

Normative data on mineralization density distribution in iliac bone biopsies of children, adolescents and young adults

N. Fratzl-Zelman^{a,*}, P. Roschger^a, B.M. Misof^a, S. Pfeffer^a, F.H. Glorieux^b, K. Klaushofer^a, F. Rauch^b

^a Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 4th Med. Dept., Hanusch Hospital, Heinrich Collin Str. 30, 1140 Vienna, Austria

^b Genetics Unit, Shriners Hospital for Children and McGill University, Montreal, Quebec, Canada H3G 1A6

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ABSTRACT

Bone mineralization density distribution (BMDD) as assessed by quantitative backscattered electron imaging (qBEI) in iliac crest bone biopsies has become in the last years a powerful diagnostic tool to evaluate the effect of metabolic bone diseases and/or therapeutic interventions on the mineralization status of the bone material. However until now, normative reference data are only available for adults. The aim of the present study is to close this gap and establish normative data from children and compare them with reference BMDD data of adults.

qBEI analyses were performed on bone samples from 54 individuals between 1.5 and 23 years without metabolic bone diseases, which were previously used as study population to establish normative histomorphometric standards.

In the trabecular compartment, none of the BMDD parameters showed a significant correlation with age. The BMDD was shifted towards lower mineralization density (CaMean -5.6% , $p < 0.0001$; CaPeak -5.6% , $p < 0.0001$; CaLow $+39.0\%$, $p < 0.001$; CaHigh -80.7% , $p < 0.001$) and the inter-individual variation was higher compared to the adult population.

The cortices appeared to be markedly less mineralized (CaMean -3.1% , $p < 0.0001$) than cancellous bone due to higher amounts of low mineralized secondary bone. However, the cortical BMDD parameters showed a strong correlation ($r = 0.38$ to 0.85 , with $p < 0.001$ to < 0.0001) with cancellous BMDD parameters.

In conclusion, this study provides evidence that BMDD parameters in growing healthy subjects are relatively constant and that these data can be used as normative references in pediatrics osteology. The larger inter-individual variability compared to adults is most likely related to alterations of the bone turnover rate during growth.

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Introduction

There is rising interest to include aspects of bone material quality [1–4] into the diagnosis of bone diseases, the estimation of fracture risk and treatment decisions. At present, assessment of bone material quality requires the examination of bone biopsy samples. One of the key parameters of material quality is mineral density content of the bone matrix, which determines the stiffness and toughness of bone material [3]. The local mineralization density of bone tissue can be measured by various techniques, such as microradiography [5,6], synchrotron radiation microcomputed tomography [7,8] and quantitative backscattered electron imaging (qBEI) [9].

The bone mineralization density distribution (BMDD) provides important information about the effect of metabolic bone diseases and therapeutic interventions on the mineralization of the bone material

[9–12]. Reference data for trabecular BMDD in adults are available for the age range from 25 to 90 years [13]. These show a remarkably small inter-individual variation (coefficient of variation of less than 1.7%) in BMDD, suggesting that even small deviations have biological relevance. Indeed, low matrix mineralization was found in osteoporosis [14,15] and mild primary hyperparathyroidism [16] while a shift towards higher mineralization was found in osteogenesis imperfecta [17,18].

At present, the use of BMDD in pediatric populations is hampered by the lack of reference data for the age range below 25 years. In previous studies we therefore compared BMDD values of children and adolescents either with adults [19] or with individually age-matched controls [9,18,20]. However, comparison with adult data is certainly not ideal, as preliminary data suggest that trabecular bone matrix is less mineralized in children than in adults [7,21]. Comparison with age-matched controls is cumbersome and requires access to the raw data of control subjects. Establishing pediatric reference data for BMDD is therefore important for assessing pediatric bone diseases by qBEI and would also yield new insights into normal bone

* Corresponding author. Ludwig Boltzmann Institute of Osteology, UKH Meidling, Kundratstrasse 37, A-1120 Vienna, Austria. Fax: +43 1 60150 2651.

