

Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality

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Abstract

The introduction of three-dimensional high-resolution peripheral *in vivo* quantitative computed tomography (HR-pQCT) (XtremeCT, Scanco Medical, Switzerland; voxel size 82 μm) provides a new approach to monitor micro-architectural bone changes longitudinally. The accuracy of HR-pQCT for three important determinants of bone quality, including bone mineral density (BMD), architectural measurements and bone mechanics, was determined through a comparison with micro-computed tomography (μCT) and dual energy X-ray absorptiometry (DXA). Forty measurements from 10 cadaver radii with low bone mass were scanned using the three modalities, and image registration was used for 3D data to ensure identical regions were analyzed.

The areal BMD of DXA correlated well with volumetric BMD by HR-pQCT despite differences in dimensionality ($R^2=0.69$), and the correlation improved when non-dimensional bone mineral content was assessed ($R^2=0.80$). Morphological parameters measured by HR-pQCT in a standard patient analysis, including bone volume ratio, trabecular number, derived trabecular thickness, derived trabecular separation, and cortical thickness correlated well with μCT measures ($R^2=0.59$ – 0.96). Additionally, some non-metric parameters such as connectivity density ($R^2=0.90$) performed well. The mechanical stiffness assessed by finite element analysis of HR-pQCT images was generally higher than for μCT data due to resolution differences, and correlated well at the continuum level ($R^2=0.73$).

The validation here of HR-pQCT against gold-standards μCT and DXA provides insight into the accuracy of the system, and suggests that in addition to the standard patient protocol, additional indices of bone quality including connectivity density and mechanical stiffness may be appropriate to include as part of a standard patient analysis for clinical monitoring of bone quality.

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1. Introduction

Osteoporosis (OP) is a common multi-factorial disorder of reduced bone mass and micro-architectural deterioration of bone tissue, manifesting clinically as a concurrent increase in fragility and fracture risk [1,2]. Although the clinical diagnosis of OP is primarily based on hip bone mineral density (BMD) measurements from dual energy X-ray absorptiometry (DXA) compared to a reference population database, as outlined by the World Health Organization [3], there is an increasing body of evidence suggesting a combination of factors including bone architecture, turnover,

damage accumulation and mineralization play an important role in the assessment of fracture risk [4] – jointly referred to as *bone quality* [5]. Recently, a new technology for *in vivo* clinical assessment of bone structure named three-dimensional high-resolution peripheral quantitative computed tomography (HR-pQCT) was introduced (XtremeCT, Scanco Medical, Switzerland) that has potential to measure important components of bone quality including BMD, micro-architectural morphology and bone mechanics. The ability of the HR-pQCT system to assess these important parameters needs to be more fully explored.

The measure of BMD is the current gold standard for clinical assessment of bone fracture risk, and it is clear that fracture risk generally increases with decreasing BMD [6]. While DXA is able to measure BMD at many important sites

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(hip, lumbar spine, wrist), it is limited to areal measurements of BMD (aBMD; g/cm^2) which makes it difficult to elucidate the effects of bone physical size and spatial variations in remodeling zones (i.e., cortical versus trabecular compartments) [7] in patients undergoing treatment or monitoring of OP. A volumetric assessment of BMD (vBMD; g/cm^3) distinguishes BMD in different compartments and is independent of physical size [8]. However, it is not clear what influence beam hardening and resolution may have on vBMD measurements.

Bone micro-architecture is an important factor underlying observed changes in BMD, and the emergence of non-destructive assessment by micro-computed tomography (μCT) [9,10] has been important for advancing our understanding of its impact. The analysis of bone morphology by μCT typically includes metric measures such as bone volume ratio (BV/TV), bone surface ratio (BS/BV), and direct 3D measures of trabecular thickness (Tb.Th*), trabecular separation (Tb.Sp*), trabecular number (Tb.N*) [11]. Non-metric measures provide insight into structure composition including structural model index (SMI) [12], degree of anisotropy (DA) [13], and connectivity density (Conn.D) [14]. The high-resolution data needed to assess these parameters have typically limited their application to human research for *in vitro* studies using cadavers, or studies using iliac crest biopsies [15]. As a first step towards *in vivo* structure measurements, a precursor to HR-pQCT introduced in the late 1990s provided the ability to perform measurements of peripheral sites (distal radius and tibia) at isotropic voxel sizes of $165\text{ }\mu\text{m}$ [16]. Because the resolution was close to the thickness of typical human trabeculae, direct μCT measures were not recommended and special structure extraction methods were developed [17]. The approach included a derived bone volume ratio (BV/TV^d), calculated by dividing the measured apparent trabecular bone density by $1200\text{ mg}/\text{cm}^3$ hydroxyapatite (HA) representing mineralized bone, and a direct measurement of Tb.N [11] of the structure. Thus, using BV/TV^d and Tb.N, measurements of trabecular thickness and separation were calculated [17]. This so-called semi-derived approach minimized the issue of resolution dependence of the directly measured parameters, of which trabecular thickness is particularly sensitive [18], yet they included true 3D structure information (i.e., Tb.N*) in their calculation, thus avoiding some of the limitations of traditionally derived morphological analysis from 2D histological sections [19]. The semi-derived analysis [17] has been carried through to the new HR-pQCT for standard patient analysis [20,21]; however, having an isotropic voxel size of $82\text{ }\mu\text{m}$ raises the question of whether typical metric and non-metric μCT measures may now provide reasonable results on these new high resolution *in vivo* data.

