Osteoporos Int (2005) 16: 783–790
DOI 10.1007/s00198-004-1759-2

ORIGINAL ARTICLE

Cortical and trabecular bone density and structure in anorexia nervosa

**Gabriella Milos · Anja Spindler · Peter Rüegsegger
Burkhardt Seifert · Sabina Mühlebach
Daniel Uebelhart · Hans Jörg Häuselmann**

Received: 3 June 2003 / Accepted: 27 August 2004 / Published online: 28 September 2004

Abstract The aim of the study was to examine bone density and architecture with three different measurement methods in a sample of young women with anorexia nervosa (AN) and in an age-matched control group of women. Three-dimensional periphery quantitative computer tomography (3D-pQCT) at the ultradistal radius, a new technology providing measures of cortical and trabecular bone density and architecture, was performed, as well as quantitative ultrasound (QUS) at the heel, and dual energy X-ray absorptiometry (DXA) at the spine and hip. Thirty-six women with AN aged 18–30 years (mean duration of AN: 5.8 years) and 30 age-matched women were assessed. Bone mineral density measured by DXA at the spine and hip, and broadband ultrasound attenuation measured by QUS at the heel were significantly lower in patients than controls. 3D-pQCT demonstrated a highly significant deficit in the absolute number of bone trabecules and a significant reduction of cortical thickness. Severity of underweight was significantly associated with bone deficits at the hip measured by DXA. 3D-pQCT revealed mostly deficits of cortical bone related with age of onset of eating disorder. Using three different methods to measure bone density and bone structure at the hip, spine, heel and ultradistal radius, significant deficits in bone mineral density both in trabecular and cortical bone, as well in trabecular structure could be demonstrated in the AN patients.

Keywords Anorexia nervosa · Cortical and trabecular bone · DXA · Osteoporosis · pQCT · QUS

G. Milos (✉) · A. Spindler · S. Mühlebach
Department of Psychiatry, University Hospital of Zurich,
Culmannstrasse 8, 8091 Zurich, Switzerland
E-mail: gabriella.milos@psy.usz.ch
Tel.: +41-1-2555280
Fax: +41-1-2554530

P. Rüegsegger
Department of Biomedical Technology,
ETH and University of Zurich, Zurich,
Switzerland

B. Seifert
Social and Preventive Medicine Institute,
University of Zurich, Zurich, Switzerland

D. Uebelhart
Department of Rheumatology, University Hospital,
Zurich, Switzerland

H. J. Häuselmann
Center for Rheumatology and Bone Disease,
Klinik Im Park, Zurich, Switzerland

Introduction

The pathogenesis of bone deficits in patients with anorexia nervosa (AN) appears to be multi-factorial. Low body weight, inadequate calcium, vitamin D and protein intake, as well as alteration of hormone regulation (sexual hormones, cortisol and insulin-like growth factor I system) seem to be related with decreased bone mineral density (BMD) [1,2]. However, the exact pathophysiology of bone deficits in AN has still not been totally clarified.

The standard method for measuring bone density is dual energy X-ray absorptiometry measurement (DXA). Using this method at the vertebral body and femoral neck, several studies of the recent decades reported that ca. 50% of AN patients showed bone density measurements that were more than 2 SD below those of healthy age-matched controls [3,4,5,6]. Generally, DXA measurement of the vertebral body is considered most appropriate to follow trabecular bone deficits, whereas measurement of the femoral neck and proximal part of the distal forearm is better to follow cortical bone deficits. However, the DXA measurement can neither sufficiently differentiate between cortical and trabecular bone [7,8], nor give details with respect to bone architecture.

The quantitative ultrasound (QUS), a fast and radiation-free method, has also been applied in samples with AN women [9,10]. It has been shown that QUS performed at the heel can predict fracture risk at the hip

and the spine as reliably as DXA [11] and that it differentiates between bone of healthy controls and of AN patients [9]. However, QUS generates global measures of bone density and does not distinguish between different bone compartments or provide information about architecture of the bone.

Recently, a new three-dimensional method was introduced for the measurement of bone density and structure, namely the three-dimensional peripheral quantitative computer tomography (3D-pQCT). 3D-pQCT is a non-invasive method that examines bone microstructure in the peripheral bone, typically at the ultradistal radius [12,13]. So far, only one study has utilized this technology in AN patients [9], showing only modest differences between healthy controls and AN subjects. However, the machine used in the study by Resch et al. differentiated between two parameters only (trabecular area and total area of the region of interest), thus examined neither cortical bone nor any parameters of bone structure.

