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Original Article

Peripheral quantitative computed tomography of the distal and proximal forearm in children and adolescents: bone densities, cross-sectional sizes and soft tissues reference data

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Abstract

Objectives: Peripheral quantitative computed tomography (pQCT) is gaining popularity in the field of paediatric bone densitometry, however, very little is known about reference limits. The purpose of this study was to develop country-specific reference data for bone densities, cross-sectional sizes, strength and regional tissue distribution measured by pQCT at the distal and proximal forearm in children and adolescents aged 5-19 yrs. **Methods:** Stratec XCT 2000L apparatus was used. Measurement sites were 4% and 66% of the forearm length on non-dominant arm. Studied group comprised 221 participants (103 girls) aged 4.5-19.5 yrs. The LMS method was used to fit percentile curves for each outcomes. **Results:** Smoothed percentile curves were developed for following outcomes: trabecular volumetric bone mineral density, total volumetric bone mineral density, distal total bone cross-sectional area, cortical volumetric bone mineral density, cortical cross-sectional area, proximal total bone cross-sectional area, polar strength strain index, fat cross-sectional area and muscle cross-sectional area. **Conclusions:** In this study we present reference data for bone densities, cross-sectional size and strength as well as for regional tissue distribution measured by pQCT at the distal and proximal forearm in children 5-19 yrs in a way allowing simple calculation of reliable Z scores.

Keywords: pQCT, Radius, Forearm, Children, Reference Data

Introduction

Peripheral quantitative computed tomography (pQCT) is gaining popularity in the field of paediatric bone densitometry. pQCT analyses 3-D cross-sectional images of long bones at certain levels, so pQCT is able to measure cortical and trabecular bone separately, to determine volumetric bone mineral density and to estimate bone strength[1-3]. It is also possible to analyze soft tissue compartment, muscle and fat cross-sectional area could be determined. Finally, pQCT, providing both information about bone and muscle, allows assessment of the functional muscle-bone unit[4,5]. Moreover, pQCT scans deliver only a very low radiation dose and avoid systemic irradiation[1,2]. Effective dose for patient is less than dose received daily from natural sources of radiation[6].

Until today, there is no world-wide reference data for pQCT[7] and very little is known about volumetric bone mineral density reference limits[3,8]. Only 5 studies provide local reference data for two populations: Dortmund (German)[9-12] and Greater Manchester (England)[13]. Methodological differences between these studies exist as well as differences in studied populations, so, at the moment, country-specific reference data are needed.

The purpose of this study was to develop country-specific reference data for bone densities, cross-sectional size and strength as well as for regional tissue distribution and bone cross-sectional area by muscle area measured by pQCT at the distal and proximal forearm in children and adolescents aged 5-19 yrs.

The authors have no conflict of interest.

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Table 1. Characteristics of studied group.

	Male (n=118)				
	Median	**Minimum**	**Maximum**	**Q1**	**Q3**
Age [yrs]	11,5	4,5	19,3	7,8	15,4
Height [cm]	151,0	106,0	187,0	130,0	177,0
Weight [kg]	42,0	16,5	88,0	25,0	60,8
BMI [kg/m^2]	17,5	14,0	25,3	15,6	20,0
	Female (n=103)				
Age [yrs]	11,7	4,8	19,4	8,6	15,4
Height [cm]	154,0	110,0	173,0	135,0	163,0
Weight [kg]	42,5	17,0	69,0	27,2	53,0
BMI [kg/m^2]	17,7	13,4	23,9	15,8	19,7

BMI - body mass index.

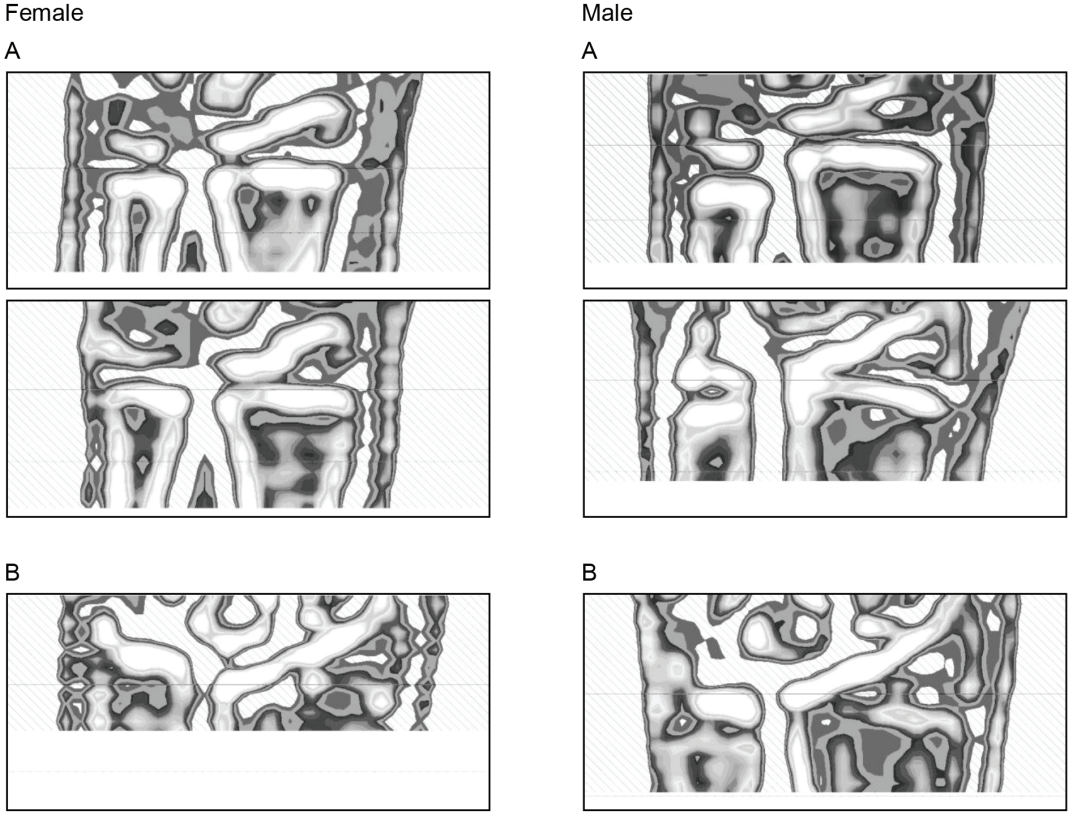

Figure 1. Scheme of determination of start position: left panel refers to female, right to male; A - growth plate is visible, B - growth plate is fused; upper line - reference line (start position); lower line - measurement line (4% length of forearm).