The assessment of bone quality through *in vivo* imaging provides the opportunity, in addition to BMD and morphology, to measure mechanical characteristics directly on a subject-specific basis using the finite element (FE) analysis approach [22]. The FE approach has been previously shown

to be effective at predicting continuum level mechanical properties of trabecular bone [22–26]. Using FE models derived from 3D image data at an isotropic voxel size of $165\text{ }\mu\text{m}$ it is possible to reasonably predict a Colles-type fracture of the radius [27] better than by morphological parameters alone [28]. The application of the FE methodology to the $82\text{ }\mu\text{m}$ voxel data of the HR-pQCT system will be important not only for directly monitoring patient-specific bone mechanics, but also for distinguishing the relative role of cortical versus trabecular bone on load distribution during treatments [29].

The purpose of this study, therefore, is to compare the newly developed HR-pQCT system to current gold-standards. Bone mineral density will be compared using the current clinical standard DXA, and morphology and stiffness comparisons will use μCT as the gold-standard [30,31]. The comparisons will be based on measurements of intact human cadaver radii using HR-pQCT *in vivo* protocols. The validation of the HR-pQCT system will include a comparison of the results from the standard patient analysis (semi-derived parameters) as well as explore the possibility of using other μCT metric and non-metric parameters that can provide insight into bone quality.

2. Methods

2.1. Specimens

Fresh human cadaver forearms (5 males and 5 females) with ages ranging from 55 to 93 were obtained from the Gross Anatomy Lab at the University of Calgary. No specific criteria were used for selection and no medical history was available, so it could not be determined if previous bone diseases were present. The radii were frozen until 48 h before scanning and were allowed to thaw at room temperature. All procedures were approved by the Office of Medical Bioethics at the University of Calgary.

2.2. Scanning by HR-pQCT, DXA and μCT

The sequence of scanning was the same for all specimens. The thawed intact forearms were scanned by HR-pQCT (XtremeCT, Scanco Medical, Switzerland) and DXA (Hologic, Discovery, QDR series, USA), and subsequently by μCT (vivaCT 40, Scanco Medical, Switzerland) after the radii were dissected of soft tissues (due to gantry size limitations). Each of the 10 intact cadaver radii were scanned at four adjacent locations (Figs. 1 and 2) resulting in a total of 40 measurement sites. The second most distal location is similar to the standard location for an *in vivo* measurement (Fig. 2), and will be referred to as the standard location. The use of adjacent locations provided a broad range of cortical and trabecular bone properties due to site-specific variations.

The scanning parameters for the HR-pQCT used default *in vivo* settings by the manufacturer (60 kVp, 1000 μA , 100 ms integration time) resulting in 2.8 min/scan. The landmark for

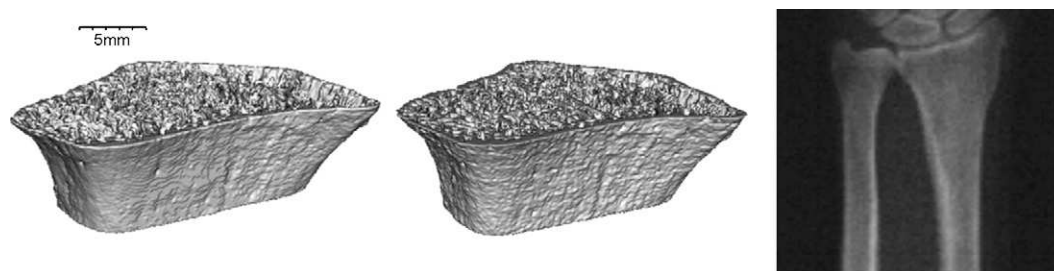


Fig. 1. Representative images of the three imaging modalities utilized for comparison in this study. The system being validated was xCT (center). The gold standard utilized for bone structure and mechanical stiffness was μ CT (left). The gold standard utilized for BMD measurements was DXA (right).

the first scanning section of the distal radius was chosen as the most proximal location on the sub-chondral plate. Each measurement included 110 slices corresponding to a 9.02 mm thick cross-section of the forearm at an isotropic voxel size of 82 μ m.

Areal BMD scanning was performed by DXA (Hologic, Discovery, QDR series) scanning the entire distal forearm (Fig. 1). The scan included all the regions measured by HR-pQCT, and the four corresponding sub-regions were defined using the same landmark used for HR-pQCT for evaluation.

Finally, the radii were scanned by μ CT (vivaCT 40, Scanco Medical, Switzerland) in saline solution using an *in vitro* scanning protocol (55 kVp, 109 μ A, 300 ms integration time) resulting in an isotropic 19 μ m voxel size. A 38 mm section of the distal radius was scanned, thereby encompassing the entire region scanned by HR-pQCT (plus 2 mm extra). The overlap ensured identical regions could be evaluated by both μ CT and HR-pQCT.

2.3. Evaluation of HR-pQCT

The HR-pQCT images were evaluated using two approaches which included a standard patient evaluation

protocol, followed by an evaluation using methodologies normally reserved for high-resolution μ CT data (i.e., direct and non-metric methods).

