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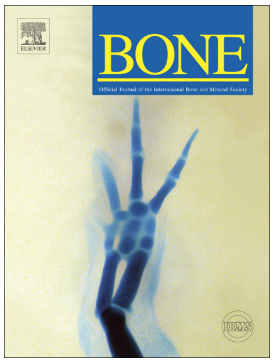

Studying Bone Mineral Density in Young People: The Complexity of choosing a pQCT Reference Database

A.D. Lalayiannis, M. Fewtrell, L. Biassoni, S. Silva, N. Goodman, R. Shroff, N.J. Crabtree

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Studying Bone Mineral Density in Young People: The Complexity of choosing a pQCT Reference Database

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Keywords (6 max)

Peripheral quantitative CT (pQCT); Bone Mineral Density; Healthy Reference Data; Bone mineralization; Bone Mineral Content

Abstract (307 words)

Background

Many chronic illnesses affect bone health, and commonly lead to mineralization abnormalities in young people. As cortical and trabecular bone may be differentially affected in certain diseases, an imaging technique that allows for detailed study of the bone structure is required. Peripheral quantitative computed tomography (pQCT) overcomes the limitations of dual energy X-ray absorptiometry (DXA) and is perhaps more widely available for use in research than bone biopsy. However, in contrast to DXA, where there are large reference datasets, this is not the case for pQCT.

Methods

Fifty-five children and young adults aged 7 to 30 years had the non-dominant tibia scanned at the 3% & 4% sites for trabecular bone mineral density and the 38% site for cortical bone mineral density and bone mineral content. Image acquisition and analysis was undertaken according to the protocols of two of the largest reference datasets for tibial pQCT. The Z-scores generated were compared to examine the differences between protocols and the differences from the expected median of zero in a healthy population.

Results

The trabecular bone mineral density Z-scores generated by the two protocols were similar. The same was true for cortical mineral content Z-scores at the 38% site. Cortical bone mineral density was significantly different between protocols and likely affected by differences in the ethnicity of our cohort compared to the reference datasets. Only one reference dataset extended from childhood to young adulthood. Only trabecular bone mineral density, periosteal and endosteal circumference Z-scores from one methodology were not significantly biased when tested for deviation of the median from zero.

Conclusions

pQCT is a useful tool for studying trabecular and cortical compartments separately but, there are variations in pQCT scanning protocols, analysis methodology, and a paucity of reference data. Reference datasets may not be generalizable to local study populations, even when analysed using identical analysis protocols.

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1. Introduction

Monitoring and studying the effects of diseases on bone is challenging. Bone health is not affected uniformly by all chronic diseases [1]. Bone biopsy is considered the gold standard technique for assessing bone health, allowing for the evaluation of all aspects of dynamic bone metabolism by histomorphometry; turnover, mineralisation and volume [2]. However, it is invasive, not easily repeatable, and it is only performed in certain centres around the world.

Routinely used serum biomarkers such as calcium, phosphate, alkaline phosphatase and parathyroid hormone correlate poorly with bone turnover and mineralization in some chronic diseases such as chronic kidney disease [3]. There is no sufficiently sensitive and specific set of biomarkers that can be relied upon for accurate assessment of bone [4, 5].

Imaging modalities provide an effective, accessible way of estimating bone mineral density clinically. The most widely available is Dual-energy X-ray Absorptiometry (DXA), with robust normative data for age, sex, and race [6]. However, DXA provides information for bone mineral content over a projected area (g/cm^2) which may be misleading in shorter people or children with stunted growth [7]. Additionally, the image produced is a superimposition of trabecular and cortical bone, thus failing to explain changes in each bone compartment [8]. An imaging modality that overcomes this problem is peripheral Quantitative Computed Tomography (pQCT). In this article we highlight the different image acquisition and analysis variations that are available with this modality, as well as present the largest reference datasets for comparison. The aim of our study was to compare tibial pQCT measurements from a sample of the local healthy population of children, adolescents, and young adults to two of the largest reference datasets available.

1.1 Peripheral Quantitative Computed Tomography (pQCT)

pQCT examines a slice of the bone and measures volumetric bone mineral density (volumetric BMD in mg/cm^3) for both cortical and trabecular compartments separately [9] without the need to adjust for body size during image acquisition. Other parameters measured are bone mineral content, bone area and other derived geometric measurements (cross-sectional area, periosteal and endosteal circumferences, cross-sectional moment of inertia) [10]. pQCT has been

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used to study the effects of chronic illness on bone density and dimensions in children with a variety of underlying diseases, such as chronic kidney disease [11, 12], cystic fibrosis [13], inflammatory bowel disease [14, 15], arthritides [16], acute lymphoblastic leukaemia survivors [17], diabetes [18], nephrotic syndrome [19, 20], anorexia nervosa [21, 22] and Duchenne muscular dystrophy [23].

Analysing bone by pQCT is an operator dependent process that requires two steps: scanning and image acquisition followed by analysis of the images. Both steps have several variables controlled by the operator.

1.2 Acquisition

Image acquisition requires choosing the long bone to be imaged, selecting the appropriate site(s) to be scanned and setting the scanning parameters.

Most limb long bones can be scanned in the pQCT scanner, but the radius and tibia are used most often. The femur can be imaged in some scanners (e.g. XCT 3000, Stratec). They are easily accessible and allow the patient to rest comfortably in a chair or on an examination table whilst the scanning takes place. The long bone is measured, and the length entered into the software. For the tibial length, the tibial plateau to the middle of medial malleolus is used. For radial scans, the distance from the external anatomical landmarks of the olecranon of the ulna to the styloid process of the radius is used. Trabecular BMD is best assessed at a metaphyseal site and cortical BMD at a diaphyseal site. The cortex is very thin at the metaphyseal sites and the partial volume effects of the voxels may skew the BMD estimation [24]. Conversely, the trabecular cross-sectional area is small at the diaphyseal sites.

The loci to be scanned are calculated from a fixed length from an anatomical landmark or as a percentage of the total length. Thus a 50% locus would mean 50% of the tibial length. Various sites have been used in the literature, but they are commonly 3% or 4% for trabecular bone measures (metaphyseal sites), 38% or 50% for cortical measures (diaphyseal sites) and 66% for muscle and fat area estimation for the tibia (Figure 1). For the radius the corresponding sites are 4% for trabecular measures and 50% or 66% for cortical measures for the radius.