Materials and methods

Studied group

Inclusion criteria were as follows: children and adolescents from Warsaw area preschools and schools, aged from 4,5 to 19,5 yrs, with body height between 5th and 95th percentile, body weight between 5th and 90th percentile and body mass index between 5th and 85th percentile, based on Polish growth references for school and preschool children[14,15]. Exclusion criteria were: presence of disease which may affect bone metabolism and more than 2 previous fractures. 314 children and adolescents declared to participate in the study,

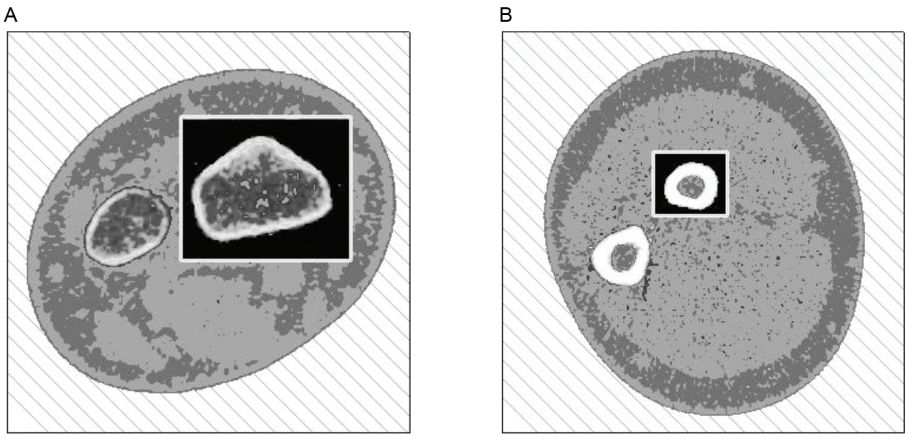

Figure 2. Cross-sectional images of left forearm: A - "4%" site, B - "66%" site; box indicates radial bone.

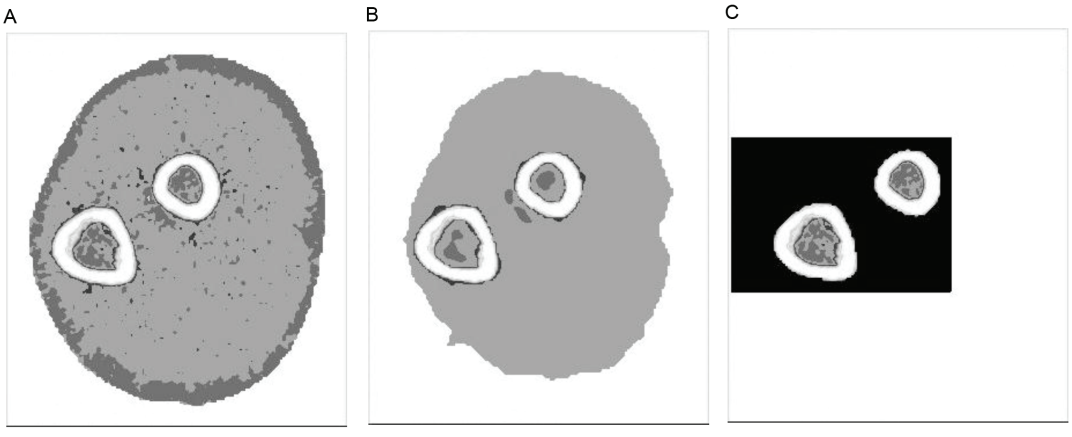

Figure 3. Scheme of determination of fat and muscle cross-sectional area (66% length of forearm); A - total forearm area, B - muscle+bone area, C - bone area. Fat area = "total forearm area" minus "muscle+bone area". Muscle area = "muscle+bone area" minus "bone area".

93 of them did not fit inclusion criteria or met exclusion criteria. Finally, 221 participants (118 boys and 103 girls) were included in the study. The protocol was approved by the local Institutional Review Board. Informed written consents were obtained from all participants and their legal guardians. Characteristics of studied group were presented in Table 1.

Measurements

All measurements were done with the Stratec XCT 2000L (Stratec Medizintechnik, Pforzheim, Germany) apparatus with software ver. 6.20 on non-dominant arm[7]. Dominance was determined by subject's report. Measurement sites were 4% and 66% of the forearm length. Forearm length was measured with the ruler from the ulnar styloid process to the olecranon. The scout view was used to determine start position as follows: if the growth plate was visible the reference line was placed through the most distal portion of the growth plate; if the growth plate had fused the reference line was placed through the middle of horizontal part of the articular surface of the radius (Figure 1). The scan lines were automatically placed at a distances of 4% and 66% of the forearm length, proximal to the reference line[6]. At the 4% site trabecular volumetric bone mineral density (mg/cm^3), total volumetric bone mineral density (mg/cm^3) and total bone cross-sectional area (mm^2) were measured with using the CALCBD analysis algorithm, contour mode 1, peel mode 1 and threshold 280 mg/cm^3. Area was set as 45% (central) in the case of trabecular volumetric bone mineral density determination. At the 66% site CORTBD algorithm

