premenarchal ($n=3$; lack of menarche before 16 years of age). No patient had any history of fractures and all remained non-traumatic during the study. Patients with additional factors potentially affecting bone metabolism (chronic diseases and medication) were not included to the study. Management included hypercaloric diets, psychotherapy and oral administration of multivitamin tablets during the hospitalization (Multivitamin, 3 tablets a day) containing 500 IU of vitamin D_3, 1250 IU of vitamin A, 2.5 mg of vitamin E, 5 mg of vitamin B_1, 2.5 mg of vitamin B_2, 1.5 mg of vitamin B_6, 25 mg of vitamin C, 60 mg of nicotinamide and 10 mg of calcium pantothenate. After 4–12 weeks of hospitalization the patients were discharged from the hospital and controlled as outpatients. According to Ward et al. [7], the recovery criteria were a body mass index (BMI [kg/m^2]) of more than 18.5 kg/m^2 and resumption of menses for at least 6 months.

The evaluation of nutritional, skeletal and hormonal status was performed at baseline; the second and third measurements were performed after a mean period of 7.8±2.4 months and 19.4±5.6 months of follow-up, respectively.

The study was approved by the Ethics Committee of the Medical University of Silesia and written consent was obtained from all participants before participation.

Methods

Anthropometric measurements

Subjects' height was measured using a single stadiometer, weight was measured on electronic scale and BMI value was calculated.

Bone mineral density

BMD was assessed by dual-energy X-ray absorptiometry (DXA). DXA measurements of bone mineral density (g/cm^2) of lumbar spine (s-BMD) and total body (TB-BMD) were performed with Lunar DPX-L (USA). The results of BMD were compared to reference population and expressed as *Z*-scores. The coefficient of variation (%CV=[SD/mean]×100) for BMD measurements was 1.1% for spine and 0.6% for TB. Due to ethical reasons serial BMD measurements were not performed and CVs were not obtained in patients with AN.

Qualitative characteristics of bone status

These were assessed by quantitative ultrasound (QUS) of hand proximal phalanges. QUS measurements were performed in 14 patients with DBM Sonic 1200 (IGEA, Italy), measuring amplitude-dependent speed of sound (Ad-SoS [m/s]) in proximal phalanges of fingers II–V of the right hand. The coefficient of variation was 0.64% and was calculated on the basis of 75 measurements

Table 2
Results of BMD and QUS measurements

	Baseline	Second measurement	Third measurement	Δ per month
Spine BMD (g/cm^2)	1.026±0.145	1.011±0.141	1.003±0.130	−0.003±0.010
Z-score	−1.28±1.49	−1.46±1.38	−1.64±1.31	−0.077±0.168
Total body BMD (g/cm^2)	1.037±0.080	1.035±0.083	1.032±0.078	0.001±0.005
Z-score	−0.67±0.98	−0.79±0.95	−1.06±1.00 *	−0.051±0.072
Ad-SoS (m/s)	2083±72	2065±56	2077±45	−2.72±11.94
Z-score	0.26±1.75	−0.40±1.12	−0.50±0.88 **	−0.127±0.280

Data expressed as mean±SD.
* Significant versus baseline, $p<0.005$.
** Significant versus baseline, $p<0.05$.