In the present study, we introduced the technology of a new multislice 3D-pQCT machine [14] in the investigation of bone density and bone structure of women affected by AN. Using a resolution of approximately 160–200 μm, this technology produces measurements of ten different parameters distinguishing between cortical bone and trabecular bone and examining different aspects of bone architecture (ratio of vertical rods and horizontal plates). More specifically, the method measures six different parameters of trabecular bone, three of which provide details of volumetric trabecular bone density (density of trabecular area of the bone, density of the sub cortical area of trabecular bone, and density of the central part of the trabecular bone), and three provide details of trabecular bone structure (trabecular number, trabecular separation, and trabecular thickness). In addition, the method measures two parameters of cortical bone (cortical bone density and absolute thickness of cortical bone) and two parameters of the overall bone (mean entire bone density and relative bone volume as part of the total volume in percent).

In this cross-sectional study, we examined a group of young female AN patients and compared them to age-matched female controls using the well-established DXA method, QUS methodology, and multislice 3D-pQCT technology. In addition, the study was aimed at identifying anamnestic and clinical characteristics of AN that might be associated with bone deficiencies. Bone density and bone structure of AN patients were examined for associations with patient characteristics relating to type of AN, duration of underweight, lifetime minimum BMI and characteristics relating to patient behavior.

Materials and methods

Patient group (PG)

Between February 1999 and February 2000, a total of 36 women with AN according to the Diagnostic and Statistical Manual of Mental Disorders 4th edn [15], with an age range of 18–30 years were recruited via the Eating Disorder Unit (inpatients and outpatients) of the University Hospital of Zurich. Pregnant women were excluded from the study. Age, body mass index (BMI), and age at menarche of the PG are described in Table 1. All AN patients had a BMI <17.5 (diagnostic criterion for AN) at the time of recruitment. However, during the time period between the initial recruitment and the physical examinations, some weight changes occurred so that the BMI at the time of examination ranged from 13.1 to 17.9 kg/m^2 (mean $\pm$ SD: 16.0 ± 1.3). The average age at AN onset was 17.6 years (SD 3.2) and the average duration of the disorder was 5.8 years (SD 3.5). The mean duration of underweight (BMI <17.5) was 56 months (SD 37) and the mean minimum lifetime BMI was 13.2 (SD 1.7). The mean duration since the last

Table 1 Characteristics of patient and control group. Data are presented as mean $\pm$ SD unless otherwise noted

Variable	Anorexia nervosa ($n=36$)	Control group ($n=30$)	Statistic	*df*	*P*
Age, years	23.4 ± 3.7	22.8 ± 2.1	$t=0.8$	56.4	0.38
Body mass index, kg/m^2	16.0 ± 1.3	20.5 ± 1.5	$t=13.2$	64	<0.0001
Age at menarche, years	13.2 ± 1.2	13.3 ± 1.5[a]	$t=0.5$	63	0.59
Use of calcium products, %	38.9	6.7	$\chi^2=9.3$	1	0.002
Vitamin D use, %	19.4	3.3	Fisher exact	NA	0.063
Sexual hormone use, %	63.9	53.3	$\chi^2=0.8$	1	0.39
Sexual hormone intake duration, months	41.5 ± 42.4[b]	25.5 ± 20.3[b]	$F=1.6$[c]	1,32	0.22
Total physical activity, h/week	16.1 ± 17.4	10.3 ± 11.7	MWU $Z=0.9$	NA	0.40
Weight bearing activity, h/week	8.2 ± 7.2	4.6 ± 3.8	MWU $Z=2.3$	NA	0.021
Smoking, %	27.8	40	$\chi^2=1.1$	1	0.29
Smoking, cigarettes/week	95.7 ± 92	34.5 ± 40.5	$t=1.9$	1,11.9	0.076
Alcohol use, %	13.9	23.3	$\chi^2=1.0$	1	0.32
Hashish use, %	2.8	3.3	Fisher exact	NA	1.00
Time since last period, months	24.3 ± 25.5[d]	–	–	–	–

[a] $n=29$, 1 CG S did not recall age at menarche
[b] PG $n=22$, CG $n=13$, 4 Ss did not report sexual hormone intake duration
[c] ANCOVA with age as covariate
[d] $n=31$, 5 PG Ss with missing data

period was 24 months (SD 26). There was no primary amenorrhea in the patient group.