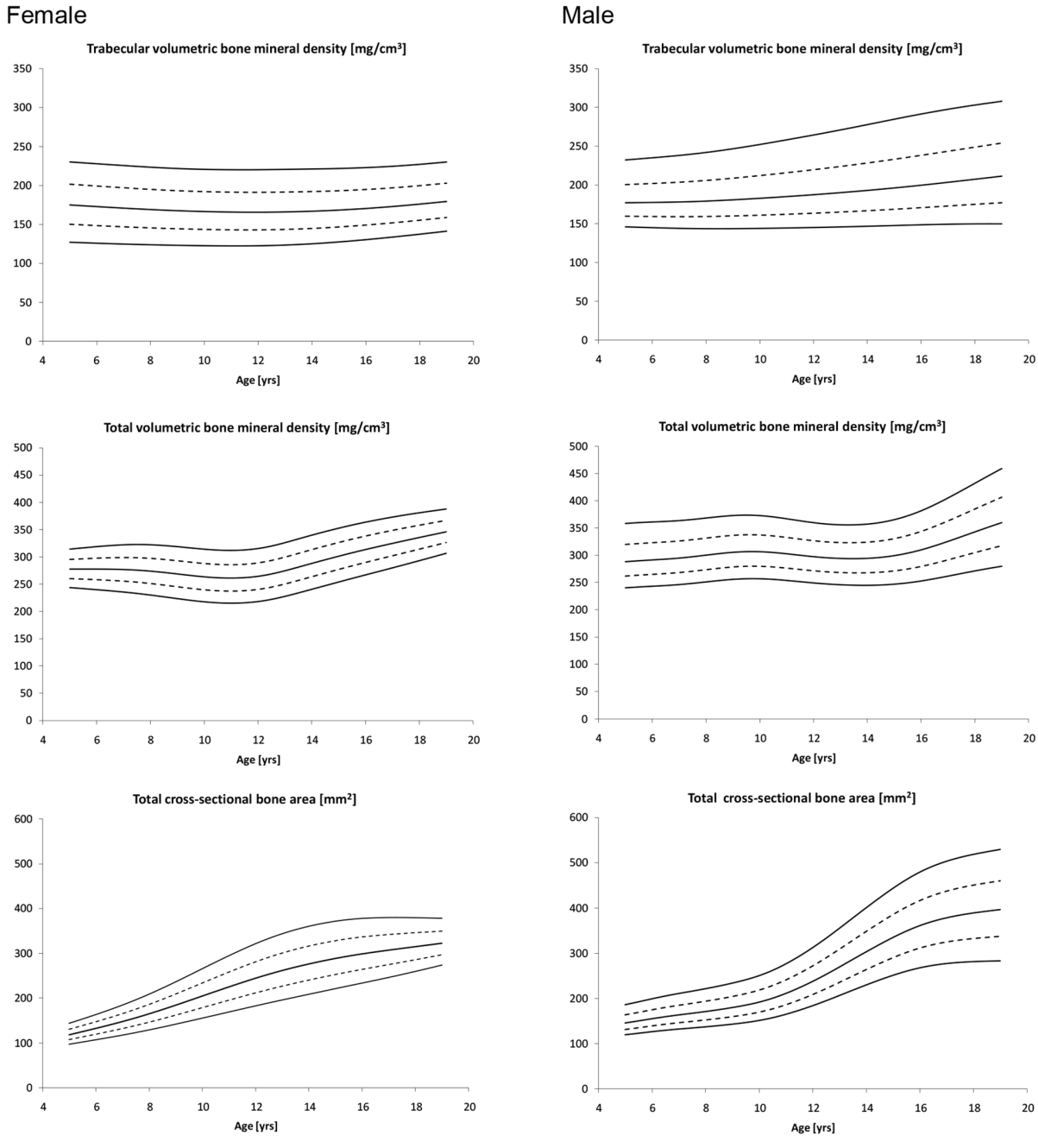

Figure 4. Reference ranges for distal radius (4%) outcomes. Left panel refers to female and right to male. Median (solid line in the middle) and percentiles corresponding to +/-1 SD (dashed lines) and +/- 2 SD (outer solid lines) were presented.

with separation mode 1 and threshold 711 mg/cm^3 was used for determining cortical volumetric bone mineral density (mg/cm^3), cortical cross-sectional area (mm^2) and total bone cross-sectional area (mm^2) (Figure 2). Threshold of 280 mg/cm^3 was used for polar strength strain index (mm^3) calculation. Fat cross-sectional area (mm^2) was calculated by subtraction of muscle+bone cross-sectional area from total forearm cross-sectional area. Muscle cross-sectional area (mm^2) was calculated by subtraction of bone cross-sectional area from muscle+bone cross-sectional area (Figure 3). CALCBD algorithm was used, with threshold -53 mg/cm^3, contour mode 3 and peel mode 1 for determination of total forearm area; threshold 40 mg/cm^3, contour mode 1, peel mode 2 and filter F03F05 for muscle+bone area; threshold 280 mg/cm^3, contour mode1 and peel mode 2 for bone area.

Scan speed, slice thickness and voxel size were 30 mm/s,

Table 2. Skewness (L), median (M) and coefficient of variation (S) equations of reference data in girls by age and muscle area.