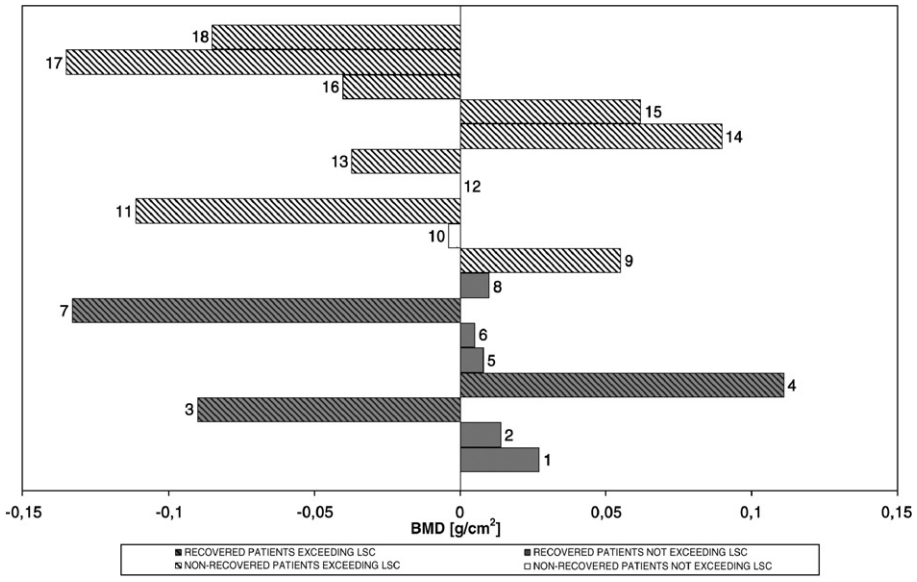

Fig. 1. Individual changes in spine-BMD value between third and baseline value in anorectic patients.

performed in 15 young subjects (five scans on each of them). The *Z*-score values for Ad-SoS were calculated using the normative data for QUS measurements performed in young individuals derived from the same region of the country [14].

In order to follow reliable changes of BMD and Ad-SoS in individual subjects, the least significant change (LSC) was calculated. The LSC or critical difference, denotes the minimum difference between two successive results in an individual that can be considered to reflect a real change. The LSC was calculated using the formula: $\%CV \times 2 \times 1.41$, which would represent a statistical difference at the 95% confidence level [22].

Biochemical analysis

All blood samples were collected in fasting state between 8.00 and 9.00 a.m. In patients with recovered menses blood samples were taken at early follicular phase. Morning urine sample was analysed for total calcium and creatinine concentrations and Ca/creatinine ratio was calculated. Serum ionised calcium was determined by AVL 984 analyser. The levels of serum and urine total calcium, serum phosphate, bone alkaline phosphatase (bALP) and urine creatinine were determined using a Cobas Integra 400 device (Roche Diagnostics). Serum osteocalcin was measured by ELISA (N-MID osteocalcin, Roche Diagnostics) and serum carboxy-terminal telopeptide of type I collagen (ICTP) by radioimmunoassay kit (Orion Diagnostica, Finland).

Hormonal status

Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were performed at the hospital laboratory using Elecsys 2010 analyser (Roche Diagnostics). All blood samples were collected in fasting state between 8.00 and 9.00 a.m.

Statistical analysis

For all obtained anthropometric, bone mineral density and biochemical values a change per month of observation was calculated. Normal data distribution were assessed using Kolmogorov–Smirnov test. All datasets were normally distributed with the exception of estradiol and LH concentrations. Results are presented as means ± SD for normally distributed variables and as

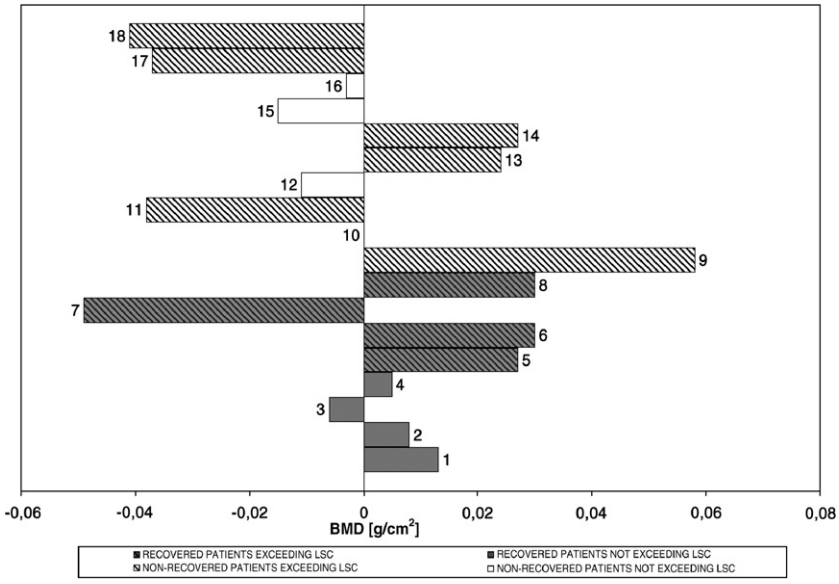

Fig. 2. Individual changes in total body-BMD value between third measurement and baseline value in anorectic patients.