Twenty-two (61%) patients had AN of the restricting type, and 14 (39%) AN of the binge-purge type. According to DSM IV, the restricting subtype describes a presentation in which weight loss is accomplished primarily through dieting or fasting. In contrast, the binge-eating/purging subtype is diagnosed when the individual has regularly engaged in binge eating or purging (or both). Purging behavior is characterized by vomiting or the misuse of laxatives, diuretics, or enemas. Patients of the restrictive type had a lower BMI (mean ± SD: 15.6 ± 1.4) than patients of the binge-purge type [mean ± SD: 16.5 ± 0.7, $t(34) = 2.3$, $P < 0.05$]. They also had a shorter duration of AN (mean ± SD: 4.8 ± 3.5) than patients of the binge-purge type [mean ± SD: 7.5 ± 2.9, $t(34) = 2.4$, $P < 0.05$]. The two groups did not differ in age, age at first period, age at eating disorder onset, and minimum lifetime BMI.

Control group (CG)

A total of 30 female medical and psychology students were recruited through university lectures for the control group. Inclusion criteria were: age 18–30, no current or lifetime eating disorder, no underweight (i.e. current BMI > 18) and no regular intake of medication (except oral contraceptives), no bone or other general diseases. The CG mean current age and mean age at time of first period did not differ from the PG (see Table 1). The age range in the CG was smaller (20–28) and current age variances of the two groups were not homogenous ($P < 0.001$).

Procedures and assessments

The diagnosis of AN was made according to DSM IV [15] at the Psychiatric Department (section of eating disorders) of the University Hospital Zurich. All participants were examined by a rheumatologist. Participants completed a questionnaire including socio-demographic data, the history of eating disorder, (under)weight, and menstruation, the use of medication, hormonal substitution or contraceptives, as well as the intake of vitamin, mineral, or calcium products. The questionnaire also included questions on participants' movement patterns such as postural and non-postural sports, and hours of sport per week.

Measurement of bone mineral density and bone micro-architecture

All patients and controls were examined with the following imaging methods.

Dual energy X-ray absorptiometry (DXA) of the lumbar spine (L2–L4), the total proximal hip area and the non-dominant femoral neck was performed using an Hologic QDR 4500 device (Hologic Corp., Inc., Waltham, Mass., USA). Areal bone density was expressed in g/cm^2. In addition, all values were expressed as *T*-scores as absolute numbers defined 1994 by the World Health Organization (WHO) (amount of standard deviations below or above the mean bone density value of young white Caucasian women at peak bone mass). The short-term precision of the DXA measurement after reposition (coefficients of variation, CV) was 1% at the level of the lumbar spine and 1.5% at the total proximal hip area. The technical short-term precision of the Hologic QDR 4500 is 0.34%.

Quantitative ultrasound (QUS) measurement was performed at the level of the calcaneus with the dry QUS system of the Hologic Sahara device using an oil-based coupling gel (Hologic Corp.). The speed of sound (SOS) was measured in m/s and the broadband ultrasound attenuation (BUA) in dB/MHz. In addition, the quantitative ultrasound index (QUI), which is an index automatically calculated by the machine, was listed. The stability of the system was regularly measured and considered to be good; the precision was 5% for BUA and 0.3% for SOS [16].

Multislice three-dimensional periphery quantitative computer tomography (3D-pQCT) of the ultradistal radius of the non-dominant forearm using multislice prototype three-dimensional quantitative computer tomography (3D-pQCT) (Scanco Medical Bassersdorf Switzerland) [17,18]. The measurement protocol included acquisition of a three-dimensional stack of 60 high-resolution CT slices at the most distal end of the radius using a total observation region of 10 mm. Slice thickness was 0.28 mm, pixel matrix 512×512 and pixel size 0.17 mm. To obtain cubic voxels, the consecutive cross-sectional slices were measured in steps of 0.17 mm in the axial direction. Measuring parameters are defined as follows:

- D100: mean entire bone (cortical and trabecular) density of the ultradistal part of the radius in grams hydroxyapatite equivalence per cm^3 (grHA/cm^3).
- Dcomp: bone density of the cortical part of the bone (grHA/cm^3).
- C.Th: absolute thickness of cortical bone in mm.
- Dtrab: density of the trabecular area of the bone (grHA/cm^3).
- Dmeta: density of the sub cortical area of the trabecular bone (grHA/cm^3).
- Dinn: density of the central part of trabecular bone (grHA/cm^3) (Dinn and Dmeta together correspond the total trabecular bone).
- BV/TV: relative bone volume as part of the total volume in percent.
- Tb.N: absolute number of trabecules per mm area.
- Tb.Th: mean thickness of bone trabecules in mm.
- Tb.Sp: mean separation distance between trabecules in mm.