	L	M	S
Trabecular volumetric bone mineral density [mg/cm^3]	-4,7054*10^{-2}*age+0,7790	-6,7914*10^{-4}*age^4+3,7216*10^{-2}*age^3-0,4842)*age^2+0,3331*age+181,275	-7,2344*10^{-8}*age^6+5,8015*10^{-6}*age^5-1,8336*10^{-4}*age^4+2,9012*10^{-3}*age^3-2,4245*10^{-2}*age^2+0,1015*age-0,0195
Total volumetric bone mineral density [mg/cm^3]	1,4294*10^{-2}*age+0,2775	5,2215*10^{-4}*age^6-3,6555*10^{-2}*age^5+1,0059*age^4-13,7658*age^3+98,3137*age^2-349,131*age+763,078	1,9631*10^{-6}*age^4-7,5057*10^{-5}*age^3+2,7772*10^{-4}*age2+1,0968*10^{-2}*age+0,00928
4% Total cross-sectional bone area [mm^2]	-1,2425*10^{-2}*age+0,3869	-7,2168*10^{-2}*age^3+2,0866*age^2-1,0446*age+79,0822	7,0761*10^{-6}*age^4-3,8012*10^{-4}*age^3+6,1775*10^{-3}*age^2-3,2287*10^{-2}*age+0,1493
Cortical volumetric bone mineral density [mg/cm^3]	-0,1810*age+4,9841	17,2609*age+841,775	-2,2825*10^{-4}*age^2+3,5655*10^{-3}*age+0,0286
Cortical cross-sectional area [mm^2]	-5,0939*10^{-2}*age+0,9738	-9,4880*10^{-3}*age^3+0,2078*age^2+3,1212*age+5,2583	-8,6184*10^{-3}*age+0,2409
66% Total cross-sectional area [mm^2]	0,2192*age-2,9652	-6,8482*10-3*age3+5,9359*10-2*age2+5,7608*age+44,0923	3,0520*10^{-6}*age^4-1,8990*10^{-4}*age^3+3,2241*10^{-3}*age^2-1,4706*10^{-2}*age+0,1250
Polar strength strain index [mm^3]	-6,3841*10^{-4}*age^3+3,0935*10^{-2}*age^2-0,5973*age+3,7361	-4,6018*10^{-2}*age^3+1,3122*age^2+4,2307*age+35,7862	1,0360*10^{-6}*age^5-6,1436*10^{-5}*age^4+1,3245*10^{-3}*age^3-1,3122*10^{-2}*age^2+6,1718*10^{-2}*age+0,0670
Fat cross-sectional area [mm^2]	5,0047*10^{-7}*age^6-4,2583*10^{-5}*age^5+1,4372*10^{-3}*age^4-2,4129*10^{-2}*age^3+0,2072*age^2-0,8350*age+2,1355	1,5742*10^{-2}*age^4-0,6622*age^3+8,1653*age^2+1,2784*age+743,352	-1,3579*10-6*age5+8,8712*10-5*age4-2,1344*10-3*age3+2,1397*10-2*age2-6,4694*10-2*age+0,1498
Muscle cross-sectional area [mm^2]	-6,4471*10^-5*age^5+3,7126*10^{-3}*age^4-7,8760)*10^{-2}*age^3+0,8015*age^2-4,5671*age+14,025	-3,2718*age^2+180,288*age+198,005	5,9588*10^{-7}*age^4-5,3147*10^{-5}*age^3+8,6670*10^{-4}*age^2+1,3680*10^{-3}*age+0,0465
Cortical cross-sectional area [mm^2] by muscle area	-6,2015*10^{-4}*muscle area+1,5223	-1,2298*10^{-8}*muscle area^3+4,9842*10^{-5}*muscle area^2+1,4531*10^{-2}*muscle area+7,2278	2,3185*10^{-17}*muscle area^5-0,1970*10^{-12}*muscle area^4+6,2540*10^{-10}*muscle area^3-9,5351*10^{-7}*muscle area^2+7,2600*10^{-4}*muscle area-0,1113

2,3 mm and 0,5x0,5 mm, respectively.

All measurements were done between May 2013 and Jun 2016 by the same operator on the same unit. Routine quality assurance procedures were carried out, basing on phantom supplied by manufacturer. Phantom comprises two "parts": standard and cone. Standard phantom was measured each day when patients were measured. Cone phantom was measured monthly. Measurement errors were (CV%, standard phantom): 0,35% for total density, 0,44% for trabecular density and 0,37% for cortical density in the whole study period.

Quality of each slice was rated from 1 (no movement) to 5 (extreme movement) by the same operator according to visual scale. Slices rated >3 were excluded from analysis as suggested by others[16]. In the case of 4% site no exclusion was needed, in the case of 66% site 15 measurements were excluded.

Body weight and height were measured in the standing position using medical scale with stadiometer (Tryb, Bydgoszcz, Poland). Body mass index was calculated as body weight divided by height in meters squared. Age of each participant was calculated from birth and observation dates.

Statistics

The LMS method[17] was used to fit percentile curves for each outcomes in both sexes. LMSchartmaker v. 2.54 (Medical Research Council, UK)[18] was used to derive the smoothed percentiles. The LMS method uses polynomial

Table 3. Skewness (L), median (M) and coefficient of variation (S) equations of reference data in boys by age and muscle area.