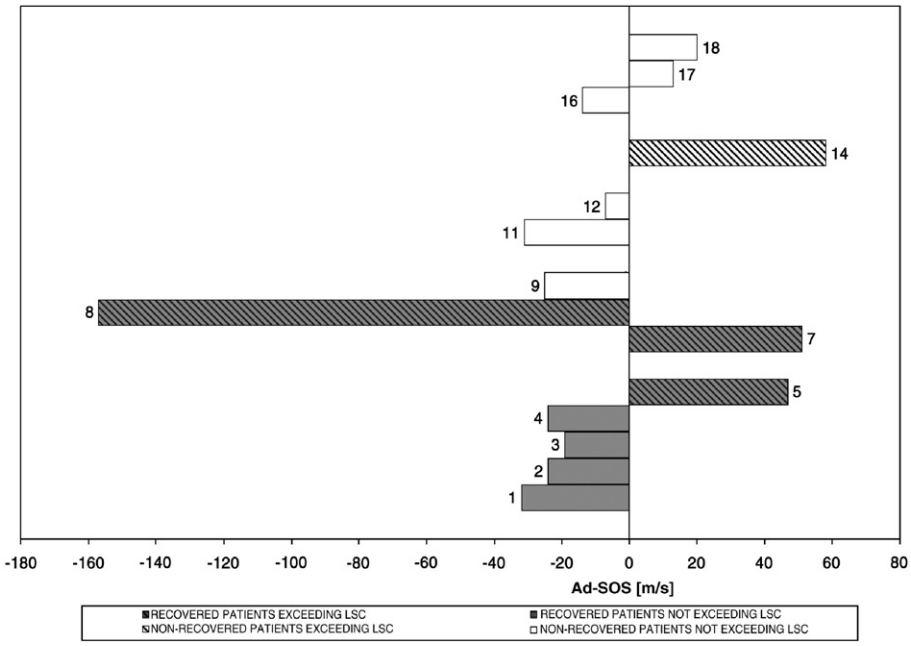

Fig. 3. Individual changes in Ad-SOS value between third measurement and baseline value in anorectic patients.

medians and quartiles for not normally distributed ones (LH and estradiol concentrations). Statistical analyses were performed using Statistica 5.5 for Windows. Comparisons of densitometric, hormonal and biochemical data between baseline and follow-up measurements were performed using ANOVA and post-hoc Newman–Keuls test or Friedman test if data distribution was not normal. Comparisons between non-recovered and recovered anorectic groups were performed using Student's *t*-test for independent samples or Mann–Whitney *U* test if data distribution was not normal. Correlations were analyzed by Pearson's linear correlation test or Spearman's test if data distribution was not normal. All results were considered as statistically significant at $p<0.05$.

Results

Clinical and anthropometric data

Mean weight and BMI increased significantly at follow-up in comparison with baseline values (Table 1). The percentage increase of weight was 24.4±20.6% and of BMI 21.4±22.9%. Menses recovered in 11 of the patients (mean period of regular menses was 11.9±6.2 months; range 6–22 months). Five patients still had secondary amenorrhea and two were premenarchal. Mean period of amenorrhea was 23.1±17.9 months. Eight subjects were considered as the recovered subgroup and nine patients were assigned to the non-recovered subgroup due to above-mentioned criteria of Ward et al. [7].

Table 3
Simple linear correlations of changes (Δ) in anthropometric, densitometric and hormonal parameters between baseline and third measurement

	Δ Ad-SOS	Δ spine BMD	Δ total body BMD
Δ Weight	0.04	0.53*	0.52*
Δ Height	0.09	0.60*	0.57*
Δ BMI	0.08	0.49*	0.46
Amenorrhea duration	−0.12	−0.57*	−0.44
Δ bALP	0.59*	0.68*	0.58*
Δ Osteocalcin	0.95*	0.31	0.68*
Δ Estradiol	0.94*	0.53*	0.05

* $p<0.05$.

Bone mineral density data

BMD data are provided in Table 2. The mean values of TB-BMD, s-BMD and Ad-SOS remained unchanged during the period of observation. However, mean values of *Z*-scores for TB-BMD and Ad-SOS at follow-up were significantly lower in comparison with baseline and *Z*-scores for s-BMD decreased non-significantly ($p=0.08$). There were no significant differences in patterns of change in bone mineral density in the 9 anorectic girls considered as recovered versus the other non-recovered 9 girls (data not shown).