The average short-term precision of the multislice high-resolution 3D-pQCT after repositioning is 1.1%

for Dtrab and 1.6% for structural parameters such as TbN [17,18].

DXA and QUS were conducted at the Department of Rheumatology, University Hospital Zurich, 3D-pQCT at the Institute for Biomedical Engineering, University of Zurich and Federal Institute of Technology (ETH). All three exams were conducted in a time span of maximal 8 weeks. The study was approved by the Ethics Committee of the Psychiatric Department of the University Hospital Zurich. All participants gave written consent.

Statistical analysis

Group differences were determined by *t*-tests in the case of normally distributed variables, Mann-Whitney *U*-tests for ordinal variables, chi-square and Fisher's exact tests for nominal variables. Bone density parameters were tested for group differences by means of ANCOVA with age as a covariate. Partial correlations were conducted testing associations between AN-related characteristics and bone parameters while controlling for age. Because of non-normality, the variables "lifetime duration BMI <17.5" and "time since last period" were log-transformed before testing for partial correlations. $P<0.05$ were considered statistically significant.

Results

Comparison of patient and control group characteristics (Table 1)

Significantly more patients used calcium products as dietary supplements than participants in the CG. Marginally more patients than controls used vitamin D. No differences between the two groups were found regarding the proportion of participants using sexual hormones (contraceptives or hormonal substitution) or the duration of hormone intake. While the total amount of all physical activities, comprising occupational activities, house and garden work, did not differ between the two groups, patients reported a significantly higher amount of postural activities than controls. No differences were found between both groups regarding the consumption of tobacco, alcohol, or hashish, but the PG smokers smoked marginally more cigarettes per week than the CG smokers.

Comparison of patient and control groups regarding bone density and structure as measured by the three methods

Dual energy X-ray absorptiometry (DXA)

The World Health Organization (WHO) has provided guidelines for assessing osteoporosis and osteopenia in postmenopausal women when using DXA technology. The WHO criteria are commonly used in clinical practice, even with younger patients. They are also used to determine severity of bone deficiency in research on eating disorder patients [19,20]. Thus, to allow comparisons with the literature and to provide information on the clinical severity of bone deficiencies in the study sample, we examined our data using the WHO criteria.

According the WHO definitions of osteopenia (DXA *T*-score ≤ -1.0 SD and > -2.5 SD) and osteoporosis (DXA *T*-score ≤ -2.5 SD) the DXA examination of the femur/hip yielded eight AN patients (22%) with normal bone density, 23 (64%) with osteopenia and five (14%) with osteoporosis. DXA measurements of the lumbar spine identified six AN patients (17%) with normal bone density, 17 (47%) with osteopenia and 13 (36%) with osteoporosis. In the CG, DXA examination of the femur yielded 27 women (90%) with normal bone density and three women (10%) with osteopenia. Measurements of the lumbar spine identified 22 control women (74%) with normal bone density, seven (23%) with osteopenia and one (3%) with osteoporosis. Using a combination of the two localizations, only 11% ($n=4$) AN patients had normal bone density in both localizations, whereas 50% ($n=18$) had osteopenia in at least one of the two localizations (but no osteoporosis) and 39% ($n=14$) had osteoporosis in either or both localizations. In the CG 67% ($n=20$) showed normal bone density in both localizations, 30% ($n=9$) had osteopenia in either or both localizations (but no osteoporosis) and 3% ($n=1$) had osteoporosis in either or both localizations. Seven women (19.4%) in the patient group and four women (13.3%) in the control group reported a history of fracture.

All DXA parameters showed highly significant differences between the two groups regarding the measurements of hip (femoral neck and total proximal hip area) and spine bone mineral density (see Table 2). The DXA *t*-values of the PG were significantly lower than 0, indicating that bone density in all three locations was lower than the expected mean of the reference population (see Table 2). DXA *t*-values of the CG were comparable to population means for femoral neck, but were marginally lower for total hip, and significantly lower for lumbar spine (Table 2).

Quantitative ultrasound (QUS)

In the QUS heel measurements only the BUA was significantly lower in AN patients than in the control, whereas QUI and SOS were comparable between the two groups (Table 2).