E-mail address: nadja.fratzl-zelman@osteologie.at (N. Fratzl-Zelman).

development. We therefore performed qBEI analyses of complete transiliac bone biopsy specimen from children, adolescents and young adults without metabolic bone diseases.

Materials and methods

Subjects

The study population comprised 54 Caucasian subjects aged from 1.5 to 23 years (32 female, 22 male). Bone biopsies were performed during surgery for various orthopedic conditions, such as lower limb deformities, scoliosis, clubfeet and other problems that require corrective surgery (exostoses, cubitus valgus and equinovarus of the foot). All subjects were ambulatory, had normal renal function as assessed by measurement of serum creatinine and had no evidence of any metabolic bone disease. None was immobilized prior to surgery or

received medications known to affect bone metabolism. This cohort is part of a larger population that has been presented before to establish reference data for histomorphometric parameters in cancellous and cortical bone [22–24].

Quantitative backscattered electron imaging (qBEI)

Quantitative backscattered electron imaging was employed to visualize and quantify the local mineralization density in transiliac bone biopsy samples. The physical principle of the technique is based on a quantification of the intensity of electrons backscattered from the surface of a sectioned bone area. The obtained signal is proportional to the weight fraction of calcium (Ca wt.%) present locally in the embedded bone tissue. Full details of the technique have been described elsewhere [10,13]. Blocks containing polymethylmethacrylate-embedded undecalcified iliac bone samples were prepared for