In Figs. 1–3 changes in densitometric and ultrasound results in individual patients are presented. Using the LSC values for skeletal measurements a significant increase exceeding LSC in s-BMD, TB-BMD and Ad-SOS was noted in 4, 6 and 3 subjects, respectively. The respective numbers of subjects with a significant decrease exceeding LSC was 7, 4 and 1.

We observed significant positive simple linear correlations between changes (Δ) in anthropometric parameters (weight, height, BMI) and changes (Δ) in s-BMD and TB-BMD values (Table 3). No significant correlations between changes in anthropometric parameters and changes in Ad-SoS were observed. Changes in s-BMD correlated inversely with the duration of amenorrhea. We also observed positive correlations in changes (Δ) in densitometric values and changes (Δ) in bone formation markers (bALP, osteocalcin) and serum estradiol concentrations (Table 3). Ad-SOS correlated positively with bALP, osteocalcin and estradiol concentrations, s-BMD with bALP and estradiol and TB-BMD with bALP and osteocalcin concentrations. Correlations of densitometric parameters with changes in other biochemical and hormonal parameters were not significant.

Table 4
Results of laboratory measurements

	Baseline	Second measurement	Third measurement	Δ per month	*P* value
Osteocalcin (ng/ml)	19.51 ±9.45	17.89 ± 8.75	23.88 ± 13.43	1.03±4.23	0.22
bALP (U/l)	16.36 ±8.57	29.76±24.09	42.83±26.36	2.11±3.39	0.01
ICTP (μg/l)	12.30 ±13.04	9.23±13.16	5.93±1.81	−0.62±1.41	0.07
FSH (mIU/ml)	2.58 ±2.85	4.18±3.31	6.55±2.91	0.54±1.00	0.001
LH (mIU/ml)	0.16 (0.07–0.45)	2.25 (0.15–5.03)	4.83 (2.10–6.24)	0.49±0.60	0.002
Estradiol (pg/ml)	11.53 (10.00–62.60)	40.91 (10.09–68.5)	56.78 (35.16–86.40)	2.53±6.12	0.06
Ca^{2+} (mmol/l)	1.12 ±0.05	1.14±0.04	1.12±0.20	0.001±0.007	0.21
Total Ca (mmol/l)	2.59 ±0.20	2.48±0.24	2.42±0.13	−0.018±0.036	0.05
Total phosphate (mmol/l)	1.37 ±0.22	1.21±0.16	1.23±0.19	−0.01±0.05	0.06
Ca/creatinine ratio	0.16 ±0.09	0.14±0.09	0.14±0.08	−0.0005±0.009	0.89

Data expressed as mean±SD except for LH and estradiol, distributed not normally, expressed as median and quartiles.

Biochemical and hormonal data

These data are shown in Table 4. FSH, phosphate, ionised and total calcium serum concentrations were within normal ranges for age and Tanner stage in the healthy female population at all three points of study [23]. Morning urine Ca/creatinine ratio did not exceed the 0.21 value that is considered significant for hypercalciuria [24]. Serum total calcium and phosphate concentrations showed a non-significant ($p=0.05$ and $p=0.06$, respectively) trend to decrease while the serum FSH level increased significantly. Serum levels of LH and estradiol were below normal range at baseline, but they returned to normal during the observation period. Baseline serum ICTP concentrations were above normal range (4–7 μg/l) with a non-significant ($p=0.07$) trend to decrease during the observation. Concentration of osteocalcin at baseline was below normal ranges (23.2–77.4 ng/ml) for the healthy female population at the same age and Tanner stage, but returned to normal ranges at the third measurement [25]. Mean activity of bone alkaline phosphatase was low at baseline, but its activity increased significantly reaching normal value for healthy female population (21.8–71.4 U/l) at the same age and Tanner at second measurement [25]. There were no statistically significant differences in biochemical and hormonal parameters values between non-recovered and recovered subgroups at baseline, second and third measurement (data not shown).

Discussion

In the current study serious skeletal abnormalities in patients with anorexia nervosa were monitored. Instead of an increase in BMD and Ad-SoS observed in normal healthy population at the same age, we noted aggravation in skeletal status. Impaired bone accrual that normally occurs during adolescence may disturb an attainment of peak bone mass and increase the risk of osteoporosis and fractures in their adult life.