Three-dimensional peripheral quantitative computer tomography (3D-pQCT)

The 3D-pQCT measurements of the ultradistal radius yielded the most significant differences between the groups in the number of trabecules per mm (Tb.N) and the interconnected average separation distance between

Table 2 Bone density and structure in patients and control group as measured by the three methods

	Anorexia nervosa ($n=36$)			Control group ($n=30$)			$F(1,63)$[b]	P
	Estimated mean ± SE	t[a] $df=35$	P	Estimated mean ± SE	t[a] $df=29$	P		
DXA								
Femoral neck BMD	0.72 ± 0.02	–	–	0.88 ± 0.02	–	–	43.5	< 0.0001
T-score femoral neck	−1.21 ± 0.16	8.6	< 0.0001	0.30 ± 0.17	1.7	0.11	42.5	< 0.0001
Total hip BMD	0.76 ± 0.02	–	–	0.98 ± 0.02	–	–	71.0	< 0.0001
T-score total hip	−1.47 ± 0.14	11.5	< 0.0001	0.30 ± 0.15	1.8	0.08	71.6	< 0.0001
Lumbar spine BMD	0.86 ± 0.02	–	–	1.01 ± 0.02	–	–	31.3	< 0.0001
T-score lumbar spine	−1.92 ± 0.16	12.3	< 0.0001	−0.60 ± 0.18	2.9	0.006	29.6	< 0.0001
QUS calcaneus								
QUI	108.4 ± 3.3	–	–	114.9 ± 3.6	–	–	1.8	0.19
BUA	71.5 ± 2.6	–	–	89.2 ± 2.9	–	–	20.9	< 0.0001
SOS	1585.6 ± 6.3	–	–	1583.5 ± 6.9	–	–	0.0	0.83
3D-pQCT radius								
D100	359.4 ± 10.3	–	–	397.7 ± 11.3	–	–	6.3	0.015
Dcomp	888.4 ± 12.6	–	–	908.4 ± 13.8	–	–	1.1	0.29
C.Th	0.92 ± 0.03	–	–	1.03 ± 0.03	–	–	7.3	0.009
Dtrab	173.4 ± 7.0	–	–	196.1 ± 7.7	–	–	4.7	0.033
Dmeta	217.4 ± 6.8	–	–	240.1 ± 7.5	–	–	5.0	0.028
Dinn	141.7 ± 7.4	–	–	164.3 ± 8.1	–	–	4.3	0.043
BV/TV	0.14 ± 0.006	–	–	0.16 ± 0.006	–	–	4.8	0.032
Tb.N	1.57 ± 0.01	–	–	1.66 ± 0.01	–	–	30.8	< 0.0001
Tb.Th	0.09 ± 0.003	–	–	0.10 ± 0.003	–	–	1.6	0.21
Tb.Sp	0.55 ± 0.007	–	–	0.51 ± 0.008	–	–	16.3	0.0001

[a]One-sample *t*-testing difference from 0
[b]ANCOVA with age as covariate

trabecules (Tb.Sp). Furthermore, still significant but considerably smaller differences were found in the average bone density of the entire bone diameter (cortical and trabecular) (D100), the thickness of the bone cortex (C.Th), the density of the total trabecular area (Dtrab), the sub cortical trabecular bone area (Dmeta), the inner area of trabecular bone (Dinn), and the relative bone volume as part of the total volume in percent (BV/TV) (Table 2).

Associations between AN-related characteristics and parameters of bone density and structure

Comparing patients with restrictive AN and binge-purge AN, no differences in any of the bone parameters as measured by the three methods were found. Table 3 lists the potential correlations of several AN-related characteristics (minimum lifetime BMI, duration BMI < 17.5, age of AN onset and duration of amenorrhea) with the parameters of the three methods (calculated within the patient group). The minimum lifetime BMI showed the highest significant correlations with DEXA of the total hip area, followed by the femoral neck. There was no significant correlation of lumbar spine with this parameter and only a modest but significant correlation with all QUS measurements. Furthermore, duration of underweight was also significantly correlated in particular to the DXA measurement of the total hip area and to a lesser degree but still significant to DXA of the femoral neck and spine, SOS and QUI at the heel and C.Th, Tb.N by 3D-pQCT at the ultradistal radius. With regard to the 3D-pQCT measurements Dcomp and C.Th showed significant correlations with duration of underweight and age of AN onset. Finally, D100 and Tb.N evidenced only modestly significant correlations with duration of underweight and age of AN onset. An analysis of covariance was conducted comparing women who developed AN before completion of puberty (17 years of age) with women whose AN onset was after completion of puberty with regard to DEXA, US and 3D-pQCT parameters while controlling for current age. Women with an onset age before 17 ($n=17$) had significantly lower C.Th (EM = 0.85, SE = 0.04) and Dcomp (EM = 852.8, SE = 20.3) values than women with onset at 17 or older ($n=19$) [C.Th: EM = 0.98, SE = 0.04, $F(1,33)=4.3$, $P=0.045$; D.Comp: EM = 920.8, SE = 19.0, $F(1,33)=5.2$, $P=0.030$]. None of the other bone parameters differed between these two groups (Table 3).