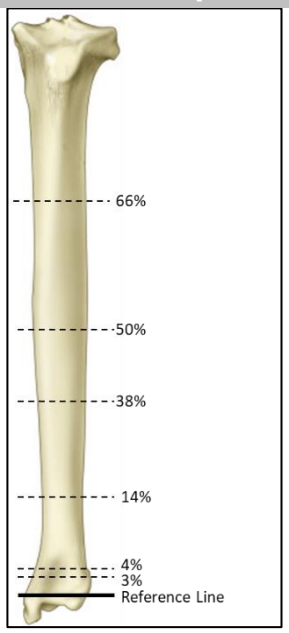

Figure 1. Commonly used sites (dotted lines) for image acquisition on a tibia. 3 & 4% are metaphyseal bone for more accurate trabecular BMD estimation. 14, 38 & 50 % are diaphyseal sites for more accurate cortical BMD estimation. 66% is used for fat and muscle area estimation. Solid line represents the reference line placed by the operator to determine the percentage distance from this point.

The software requires other variables such as scanner speed, voxel, and image slice size to be inputted. These are generally set in the software as standard and must be manually altered. The commonest settings used are 15-25mm/s speed, 0.4-0.5mm voxel size and 2-2.3mm slice size. Pediatric studies tend to use smaller voxel and slice sizes and faster speeds (0.4mm voxel, 2mm slice, 25mm/s speed) as this reduces the radiation exposure and shortens the overall scanning time.

Prior to initiating the scan, the operator must position the patient, set the variables as required, and then obtain a scout view of the distal end of the long bone. A 'reference line' is placed automatically but can be altered manually by the operator. The reference line is the level from which the distance to the image acquisition site will be calculated. Position of the reference line can vary significantly from study to study, and in certain cases, is not reported at all. The most frequently encountered reference line placement descriptions are at the proximal border of the distal endplate in patients with fused growth plates (Figure 2a) and at the proximal border of the distal tibia growth plate in children with open growth plates (Figure 2b). Placement of the reference line is a crucial step for the operator, as it influences the distance to imaging sites. There is a need to avoid potential healing fractures or bisphosphonate treatment sclerotic lines, and in any repeated scans (e.g. longitudinal studies) the same site needs to be imaged for consistency.

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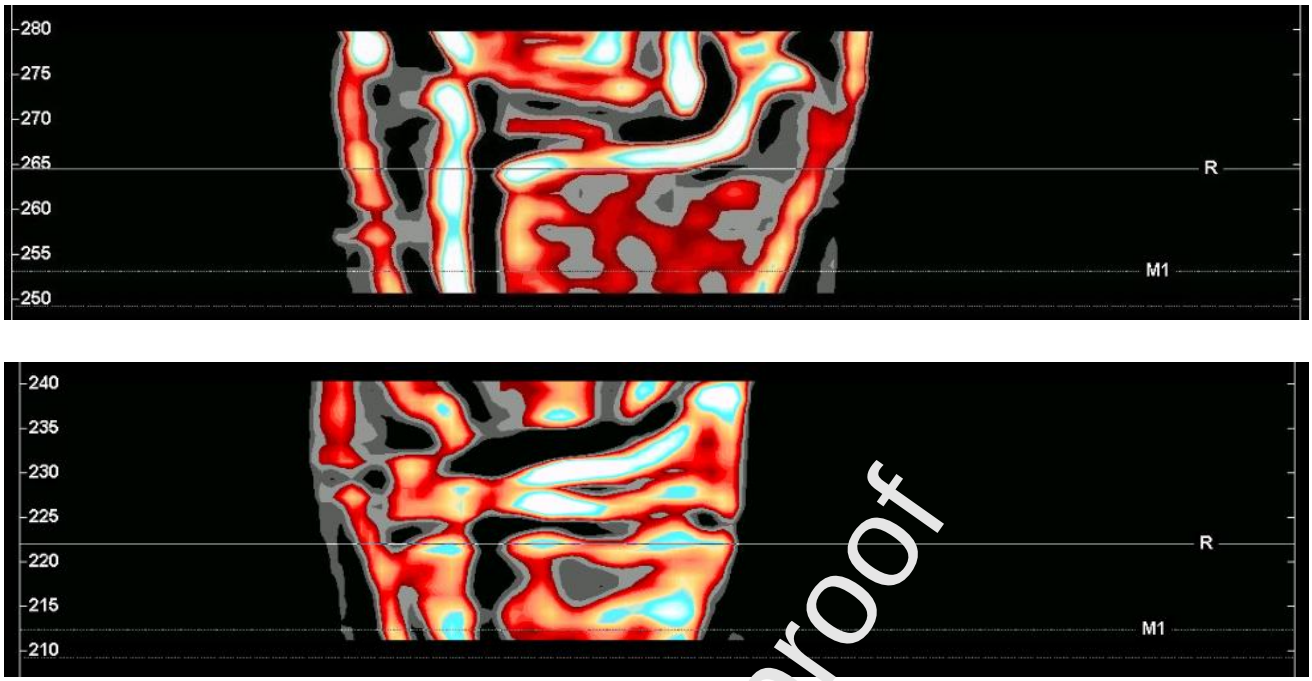

Figures 2a &2b. Reference line (denoted by R) placement at the proximal border of the distal endplate in an adult with a fused growth plate, and at the proximal border of the distal tibia growth plate in a child with open growth plates. M1/dotted line denotes the first point of image acquisition.

Image acquisition takes only a few minutes (typically around 90s per site), and once the images at the different sites have been obtained, the operator can proceed immediately to analysis, providing there is no significant motion artefact.

1.3 Image analysis

Once the scanning has completed and the images have been obtained, they are available immediately for analysis by the software. The appropriate image is selected and the region of interest (ROI) must be set round the bone (in this case the tibia, avoiding the fibula). There are different analysis modes to choose from, and each scanning protocol may use different ones.