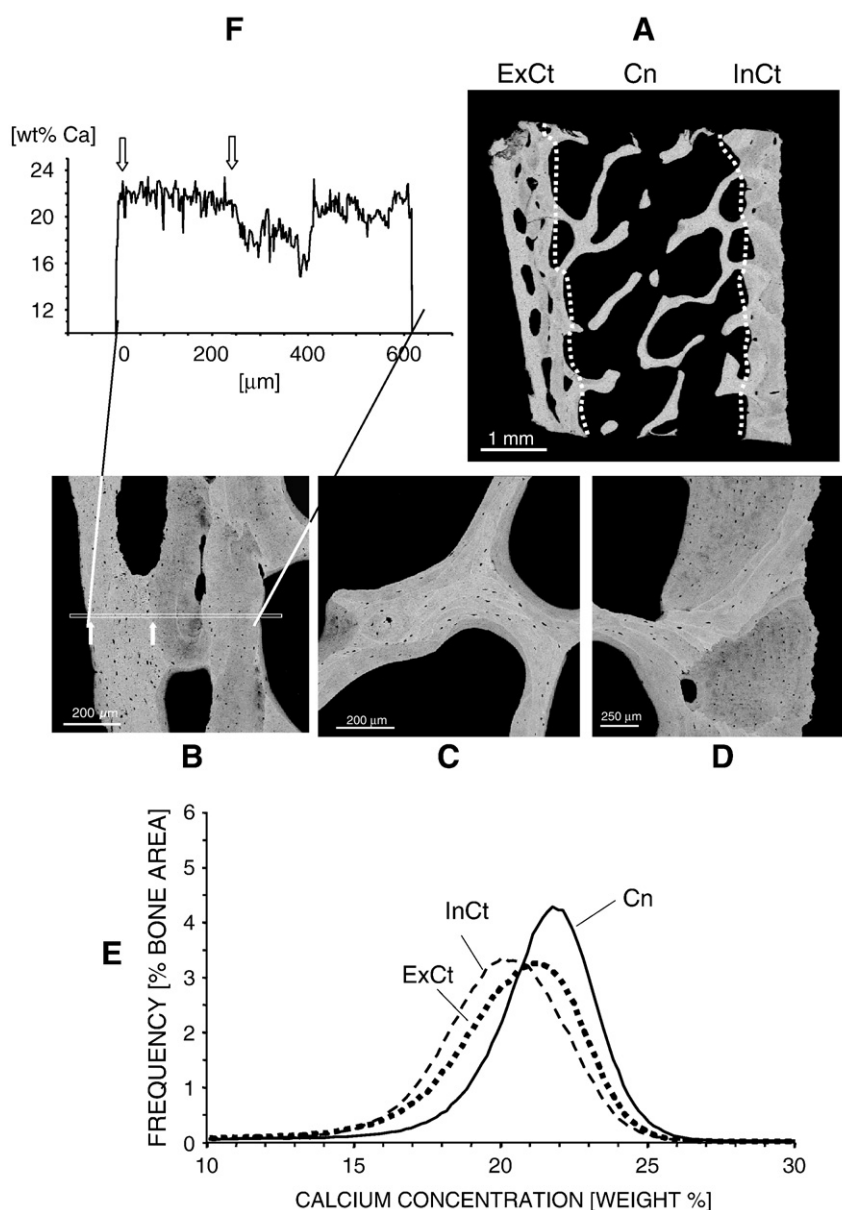


Fig. 1. Backscattered electron images and corresponding BMDD of a transiliac bone biopsy of a 12-year-old study participant. Overview of the entire biopsy sample showing the asymmetric aspect of the two cortices: (A) The external cortex (ExCt) is characterized by periosteal primary bone and endocortical cancellation while the internal cortex (InCt) is characterized by periosteal eroded surfaces and endocortical encroaching of trabecular features. (B) Detail of external cortex. (C) Detail of cancellous bone. (D) Detail of internal cortex. (E) BMDD curves corresponding to the ExCt and internal InCt as well as cancellous bone (Cn). (F) Calcium content profile through the external cortex from the periosteal to the endocortical surface as indicated by the white line. Arrows indicate the region of primary periosteal bone.

qBEI by grinding and polishing in order to obtain plane and parallel surfaces. The sample surface was then carbon coated. Quantitative BEI was performed in a digital scanning electron microscope (DSM 962, Zeiss, Oberkochen, Germany) equipped with a four-quadrant semiconductor backscattered electron detector. The microscope was operated at 20 keV electron energy and a probe current of 110 pA.

A series of backscattered electron images using a pixel resolution of 2 μm (as shown in Figs. 1B to D) were performed covering the entire biopsy area including both cortices and the cancellous bone compartment. Mineralized bone tissue were segmented from soft tissue including osteoid and marrow space filled with embedding medium by grey level thresholding [10,11]. Cancellous bone was separated from the cortical bone as indicated by the dashed line in Fig. 1A. Thereby the line was following the bone tissue where the compact bone character is changing into a cancellous one (subjectively). In the age range of 1.5 to 14 years morphological features as visualized by qBEI allowed us to assign the two cortices to external (Ex.Ct) and to internal (In.Ct) cortices (see Fig. 1) [24]. However, for ages above 14 years the above mentioned morphological features disappeared and hampered the discrimination between external and internal cortices. Hence, for the entire age range 1.5 to 23 years only one set of the BMDD data for cortical bone, the average of the two cortices was determined.

From the grey level images obtained by the area scans, grey level frequency histograms were derived and converted by calibration [10] to BMDD. BMDD curves indicate the frequency of pixels corresponding to specific calcium contents occurring throughout the sample area. BMDD curves were evaluated separately for the trabecular compartment and the two cortical compartments (Fig. 1 E).