Discussion

In this cross-sectional study, three different techniques, DXA, QUS and 3D-pQCT, were used to determine bone mineral density and structure in a group of female AN patients and an age and sex-matched control group. DEXA and QUS revealed that bone density in AN patients was significantly lower than in controls.

Table 3 Age-controlled correlations between AN-related characteristics and bone parameters. $n = 36$ for all correlations with the exception of "time since last period" (five cases with missing data, $n = 31$)

	DXA *T*-scores			QUS calcaneus			3D-pQCT ultradistal radius								
	Femoral neck	Total hip	Lumb. spine	BUA	SOS	QUI	D100	Dcomp	C.Th	Dtrab	Dmeta	Dinn	BV/TV	Tb.N	Tb.Th	Tb.Sp
Min. lifetime BMI, kg/m^2	0.51**	0.54***	0.25	0.42*	0.35*	0.42*	0.06	0.08	0.14	0.07	0.07	0.07	0.07	0.24	0.01	−0.15
Lifetime duration BMI < 17.5, months[a]	−0.36*	−0.44**	−0.35*	−0.21	−0.39*	−0.39*	−0.24	−0.27	−0.36*	−0.19	−0.16	−0.21	−0.20	−0.36*	−0.13	0.29
Age of AN onset, years	0.31	0.29	0.16	0.17	0.26	0.27	0.40*	0.52**	0.52**	0.13	0.09	0.15	0.13	0.28	0.09	−0.22
Time since last period, months[a]	0.06	0.09	−0.07	−0.17	0.06	−0.01	−0.15	−0.30	−0.21	0.15	0.16	0.14	0.15	0.08	0.16	−0.13

< p > [a]Variable was logarithmically transformed < /p >
$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

When combining the DEXA measurements at the lumbar spine and the hip, nearly 40% of the AN patients fulfilled the WHO definition of osteoporosis (*T*-score ≤ -2.5). This proportion is comparable with results of other studies [3,19,20]. Only 11% of the AN patients had normal bone density; this finding underlines the severity of bone deficits in young women suffering from AN.

The DXA measurements showed highly significant differences between AN and controls in all three tested localizations (femoral neck, total hip, lumbar spine). Of the three QUS parameters measured at the heel, only BUA revealed a significant difference between AN patients and the control group. This pattern is consistent with findings reported by others [9,10].

Based on the localization of affected bone, the current viewpoint is that AN causes deficits both in cancellous and cortical bone [2,21]. The results of our study support this view. They also provide some information how these deficits are represented in the bone microarchitecture. The multislice high resolution 3D-pQCT technique of the ultradistal radius showed that AN patients had a significantly lower number of trabecules per mm (Tb.N) and a significantly larger interconnected average separation distance between trabecules (Tb.Sp) than controls. Patients and controls did not differ regarding mean trabecular thickness (Tb.Th). These results suggest that trabecules require a minimum thickness to subsist and that the nutritional deficiencies of AN patients cause entire trabecules to disappear, or prevent the development of trabecules, producing a clinical picture in which trabecules are more widely separated but not, or only slightly, reduced in thickness. When conducting transiliac bone biopsies of patients with non-traumatic vertebral compression fractures and hip fractures without severe trauma, Parfitt et al. [22] found comparable changes in the microarchitecture supporting this mechanism of trabecular deficiency.

With regard to cortical bone, our study revealed that AN patients had significantly lower cortical thickness at the ultradistal radius than controls. In addition, all cortical parameters, the 3D-pQCT measurements of the entire bone diameter (D100), the bone density of the cortical part of the bone (Dcomp), and the absolute thickness of the cortical bone (C.Th), were significantly associated with age of AN onset. As none of the trabecular measures showed such associations, our results indicate that a younger age at onset of AN mostly affects the development or maintenance of cortical bone, specifically of the ultradistal radius.