First, the standard patient protocol involved segmenting the periosteal surface of the radii using a semi-automated contouring scheme followed by a threshold-based algorithm to separate the cortical and trabecular regions (Fig. 3) [16]. User input required for analysis is normally less than 5 min/sample. Extraction of the mineralized phase occurs fully automatically, and uses a Laplace–Hamming filter followed by global thresholding. The indices determined by the analysis include measures of vBMD (mgHA/cm^3) in the trabecular region (D_{trab}), cortical region (D_{comp}) and the combined total vBMD (D_{100}). The derived BV/TV^d was determined by dividing the apparent trabecular bone density by $1200 \text{ mg}/\text{cm}^3$ HA which represents mineralized bone [16], and the Tb.N^* was determined using 3D ridge extraction methods [11]. The trabecular thickness (Tb.Th) and separation (Tb.Sp) are derived from the measures of BV/TV^d and Tb.N^* using standard morphological relations [19]. The cortical thickness (Ct.Th) was calculated by dividing the cortical volume by the outer bone surface. The standard patient analysis indices used in the validation include vBMD (D_{100}), BV/TV^d , Tb.N^* , Tb.Th, Tb.Sp

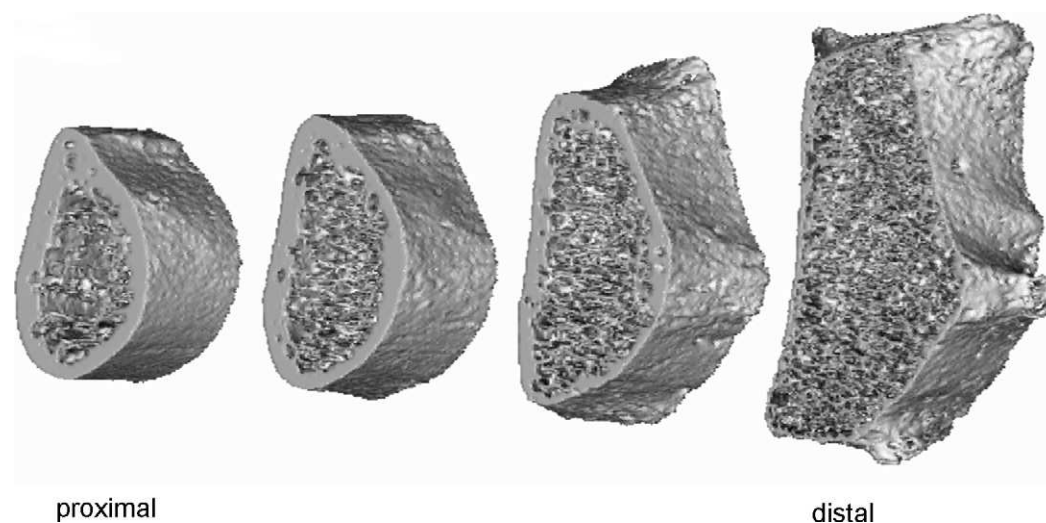


Fig. 2. Each of the 10 cadaver radii were evaluated at four locations immediately adjacent to each other (exploded view shown). The second most distal location is similar to the standard location for an *in vivo* measurement, and will be referred to as the standard location. The cortical and trabecular regions varied significantly along the radius providing a wide variation of structure types for validation.

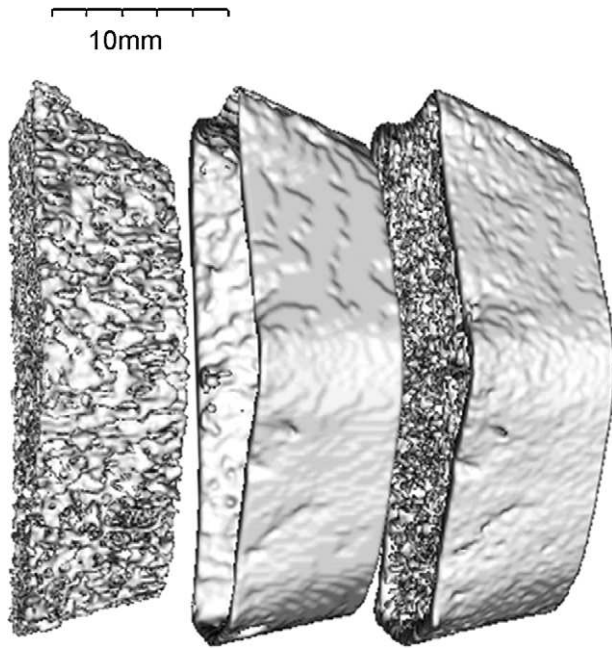


Fig. 3. An example of automatic segmentation of a distal radius cross section into trabecular and cortical bone. This method was utilized to separate the trabecular and cortical bone for all xCT measurements.

and Ct.Th. This analysis is referred to here as the HR-pQCT standard evaluation.

The second analysis used direct voxel-based measurements of structure as well as non-metric indices, and will be referred to as a μ CT-based HR-pQCT analysis. The input for this analysis was the same segmented cortical and trabecular regions output from the standard patient analysis. Bone volume ratio (BV/TV) was determined by directly summing the voxels representing bone and dividing by the total volume, and bone surface ratio (BS/BV) used an isosurface approach [32,33]. Trabecular number (Tb.N^{*}), thickness (Tb.Th^{*}) and separation (Tb.Sp^{*}) were based on direct methods [11], as well as cortical thickness (Ct.Th^{*}). Other non-metric indices included degree of anisotropy (DA) [13], structural model index (SMI) [12] and connectivity density (Conn.D) [14]. The μ CT-based analysis assumes that the image resolution is sufficient to determine structure directly, and the purpose of applying this analysis to HR-pQCT image data was to determine if some or all of these indices provide reasonable results. The second analysis also included a measurement of total bone mineral content (BMC). The total BMC was calculated by using the total vBMD and multiplying by the volume that region.

