Measurement of subregional vertebral bone mineral density in vitro using lateral projection dual energy X-ray absorptiometry (DXA): Validation with peripheral quantitative computed tomography (pQCT).

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Abstract

Although a strong relationship exists between areal bone mineral density (aBMD) derived from dual energy X-ray absorptiometry (DXA) and bone strength, the predictive validity of aBMD for osteoporotic vertebral fractures remains suboptimal. The diagnostic sensitivity of DXA may be improved by assessing aBMD within vertebral subregions, rather than relying on an estimate derived from the total area of the vertebra. The objective of this study was to validate a method of measuring subregional vertebral aBMD in vitro using lateral-projection DXA against subregional volumetric BMD (vBMD) measured with peripheral quantitative computed tomography (pQCT). A mixed set 49 lumbar and thoracic vertebrae from 25 donors were scanned using lateral-projection DXA and pQCT. ABMD and apparent vBMD were measured in 7 vertebral regions (1 total area and 6 subregions) from the lateral DXA scan. VBMD was calculated in anatomically equivalent regions from pQCT scan data, using a customised software program designed to increase efficiency of the analysis process. Significant differences in densitometric parameters between subregions were observed by DXA and pQCT ($p<0.01$). Subregional vBMD derived from pQCT was explained by a significant proportion of the variance in DXA-derived aBMD ($R^2=0.51$-0.67, $p<0.05$) and apparent vBMD ($R^2=0.64$-0.75, $p<0.05$). These results confirm the validity of measuring aBMD in vertebral subregions using lateral-projection DXA. The clinical significance should now be explored.

Introduction

In the clinical environment, evaluation of bone mineral density and monitoring skeletal responses to therapy are most commonly performed using dual energy X-ray absorptiometry (DXA). Although other technologies such as quantitative computed tomography (QCT) [1], transmission ultrasound [2], and more recently magnetic resonance imaging [3] also provide surrogate measures of bone strength for inferring fracture risk, DXA remains the most common tool for this purpose owing to its speed, low radiation dose, moderate cost, ease of operation and established funding schemes within healthcare systems [4]. Despite the wide acceptance of DXA as a definitive tool for the diagnosis of osteopenic disorders and to guide decisions regarding therapy, a reliance on standard postero-anterior (PA) projection DXA data for the spine carries limitations, particularly low diagnostic sensitivity and predictive validity for vertebral fractures [5-7].

Although areal BMD (aBMD) is strongly associated with fracture risk both at appendicular and axial sites [8], standard DXA parameters derived from a lumbar spine scan cannot be used with certainty to predict which patients will sustain a low trauma osteoporotic vertebral fracture [6]. Data from a range of epidemiological studies highlight marked overlap in DXA data between individuals with and without osteoporotic vertebral fractures [9], making a judgement of fracture risk difficult. Thus, greater certainty in predicting which patients are at risk of sustaining a vertebral fracture and those at risk of entering the vertebral fracture cascade is critical for clinicians. Indeed, the use of fracture

risk tools such as FRAX^{TM} [10] and trabecular bone score [11] may assist in this regard, as well as other clinical risk factors such as age, strength, and balance [9].

In routine clinical practice, DXA-derived aBMD and bone mineral content (BMC) are based on average lumbar spine measures taken between L1-L4 vertebrae, or for lateral scans L2-L4 vertebrae. Rarely are data considered for single vertebral levels [12], yet even in these circumstances the aBMD and BMC data are calculated based on the whole vertebral body area. This approach precludes characterisation of any heterogeneity in the distribution of BMC *within* the vertebral body, despite a large volume of literature confirming that BMC is not homogenously distributed throughout the vertebral centrum [13-27] and that the distribution of bone mass relates to vertebral strength and fracture mechanics [24, 28, 29]. We developed a protocol to measure aBMD within vertebral subregions using lateral-projection DXA [15, 16, 30], and applied this protocol to a population with osteoporosis and without vertebral fractures. The subregional approach demonstrated greater diagnostic sensitivity for vertebral fracture compared with standard DXA parameters [16], suggesting that subregional aBMD profiles may be a key factor in better informing vertebral fracture risk. Although the pilot data were encouraging, robust validation of the subregional aBMD protocol is required before extending clinical investigations. Recently, we described the technical capabilities of DXA, peripheral quantitative computed tomography (pQCT) and micro-computed tomography (μCT) to derive bone parameters in anatomically-equivalent vertebral subregions as well as preliminary data regarding correspondence in data derived using these technologies [15]. Those results provided preliminary evidence for the concurrent validity of the