	L	M	S
Trabecular volumetric bone mineral density [mg/cm³]	9,5656*10⁻⁴*age³-3,1555*10⁻²*age²+0,3809*age-2,6482	0,1390*age²-0,8509*age+177,64	4,8310*10⁻³*age+0,0880
Total volumetric bone mineral density [mg/cm³]	7,4554*10⁻²*age-1,2723	-1,5887*10⁻⁴*age⁶+7,8924*10⁻³*age⁵-0,1178*age⁴+0,1383*age³+10,096*age²-72,886*age+433,296	-3,4520*10⁻⁷*age⁵+1,8482*10⁻⁵*age⁴-3,4171*10⁻⁴*age³+2,8106*10⁻³*age²-1,1544*10⁻²*age+0,1184
4% Total cross-sectional bone area [mm²]	0,1022*age-1,4502	2,3525*10⁻³*age⁵-0,1666*age⁴+4,2667*age³-48,453*age²+255,817*age-356,619	3,2780*10⁻³*age+0,09296
Cortical volumetric bone mineral density [mg/cm³]	-0,1506*age+4,8846	-2,0894*10⁻³*age⁵+0,1186*age⁴-2,4159*age³+20,809*age²-52,614*age+895,806	2,0784*10⁻⁶*age⁴-1,0158*10⁻⁴*age³+1,4460*10⁻³*age²-6,3239*10⁻³*age+0,04058
Cortical cross-sectional area [mm²]	-0,1170*age+2,5824	-6,4529*10⁻³*age⁴+0,3070*age³-5,1535*age²+40,609*age-84,533	4,1504*10⁻³*age+0,08333
66% Total cross-sectional area [mm²]	2,1771*10⁻⁵*age⁵-1,4004*10⁻³*age⁴+3,3497*10⁻²*age³-0,3580*age²+1,5207*age-1,6762	5,8698*age+47,789	-8,6581*10⁻⁸*age⁶+6,5553*10⁻⁶*age⁵-1,9212*10⁻⁴*age⁴+2,7383*10⁻³*age³-1,9780*10⁻²*age²+7,1267*10⁻²*age+0,02589
Polar strength strain index [mm³]	8,9866*10⁻⁶*age⁶-6,6826*10⁻⁴*age⁵+1,9536*10⁻²*age⁴-0,2828*age³+2,1120*age²-7,9142*age+14,238	-1,0859*10⁻²*age⁴+0,4839*age³-7,2744*age²+61,308*age-89,591	3,2092*10-7*age6-2,1244*10-5*age5+5,5453*10-4*age4-7,2900*10-3*age3+5,0324*10-2*age2-0,1583*age+0,2663
Fat cross-sectional area [mm²]	-4,8926*10⁻³*age²+0,2484*age-2,5775)	-1,3566*10⁻³*age⁶+9,6426*10⁻²*age⁵-2,6721*age⁴+36,090*age³-244,512*age²+778,031*age-182,931	1,8325*10⁻²*age+0,07894
Muscle cross-sectional area [mm²]	0,09831*age-0,8615	10,993*age²-47,736*age+1170,66	-1,2280*10⁻⁵*age³+1,0728*10⁻⁵*age²+1,1745*10⁻²*age+0,02019
Cortical cross-sectional area [mm²] by muscle area	-4,4129*10⁻⁴*muscle area+2,7986	-5,9937*10⁻⁶*muscle area²+7,9284*10⁻²*muscle area-10,692	-3,5254*10⁻⁷*muscle area+0,1151

Table 4. Skewness (L), median (M) and coefficient of variation (S) equations of reference data in girls and boys by height.

	Female		
	L	M	S
Cortical cross-sectional area [mm²]	0,3305	4,8214*10⁻⁹*height²+0,8960*height-79,477	3,1134*10⁻¹²*height²-2,2169*10⁻³*height+0,4816
Polar strength strain index [mm³]	7,2758*10⁻¹²*height²-2,8787*10⁻²*height+4,6517	-1,2986*10⁻⁴*height³+8,7002*10⁻²*height²-13,455*height+685,72	0,1729
66% Total cross-sectional area [mm²]	0,05462	3,3791*10⁻³*height²-2,0258*10⁻²*height+34,07	5,8106*10⁻¹⁰*height⁵-3,9834*10⁻⁷*height⁴+1,0792*10⁻⁴*height³-1,4458*10⁻²*height2+0,9595*height-25,1802
	Male		
	L	M	S
Cortical cross-sectional area [mm²]	1,2903	5,3114*10⁻⁵*height³-1,9262*10⁻²*height²+2,9840*height-139,09	0,1378
Polar strength strain index [mm³]	0,6254	3,6572*10⁻⁴*height³-0,1400*height²+20,4848*height-962,86	-1,5649*10⁻¹³*height²+7,2711*10⁻⁴*height+0,06812
66% Total cross-sectional area [mm²]	-0,6158	1,1682*10⁻⁴*height³-4,7410*10⁻²*height²+7,2914*height-309,33	0,1375