2.4. Evaluation of DXA and μ CT

The gold-standard for density measurements using DXA determined two main output parameters including aBMD as well as total bone mineral content (BMC). Although the aBMD is the normal clinical measure, its comparison

with vBMD from HR-pQCT can be influenced by size effects. Therefore, BMC determined from both DXA and HR-pQCT should provide an unbiased comparison for validation.

The evaluation of the μ CT images was performed on the corresponding regions of interest (ROI) from the HR-pQCT scans by performing 3D image registration. Rigid registration was performed using Mattes mutual information [34,35] with a linear interpolator in a multi-resolution scheme. The procedure applied to μ CT image data is described in detail elsewhere [36]. After identifying the corresponding ROI on the μ CT images, semi-automated contouring was applied on the endosteal cortical surface to isolate the trabecular and cortical regions. Although it would have been possible to identify trabecular and cortical regions using the HR-pQCT results, it was decided that it was important to independently re-determine these regions, particularly for evaluation Ct.Th measurements. The μ CT data was resampled from 19 μ m to 38 μ m due to hardware constraints, and Gaussian filtered ($\sigma=1.2$, support=2) and globally thresholded (11.2%) to extract the mineralized phase. Lastly, a morphological analysis was performed as per the μ CT-based procedure outlined earlier.

2.5. Mechanics

A micro-finite element (μ FE) analysis was performed on 3.12 mm³ cube of bone extracted from the centre of the radii [37,38]. A larger sample containing cortical and trabecular bone could not be chosen due to the resolution of the μ CT data. The same registration method described earlier was used to ensure identical regions in HR-pQCT and μ CT. The image data was converted to μ FE by a direct voxel-conversion approach, and an elastic modulus of 10 GPa [22] and Poisson's ratio of 0.3 [25] defined the isotropic homogeneous material properties. The HR-pQCT models consisted of 38³ voxels (82 μ m voxel size), and the μ CT models consisted of 164³ voxels (19 μ m voxel size). A uniaxial compression test (1% strain) was performed in the axial direction and solved using a custom element-by-element solver [39]. The continuum-level parameter tested was the reaction load due to the applied strain, and the tissue-level parameters were average strain energy density (SED) and von Mises stress.

2.6. Statistical analysis

A regression analysis was performed to determine the R^2 coefficient and the linear equation relating each of the HR-pQCT measurements to the corresponding gold-standard measurement. The mean value for each measurement was determined, and a paired two sample t -test (SPSS, Chicago, IL) was used to test for significant differences between the HR-pQCT measurements and the gold standard measurements. A Bland–Altman plot and error histogram was determined for qualitative analysis of accuracy [40].

Table 1

Linear calibration equations and correlations between HR-pQCT and either μ CT or DXA gold-standards

	Parameter	Slope	Intercept	R ²
Mineralization (HR-pQCT vs. DXA)	BMD	0.62	0.15	0.69
	BMC	1.4	-0.043	0.80
Morphology (HR-pQCT vs. μ CT)	BV/TV ^d	1.5	0.012	0.86
	Tb.N*	0.56	0.37	0.95
	Tb.Th	1.0	0.12	0.59
	Tb.Sp	0.47	0.59	0.84
	Ct.Th	0.82	0.19	0.96
	BV/TV	0.78	0.003	0.70
	Tb.N*	0.52	0.37	0.94
	Tb.Th*	0.96	0.032	0.10
	Tb.Sp*	0.28	0.80	0.57
	Ct.Th*	0.93	-0.073	0.98
	Conn.D	1.0	-0.23	0.91
	BS/BV	0.86	5.6	0.18
	SMI	0.41	2.1	0.075
	DA	0.53	1.1	0.67
Mechanics (HR-pQCT vs. μ CT)	Reaction	0.42	-5.0	0.73
	SED	0.42	0.004	0.50
	von Mises	0.46	2.5	0.51

Measures representing mineralization, morphology and mechanics are identified along with the gold-standard used for comparison (in brackets). Parameters highlighted in gray represent HR-pQCT-standard evaluation measurements. Note that the correlation for BMD is between volumetric BMD from HR-pQCT and areal BMD from DXA.

3. Results

The bone architecture varied within each subject depending on the axial position of the measurement (Fig. 2) which generated a broad range of structure types. When the cortex is thin, it is more challenging to automatically separate the trabecular and cortical compartments in the HR-pQCT images, yet qualitatively the automated method was still successful (Fig. 3).