Seemann et al. [21] observed that patients with onset of AN before puberty had bone deficits in vertebral and femoral locations, whereas patients with adult onset showed bone loss mostly in vertebral localizations. The authors inferred that AN affects different regions at different ages depending on the stage of bone growth and development. For instance, before puberty appendicular growth is more rapid than axial, whereas during puberty appendicular growth slows and axial

growth accelerates [21]. In our study, the minimum lifetime body mass index (BMI), which represents the severity of underweight since onset of AN, was associated with hip DXA measurements but not with spine DXA. As the hip localization is principally representative of cortical bone, and the spine localization of trabecular bone, these results could suggest that the degree of underweight has a greater effect on the cortical than the trabecular part of bone. The duration of underweight (months with BMI < 17.5 kg/m^2) was associated with lower levels of bone density as measured by DXA in all examined localizations, but especially for total hip. Also, lower levels of 3D-pQCT cortical thickness (C.Th) and lower number of trabecules (Tb.N) were linked with the duration of underweight. The 3D-pQCT findings indicate that duration of underweight affects both, the cortical and trabecular bone compartments.

Previous studies comparing the bone density between the two different forms of AN, i.e. the restrictive and the binge-purge type, have yielded inconsistent findings [19,23,24,25]. Our study did not reveal any differences between the two forms of AN in any of the bone parameters measured by the three methods. Our lack of findings is consistent with suggestions by Goebel et al. that the degree of underweight is an important predictor of low bone density, whereas other behaviors like bingeing and purging seem to be of relatively minor importance [25]. It should be noted, too, that the discrimination between the two AN types is based on behavioral symptoms (e.g. purging) that frequently change during the course of the illness [26,27]. Because the distinction between the two types is based on current symptomatology, it may yield groups that are not homogenous with regard to history of restrictive or binge-purge behavior. This heterogeneity is bound to mask differences in effects that restrictive eating and binge-purging might have on bone parameters.

Several limitations of the study are of note. While AN patients and controls were generally comparable with respect to demographic parameters and lifestyle characteristics, the age range of the control group was smaller and variances were not homogenous in the two groups. However, patients and controls did not differ regarding mean age. In addition, the DXA *t*-values of the control group were comparable to population means for femoral neck, but were marginally lower for total hip, and significantly lower for lumbar spine. It is conceivable that our control group was subject to a genetic predisposition selection bias. Because of the currently available technology it was not possible to examine the same skeletal regions with the three methods used in this study. Direct comparisons between the examined regions are curtailed because of differences in bone characteristics at the different skeletal sites (axial/appendicular, proximal/distal, bone region with and without postural effect). It is conceivable that future technology will enable the examination of different bone regions with the same methodology.

In summary, we found significant bone deficits in AN patients compared to controls in all examined skeletal locations independent of the method used. In AN patients specific anamnestic characteristics were linked with deficiencies in different bone localizations in both cortical and trabecular compartments. This is the first study to show the effect of AN on cortical and trabecular bone, not simply by assessing the localization of deficits, but by specifically examining the bone microarchitecture using the new technology of 3D-pQCT. In conclusion, using different techniques to assess bone mineral density and bone structure, our results confirm the high prevalence and high degree of site-specific bone deficiencies in young women with AN.

Acknowledgements The authors thank Andres Laib for his technological advice. This research was sponsored by an educational Grant of Merck Sharp & Dohme-Chibret AG Switzerland.