Contour Modes

The contour mode is the way in which the program separates soft tissue from bone and defines the way the outer contour of the bone is detected. The result will give total bone area. A density 'threshold' level is set, above which the software considers the pixels as 'bone', and below

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as 'soft tissue'. Usually this is set at 169mg/cm^3 for trabecular bone and 710mg/cm^3 for cortical bone.

Peel Modes

The peel mode is the way in which the image analysis separates cortical from trabecular bone. The cortical bone is 'peeled' away, allowing trabecular bone to be analysed. The remaining cross-sectional area of pixels defined as trabecular bone can be defined further depending on the different peel modes.

Table 1 shows the available contour and peel modes as summarized by Veitch et al [25].

Analysis	
Contour Mode	**Effect on image within ROI**
C1	*Threshold Algorithm*: allows the operator to select a threshold value which is used to separate the soft tissue from the outer edge of the bone – working from the outside inwards eliminating voxels above the set threshold (Threshold set at 169 mg/cm^3 for standard metaphyseal analysis)
C2	*Iterative Contour selection*: eliminates the soft tissue outside the bone. The threshold is automatically set by the software. The algorithm performs an iterative contour detection procedure by finding the first voxel of the outer bone edge. This voxel is compared to a set of neighbouring voxels using a set algorithm to determine the bone edge. This process continues all around the bone, returning to the starting point and thus defining the outer cortical shell
C3	As C2 but threshold operator defined (e.g. higher threshold of 710mg/cm^3 can be used to identify cortical bone only)
Peel Mode	
P1	*Default mode: w*orking from the outside edge of the bone the algorithm concentrically "peels" away a defined percentage of the outside area. A manually set percentage of cross-sectional area of trabeculum is used for analysis. This is usually set at 45%, to avoid the endosteum
P2	Operator defined inner threshold to separate trabecular and sub cortical bone. Voxels above the threshold are assigned as cortical and those lower than the threshold are assigned trabecular status.
P3	P2 combined with P1. If the amount of bone left after P2 is greater than the manually set percentage, then additional bone is peeled away. Used to

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	eliminate the possibility of including sub cortical bone as trabecular bone.
P4	As per P3 but the operator defines a percentage of the detected bone area to be automatically peel off the trabecular area. Normally 5%
P5	Automatically detects threshold level from analysis of the steepest density gradient. Higher densities are defined as cortical, and lower densities as trabecular
P6	As per P5, but also 'peels' away cortical bone leaving a percentage of trabecular bone
P7	As per P5, but 'peels' away an extra 5% of the inner contour
P20	All pixels within a manually set percentage of the cross-sectional area in the ROI (e.g. 45%) analysed. Lowest densities defined as trabecular bone; higher densities defined as cortical

Table 1. Contour and peel modes available to the scanning operator for analysis of the obtained images. All combinations of C and P modes can be used with each other.

The different variables in the software such as peel and contour modes as well as the threshold limits set for cortical and/or trabecular bone can significantly influence the results of the analysis on the same image acquired. Table 2 shows an example of the same 4% metaphyseal tibial site image, with results produced under 3 different analyses. The BMDs and areas produced vary considerably. The starkest contrast is seen with analysis 3, where the trabecular BMD is markedly higher. This is because the 5% peel on the inner contour is not sufficient to remove all cortical bone as can be seen in the corresponding image. This inner rim of cortical bone has contributed to the overall 'trabecular BMD', thus increasing it considerably. Figure 3 provides an example of cortical bone analysed at a 38% diaphyseal site. All pixels above 711mg/cm^3 have been identified as cortical bone.

Tibial 4% metaphyseal site imaged	**Analysis 1**	**Analysis 2**	**Analysis 3**

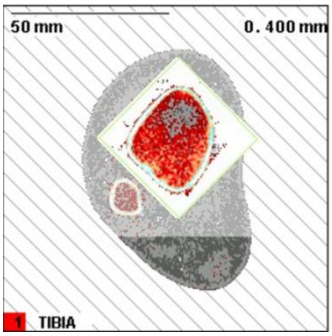	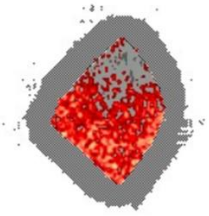		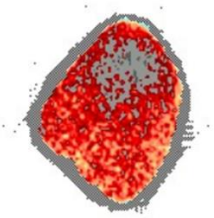
Contour Mode	C1	C2	C1
Peel Mode	P1	P1	P4
Threshold (mg/cm^3)	180	169	200
Trabecular Area	45%	45%	95%
Trabecular bone mineral density (mg/cm^3)	197.2 (±3.0)	195.4 (±3.0)	218.7(±3.0)
Trabecular bone area (mm^2)	313.8	244.7	520.2

Table 2. Example of an image slice obtained at the 4% metaphyseal site of a tibia. To obtain the BMD of the trabecular compartment and the trabecular area, the ROI (green box) has been set around the tibial bone. The analysis modes are different for each column, and the different results reported as shown. The grey area of the bone has been excluded by the software for the analysis.

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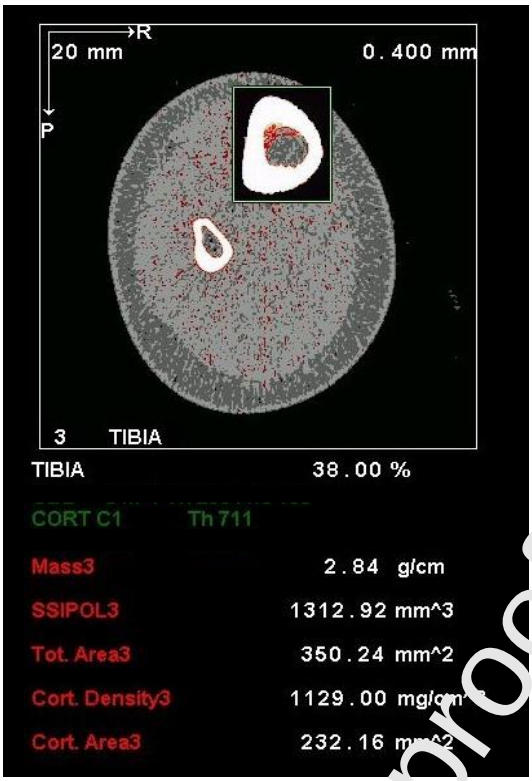

Figure 3. Example of an image slice obtained at the 38% site of a tibia. To obtain the BMD of the cortical compartment as well as the area and mineral content (in g/cm), the ROI (green box) has been set around the tibial bone. The analysis mode has been set as C1, which identified all pixels above the set threshold of 711mg/cm^3. The software assumes all pixels above that density to be cortical bone for the purposes of analysis. The peel mode has not been used as the trabecular bone was not analysed on this image.