The correlation of indices measured by HR-pQCT for density (against DXA), morphology and mechanics (against μ CT) are presented (Table 1). Note that shaded values are indices that are currently the HR-pQCT-standard values. An R^2 value near 1.0 indicates good correspondence between the HR-pQCT and its respective gold-standard. A slope near unity indicates accurate HR-pQCT measurements, and slopes greater or less than unity indicate underestimation or overestimation, respectively, of the HR-pQCT measure compared to the gold-standard. A regression analysis, Bland–Altman plots and error analysis is provided for selected indices that include mineralization (Fig. 4), HR-pQCT-standard evaluation parameters (Fig. 5), and mechanics and some μ CT-based parameters (Fig. 6). The Bland–Altman and error analysis plots were not determined for the BMD measurements (Fig. 4) because of the differences in dimensionality (i.e., aBMD versus vBMD), so BMC is also presented to assess mineralization measurements. The HR-pQCT-standard indices correlated well with the gold-standards ($R^2 > 0.83$) with the possible exception of Tb.Th ($R^2 = 0.59$) which although not highly correlated, had a regression slope near unity indicating reasonable accuracy. The assessment of Ct.Th was slightly overestimated by HR-pQCT (slope 0.81),

however it was correlated highly with the gold-standard ($R^2 = 0.95$). The μ CT-based parameters had varied results where Tb.Th* performed particularly poorly ($R^2 = 0.08$) likely due to the limitations of resolution. However, the anisotropy was reasonably well correlated ($R^2 = 0.66$) albeit overestimated by HR-pQCT, and most notably the Conn.D highly correlated ($R^2 = 0.90$) and estimated quite accurately (slope 1.04). In general, the mechanical parameters by μ FE were overestimated by HR-pQCT, and the continuum-level parameters such as reaction force ($R^2 = 0.90$) correlated better than tissue-level parameters such as strain energy density ($R^2 = 0.49$) and von Mises stress ($R^2 = 0.51$).

The mean and standard deviation of all mineralization, morphology and mechanical parameters for all specimens tested measured on the HR-pQCT, μ CT and DXA are presented (Table 2), and HR-pQCT measurements that are significantly different than the corresponding gold-standard are indicated ($^{\dagger}p < 0.05$).

4. Discussion

This study validated a new three-dimensional high-resolution peripheral quantitative computed tomography scanner (HR-pQCT) that provides *in vivo* clinical imaging at an isotropic voxel size of 82 μ m. The standard patient evaluation, including mineralization and bone morphological parameters, was compared to gold standard methods. Furthermore, a novel contribution of this study is the exploration of additional parameters, including bone morphological measurements and approaches to estimate mechanics (which are

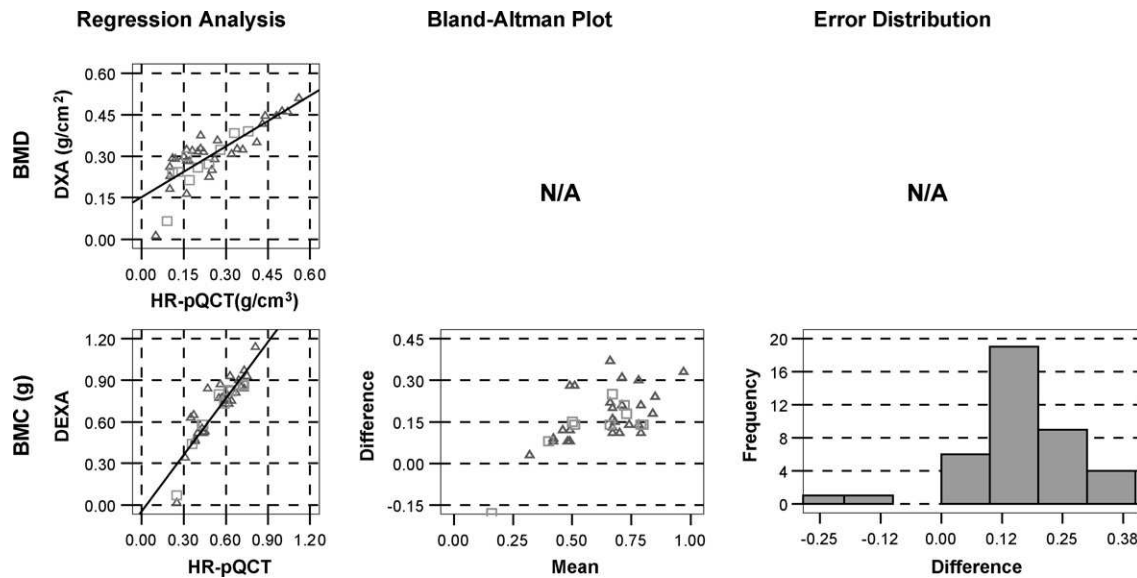


Fig. 4. Mineralization from xCT compared to gold-standard DXA. The standard location for an *in vivo* measurement is represented by a square, the other locations are represented by triangles. The regression analysis determined correlation between the measurements, and the linear equation that relates the two systems. The Bland–Altman plot provided a qualitative assessment of the two measurement systems, and the error distribution indicated whether the error was normally distributed (i.e., considered random error). Bland–Altman and error distribution plots were not appropriate for comparing aBMD from DXA with vBMD from xCT due to the dimensionality difference, therefore BMC is provided for comparative purposes.

not currently included in the standard patient evaluation), that are well established in other applications of μ CT technology. These data provide a basis for deciding what evaluations are appropriate for HR-pQCT, and can help provide bounds to guide researchers using this new technology.