subregional DXA protocol. Given our initial findings, and in order to extend the level of evidence, in the current paper our aim was to further validate the subregional DXA analysis method against pQCT methods in a much larger sample of cadavera using customised pQCT analysis software.

Materials and methods

Specimens

A set of lumbar (L1-L4) and thoracic (T10-T12) spine specimens was harvested from embalmed (N=11; 6 male, 5 female) and fresh (N=14; 4 male, 5 female, 5 unknown) cadavers (N=25 donors in total). Two intact vertebral bodies from each donor were used in this study. The mean (SD) age at death was 77.7 (9.5) years (N=20). The eleven intact cadavers were embalmed with 20-40L of embalming fluid (55% ethanol, 5% formaldehyde, 5% phenol, 20% propylene glycol and 15% water) and stored at 4°C for 3 months prior to harvesting of the spine. Previous investigators have found no effect of formalin fixation on vertebral BMD estimations by DXA on embalmed specimens compared to fresh specimens [31]. Therefore, data derived from fresh and embalmed specimens were pooled for analytic purposes in this study. The fourteen fresh specimens were frozen at -17 °C. In all specimens the ribs and ilia were removed leaving intact vertebral bodies and connective tissues. Embalmed specimens were sealed in water-tight shrink-wrap thermoplastic while fresh specimens remained in gauze wrap. Prior to any scanning, lateral radiographs were acquired from each specimen to screen for vertebral fractures and any other overt bone pathology and to verify vertebral levels in conjunction with a PA-projection DXA image. One L2 vertebral body was excluded due to the

presence of a fracture, thus N=49 vertebrae. The cadavers used in this study were donated by the next-of-kin of the deceased for use in medical research under the terms and conditions contained within the Anatomy Act of South Australia. The specific terms that apply to this study are that the research be approved by the institutional research committees. Approval to use the specimens for research purposes was granted by the Human Research Ethics Committee at the Royal Adelaide Hospital, South Australia, and Curtin University, Western Australia.

Procedure

The two vertebral bodies from each specimen were scanned with DXA and pQCT. We chose to use pQCT in this study, rather than QCT for a number of reasons. First, the intent of the study was to further validate the novel DXA protocol and not to explore the potential *in vivo* or clinical use of pQCT or QCT. The spatial resolution of pQCT is intermediate between that of DXA and μCT, thus enabling the assessment of whether DXA analyses correlate with higher resolution analyses. Second, our group has better access to, and experience with pQCT hardware and software. The scanning and analysis procedures have been described in detail elsewhere using a protocol developed by our group [15, 30], and are outlined briefly below.

Dual energy X-ray absorptiometry (DXA)

All scanning was performed using a Hologic (Hologic Inc., Bedford, MA; USA) QDR4500A fan beam densitometer, with a spatial resolution of 1.01 mm, running operating software version 9.10D. Spine samples were placed supine in a water bath