In earlier cross-sectional studies performed on anorectic adolescents [6,26–29] a reduction of BMD was present even in girls with short course of AN. On the other hand Wentz et al. [30] reported that spine BMD in anorectic girls was comparable to age-matched healthy controls. We observed a lack of expected increase in densitometric and ultrasound values that is commonly present in normal healthy young population and an obvious decrease in *Z*-scores. This lack of bone gain was in spite of increase in weight with treatment. Our findings confirm previous results of Soyka et al. [19]. In their prospective study a group of 19 anorectic adolescents with clinical and anthropometric features similar to our group was observed. Baseline mean spinal BMD and TB-BMD values were significantly lower than in healthy controls. Densitometric measurements did not increase during 12 months of observation. Our patients were evaluated for almost 20 months and no improvement in these parameters was stated either. In an earlier study by Bachrach et al. [16] BMD at the spine measured by an older method (dual-photon absorptiometry) remained stable during 12 months of observation, but TB-BMD increased. It should be noted, however, that despite gains in BMD, 8 patients still had osteopenia of the spine and/or the whole body. Some of their patients in this study were given estrogen replacement, so direct comparison is not possible. Compston et al. [31] demonstrated failure to increase BMD during weight gain in both the spine and the total body measurements. In fact, total body BMD at 3 months was significantly lower than baseline in this study. Women and adolescents with history of anorexia nervosa should be followed longitudinally to maximize premenopausal bone replacement. Retrospective studies revealed persistently reduced cortical and slightly reduced trabecular BMD even in patients with good disease outcome 11 years after first admission [2,27]. In our study the aggravation in densitometric and ultrasound quantitative measurements concerned mainly cortical skeletal sites (TB-BMD and hand phalanges consist mostly of cortical bone [32]). This suggests that cortical bone is more sensitive to undernutrition during puberty. Seeman et al. [33] postulated that AN affects different regions at different ages, depending on the bone growth and development. It is known that before puberty appendicular growth is more rapid than axial, while during puberty, axial growth accelerates and appendicular growth slows. AN in our patients began during puberty affecting predominantly appendicular growth of bone and cortical bone mass accumulation. This hypothesis is supported

by the observation of Milos et al. [34] who demonstrated that the degree of underweight has a greater effect on the cortical than on the trabecular part of bone.

The negative values of Ad-SOS *Z*-score that significantly decreased over the time of observation similar to changes in densitometric variables indicate that QUS measurements at hand phalanges may be an appropriate method in the evaluation of skeletal status in patients with AN. Earlier cross-sectional studies confirm decreased values of QUS measurements in adolescent [29] and adult [34] female anorectic subjects. Moreover, changes in Ad-SOS *Z*-score values correlate with changes in TB-*Z*-scores ($r=0.487$; $p=0.04$). In some cases we observed discrepancies between the results of DXA and QUS measurements. However, it should be noted, that Ad-SoS measurements (in contrast to DXA) reflect changes in bone mineral density as well as bone microarchitecture. There are only few literature data about this important qualitative parameter in AN, but Milos et al. [34], using 3D-pQCT technique, showed a highly significant deficit in the absolute number of bone trabecules and a significant reduction of cortical thickness on the group of young adult anorectic women.

We consider that study results support general conclusion that QUS measurements at hand phalanges may be an appropriate method for the assessment of skeletal status in subjects with anorexia nervosa. However, some differences in BMD and QUS methodologies should be taken into consideration (different skeletal site, BMD measures only quantity of bone tissue and QUS depends also on qualitative features of bones, spine is weight-bearing bone and phalanges are not) and ideal consistency cannot be expected. Despite these essential differences we were able to show general comparable trends in longitudinal changes for BMD and QUS variables.

Optimizing peak bone mass accrual during adolescence is essential, and an episode of anorexia nervosa during adolescence interferes with that process. Several previous studies have indicated that the onset of AN in adolescence results in a more severe deficit in bone mass than in those with disease onset during adulthood [33,35]. The determinants of change in BMD in adolescents with AN have not been examined extensively. Earlier studies demonstrated that duration of amenorrhea, low estrogen levels and weight loss are the major determinants of skeletal status [18,19,26,36,37]. However, in spite of an increase of weight and BMI and increasing trend of serum estrogen concentration, the densitometric parameters did not improve in our patients. This may support the hypothesis of Soyka et al. [19] that change in BMD is a slow process, and the impact of recovery may not be apparent over a relatively short time frame. Low estrogen levels and low body mass are probably not the only contributors of osteopenia in AN, therefore estrogen replacement did not have a significant effect on osteopenia in young women with AN [38–40]. Recent studies showed that reduced bone size may contribute to low BMD in AN [33,41] and anabolic therapy with s-DHEA may be effective [35].