References

1. Becker AE, Grinspoon SK, Klibanski A, Herzog DB (1999) Eating disorders. N Engl J Med 340:1092–1098
2. Klibanski A, Biller BM, Schoenfeld DA, Herzog DB, Saxe VC (1995) The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. J Clin Endocrinol Metab 80:898–904
3. Biller BM, Saxe V, Herzog DB, Rosenthal DI, Holzman S, Klibanski A (1989) Mechanisms of osteoporosis in adult and adolescent women with anorexia nervosa. J Clin Endocrinol Metab 68:548–554
4. Grinspoon S, Miller K, Coyle C, Krempin J, Armstrong C, Pitts S, Herzog D, Klibanski A (1999) Severity of osteopenia in estrogen-deficient women with anorexia nervosa and hypothalamic amenorrhea. J Clin Endocrinol Metab 84:2049–2055
5. Rigotti NA, Nussbaum SR, Herzog DB, Neer RM (1984) Osteoporosis in women with anorexia nervosa. N Engl J Med 311:1601–1606
6. Bachrach LK, Guido D, Katzman D, Litt IF, Marcus R (1990) Decreased bone density in adolescent girls with anorexia nervosa. Pediatrics 86:440–447
7. Boyanov MA, Popivanov PR, Roux C (2001) Separate assessment of forearm cortical and trabecular bone density from standard densitometry data. Ann Med 33:497–506
8. Hergenroeder AC (1995) Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. J Pediatr 126:683–689
9. Resch H, Newrkla S, Grampp S, Resch A, Zapf S, Piringer S, Hockl A, Weiss P (2000) Ultrasound and X-ray-based bone densitometry in patients with anorexia nervosa. Calcif Tissue Int 66:338–341
10. Kutilek S, Bayer M (2001) Ultrasound parameters of calcaneal bone density in girls with anorexia nervosa. Eat Weight Disord 6:220–224
11. Langton CM, Ballard PA, Bennett DK, Purdie DW (1997) A comparison of the sensitivity and specificity of calcaneal ultrasound measurements with clinical criteria for bone densitometry (DEXA) referral. Clin Rheumatol 16:117–118
12. Ruegsegger P, Durand EP, Dambacher MA (1991) Differential effects of aging and disease on trabecular and compact bone density of the radius. Bone 12:99–105
13. Hernandez ER, Revilla M, Seco-Durban C, Villa LF, Cortes J, Rico H (1997) Heterogeneity of trabecular and cortical postmenopausal bone loss: a longitudinal study with pQCT. Bone 20:283–287
14. Muller R, Hildebrand T, Hauselmann HJ, Ruegsegger P (1996) In vivo reproducibility of three-dimensional structural proper-

ties of noninvasive bone biopsies using 3D-pQCT. J Bone Miner Res 11:1745–1750
15. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington D.C.
16. Krieg MA, Cornuz J, Ruffieux C, Sandini L, Buche D, Dambacher MA, Hartl F, Hauselmann HJ, Kraenzlin M, Lippuner K, Neff M, Pancaldi P, Rizzoli R, Tanzi F, Theiler R, Tyndall A, Wimpfheimer K, Burckhardt P (2003) Comparison of three bone ultrasounds for the discrimination of subjects with and without osteoporotic fractures among 7562 elderly women. J Bone Miner Res 18:1261–1266
17. Laib A, Hauselmann HJ, Ruegsegger P (1998) In vivo high resolution 3D-QCT of the human forearm. Technol Health Care 6:329–337
18. Laib A, Hildebrand T, Hauselmann HJ, Ruegsegger P (1997) Ridge number density: a new parameter for in vivo bone structure analysis. Bone 21:541–546
19. Zipfel S, Seibel MJ, Lowe B, Beumont PJ, Kasperk C, Herzog W (2001) Osteoporosis in eating disorders: a follow-up study of patients with anorexia and bulimia nervosa. J Clin Endocrinol Metab 86:5227–5233
20. Grinspoon S, Thomas L, Miller K, Herzog D, Klibanski A (2002) Effects of recombinant human IGF-I and oral contraceptive administration on bone density in anorexia nervosa. J Clin Endocrinol Metab 87:2883–2891
21. Seeman E, Karlsson MK, Duan Y (2000) On exposure to anorexia nervosa, the temporal variation in axial and appendicular skeletal development predisposes to site-specific deficits in bone size and density: a cross-sectional study. J Bone Miner Res 15:2259–2265
22. Parfitt AM, Mathews CH, Villanueva AR, Kleerekoper M, Frame B, Rao DS (1983) Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest 72:1396–1409
23. Andersen AE, Woodward PJ, LaFrance N (1995) Bone mineral density of eating disorder subgroups. Int J Eat Disord 18:335–342
24. Andersen AE, Watson T, Schlechte J (2000) Osteoporosis and osteopenia in men with eating disorders. Lancet 355:1967–1968
25. Goebel G, Schweiger U, Kruger R, Fichter MM (1999) Predictors of bone mineral density in patients with eating disorders. Int J Eat Disord 25:143–150
26. Nielsen S, Palmer B (2003) Diagnosing eating disorders—AN, BN and the others. Acta Psychiatr Scand 108:161–162
27. Fairburn CG, Harrison PJ (2003) Eating disorders. Lancet 361:407–416