For statistical analysis, five parameters characterizing the BMDD curve were determined, as described in detail elsewhere [9,13] (see Fig. 2):

- CaMean, the weighted mean calcium concentration of the bone area obtained by the integrated area under the BMDD curve;
- CaPeak, the peak position of the histogram, which indicates the most frequently occurring calcium concentration (calcium value with the highest number of pixels) in the bone area;
- CaWidth, the full width at half maximum of the distribution, describing the variation in mineralization density;
- CaLow, the percentage of bone area that is mineralized below the 5th percentile of the reference BMDD of normal adults [13], that is below 17.68 wt.% calcium. This parameter corresponds to the amount of bone area undergoing primary mineralization.
- CaHigh, the percentage of bone area that is mineralized above the 95th percentile of the reference BMDD of normal adults that is above 25.30 wt.% calcium. This parameter corresponds to the

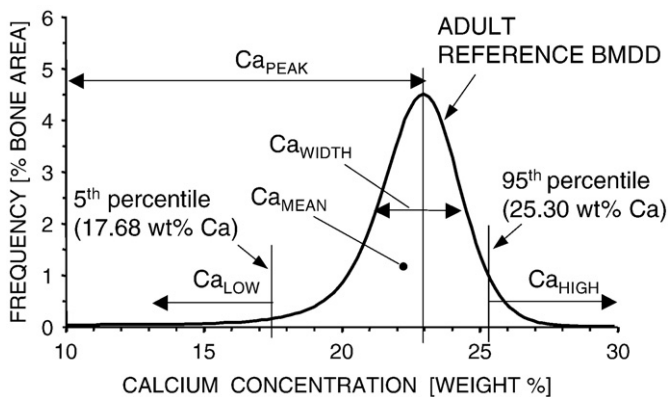


Fig. 2. The BMDD curve of normal human adult trabecular bone. All BMDD parameters used in this article (CaMean, CaPeak, CaWidth, CaLow, CaHigh) are indicated in this curve. Note that CaLow and CaHigh are defined in relation to this reference BMDD of adults [9].

Table 1

Cancellous BMDD parameters in the present study population compared to reference data for cancellous BMDD in adults [13] and to cortical BMDD parameters of the present study population.

	Adults Cancellous bone (n = 52)	Present study population	
		Cancellous bone (n = 54)	Cortex (n = 53)
CaMean [wt.% Ca]	22.20 (0.45)	20.95 ^a (0.57)	20.31 ^b (0.93)
CaPeak [wt.% Ca]	22.94 (0.39)	21.66 ^a (0.52)	21.14 ^b [20.62; 21.75]
CaWidth [Δ wt.% Ca]	3.29 [3.12; 3.47]	3.47 [3.12; 3.64]	3.81 ^b [3.38; 4.38]
CaLow [%]	4.52 [3.87; 5.79]	6.14 ^a [4.90; 7.99]	9.06 ^b [6.22; 15.00]
CaHigh [%]	4.62 [3.52; 6.48]	0.89 ^a [0.43; 1.47]	0.46 ^{b1} [0.28; 1.22]

Data shown are mean (SD) or median [25%; 75%].

^a significant difference ($p < 0.0001$) in cancellous bone results between adults and the present study population.

^{b, b1} significant difference ($p < 0.0001$, $p < 0.01$) to cancellous bone result in the present study population.

amount of bone having achieved plateau level of normal mineralization and includes also the contribution of highly mineralized cement lines [9].

Statistical analyses

Statistical analysis was performed using SigmaStat for Windows Version 2.03 (SPSS Inc.). The relationship between BMDD parameters and age was tested by Spearman rank order correlation tests. Comparisons of BMDD parameters between the present study group and those of an adult reference population were done by *t*-test or nonparametric by Mann–Whitney test when appropriate. Differences of BMDD parameters between spongiosa, and cortical bone were tested by Friedman repeated measures ANOVA on ranks followed by Tukey pairwise comparison. Not normally distributed data are given by median and interquartile range in Table 1. Correlations between results in trabecular and cortical bone were calculated by Pearson or Spearman correlation coefficients when appropriate (Table 2).

Results

Trabecular bone

In trabecular bone, none of the BMDD parameters varied significantly with age (Fig. 3A). Statistical *t*-test between the male and female group did not reveal differences in BMDD parameters either. The corresponding *p*-values ranged from 0.31 to 0.95. Consequently, a single reference BMDD of trabecular bone could be constructed for the entire age range from 1.5 to 23 years (Fig. 3B). Table 1 presents these results in numerical format.