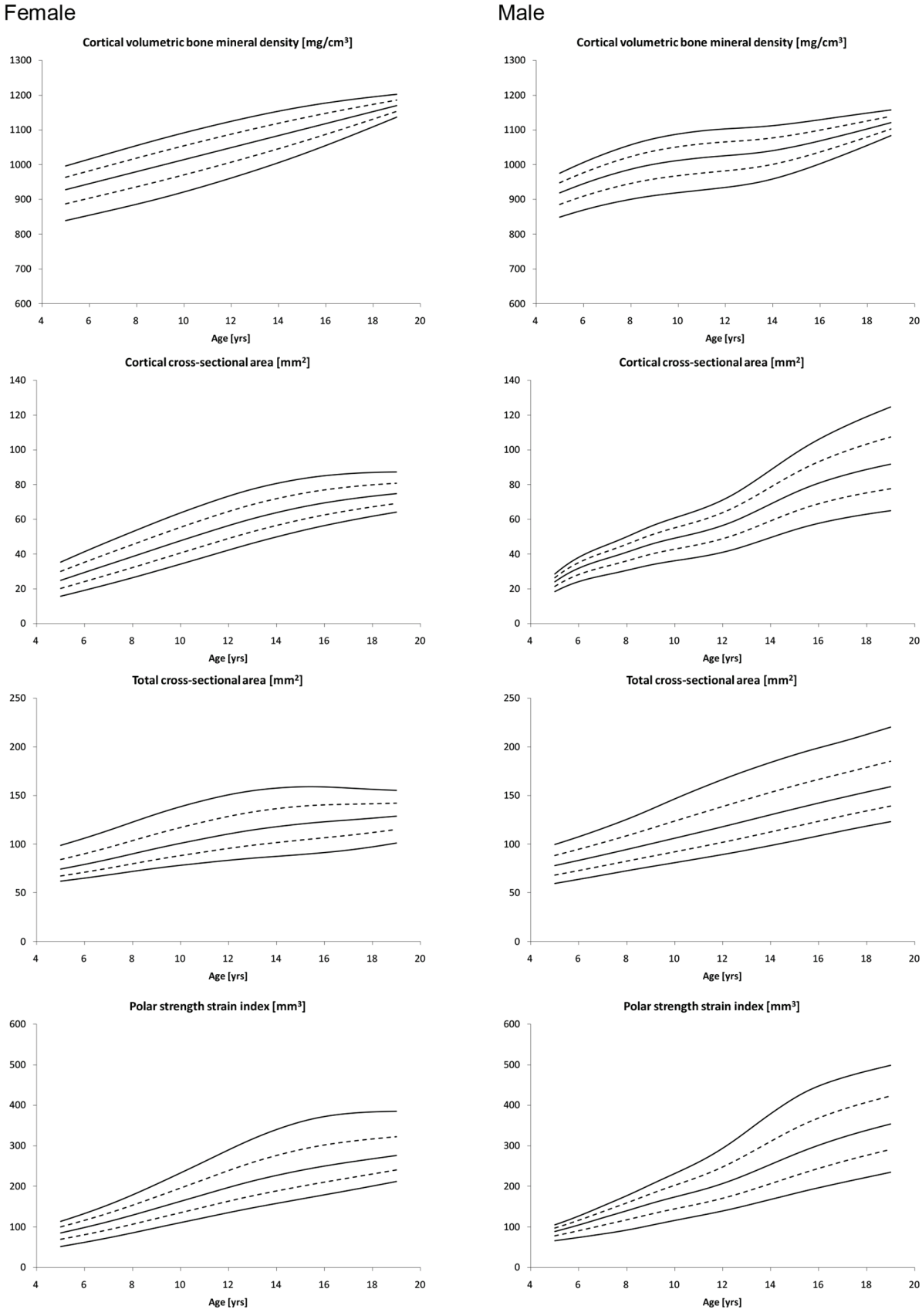

Figure 5. Reference ranges for proximal radius (66%) bone outcomes. Left panel refers to female and right to male. Median (solid line in the middle) and percentiles corresponding to +/-1 SD (dashed lines) and +/- 2 SD (outer solid lines) were presented.

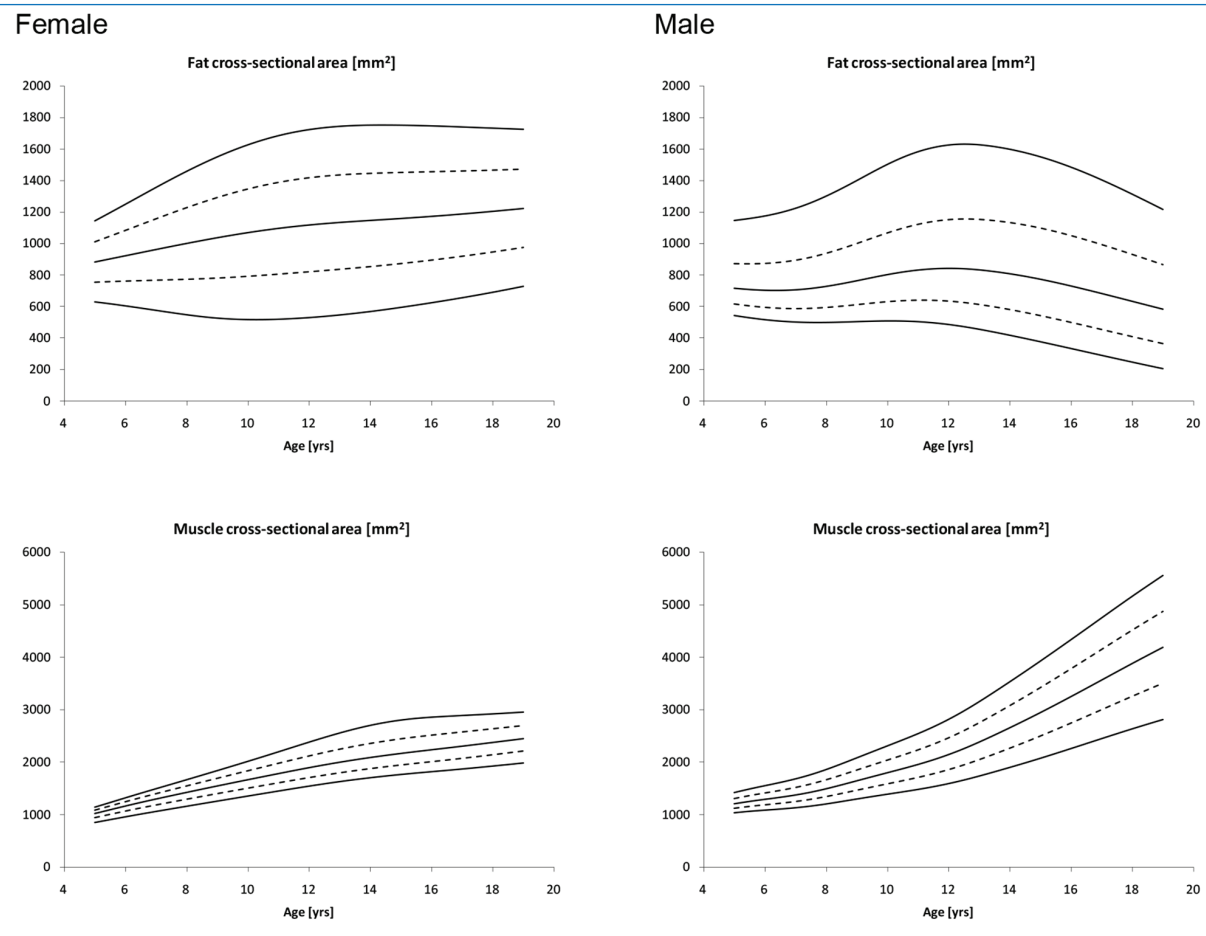

Figure 6. Reference ranges for proximal forearm (66%) regional tissue distribution. Left panel refers to female and right to male. Median (solid line in the middle) and percentiles corresponding to +/-1 SD (dashed lines) and +/- 2 SD (outer solid lines) were presented.