There are some limitations in the methods that should be outlined. The data presented here were based on *in vitro* measurements despite the intention to validate *in vivo* HR-

pQCT measurements. The primary difference is the lack of movement artifact in our images; however, in our experience with *in vivo* HR-pQCT imaging this is not a significant problem as the scan time is less than three minutes, and movement artifact concerns have not been raised in recent publications using HR-pQCT [20,21]. The use of intact and fresh cadavers closely mimicked the *in vivo* measurement protocol, and permitted invasive follow-up measurements

Table 2

Mean and standard deviation values measured on 40 sites by HR-pQCT, μ CT or DXA, for measures of mineralization, morphology and mechanics

	Parameter	Units	DXA	HR-pQCT	μ CT
Mineralization	aBMD	[mg/cm ²]	0.30 ± 0.01	-	-
	vBMD	[mg/cm ³]	-	0.2 ± 0.1 [g/cm ³] [†]	-
	BMC	[mg]	0.7 ± 0.2	0.5 ± 0.1 [†]	-
Morphology	BV/TV ^d	[%]	-	7.5 ± 0.3 [†]	-
	Tb.N*	[mm ⁻¹]	-	1.0 ± 0.4 [†]	-
	Tb.Th	[mm]	-	0.08 ± 0.03 [†]	-
	Tb.Sp	[mm]	-	1.2 ± 0.8	-
	Ct.Th	[mm]	-	0.6 ± 0.4 [†]	-
	BV/TV	[%]	-	15.9 ± 0.6 [†]	12.8 ± 0.5
	Tb.N*	[mm ⁻¹]	-	1.1 ± 0.5 [†]	0.9 ± 0.2
	Tb.Th*	[mm]	-	0.18 ± 0.01 [†]	0.20 ± 0.04
	Tb.Sp*	[mm]	-	1 ± 1	1.1 ± 0.4
	Ct.Th*	[mm]	-	0.8 ± 0.4 [†]	0.7 ± 0.4
	Conn.D	[mm ⁻³]	-	2 ± 1 [†]	2 ± 1
	BS/BV	[mm ⁻¹]	-	12 ± 1 [†]	16 ± 2
	SMI	[1]	-	2.2 ± 0.5 [†]	3.0 ± 0.8
Mechanics	DA	[1]	-	1.7 ± 0.4 [†]	2.0 ± 0.3
	R. force	[N]	-	70 ± 70 [†]	20 ± 30
	von Mises	[MPa]	-	20 ± 10 [†]	13 ± 7
	SED	[J/mm ³]	-	0.09 ± 0.06 [†]	0.04 ± 0.04

Parameters highlighted in gray represent HR-pQCT-standard evaluation measurements. The [†] symbol indicates the HR-pQCT measurement is significantly different from the gold standard ($p < 0.05$).