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1.4 Reference data

It is known that skeletal development through childhood and adolescence to young adulthood is a dynamic process. Mineral accrual starts in infancy and peaks in the third decade of life. This zenith of mineral content is termed peak bone mass (PBM) [26]. Bone mineral density and mineral content is also affected by growth velocity, pubertal status, and sex.

When obtaining pQCT measurements, as the image acquired is a 'three dimensional slice', no adjustment for size is necessary. In contrast, DXA uses 2D imaging to assess a 3D volume and generates an areal (not volumetric) bone density value (g/cm^2)[8]. Consequently, children with smaller bones/stature for their age will have a reduced areal bone density compared to children with larger bones/taller stature, despite having the same volumetric bone densities. pQCT measures a true volumetric bone density (mg/cm^3) which is not altered by differences in bone size. However, height and bone length are important considerations for any reference data set as growth is a

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fundamental factor of childhood and the length and size of the bone are closely related to its strength [27]. Biomechanically, a longer bone needs to withstand greater forces than a shorter bone. Hence the need to assess bone parameters, particularly those important in bending, in relation to height [27].

Many studies have developed normal reference data from a healthy population of children [28-31] or children and young adults [32-37]. These have most commonly included the radius and tibia as the preferred image acquisition sites. *Table 3* shows the key studies that have produced reference data.

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Authors, Year	**Population** (n=)	**Race** (n=)	**Age of population** (years)	**Soft ware for Anal ysis** (XCT 2000)	**Skeletal Sites and long bone**	**Reference line Placement**	**Voxel size, Slice size and Speed**	**Trabecular analysis** (Contour & Peel modes, density detection thresholds)	**Cortical Analysis** (Contour & Peel modes, density detection thresholds)	**Comments**
Radius										
Neu et al, 2001 [32]	371 children 107 adults	All Caucasian	6-23 29-40	v5.40	4%; non-dominant radius	Open growth plate: most distal portion of the growth plate; Fused growth plate: through the middle of the ulnar border of the articular cartilage	0.4mm; 2mm; 15mm/s	NR	NR	Only radial 4% site imaged so CortBMD analysed at 4% site, may be subject to partial volume effects
Rauch et al, 2008[33]	469	All Caucasian	6-40	v5.40	65%; non-dominant radius	Placed at the ulnar styloid process	0.4mm; 2mm; 15mm/s	NR	threshold 710 mg/cm^3	Age and sex dependent reference curves produced; This dataset is used by the software to produce Z-scores automatically
Ashby et al, 2009 [35]	629	All Caucasian	5-25	v5.50	4%; 50% non-dominant radius	Open growth plate: Line to bisect the medial border of the distal metaphysis	0.4mm; 1.2mm or 2mm; 25mm/s	C2; P1,	C1; threshold 710 mg/cm^3	Centile plots produced for many bone measurement indices based on

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						Fused growth plate: placed to bisect the medial border of the articular surface of the radius				the radial sites
Jaworski et al, 2018 [31]	221	NR	4-19	v6.20	4%;66% non-dominant radius	Open growth plate: through the most distal portion Fused growth plate: through the middle of the horizontal part of the articular surface of the radius	0.5mm; 2.3mm; 30mm/s	C1; P1; threshold 280 mg/cm^3	C1; threshold 711 mg/cm^3	Age and sex specific reference curves produced
Tibia										
Binkley et al, 2002 [34]	231	Caucasian (226) Asian (3) Native American (2)	5-22	v5.40	20%; Left tibia	No scout view placed	0.4mm; 2mm; 20mm/s	C2; P2; threshold 400mg/cm^3	C1; P1; Threshold 710 mg/cm^3	Only 20% tibial site used
Moyer-Mileur et al, 2008 [30]	416	Caucasian (391) Hispanic (9) Pacific Islander (7) Asian (5) Black (4)	5-18	v5.4	4%; 66% non-dominant tibia	Open growth plate: most proximal line of the growth plate; Fused growth plate: through endplate	0.4mm; 2mm; 30mm/s	C1; P1; threshold 180 mg/cm^3	C1; P2; threshold 711 mg/cm^3	Height and gender specific curves reported Cortical parameters reported from the 66% site

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Leonard et al, 2010 [36]	665	Caucasian (359) Black (306)	5-35	v5.50	38%; 66% non dominant tibia	NR	0.4mm; 2.3mm; 25mm/s	NR	C1; P2; Threshold 711 mg/cm^3	Largest cohort of healthy participants, including mix of Caucasian, Black, Asian and Hispanic
Roggen et al, 2015 [28]	432	All Caucasian	5-19	v6.20	4%;14%; 38% dominant tibia	Open growth plate: proximal border of the distal growth plate; Fused growth plate: distal end plate	0.5mm; 2mm; 30mm/s	C1; P1; threshold 180 mg/cm^3	C1; P1; threshold 711 mg/cm^3	Gender specific centile curves produced for trabecular and cortical measures. All participants were Caucasian.
Baker et al, 2013 [37]	500	Black (255) Caucasian (221) Asian (20) Pacific Islander (3) Native American (1)	21-78	v6.00	3%;38%; 66% left tibia	Proximal border of the distal endplate	0.4mm; 2.3mm; 25mm/s	C3; P4; threshold 169 mg/cm^3	Threshold 710 mg/cm^3	Largest healthy adult cohort with mix of races

Table 3. Notable pQCT studies providing reference data, NR; Not reported.