Compared to the adult reference population [13], the present study population had lower average mineralization density, as expressed by 5.6% lower results for CaMean and CaPeak (Table 1, Fig. 3B). Correspondingly, samples from the younger population contained 39% more areas with low mineralization, and 81% less areas with fully mineralized bone ($p < 0.001$ in both cases; Table 1). The average intraindividual heterogeneity of mineralization (i.e., CaWidth) was not significantly different between groups ($p = 0.52$). However, the

Table 2

Correlations of cortical with cancellous BMDD parameters of the present study population.

	Cortical vs. cancellous bone
CaMean	$r = 0.85$, $p < 0.0001$
CaPeak	$r = 0.83$, $p < 0.0001$
CaWidth	$r = 0.38$, $p < 0.01$
CaLow	$r = 0.75$, $p < 0.0001$
CaHigh	$r = 0.72$, $p < 0.0001$

Data shown are the correlation coefficient *r* and the corresponding *p*-value.

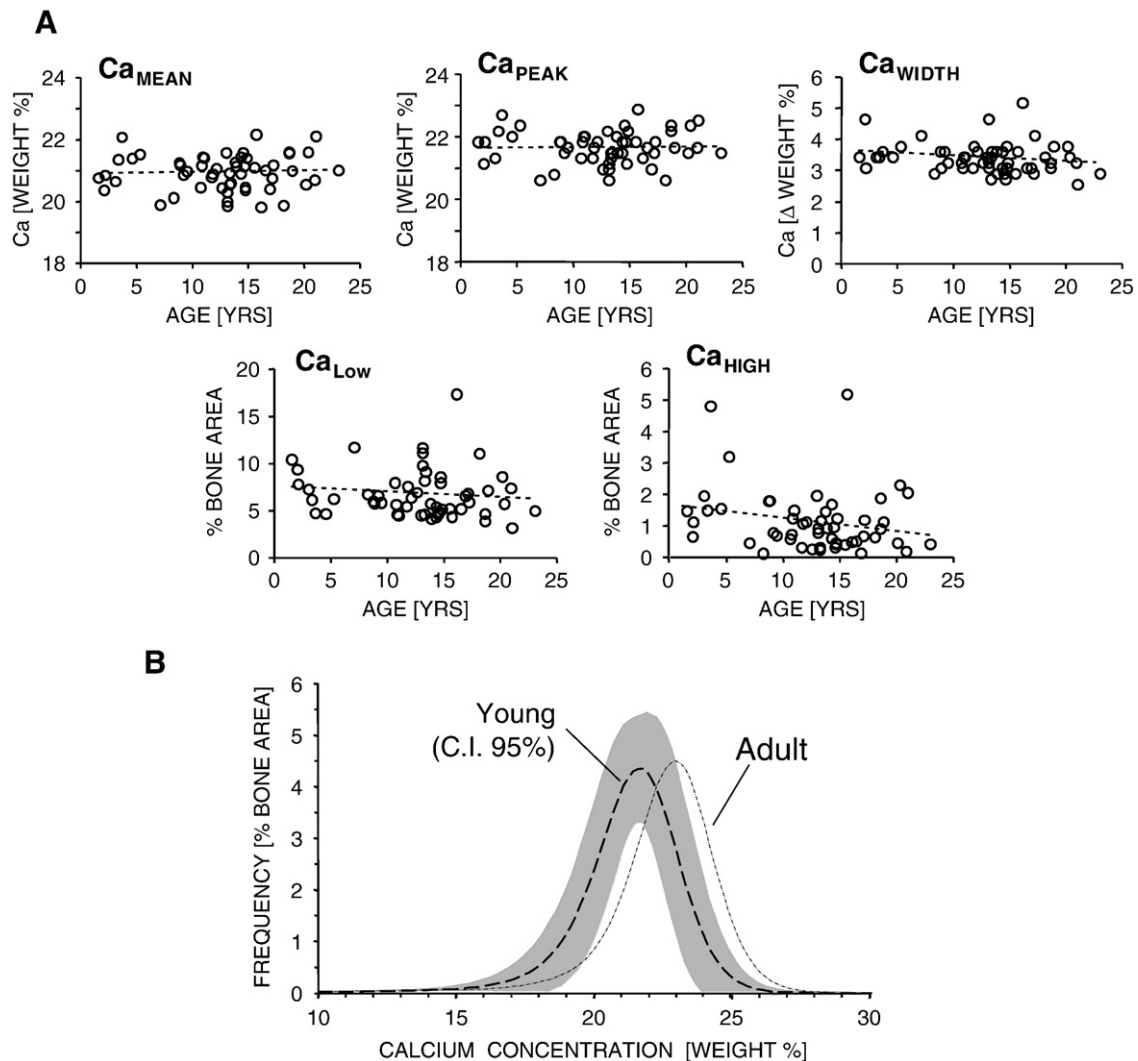


Fig. 3. Relationship of BMDD parameters with age. (A) The dotted lines indicate the linear fits. However none of the parameters showed a significant correlation with age. (B) Reference BMDD curve for trabecular bone for the age range from 1.5 to 23 years. The grey band indicates the 95% confidence interval (C.I.) for the individual histogram values. For comparison the reference BMDD curve of adults (25 to 90 years of age) is shown too.

interindividual variability of mineralization density was slightly higher in the present study population than in adults. E.g., the interindividual coefficient of variation in CaPeak was 1.7% in the adult group and 2.4% in our present cohort (Table 1).

Cortical bone

BMDD parameters of cortical bone (mean of both cortices) were significantly correlated to those of trabecular bone (Table 2). Nevertheless, the mineralization density of cortical bone was markedly lower and more heterogeneous than that of trabecular bone (Table 1, Fig. 1). This was explained by the observation that cortical bone contained more areas undergoing primary mineralization and fewer areas of fully mineralized bone (Fig. 1, Table 1).