Anorectic osteopenia in adult women is thought to be associated with "low turnover", while in non-anorectic estrogen-deficient populations bone formation and resorption increase simultaneously [36]. Data concerning bone turnover markers in adolescent anorectic patients are few and contradictory. When assessing bone metabolism in growing children, levels of bone turnover markers may reflect changes in mineralization, growth of bones or both. In our study baseline bone markers concentrations were low. We observed a 168% increase in bALP activity, while osteocalcin concentration did not change. On the other hand, serum bone resorption marker ICTP showed the trend to decrease. This dissociation of bone turnover at baseline (low bone formation and high bone resorption) is consistent with earlier reports [15,16]. Our findings are very close to the results by Audi et al. [26], who compared bALP, osteocalcin and ICTP concentrations in anorectic patients in three phases of the disease – acute (I), partially recovered (II) and fully recovered (III) in a cross-sectional study. Ranges of clinical data of their patients were similar to ours and ICTP concentrations were established by the same method. The highest ICTP serum concentrations with concomitant low osteocalcin and bALP concentrations were shown in the acute phase of AN, while in fully recovered patients they observed an increase of bone formation markers and a decrease of ICTP serum concentration. Other authors have demonstrated a significant increase of bone formation and resorption markers during 11 weeks of inpatient dietary treatment [18] and during longitudinal 12-month observation [19] of anorectic adolescent girls. In the study by Compston et al. [31], there was a consistent increase in markers of formation, whereas NTX/creatinine showed a trend to increase in the first 6 months of observation, with a subsequent decrease, so that at 12 months the value was significantly lower than at baseline. These authors hypothesised that more significant increase with weight gain in formation markers would be consistent with modeling required for linear growth but since the change in BMD during weight gain is disproportionately small for associated increase in linear growth, the changes in biochemical markers may indicate that mineralization of newly formed bone is reduced. Our findings support this theory. This may result from low calcium and vitamin D_3 intake. Our patients, apart from the period of hospitalization, were not given calcium and vitamin D_3 supplementation. In a study by Fonseca et al. [42] serum 25-OHD_3 concentrations were below normal in 88% of anorectic patients. Compston et al. [31] reported that although mean value of serum 25-OHD_3 in anorectic adolescents was within normal ranges, its value at 6 months of observation was significantly lower than at baseline. This was attributed to reduced levels of sunlight exposure during hospitalization. Polish populational studies showed that the daily intake of vitamin D_3 in healthy children at the age between 9 and 14 years is less than one fourth (23%) of recommended [43]. De la Piedra et al. [44] hypothesised that bALP is not a real indicator of bone mineralization in a mechanism similar to that observed in osteomalacia. On the other hand an increase in bALP may be a predictor of an increase in BMD and recovery of bone accrual [19].

Our study has several limitations: the small sample size and relatively short period of follow up that may mask the effect of recovery on skeletal status. The period of follow-up was variable, but it should be taken into consideration that the

current study was performed in anorectic patients seen in everyday clinical practice. All studied girls were outpatients and there were several factors like school duties, difficulties with traveling, etc., so it was not possible to obtain the same regular periods of follow-up. However, we did not observe any correlation between the duration of observation and results of densitometric, biochemical and hormonal parameters. The duration of the disease in our patients spanned over a wide range, but this is normally observed in everyday clinical practice due to the natural course of anorexia nervosa that has different dynamics in different patients. A question about variability of duration of the disease arises also in other studies performed in anorectic adolescents [6,18,19,26,31]. Another limitation of the study is also a different number of subjects evaluated by densitometric and ultrasound methods.

We conclude that AN seriously affects skeletal status in adolescent girls. Total body and spine BMD and hand Ad-SoS did not show the expected age-related increase during the course of the study and did not improve in response to weight recovery. Hence, respective *Z*-scores progressively decreased. This indicates *Z*-scores are more useful tool in evaluating bone mineral density changes in anorectic patients than absolute BMD values. Bone turnover markers analysed together with densitometric parameters suggest that AN influences both bone formation and resorption processes. QUS measurements at hand phalanges may be an appropriate method in the evaluation of skeletal status in patients with AN.

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