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It is apparent that there is much variation in the measurement site, technique, software used, and population sample. The diversity is a major drawback to generalizing these results to local populations rendering results incomparable. The anatomical measurement sites reported using the Stratec XCT 2000 include the 4%, 20%, 50% and 66% loci in the radius and the 3%, 4%, 14%, 20%, 38%, and 66% loci in the tibia. In fact, the literature is so heterogenous, that the International Society of Clinical Densitometry (ISCD) published an Official Positions Statement in 2013 in an attempt to standardize the measurements obtained and techniques used [38].

Our hypothesis is that the scanning protocol and analysis used can have a significant impact on the Z-scores generated even in the same healthy population. Careful consideration of which reference dataset is applicable to the study population is needed in the planning stage of any project. Our aim was to compare tibial pQCT measurements from a sample of the local healthy population of children, adolescents, and young adults to two of the largest reference datasets available.

2. Methods

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2.1 Study Participants

Healthy children and young adults were recruited from our tertiary pediatric hospital. The cohort comprised of children attending minor surgery lists (such as otolaryngology or plastic surgery) and their siblings who were confirmed to have no underlying systemic illness or infections and were not on any medications, and healthcare staff. Our inclusion criteria were: age from 5 to 30 years. Children under 5 were not included because of their inability to tolerate sitting still for the duration of the image acquisition. Also, this is generally the youngest age limit in the reference databases available. The older age limit was chosen as PBM is not achieved until the third decade of life. We excluded anyone with a pre-existing medical condition, or any conditions affecting growth, bone health or who would not have tolerated the scanning procedures.

A total of 72 people were identified and 65 agreed to participate. 10 were excluded after consenting due to inability to participate. 55 healthy volunteers underwent the investigations and were included in the analysis. Informed written consent was obtained from all parents or caregivers and adult participants. Assent was obtained from children when appropriate. The study was approved by the NHS Health Research Authority ethics committee (17/LO/0007).

2.2 Investigation Performed

2.2.1 Peripheral Quantitative Computed Tomography

A scan of the non-dominant tibia was obtained by pQCT as per manufacturer's instructions, and ISCD guidelines [27]. The 3% and 4% metaphyseal sites as well as the 38% diaphyseal site were used for image acquisition of trabecular and cortical bone, respectively. This was to follow the scanning protocols and image analyses as published by Roggen et al [28, 29] and Leonard et al [36, 37].

All measurements from the 3% and 38% sites were expressed in age-, sex-, race- and height adjusted Z scores according to a healthy reference dataset (personal correspondence with Prof Leonard) [36]. All measurements from the 4% and 38% sites were expressed as age- or height-adjusted Z-scores as published by Roggen et al [28, 29].

2.2.2 Peripheral Quantitative Computed Tomography Procedure

The length of the tibia was measured from the tibial plateau to the middle of the medial malleolus. The tibial length and participant's height, weight, sex, and date of birth were inserted into the software when prompted. The participants were then scanned whilst supine on an examination couch, with the non-dominant lower limb extended into the pQCT scanner (XCT 2000, Stratec) (Figure 4).

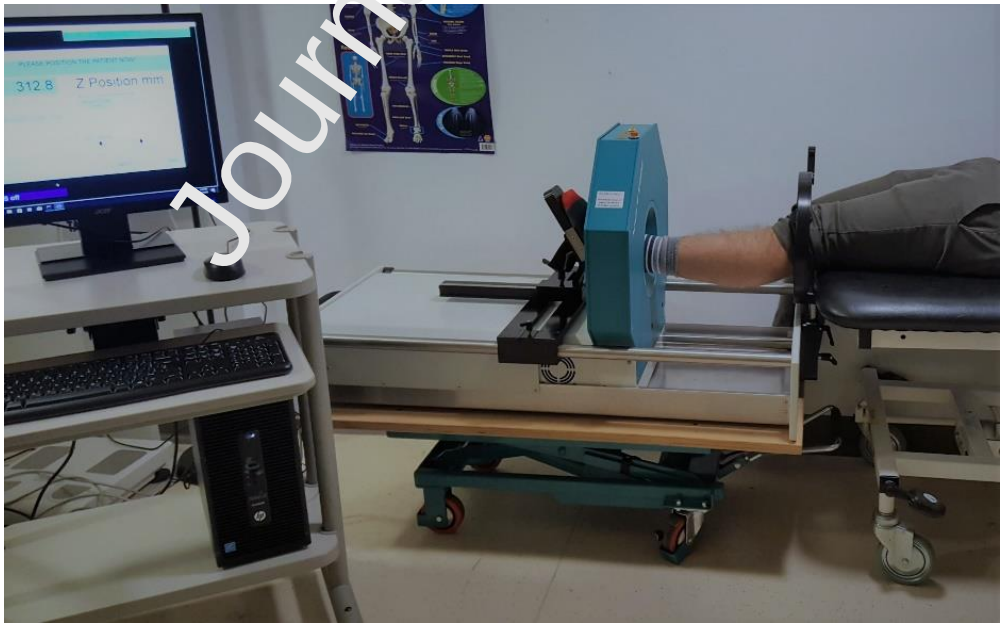

Figure 4. Young adult with left leg inserted into the pQCT scanner prior to scout view being obtained.

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The reference line was placed in the scout view image at the proximal border of the distal tibia growth plate in children with open growth plates and at the proximal border of the distal endplate in young adults with fused growth plates [28, 39]. A voxel size of 0.4mm, slice thickness of 2.3mm, and scan speed of 25mm/s were utilized.

The Stratec hydroxyapatite phantom was scanned daily for quality assurance, as well as the super-added monthly 'cone' phantom scanning as required by the software.

All pQCT scans were undertaken by ADL. All pQCT scans were scored independently and in a blinded fashion by NJC and ADL for scout view placement and movement as per Blew et al [40]. None of the scans were deemed necessary to be excluded.

The pQCT measures obtained were the trabecular volumetric BMD (mg/cm^3) at the 3% and 4% sites, and the cortical volumetric BMD (mg/cm^3) at the 38% site. The 38% site also provided measures of cortical size such as periosteal circumference (mm), endosteal circumference (mm) and cortical bone mineral content. Whilst each research group has published more measurements, such as cross-sectional area of bone and cross-sectional moment of inertia, the above measurements are common to both protocols.

2.2.3 Anthropometry

Anthropometric measures were obtained at the study visit. Height was determined using a fixed wall stadiometer, and weight with a digital scale. Height, weight and BMI measurements are expressed as Z-scores using UK reference data [41, 42] .