Though in the age range 1.5 to 14 years the two cortices, the external and internal cortices, exhibited an asymmetric aspect, the mineralization density (CaMean – 0.98%) was only slightly lower for internal compared to external cortex (data not shown). In the external cortex, bone tissue close to the periosteal surface was more highly mineralized than bone adjacent to the endocortical surface (Figs. 1B and F). The internal cortex contained remnants of highly mineralized trabeculae. The areas between these trabecular remnants were ‘filled’ with large amounts of newly formed and lower mineralized bone

(Fig. 1D). The periosteal surface of the internal cortex often presented an eroded aspect (Fig. 1D).

Discussion

The main findings of this study are that BMDD parameters of iliac trabecular bone are constant from 1.5 to 23 years of age and that matrix mineralization density is lower in cortical than in trabecular bone. In addition there was a strong correlation of the BMDD parameters between cortical and trabecular bone. These observations need to be interpreted in light of the mineralization process and of normal iliac bone development.

The lack of age dependency of BMDD parameters in these biopsies during growth of individuals, which is consistent with a previous study [17], is somewhat surprising and requires closer analysis: First, in children and adolescents, the site of biopsy is located at approximately 1 cm below the apophyseal iliac growth plate. During human skeletal development, this growth plate is one of the last to fuse [25]. It can therefore be assumed that in most of our study participants this growth plate was active when the biopsy was performed. This means that even though our study population varied widely in chronological age, the age of the bone tissue at the site of biopsy was presumably similar between study participants. Second, it