splines to fit smoothed curves: L (Box-Cox transformation power), M (median), and S (coefficient of variation) across age by maximized penalized likelihood[19]. The smoothed percentile estimates and the L, M, and S parameters were derived from raw data, separately for each outcome and sex, in a single-stage modelling. Prior to modelling, visual inspection of the data was carried out. Data were plotted against age, muscle area and height for each sex. Small amount of individual results were excluded, separately for each outcome. Finally, data were modelled by age from 4,5 to 19,5 yrs and truncated to range 5-19 yrs as suggested by others[20], since the method of penalized likelihood estimation could be imprecise at the ends of the series. Similarly, references by muscle area in girls were modelled from 895 mm^2 to 2744 mm^2 and truncated to range 900-2700 mm^2, in boys from 1079 mm^2 to 5157 mm^2 and truncated to range 1100-5100 mm^2. References by height were modelled from 110 cm to 173 cm, truncated to range 115-170 cm and from 106 cm to 187 cm, truncated to range 115-185 cm in girls and boys, respectively. For practical purposes L, M and S curves were fitted with polynomials. To avoid imprecision of calculation of L, M and S values from fitted curves, degree of polynomial was selected to achieve R^2 value at least 0,999. Upper limit of degree was set to 6. In the case of fat cross-sectional area by age in girls (L curve) and total volumetric bone mineral density by age in boys (M curve) 6th degree polynomials show R^2 0,9961 and 0,9970, respectively, which were considered as sufficient.

Results

Age- and sex-specific reference ranges for pQCT outcomes are shown graphically in Figures 1, 2, 3 and 4, for distal radius (4%), proximal radius (66%, bones), proximal forearm (66%, regional tissue distribution) and bone cross-sectional area by muscle area, respectively. Median and percentiles corresponding to +/-1 SD and +/- 2 SD lines were drawn. Left panels refer to female and right to male.

Trabecular volumetric bone mineral density (Figure 1) is relatively stable during developmental period and takes similar values in both sexes, while total area increases steadily across ages, with reaching greater values at age 19

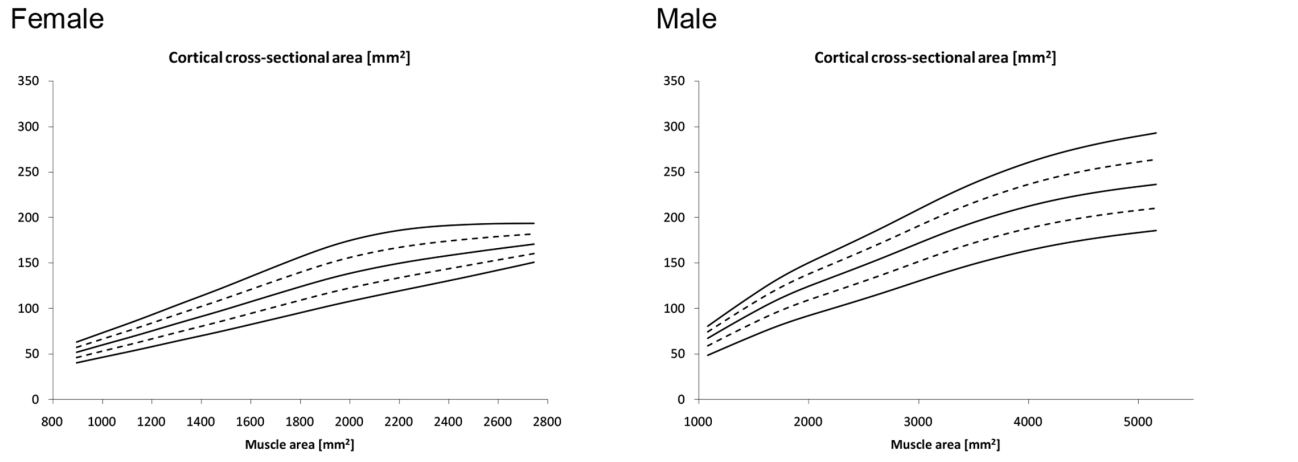

Figure 7. Reference ranges for proximal forearm (66%) bone cross-sectional area by muscle area. Left panel refers to female and right to male. Median (solid line in the middle) and percentiles corresponding to +/-1 SD (dashed lines) and +/- 2 SD (outer solid lines) were presented. Please note, that X-axis ranges are not the same for female and male.

yrs in boys than in girls. Total density increases too, however small declines in girls aged 11-12 yrs and boys aged 13-14 are visible. All bone outcomes at proximal (66%) site (Figure 2) increase steadily across ages in both sexes. In the case of volumetric cortical bone mineral density nearly the same values are reached at age 19 yrs in both sexes, while for other outcomes greater values are reached in boys, especially for total cross-sectional area. Muscle cross-sectional area (Figure 3) increases in both sexes, however, starting from similar level, it reaches nearly twice times greater level at age 19 yrs in boys than in girls. Fat cross-sectional area slightly increases with age in girls, while in boys it increases up to 12 yrs, then slightly decreases. Cortical cross-sectional area by muscle cross-sectional area (Figure 4) increases steadily in both sexes in the whole age range.