(270×180×150mm) of tap water to a depth of 18cm to simulate soft tissue composition in vivo. This procedure has been used in previous studies with validity and reliability established for both lumbar and thoracic vertebrae [17, 32-35]. A matched PA-supine lateral scan pair was performed on each specimen using the array scanning mode. At the completion of the lateral scan, both a standard analysis and a customised subregional analysis were performed. Areal BMD was calculated for the whole vertebral body area (defined as region of interest (ROI) 1) and within six subregions, three oriented sagittally (ROIs 2-4) and three transversely (ROIs 5-7) (Figure 1). Subregions were created manually by modifying the regions of interest during the analysis phase. The whole vertebral area (ROI 1) was defined by the four corners of the vertebra of interest from the lateral DXA image, including the vertebral endplate and excluding the posterior elements. Overt osteophytes were excluded from the ROIs and deleted from the bone map manually, in agreement with previous work [33, 36, 37]. The size and shape of ROI 1 was defined according to the morphology of the vertebral body. The endplates defined the superior and inferior margins, the anterior border of the centrum defined the anterior margin, while the posterior margin was defined by the junction between the vertebral centrum and pedicle of the posterior elements. Subregions 2-4 formed equal thirds in the area of ROI 1, oriented sagittaly. Subregions 5-7 formed equal thirds in area of ROI 1, oriented transversely. Measurement of subregional BMD and BMC using this protocol has been performed previously, both *in-vivo* [30] and *ex-vivo* [15, 17]. Our pilot data demonstrate good to fair short-term precision of this protocol when applied ex vivo (%CV range 1.8-6.8%) [38].The selection of these subregions was made for several reasons. First, we adopted an earlier histomorphometric framework used to quantify

variation in trabecular bone architecture in vertebral bodies [26]. Second, the decision was pragmatic to optimise the intra- and inter-user reliability of defining the subregions based on the capabilities of Hologic analysis software. Although other subregional geometries were considered in the pilot phase of this work, the final decision was influenced by optimal precision and the ability to translate the work readily into clinical application. Third, we sought to specifically include a central region of interest (ROI 6). ROI 6 was included to preferentially measure a ROI with predominantly trabecular bone, where bone fragility is believed to be particularly important in determining overall vertebral strength [39-41]. Finally, the underlying biomechanical rationale was based on i) purposive measurement of anterior and posterior vertebral aBMD, given evidence of the potential importance of anterior bone mineral parameters in the pathomechanics of osteoporotic wedge fractures [24, 42], ii) measurement of a trabecular-rich central component of the vertebral body, iii) measurement of bone mineral parameters adjacent to the vertebral endplates (ROIs 5 and 7), given the evidence of vertebral failure mechanics differing between the superior and inferior endplates [21, 43], and iv) to minimise geometric variability in subregions given that vertebral area influences aBMD.

Peripheral quantitative computed tomography (pQCT)

A Stratec XCT3000 pQCT scanner (Stratec Medizintecknik, Pforzheim, Germany) running operating software version XCT 5.50E was used to scan the vertebral bodies of interest. Specimens were placed supine in the scanner gantry, and oriented with the vertebral body perpendicular to the X-ray beam in the sagittal plane. Scanning was performed with a fixed slice thickness of 2.3mm, inter-slice distance of 2.3mm, and a

resolution of 0.5mm. Thus the voxel size was 0.5×0.5×2.3mm. For each vertebra scanned, scan slices were initiated in the inferior intervertebral space and ceased in the superior intervertebral space. During analysis, a slice 'set' was defined as the last slice passing through the inferior vertebral endplate to the first slice passing through the superior vertebral endplate. Using this set, volumetric BMD (vBMD) was calculated in vertebral ROIs which were anatomically equivalent to those defined during the subregional analysis of the lateral-DXA image, and comparable to those used in another recent study [27]. Analysis of subregional vBMD was performed with a custom software program developed using Matlab software, version R2008b (Mathworks Inc., Natick, MA, USA), and technical details have been described previously [15]. A custom analysis software package was developed, rather than using standard manufacturer (Stratec) software in order to increase the efficiency of the analysis approach and to include features which would optimise analysis such as allowing removal of osteophytes and de-rotation of scan images.

For each scan, binary data of slice density values were imported into Matlab and the user selected slices to define a scan set. The total number of slices in the set was divided into three bands which defined the superior, central, and inferior subregions (ROIs 5-7). Where the number of slices chosen for the set was not divisible by three, the superior band had the fewer number of slices assigned. Osteophytes were removed manually from the images around the cortical wall for each slice and de-rotation of the scan image was performed automatically on all slices based on the digitized AP angle of the spinous process in a single, representative slice. A user-defined rectangular global ROI was then

constructed around the vertebral body of one slice which was then automatically divided by the software into 3 sagittal subregions (posterior, middle, anterior: ROIs 2-4 respectively) of equal area by dividing the AP dimension of this global ROI by three. The subregion configuration was then mapped to all other slices included in the scan set. Subregions anatomically equivalent to DXA-defined subregions were then created by the software. A mean slice was also created using all slices in the analysis set, analogous to DXA ROI 1. Transverse subregions (inferior, central, superior: ROIs 5-7 respectively) were defined by creating a single 'mean slice' for each subregion from the set of slices included in a transverse band. Sagittal subregions (ROIs 2-4) were defined by creating a mean slice from all included slices using scan data only within the particular subregion area defined from the segmented global ROI.