2.3 Statistics

All results are presented as the median with interquartile range (IQR). SPSS 25 (IBM) and Prism, Graphpad were used for all statistical analyses. Mann-Whitney non-parametric tests were used to compare groups. A p-value of <0.05 was considered statistically significant, and two-sided testing of the hypothesis was used in all tests were appropriate. The Bland-Altman method was used to compare Z-scores for pQCT parameters generated using the two reference datasets, with a linear regression using the difference between measurements as the dependant variable, and the average of the measurements as the independent variable to analyse the correlation [43]. Non-

parametric Wilcoxon Signed Rank one sample T-testing was used to determine if the median (& its 95% confidence interval) of the Z-scores differed from a median of zero.

3. Results

3.1 Demographics of study population

A total of 55 people, aged 7 to 30, participated in the study. The median age was 16.5 years (13.3 to 24.3). Twenty-eight were female (50.9%). Twenty participants (36.3%) were aged 19 to 30 years. Most were Caucasian (76%, Black 15%, Asian 9%). The median height, weight and BMI Z-scores were 0.13 (-0.44 to 0.78), 0.36 (-0.34 to 0.87) and 0.28 (-0.35 to 0.61) respectively.

3.2 pQCT measurement Z-scores

The Z-scores for the measurements for children and adults are shown in Tables 4 and 5, respectively.

Bone Imaging measure		**Z-score as per Roggen et al**		**Z-score as per Leonard et al**	**P-value for between group difference**
		4%		**3%**	
Trabecular BMD		-0.29 (-0.94 to 0.17)		-0.17 (-1.11 to 0.62)	0.35
	38%				
Cortical BMD		0.71 (0.07 to 1.35)		-0.17 (-0.87 to 0.62)	**0.0005**
Cortical mineral content		-0.48 (-1.70 to 0.34)		-0.55 (-1.38 to 0.05)	0.85
Periosteal Circumference	Age adjusted	-1.94 (-2.88 to -0.41)	Age and height adjusted	-0.54 (-1.33 to 0.15)	**0.003**
	Height adjusted	-1.69 (-2.38 to -0.66)			**0.0008**

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Endosteal Circumference	Age adjusted	-1.98 (-2.93 to -0.71)	Age and height adjusted	-0.22 (-0.92 to 0.68)	**<0.0001**
	Height adjusted	-2.15 (-3.09 to -0.90)			**<0.0001**

Table 4. Median Z-scores of the pQCT measurements as calculated for each scanning protocol for children 7 to 18 years old. BMD; Bone mineral density

Bone Imaging measure	**Z-scores as per Leonard et al**
	3%
Trabecular BMD	-0.10 (-0.85 to 0.36)
	38%
Cortical BMD	-0.91 (-1.77 to -0.02)
Cortical mineral content	-0.54 (-1.41 to 0.03)
Periosteal Circumference (Age and height adjusted)	-0.40 (-0.72 to 0.32)
Endosteal Circumference (Age and height adjusted)	0.24 (-0.47 to 0.74)

Table 5. Median Z-scores of the pQCT measurements for adults age ≥19 years old. Roggen et al database maximum age is 18.9, so unable to calculate adult Z-scores. BMD; Bone mineral density

There was no significant difference for the TrabBMD at the 3% and 4% sites for the children, or the 38% bone mineral content. There was a significant difference for the CortBMD (Figure 5), with the Belgian derived Z-scores showing a higher median of 0.71 (0.07 to 1.35).

The Roggen et al database extends to 18.9 years old, so the Z-scores were calculated for the adults based on the Leonard et al database (Table 5).

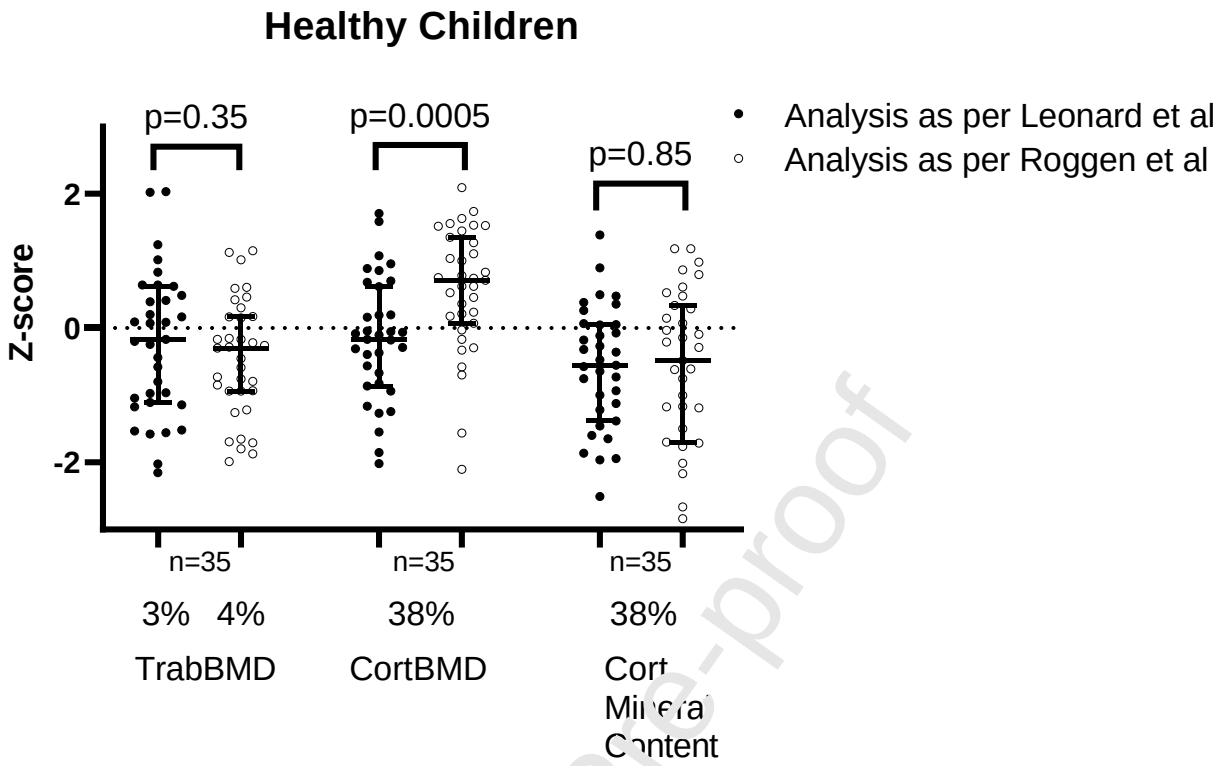

Figure 5. Median and IQR ranges for the bone measurements for healthy children as per each methodology. TrabBMD, Trabecular Bone Mineral Density; CortBMD, Cortical Bone Mineral Density