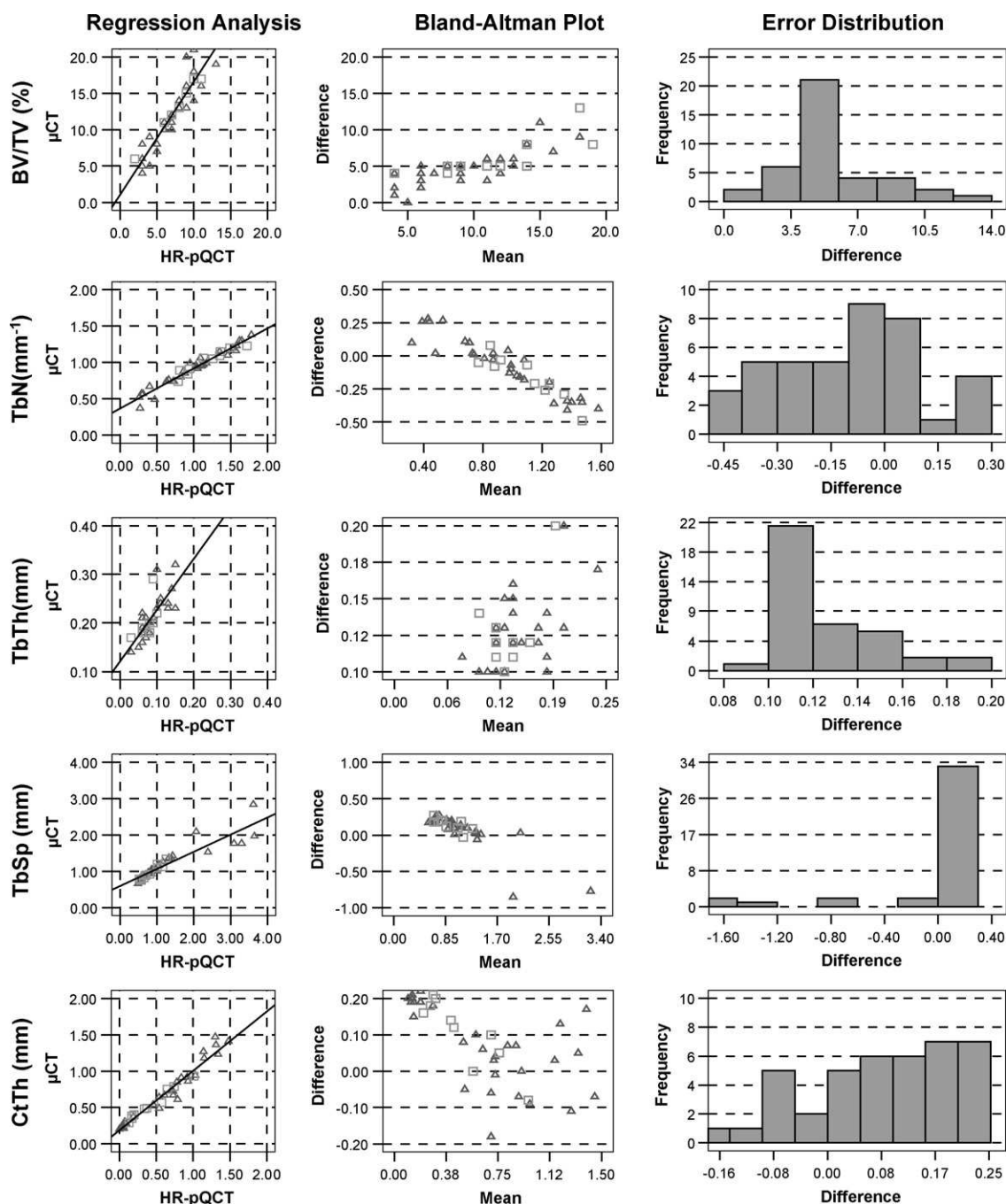


Fig. 5. Morphological parameters from xCT compared to gold-standard μ CT. The standard location for an *in vivo* measurement is represented by a square, the other locations are represented by triangles. The regression analysis determined correlation between the measurements, and the linear equation that relates the two systems. The Bland-Altman plot provided a qualitative assessment of the two measurement systems, and the error distribution indicated whether the error was normally distributed (i.e., considered random error).

by gold-standard imaging methods. The selection of DXA [41] as a gold standard for mineralization measurements, rather than more direct methodologies (e.g., ash weight or infrared imaging microscopy [42]), was based on its wide clinical use. The comparison between aBMD with vBMD, albeit having different dimensionalities, emphasizes the size-dependence of DXA measurements, whereas BMC provided a relatively unbiased comparison. The selection of

μ CT as a gold-standard for morphological and mechanics measures was chosen because of its ability to provide histological measurements [30,31], and because it allows the direct comparison of μ CT-based 3D analysis methodologies for comparison with HR-pQCT. The use of four adjacent sections from the human cadavers increased the range of variation for all of our measurements due to the site-specific differences. Three-dimensional image registration ensured

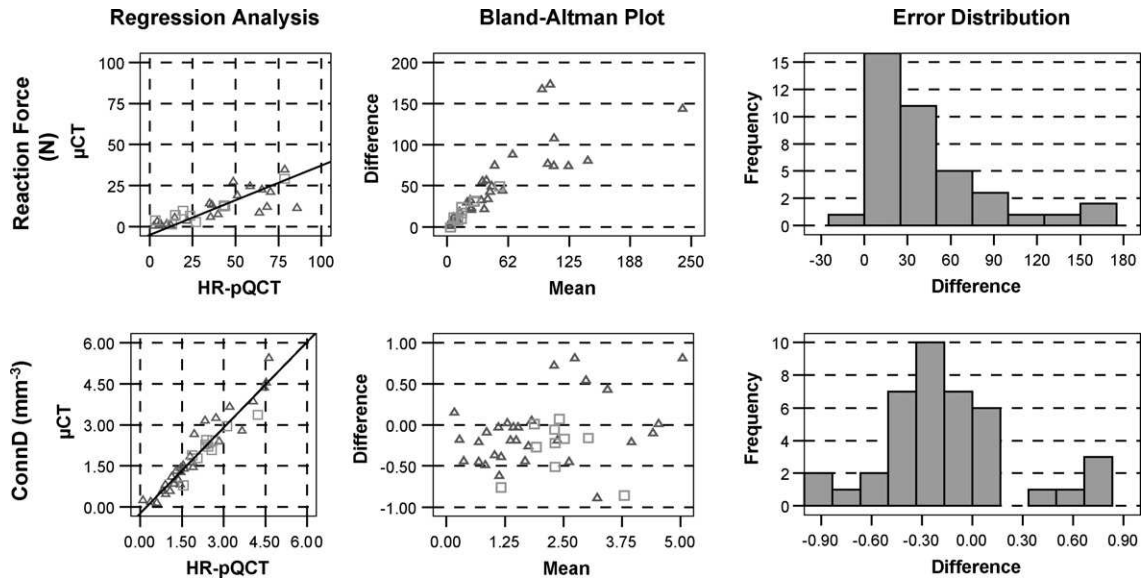


Fig. 6. Non-standard mechanical and morphological parameters from xCT compared to gold-standard μ CT. The standard location for an *in vivo* measurement is represented by a square, the other locations are represented by triangles. The regression analysis determined correlation between the measurements, and the linear equation that relates the two systems. The Bland–Altman plot provided a qualitative assessment of the two measurement systems, and the error distribution indicated whether the error was normally distributed (i.e., considered random error).

identical ROIs were compared for HR-pQCT and μ CT measurements [43]. The use of older cadavers (ages 55–93), in addition to the lack of trabecular bone in the more proximal region of the radius, is reflected by the low bone volume ratio (<8%). Therefore, these specimens represented a challenging cohort to evaluate by HR-pQCT, and the accuracy results presented here may represent a lower bounds compared to assessments made on a younger, healthy patient cohort.