A process equivalent to contour mode 1 in the Stratec software was used to strip any remaining soft tissue from the mean slice for each subregion, with a default density threshold set at 130mg/cm^3 to detect the transition from soft tissue to outer cortical wall. This threshold could be changed by the user to a lower value in circumstances where the vertebra had particularly low density and the software stripped parts of the outer cortical wall and trabecular bone as well as soft tissue from the image. As the intention was to compare pQCT density results to DXA - which does not distinguish between cortical and trabecular bone - no further segmentation to remove the cortical wall was performed; that is, all bone inside the region delineated by the outer cortical wall was included in the subsequent ROI analyses to derive a total density. Having established an outer cortical boundary, mean volumetric density was calculated from the individual density values of

each voxel contained within each subregion of the mean slices. The intra-rater and inter-rater precision of the analysis process has been established previously (mean %CV 1.65-1.90 across raters and 3.25% between raters) [15].

Data processing and data analysis

DXA parameters: areal BMD (mg/cm^2) and ap.vBMD (mg/cm^3)

DXA-derived data included areal BMD in each subregion (ROI 1-7) and the standard Hologic DXA parameters (total vertebral aBMD for the PA and lateral projections, and mid-lateral aBMD). Given the limitations of areal measures, subregional areal BMD was transformed to an apparent volumetric BMD (ap.vBMD) on the assumption that each ROI represented a section of cylinder. Transformations were based on the formula for estimating a width-corrected volumetric BMD described by Jergas et al [5] and have been described in detail previously [15].

pQCT parameter: volumetric BMD (mg/cm^3)

Volumetric BMD in each subregion was extracted directly from the pQCT analysis approach described above.

Statistical analysis

A repeated measures ANOVA was performed to evaluate differences in quantitative bone parameters between subregions for both DXA and pQCT, in keeping with earlier work [15-17]. Although there were seven regions of interest, the within-subject factor (ROI) was set *a priori* at k=4 to ensure that overlapping subregions were not compared *post hoc*. That is, sagitally-oriented subregions were not compared with transversely oriented

subregions in the same ANOVA. The first ANOVA model included ROIs 1-4 and the second ANOVA model included ROIs 1 and 5-7. The detailed *post hoc* results are not reported; instead differences between the subregions may be interpreted from the figures provided. Linear regression models were used to quantify the correspondence between DXA and pQCT parameters. The difference in R^2 values between vBMD with aBMD and vBMD with ap.vBMD was assessed using Steiger's Z test for dependent samples [44]. Using more than one vertebral body from an individual donor was considered to not effect the analysis, as the design of this study was a within-vertebral body comparison of imaging modalities. Statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc., Chicago IL) and the level of significance was set at $p<0.05$.

Results

Table 1 details the fixation status and mean (SD) standard PA-projection and lateral-projection parameters for DXA analysis of the 49 vertebral bodies used in this study. Across the sample there was a significant difference in areal BMD among the three standard DXA output parameters, with PA-projection aBMD being significantly higher than both lateral-projection aBMD ($p<0.001$) and lateral-projection aBMD in the mid-zone of the vertebral body ($p<0.001$).

There were significant differences in aBMD (DXA), ap.vBMD (DXA) and vBMD (pQCT) between ROI 1 and sagittally-oriented subregions (ROIs 2-4) ($p<0.001$) and between ROI 1 and transversely-oriented subregions (ROIs 5-7) ($p\leq0.002$), as identified by main effects in the two ANOVA models for each bone mineral parameter. Figure 2

illustrates the mean subregional densitometric values from DXA and pQCT across the subregions, from which the reader can judge the nature of the subregional heterogeneity.