has been shown that trabecular bone in the growing skeleton is subjected to a high rate of bone turnover with a small positive balance towards new bone acquisition [26]. Hence, in a situation of high bone turnover – and independently of age – the BMDD is shifted towards lower mineralization [9,12,14,16,27]. This is due to the fact that newly formed bone matrix is rapidly mineralized (within a few days) up to only 70% (primary mineralization) of fully mineralized bone matrix and the full mineralization lasts years (secondary mineralization). Consistently, a recent mathematical model showed that the most frequently occurring calcium concentration (CaPeak) decreases in situations of high bone turnover [28]. Therefore, the average degree of mineralization depends on the age of the bone tissue. High bone turnover decreases the average age of bone tissue and therefore is associated with increased CaLow and decreased CaPeak and CaMean [28,29]. Presumably, the continuing endochondral bone formation process and the subsequent high remodeling activity thus contribute to maintain bone tissue age fairly constant, which explains why the mineralization density of the bone matrix did not change with the chronological age of study participants. Once the growth process is completed, the bone tissue ‘ages’ rapidly. This means that mineralization density increases rapidly until a new steady state is achieved, the level of which depends on the prevailing remodeling rate [28]. Therefore, the mineralization density of iliac trabecular bone is higher in adults than during development.

Further, the BMDD data reflected the distinctive development of the cortices. According to previous studies, the developing ilium increases in width due to a lateral modeling drift of both cortices [23,26]. The outer cortex is expanding through periosteal bone apposition and is partially resorbed and transformed into trabecular bone on the endocortical side. In contrast, the inner cortex expands by endocortical apposition, and thus encroaches on the trabecular compartment. During this process, trabeculae are incorporated into the inner cortex and the space between trabeculae is filled up with new bone tissue.

The qBEI method shows that the primary bone produced by the periosteal osteoblasts of the external cortex is more highly mineralized than the adjacent bone tissue that has undergone intracortical remodeling and thus constitutes secondary bone. As the amount of this high-density primary bone is generally much less than that of the low-density secondary bone, the overall mineralization density of the external cortex is lower and more heterogeneous than that of the cancellous bone. Interestingly, this primary periosteal bone matrix with increased mineralization density, which has already described using microradiography [30], is known to have a different lamellar structure than secondary bone [23]. It seems that the mineralization process of this bone matrix is accelerated. Otherwise, the mineralization density would be distinctly lower, because of its relatively young age in this cortical bone region. Such rapid formation of highly mineralized periosteal bone tissue in the developing skeleton might represent an efficient mechanism to establish rapidly some mechanical competence by forming first a rigid scaffold, which is remodeled and optimized later on. This strategy seems to be analogous to the formation of highly mineralized callus tissue in fracture healing [31,32].

In contrast, the internal cortex of the ilium does not contain primary bone in growing individuals, as the formation drift occurs on the endocortical surface [23,26]. Consequently, the internal cortex is composed of a mixture between relatively old and highly mineralized trabecular remnants and new bone matrix with low mineralization. The average mineralization density therefore is below that of trabecular bone. Thus, consistent with previous observations that were made with a different experimental approach, the average mineralization density during development is lower in cortical than in trabecular bone [17]. As discussed, this is presumably the result of modeling drifts. When the modeling drifts come to a halt at the end of bone development, the mineralization density of cortical bone should

increase. In adults, CaPeak of cortical bone is indeed similar or higher than that of trabecular bone [33].

Moreover, it is remarkable, that the cortical BMDD parameters were strongly correlated to those of trabecular bone. This suggests a certain “coupling” of bone turnover rate between cortical and trabecular bone compartments in these young individuals.

In conclusion, the present study shows that trabecular BMDD of transiliac biopsy samples remains constant from 1.5 to 23 years of age and that the cortical BMDD is different, but strongly correlated to the trabecular BMDD in each individual. Therefore, the reference values for trabecular and cortical bone as well its correlation to each other derived in this study can be used for the entire age range. It should be emphasized, that due to the higher variability in BMDD, the usage of the normative data deduced from 54 individuals might be more reliable than the usage of age-matched controls. These data should increase the use of qBEI in the assessment of pediatric bone biopsy samples.

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