The analysis provided by a standard HR-pQCT patient evaluation (HR-pQCT-standard) generally performed well in comparison to the DXA and μ CT gold standards (Table 1). As expected, the mineralization correlation was better for BMC ($R^2=0.80$) than BMD ($R^2=0.69$) because the factor of dimensionality (areal versus volumetric BMD) was removed. The underestimation of BMC (slope 1.35) by HR-pQCT may be related to imaging artifacts (e.g., beam hardening), or the measurement of HA equivalents of mineralization, not capturing the full mineralized phase. Morphological parameters including BV/TV^d, Tb.N*, Tb.Sp and Ct.Th correlated well ($R^2>0.83$). The Tb.Th did correlated less well ($R^2=0.59$), but it is notable that its regression slope was close to unity. Recalling that Tb.Th and Tb.Sp are derived indices in the HR-pQCT-standard, the determination of Tb.N* and BV/TV^d is important. The calculation of Tb.N* is based on robust 3D methods [11], but BV/TV^d is based on the approximation that compact bone has a density of 1200 mg/cm³ HA [16]. Although adjusting the 1200 mg/cm³ HA value to better approximate BV/TV^d may be tempting, the effect would be linear on dependent parameters such as Tb.Th. According to the manufacturer, Tb.Th can be most accurately measured using this value, and would be detrimentally affected by adjustment of BV/TV^d. Arguably, maintaining a consistent

value of 1200 mg/cm³ HA for all HR-pQCT-standard evaluations ensures high precision for follow-up measurements and comparisons between research sites, albeit somewhat compromising accurate absolute values. Finally, it is notable that the Ct.Th, calculated by dividing the cortical volume by the outer bone surface, was highly correlated ($R^2=0.95$), thus indicating its utility for clinical studies, such as the issue of unraveling different compartmental effects (cortical versus trabecular) of bone targeted therapies such as teriparatide [44,45].

The application of μ CT-based analysis using direct measurement techniques had mixed results. Reasonable correlations were found for BV/TV, although it was generally overestimated (BV/TV^d was underestimated by dividing the apparent density of the trabecular bone by 1200 mg/cm³ HA); and Tb.N* performed well ($R^2=0.94$) which is consistent with previous studies based on 165 μ m measurements of femoral head samples [17]. However, the 82 μ m HR-pQCT measurements did not permit accurate results from direct measure Tb.Th* and Tb.Sp* parameters, and this was particularly true for Tb.Th* because of the relatively thin trabeculae (~ 200 μ m). On the other hand, the direct measure Ct.Th* performed well ($R^2=0.97$) likely due to the cortex being a thicker structure (~ 650 μ m). Non-metric indices such as BS/BV and SMI performed poorly on the HR-pQCT data, and this is likely because they are both dependent on isosurfacing the image data. The 82 μ m HR-pQCT voxel size relative to thin average trabeculae (~ 200 μ m) is problematic for isosurfacing [33], and ideally there should be a minimum average of 4–5 voxels per structure. Nevertheless, DA is also dependent on isosurfacing, yet its performance was reasonable ($R^2=0.66$). One non-metric index that performed

particularly well was Conn.D ($R^2=0.90$) which may be a feasible parameter to estimate by HR-pQCT. This parameter is of interest because of its importance for implicating the irreversibility of bone architecture changes in osteoporosis [46,47].

The mechanics assessed by μ FE performed better for continuum level results, represented by the reaction force, than for the tissue level results such as SED and von Mises stress. Typically, the continuum level stiffness was overestimated by HR-pQCT, and this is consistent with other studies that found a trend of increasing stiffness with increasing voxel size [48]. In contrast, a study comparing 3D pQCT at 165 μ m voxel size to μ CT at 56 μ m voxel size [49] found that the lower resolution data tended to underestimate structure stiffness. However, an important note about that study is that a Gaussian filter and global threshold was used in combination with a volume conservation criterion to segment the mineralized phase for subsequent μ FE, and in lower resolution data this can result in an artificially disconnected structure (i.e., weakened). In the current study, a Laplace–Hamming filter was used for HR-pQCT structure extraction, which has been shown to be more effective at preserving bone topology [17]. The tissue level parameters were less well correlated, and this may be in part due to the stress concentrations in the hexahedron μ FE models. These stress raisers are predominantly at the surface, and in the lower resolution HR-pQCT data the proportion of voxels representing the surface increases thus skewing the results. So, while it appears that continuum level mechanics can be reasonably estimated from HR-pQCT data, further improvements in μ FE meshing and solver strategies will be needed to enable reliable tissue level mechanical characterization.

In conclusion, the bounds of the accuracy of HR-pQCT-standard as well as μ CT-based measurements have been tested against gold standards. The degree of over- or underestimation of those parameters is important when absolute values are needed; however, it is important to not lose sight of the fact that their correlation with the gold standard is a good indicator of their suitability for longitudinal studies monitoring bone changes (despite absolute accuracy discrepancies). The use of HR-pQCT for monitoring bone quality in diseases such as osteoporosis has enormous potential for clinical research because it permits full 3D analysis of peripheral skeletal sites. Based on the data assessed here it would seem that not only does the HR-pQCT-standard provide reasonably accurate results, but there are possibilities for adding other measurement indices such as Conn.D and μ FE to more completely assess bone quality for monitoring patients.

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