A significant proportion of the variance in subregional volumetric BMD derived from pQCT scans was explained by DXA-derived subregional aBMD (R^2=0.51-0.67, $p<0.05$) and ap.vBMD (R^2=0.64-0.75, $p<0.05$) (Table 2). These relationships are illustrated in Figures 3 and 4, respectively. For ROIs 1, 2, 3 and 5, the correspondence between 'vBMD *vs*. ap.vBMD' was significantly greater ($p<0.05$) than between 'vBMD *vs*. aBMD' (R^2=0.75, 0.72, 0.67, 0.66, and R^2=0.63, 0.62, 0.58, 0.51, respectively).

Discussion

Up to 75% of the variance in subregional vBMD measured with pQCT can be accounted for by subregional aBMD measured with lateral-projection DXA within an ex vivo context. Although the clinical significance of DXA-derived heterogeneity in aBMD is yet to be fully explored, pilot data which suggest improved diagnostic sensitivity for vertebral fractures are encouraging [16], and this study provides justification for further study in a clinical context.

Although a large body of evidence has been published to establish unequivocally that the distribution of bone mass varies through the vertebral body, the majority of this work has been undertaken using technologies other than DXA [9]. Over time, we have examined the application of DXA in this context given its universal acceptance and widespread availability as a diagnostic clinical tool [9, 15-17, 30, 45]. Consistent with our earlier

work, and that of others, in this study we have demonstrated the capacity of lateral-projection DXA to measure heterogeneity in aBMD between intra-vertebral subregions (Figure 2A), and in particular that anterior and central subregions have lower aBMD than their adjacent subregions and aBMD measured over the whole vertebral area. Across both imaging modalities the middle (ROI 3) and central (ROI 6) subregions demonstrated consistently lower bone mineral values than the posterior and adjacent subregions, respectively. Consistency in this pattern likely reflects the predominantly high proportion of trabecular bone measured in these subregions by both DXA and pQCT modalities without the overriding influence of the cortical shell and endplate bone in other subregions. These findings may have important biological implications for vertebral fragility and should be explored in future work. Although the overall *pattern* of heterogeneity in subregional densitometric parameters across the outcome measures was not always consistent for every subregion (Figure 2A-C), attributable to the inclusion of more cortical bone in the DXA-derived data compared to the pQCT-derived data as well as geometric differences, these data confirm the heterogenous distribution of bone mass throughout the vertebral body, which can be readily identified using DXA or pQCT. These data also highlight the influence of the assumed volume of cylindrical geometry in determining ap.vBMD [5]. Specifically, the mean values in ap.vBMD over the sagittally- (ROIs 2-4) and transversely- (ROIs 5-7) oriented subregions were not equivalent to the value in ap.vBMD of ROI 1. This is expected, due to the geometric assumptions for deriving the cylindrical volumes in different orientations. Nonetheless, the reported subregional ap.vBMD values clearly show differences between the subregions, for which the biological significance should be explored.

As illustrated in Figures 3 and 4, there was less variability in data points for "volumetric *vs*. apparent volumetric" data (vBMD *vs*. ap.vBMD) than for "volumetric *vs*. areal" data (vBMD *vs*. aBMD), particularly for ROIs 1, 2, 3 and 5, evidenced by the width of the 95% CI which would account for the statistically-significant differences between the R^2 values for these ROIs. With respect to ROIs 4, 6 and 7, a trend was identified for the "volumetric *vs*. apparent volumetric" data to have higher R^2 values than the "volumetric *vs*. areal" data. The absence of a statistically-significant difference is likely related to sample size and potentially inadequate power to detect a small effect size between the R^2 parameters.