3.3 Bland-Altman method comparison of the two analysis protocols

The TrabBMD and cortical BMC Z-scores showed moderate correlation (R^2=0.26, p=0.002 and R^2= 0.19, p=0.008 respectively) with the majority of measurements within 1 Standard Deviation (SD) (Figure 6a and 6b). The CortBMD Z-scores did not show the same correlation (R^2=0.08, p=0.10) (Figure 7), with significant offset and variation.

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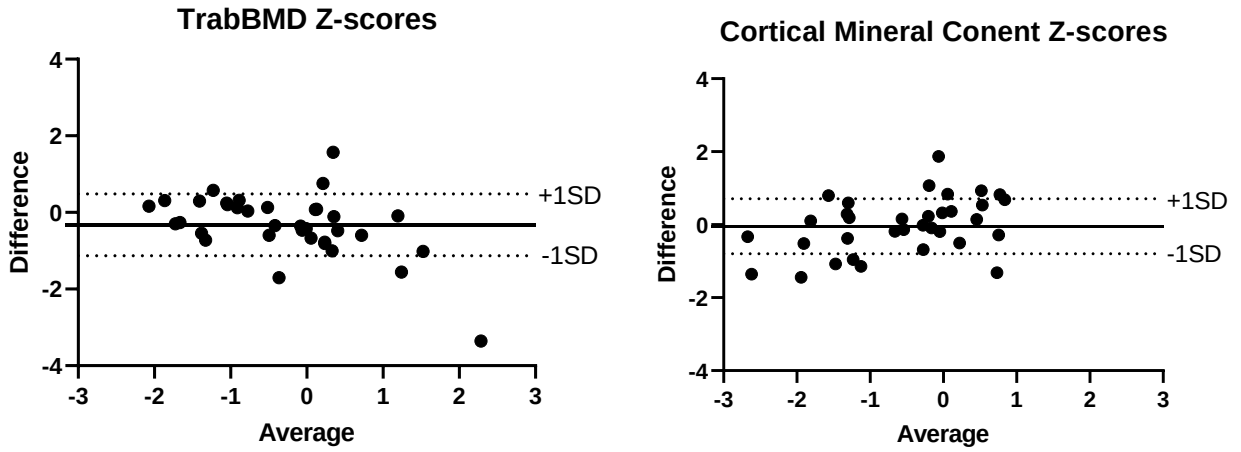

Figures 6a &6b. Bland-Altman plots of Trabecular bone mineral density (TrabBMD) and cortical mineral content Z-scores, with the bias depicted by the solid line and the 1 standard deviation points (SD) by the dotted lines. One measurement on figure 6b is outside the axis limits.

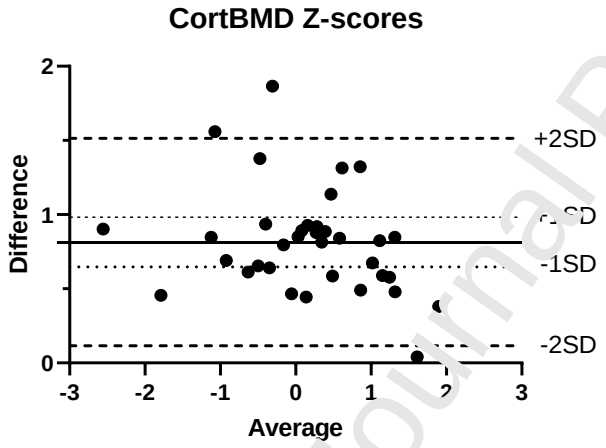

Figure 7. Bland-Altman plot of cortical bone mineral density (CortBMD) with the bias depicted by the solid line and the 1 standard deviation points (SD) by the dotted lines. The dashed lines show the 2 SD points, highlighting the significant variation of the difference.

3.4 Assessment of median Z-score deviation from zero

The assessment of deviation from a median of zero is displayed in Table 6. In our cohort the median Z-score for trabBMD, cortBMD and endosteal circumference by Leonard et al in children did not differ significantly from zero. In adults, Z-scores of trabBMD and measures of bone size did not differ significantly from zero.

		Children					Adults	
Bone Imaging measure Z-scores median (95%CI)		**Roggen et al**	**WSR Test p value**		**Leonard et al**	**WSR Test p value**	**Leonard et al**	**WSR Test p value**
Site		**4%**			**3%**			
Trabecular BMD		-0.29 (-0.75 to -0.14)	**0.008**		-0.17 (-0.56 to 0.32)	0.36	-0.10 (-0.60 to 0.24)	0.33
Site		**38%**						
CortBMD 22		0.71 (0.26 to 0.89)	**0.0006**		-0.17 (-0.59 to 0.11)	0.2	-0.91 (-1.32 to -0.52)	**0.0004**
Cortical Mineral content		-0.48 (-1.17 to -0.23)	**0.01**		-0.55 (-1.02 to -0.30)	**0.0006**	-0.54 (-1.54 to -0.12)	**0.01**
Periosteal Circumference	Age adjusted	-1.94 (-2.69 to -0.17)	**<0.0001**	Age and height adjusted	-0.54 (-0.90 to -0.15)	**0.008**	-0.40 (-0.70 to 0.12)	0.12
	Height adjusted	-1.69 (-2.18 to 0.23)	**<0.0001**					
Endosteal Circumference	Age adjusted	-1.98 (-2.66 to -1.45)	**<0.0001**	Age and height adjusted	-0.22 (-0.54 to 0.30)	0.4	0.24 (-0.46 to 0.67)	0.39
	Height adjusted	-2.15 (-2.62 to -1.53)	**<0.0001**					

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Table 6. Median (95% confidence interval of the median) of Z-scores of measurements at each tibial site. Wilcoxon Signed Rank Testing (WSR) indicating if median discrepancy from a median of zero is statistically significant.