Equations for L, M and S curves by age, muscle area and height in both sexes were presented in Tables 2, 3 and 4.

Discussion

In this study we provide reference ranges for bone densities, cross-sectional sizes and strength as well as for regional tissue distribution and bone cross-sectional area by muscle area for distal (4%) and proximal (66%) forearm. We used Cole's LMS method due to its easiness and lack of discontinuities on age range intervals borders[17,19]. Reference ranges were provided for age, muscle area and height for both sexes as was usually done[8]. In fact, preliminary analysis showed that correlations of outcomes with age were stronger than correlation with height. The last one is not present for geometrical outcomes for cortical bone, which correlate equally with age and with height (data not shown). Therefore reference data for geometrical outcomes were also developed by height. Bone area by muscle area was also presented as a measure of muscle/bone relationship.

Since to date, modern reference data for two population were published. Rauch F. and Schoenau E published percentiles for German population (Dortmund)[11,12] and Ashby RL et al. for English population (Greater Manchester)[13]. Two older papers[9,10] concern the same Dortmund population, however data were analyzed in the old manner, means and standard deviations for age groups were presented.

Small methodological differences between studies exist. In the case of Dortmund and Warsaw studies reference line was placed in the same manner while in the case of Greater Manchester study, reference line was placed slightly more proximal. Scan speeds and voxel sizes differed, too.

Trabecular volumetric bone mineral density seems to be consistent across studies and sexes, in the meaning of its median as well as reference borders. The only difference concerns younger boys, in which median in Dortmund population was 10 mg/cm^3 higher and in Greater Manchester 10 mg/cm^3 lower than in Warsaw population. Total volumetric bone mineral density reference values presented the same shape in all population, with tiny decrease in boys in growth spurt period. In younger children reference values were quite similar, in girls from Dortmund median was 20 mg/cm^3 higher than in Warsaw as well as in girls from Greater Manchester, in boys only Greater Manchester population presented median 35 mg/cm^3 higher than Warsaw and Dortmund. In older children from Greater Manchester medians were 50 mg/cm^3 and 60 mg/cm^3 higher than in Warsaw, in girls and in boys, respectively. In older girls from Dortmund median was the same as in Warsaw, however reference range was broader, upper limit was 100 mg/cm^3 higher while bottom limit was 30 mg/cm^3 lower. In older boys whole reference range was higher, differences were 30 mg/cm^3, 10 mg/cm^3 and 40 mg/cm^3 for median, lower and upper limit, respectively. Total

cross-sectional bone area references in younger children presented nearly the same values in Warsaw as in Dortmund, in both sexes. In older boys the only difference was for upper limit, which was lower in Dortmund population by 40 mm^2. In older girls from Dortmund median was lower (30 mm^2) as well as bottom reference limit (50 mm^2), while upper reference limit was nearly the same. References data for total cross sectional bone area (4%) and for 66% site were not available for Greater Manchester population[13]. Cortical volumetric bone mineral density reference values in Warsaw population were close to those in Dortmund population, the only difference concerns lower limit in younger girls, which was 50 mg/cm^3 higher in Dortmund population. Cortical cross-sectional reference ranges were also similar, as well as total cross-sectional and SSI reference range. Differences were as follows: in younger boys cortical cross-sectional area median was 50 mm^2 higher in Dortmund population; in older girls total cross-sectional area bottom reference range limit was 10 mm^2 lower and in the case of SSI the same reference limit was 10 mm^3 lower. In older boys reference range for total cross-sectional area was 20 mm^2 lower for upper reference limit and median and 30 mm^2 lower for bottom reference limit. Median of muscle cross sectional area reference for younger boys in Dortmund was 600 mm^2 higher than in Warsaw, for girls difference was smaller, amount to 200 mm^2, while upper and lower limit were nearly the same for both sexes in this age. In older boys Dortmund reference data presented 300 mm^2 higher lower limit while median and upper limit was similar. In older girls differences were more pronounced. Median of reference data in Dortmund was 400 mm^2 higher, lower reference limit was 200 mm^2 higher and upper reference limit was 800 mm^2 higher. These dissimilarities may be attributed to differences in studied population as well as to methodological differences.

Studied group originated from Warsaw area. As is known, prevalence rate of overweight and obesity is slightly higher in Warsaw area children than in Poland[21,22]. Voluntary basis of participation might have introduced small selection bias, too. However, we exclude overweight and obese children, basing on Polish reference data[14,15]. We exclude also children with disease that may affect bone metabolism as well as these with more than 2 past fractures. Another limitation of the study is the partial volume effect, which may lead to a underestimation of cortical volumetric bone mineral density in the youngest children[23]. Cross-section of cortical rim contains a certain amount of voxels filled with both cortical and sub-cortical tissues. Percentage number of such voxels is greater in the case of thinner bones. As a results, cortical volumetric bone mineral density increases slightly together with increasing cortical thickness, even if the "true" volumetric density remains stable. This phenomenon could be at least partially removed by using an algorithm developed to eliminate the partial volume effect, however, this algorithm has not been yet validated in children[24]. The last limitation of the study is related to its cross-sectional design. Cross-sectional data could not necessarily reflect longitudinal changes in individual growth and development.

In conclusion, in this study we present reference data for bone densities, cross-sectional sizes and strength as well as for regional tissue distribution and bone cross-sectional area by muscle area measured by pQCT at the distal and proximal forearm in children and adolescent in a way allowing simple calculation of reliable Z scores. In consequence, the early detection of bone and regional tissue distribution abnormalities may be now implemented for everyday clinical practice.

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MJ and KG are responsible for subject enrolment; MJ and KG drafted the manuscript and completed manuscript revision; MJ is responsible for study design, outcome assessment, data collection, statistical analysis, data interpretation, literature search and funds collection; MJ takes responsibility for the integrity of the data analysis.

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