Peripheral QCT represents an appropriate tool against which concurrent validity for the subregional DXA analysis can be established since it offers the next level in spatial resolution for bone tissue after DXA and its volumetric BMD output is as predictive for vertebral failure as DXA [35, 46, 47]. In the context of this study, however, pQCT was not used to differentiate between trabecular and cortical bone – one of the main capabilities for which it is commonly used. We chose to use a 'total density' measure for pQCT (cortical and trabecular bone) to compare with DXA, since DXA cannot differentiate cortical and trabecular bone compartments. We also chose to include endplate bone in the pQCT analysis set in order to align as closely as possible with the DXA analysis. However, the amount of endplate bone included could not be controlled within our experimental setup since the slice thickness of the pQCT device was fixed and endplate morphology varied across the samples. This issue has been discussed in detail

previously [15]. Nonetheless, the custom analysis software allowed us to remove osteophytes and select which slices to include in an analysis set in order to align as closely as possible with the DXA analysis. We chose to use lateral-projection DXA for subregional analysis owing the greater diagnostic sensitivity of lateral DXA compared to PA-projection DXA for vertebral fractures [5, 7], and the capacity to define vertebral subregions from the lateral image without the over-riding influence of cortical bone from posterior vertebral elements. Correspondence between aBMD and ap.vBMD with pQCT-derived vBMD reached up to R^2=0.67 and R^2=0.75, respectively, representing moderate to good concurrent validity, comparable to earlier studies [48-51]. Our data do not indicate perfect correspondence between imaging modalities. Indeed, perfect correspondence would not be expected in light of the fundamental differences between imaging modalities (areal *vs*. volumetric) and the error component associated with each method. The linear regression models should be interpreted in the context of reasonable correspondence between modalities to substantiate concurrent validity, rather than the ability of the dependent variable (DXA) to directly predict the putative independent pQCT parameters. Not surprisingly, correspondence was greater between 'vBMD and ap.vBMD' than between 'vBMD and aBMD', and this is likely attributable to geometric similarities. That is, comparing an apparent volumetric density, rather than an areal density, with a true volumetric density.

In our earlier study we described the development of a custom software application, using Matlab, to analyse subregional vBMD [15]. The advantages of this software included the ability to de-rotate specimens, manually remove osteophytes from the cortical wall,

exclude slices with overt anomalies, use mean slices by summing density values across ROI slices (largely avoids problems with segmenting thin cortical wall in single slices), adjust the contour threshold per specimen as required, and export subregional vBMD data directly without further post-analysis processing, thereby significantly increasing analysis efficiency. These analysis features are not available with standard manufacturer software and therefore maximised the comparability between DXA analysis and pQCT analysis – the primary aim of this study – and optimised the efficiency of the analysis approach. Moreover, the vBMD values derived with Matlab in this study for ROI 6 are comparable with trabecular vBMD for the lumbar spine reported in other studies [40, 51-53].

A potential limitation in the design of this study was the use of two vertebrae from each donor, thus data points were not truly independent. However, given that the primary aim was to explore correspondence between imaging modalities for single vertebrae and their subregions, thereby utilising a repeated within-vertebra design, we do not consider this to be a significant issue. Using a large sample size, this study has demonstrated the concurrent validity of measuring subregional aBMD with lateral projection DXA. An important limitation of the comparative method used in this study was that we are unable to comment on the agreement between volumetric data (ap.vBMD and vBMD). The aim of the study was to examine the correspondence between modalities (concurrent validity), and not to explore the accuracy of the transformation of aBMD to ap.vBMD with true volumetric BMD obtained from pQCT. The intercept in Figures 4A-G suggest that the pQCT modality measures consistently higher vBMD in each ROI than the DXA

modality. It is not surprising that the regression line does not pass through the origin as the data are derived from fundamentally different imaging modalities.

As this study was an *ex-vivo* within-vertebral body comparison of two clinical imaging modalities the clinical utility of subregional aBMD measures now needs to be clarified *in vivo*. Whether subregional aBMD is a better predictor of vertebral failure than standard DXA should also be investigated. Although lateral-projection DXA has been feasible for some 20 years it is not widely used in a clinical context owing to concerns regarding its precision, inability to apply WHO diagnostic thresholds for osteoporosis, and in many cases only the L3 vertebra is available for analysis due to overlap of the vertebrae above and below from the ribs and iliac crest, respectively. However, as evidence concerning the clinical utility of lateral-projection DXA accumulates, translation into clinical practice may become more acceptable.