4. Discussion

We chose Leonard et al [36, 37] and Roggen et al [28, 29] as the two databases closest to our population, with the largest number of participants. They have published comparable scanning and analysis protocols with similar scanning sites. For the analysis of our healthy cohort, we separated it into ages under 19 and over 19, as the Roggen et al database extends up to 19 years old.

The trabecular BMD Z-scores assessed at the 3% or 4% sites are similar and there is no difference between the methodologies. This is perhaps because the 3% and 4% sites do not differ by many millimetres and are thus essentially the same locus. For a 350mm measured tibia, for example, the 3% site would target an area 1.05 cm from the reference line placement, and the 4% would target a distance 1.4cm from the reference line. It is highly likely that these areas have a very similar composition, size and density of trabecular bone. In addition, these sites may overlap depending on the operator's measurement of the tibial length. This remains a potential source of confounding bias as the trabecular density decreases significantly along the metaphysis [44].

The cortBMD Z-scores at the 38% site differed significantly, with Roggen et al Z-scores higher on average. This is perhaps as a result of having a proportion of Black and Asian participants in our cohort, and the Roggen et al database contains only Caucasian participants. It is well established that Black and mixed race people have a higher bone mineral density [45-48]. The Bland-Altman analysis of the Z-scores reflected the significant differences between the Z-scores derived by the different image analysis protocols. Cortical mineral content Z-scores were similar with each protocol and did not differ significantly. The Z-scores of the peri- and endosteal circumferences (measurements of bone size with age *or* height adjustment for Roggen et al and age *and* height adjustment for Leonard et al) differed significantly, and this may be due to our cohort population differences. The voxel size, slice size and speed of scan varied from the Roggen et al methodology, which may have affected results or resulted in different volume effects.

Adult Z-scores were not generated by the Roggen et al database as it extends to 19 years old only. It is important to note that normal bone mineralization continues until peak bone mass is reached in the late twenties or early thirties [26]. Any study design would need to carefully consider the age range of the reference database to be used, as assuming all young adults are aged 19 and interpolating the Z-scores may provide misleading results.

As our cohort is representative of a healthy cohort, the median and mean values of the Z-scores should be close to zero. When assessing the children's cohort's median Z-scores bias from zero, the Leonard Z-scores of TrabBMD, CortBMD and endosteal circumference did not differ significantly. For the cortical mineral content and periosteal circumference difference from zero, it may be that the sample of 35 children is too small and may have contributed to this result. Equally, the cohort may differ in a significant way from the reference dataset in terms of regular diet, calcium intake, physical exercise, and dietary supplements. Sample size and lifestyle factors limitation apply to the 20 adults showing that their median Z-score for CortBMD and cortical mineral content differed significantly from zero.

Additional limitations include that the Roggen et al scanning protocol specifies use of the dominant tibia, whereas in this study the non-dominant tibia was used for all measurements. There is evidence that there are differences between the dominant and non-dominant limb bone measures, but the extent to which this is significant is debated [49, 50].

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4.1 Overall limitations precluding pQCT from routine use

Peripheral QCT has many advantages and overcomes most of the criticisms encountered by DXA and also provides information on bone density, size and strength [8].

The variability in the literature of reference line placement and scanning protocol cannot be overlooked. Even if following the ISCD Official Positions Statement [38], there is still scope for sufficient human error in reference line placement and tibial length measurement to cause significant measurement error margins. There is also the challenge of standardisation between machines. Cross-calibration between scanners and research centres is vital to avoid bias of the measurements. Reducing human error by training researchers to perform pQCT according to the same protocol and minimising the number of researchers performing the scans is important.

Furthermore, the most appropriate long bone for assessment has been debated. Whilst both the tibia and radius provide easy access to a metaphyseal and diaphyseal measurement, there is

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only now consensus on using the non-dominant limb. Imaging slice location on the tibia or radius for accurate and reproducible assessments is also varied. These need to be reproducible, not just between centres, but also longitudinally for patient follow up assessments. Currently, this overwhelming heterogeneity means there is a paucity of reference data for age, height and puberty staging for comparison and Z-score generation [93-98]. The lack of universally used or globally applicable reference data, and the variability in image acquisition techniques, currently limit the use of pQCT largely to research.

4.2 High Resolution pQCT (HR-pQCT)

HR-pQCT is a newer, promising technology which is now becoming more widely available for research. First generation scanners were adept at scanning distal, metaphyseal sites [51]. Second generation scanners allow for image acquisition at distal sites as well, allowing for detailed cortical and trabecular analysis. It offers much more detail than pQCT, showing trabecular bone microarchitecture, and enables measurement of trabecular number, thickness and separation [10]. With reference databases being published [52], the same heterogeneity of scanning protocols needs to be avoided [53]. Image acquisition sites, analysis methods and guidelines for its use need to be standardized as suggested recently by Whittier et al [51].

5. Conclusion

pQCT is a useful imaging tool in studying bone, especially in chronic illnesses that affect the bone compartments in different ways. It is, however, confined mainly to the research domain. The marked lack of methodology standardization and reference data further add to the limitations in its widespread clinical application. This study highlights the complexities and difficulties in choosing a reference dataset for conducting research with pQCT.

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Conflict of Interest Statement

All authors have declared no conflict of interest.

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Author contributions

ADL: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft **MF**: Writing - Review & Editing **LB**: Writing - Review & Editing **SS**: Investigation, Data Curation, Writing - Review & Editing **NG**: Investigation, Data Curation, Writing - Review & Editing **RS**: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Funding acquisition, Supervision **NJC**: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Supervision

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"Highlights"

- ***pQCT is a useful tool for assessing trabecular and cortical compartments separately***
- ***There is a marked variation in the literature in methods and scanning protocols***
- ***There are few reference datasets to use as comparators***
- ***Reference datasets may not be generalizable to local study populations***

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