In conclusion, moderate to good correspondence was observed between DXA-derived and pQCT-derived vertebral subregional bone mineral parameters. Lateral-projection DXA is a valid tool to measure areal BMD within vertebral subregions in an ex vivo context. The biological and clinical significance of heterogeneity in subregional aBMD, as characterised by DXA, should now be explored.

Conflict of interest statement

All authors have no conflicts of interest.

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Tables

Table 1 Vertebral levels and fixation status of specimens used and mean (SD) areal bone mineral density (aBMD) results for standard DXA parameters derived from PA and lateral projection DXA scans (mg/cm^2).

Vertebral level	Embalmed	Fresh	PA-projection aBMD	lateral-projection aBMD	lateral-projection aBMD mid zone
T10^	0	1	536.0	397.0	253.0
T11^	0	1	515.0	353.0	230.0
T12	0	3	695.3 (237.3)	500.3 (145.0)	405.7 (135.5)
L1	0	6	821.0 (174.5)	528.7 (123.3)	420.3 (114.3)
L2	10	8	902.1 (190.8)	547.4 (157.3)	453.8 (183.5)
L3	11	6	930.1 (259.9)	600.4 (226.5)	613.1 (604.3)
L4	0	3	833.7 (139.5)	497.0 (158.5)	363.3 (140.0)

^ mean (SD) data for aBMD not available for these vertebral levels since n=1.

Table 2 Summary of linear coefficients of determination (R^2) established with linear regression models between DXA predictor variables areal BMD (aBMD), apparent volumetric BMD (ap.vBMD), and the dependent pQCT variable volumetric BMD (vBMD) derived with Matlab for each region of interest (ROI). The test of statistically significant differences between the R^2 values is expressed with the Z score and associated two-tailed p-value.

ROI	R^2: vBMD *vs.* aBMD	R^2: vBMD *vs.* ap.vBMD	Z score	p-value
1 (whole)	0.63	0.75	-2.08	0.037*
2 (posterior)	0.62	0.72	-2.09	0.037*
3 (middle)	0.58	0.67	-1.99	0.047*
4 (anterior)	0.66	0.72	-1.80	0.072
5 (superior)	0.51	0.66	-2.28	0.023*
6 (central)	0.65	0.64	0.23	0.818
7 (inferior)	0.67	0.69	-0.26	0.795
Mean	*0.62*	*0.69*	*n/a*	*n/a*

n/a: not applicable

* statistically significant difference in R^2 values

Figure Legends

Figure 1 DXA-derived vertebral subregions defined using Hologic software. ROI 1 (whole) was defined by the four corners of the vertebra. ROIs 2-4 (posterior, middle, anterior) formed equal thirds in the area of ROI 1, oriented sagittally. ROIs 5-7 (superior, central, inferior) formed equal thirds in area of ROI 1, oriented transversely. Reproduced with permission from Elsevier Copyright Clearance Center.

Figure 2 Mean densitometric parameters across the subregions for (A) DXA-derived areal BMD (mg/cm^2), (B) DXA-derived apparent volumetric BMD (ap.vBMD) (mg/cm^3), (C) pQCT-derived volumetric BMD (mg/cm^3). Error bars represent the standard error of the mean.

Figure 3 Scatter plots of pQCT-derived volumetric BMD (vBMD) (mg/cm^3) and DXA-derived area BMD (aBMD) (mg/cm^2) for the (A) whole vertebral area (ROI 1) and each subregion: (B) ROI 2 posterior, (C) ROI 3 middle, (D) ROI 4 anterior, (E) ROI 5 superior, (F) ROI 6 central, and (G) ROI 7 inferior. The line of best fit (solid line) and 95% confidence interval (broken lines) are derived with linear regression.

Figure 4 Scatter plots of pQCT-derived volumetric BMD (vBMD) (mg/cm^3) and DXA-derived apparent volumetric BMD (ap.vBMD) (mg/cm^3) for the whole vertebral area (ROI 1) (A) and each subregion: ROI 2 posterior (B), ROI 3 middle (C), ROI 4 anterior (D), ROI 5 superior (E), ROI 6 central (F), and ROI 7 inferior (G). The line of best fit (solid line) and 95% confidence interval (broken lines) are derived with linear regression.