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The intravertebral distribution of bone density: correspondence to intervertebral disc health and implications for vertebral strength

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Abstract

Summary—This study's goal was to determine associations among the intravertebral heterogeneity in bone density, bone strength, and intervertebral disc (IVD) health. Results indicated that predictions of vertebral strength can benefit from considering the magnitude of the density heterogeneity and the congruence between the spatial distribution of density and IVD health.

Introduction—This study aims to determine associations among the intravertebral heterogeneity in bone density, bone strength, and IVD health

Methods—Regional measurements of bone density were performed throughout 30 L1 vertebral bodies using microcomputed tomography (μ CT) and quantitative computed tomography (QCT). The magnitude of the intravertebral heterogeneity in density was defined as the interquartile range and quartile coefficient of variation in regional densities. The spatial distribution of density was

quantified using ratios of regional densities representing different anatomical zones (e.g., anterior to posterior regional densities). Cluster analysis was used to identify groups of vertebrae with similar spatial distributions of density. Vertebral strength was measured in compression. IVD health was assessed using two scoring systems.

Results—QCT- and μ CT-based measures of the magnitude of the intravertebral heterogeneity in density were strongly correlated with each other ($p < 0.005$). Accounting for the interquartile range in regional densities improved predictions of vertebral strength as compared to predictions based only on mean density ($R^2 = 0.59$ vs. 0.43 ; F -test p -value = 0.018). Specifically, after adjustment for mean density, vertebral bodies with greater heterogeneity in density exhibited higher strength. No single spatial distribution of density was associated with high vertebral strength. Analyses of IVD scores suggested that the health of the adjacent IVDs may modulate the effect of a particular spatial distribution of density on vertebral strength.

Conclusions—Noninvasive measurements of the intravertebral distribution of bone density, in conjunction with assessments of IVD health, can aid in predictions of bone strength and in elucidating biomechanical mechanisms of vertebral fracture.

Keywords

Computed tomography; Heterogeneity; Intervertebral disc; Strength; Vertebra

Introduction

Vertebral fractures, the most common type of osteoporotic fracture [1], are associated with pain, difficulty in performing activities of daily living, depression, overall decreased quality of life, and increased mortality rates [2–4]. The limitations of current methods of estimating an individual's risk of vertebral fracture, which rely heavily on measurement of the average bone mineral density (BMD) in the vertebral body, are well recognized. For example, an average BMD explains only ~60 % of the variance in vertebral strength [5], and nearly half of the individuals who have fracture do not exhibit average BMD in the osteoporotic range [6]. Noting that the intravertebral distribution of BMD is highly spatially heterogeneous, prior studies have proposed using regional measurements of BMD [7–9] or quantitative measures of the intravertebral heterogeneity in regional BMD values [7, 10–13] for improved predictions of vertebral strength and fracture risk. Recent data show that vertebral strength depends on the magnitude of the intravertebral heterogeneity in density [10]. However, the biomechanical mechanisms by which the spatial heterogeneity in BMD affects vertebral strength and fracture have not been established. Identifying these mechanisms would greatly benefit predictions of vertebral fracture and would enhance current understanding of the pathogenesis of these fractures.

Spatial heterogeneity in BMD within the vertebral trabecular centrum has been postulated to depend on the health of the adjacent intervertebral discs (IVDs) [14–18]. Numerous studies have shown marked effects of disc degeneration on the distribution of pressure in the IVD [19, 20], on the way an applied load is distributed across the endplates [21–23], and on stress and strain distributions in the vertebra [18, 21, 24–26]. While no direct evidence of bone adaptation in response to IVD degeneration has been reported, spatial distributions of

density differ in spine segments with vs. without IVD degeneration [14–16]. In vertebrae adjacent to degenerated IVDs, trabecular bone is denser and stronger in the posterior than anterior regions of the centrum [14, 15, 27], consistent [18] with measurements of disc pressure [19, 28] showing reduced and elevated pressure in the nucleus pulposus and posterior annulus fibrosus, respectively, in degenerated IVDs. These data indicate that, regardless of whether the spatial distribution of bone density within the vertebra arises from degenerative changes in the adjacent IVDs, the biomechanical consequences of the intravertebral heterogeneity in BMD may correspond closely to the interplay between vertebra and IVD.

The overall goal of this study was to determine associations among the intravertebral heterogeneity in bone density, bone strength, and IVD health. Whereas prior studies on the impact of intravertebral heterogeneity in density on bone strength have primarily used density measurements obtained from micro-computed tomography (μ CT) [10–12] or high-resolution peripheral quantitative computed tomography (HR-pQCT) [13], we sought here to increase the clinical relevance for the axial skeleton by using QCT. Therefore, the objectives of this study were (1) to compare quantitative measures of the magnitude of the intravertebral heterogeneity in density measured by QCT vs. μ CT, (2) to assess the use of quantitative measures of the magnitude of the intravertebral heterogeneity to improve predictions of vertebral strength, and (3) to determine whether the spatial distribution of bone density within the vertebra is associated with vertebral strength, the magnitude of the intravertebral heterogeneity in density, and the health of the adjacent IVDs.

Methods

Specimen preparation

Thirty spine segments consisting of L1 with adjacent IVDs were harvested from fresh-frozen spines (age range, 41–91 years; mean $\pm$ SD, 79.9 $\pm$ 11.3, 14 females and 16 males) by making a transverse cut just above the inferior endplate of T12 and another transverse cut just below the superior endplate of L2 (Fig. 1a). The posterior elements were removed in order to allow the spine segment to fit within the mechanical testing device, as described below. The spine segments were kept hydrated at all times and were wrapped in saline-soaked gauze at -20°C when not in use.

Imaging and mechanical testing

The top and bottom endplates of each specimen were potted in circular, radiolucent dishes filled with 2–4 mm of polymethylmethacrylate, and were imaged with QCT (GE Healthcare) at a two resolutions: (1) $0.32\times0.32\times0.626$ and (2) $0.7\times0.7\times0.626$ mm/voxel for volumetric BMD and areal BMD measurements, respectively. For the high-resolution scans, the voltage, current, scan field of view, and volume computed tomography dose index were 120 kVp, 240 mA, 32 mm, and 96.5 mGy, respectively, and the reconstructions were done using the bone convolution kernel and head filter. The settings for the low-resolution scans were 120 kVp, 120 mA, 50 mm, and 23.78 mGy, respectively, and the reconstructions were done using the bone convolution kernel and body filter. A three-component, calcium hydroxyapatite phantom of densities 150, 75, and 0 mg/cm³ (Image Analysis, Columbia,

KY) was included in the scan field of view and was used to convert intensity values into mineral densities. The specimens were also scanned via μ CT (μ CT80, Scanco Medical, Brüttisellen, Switzerland) at a resolution 37 μ m/voxel. The settings for voltage, current, and integration time were 70 kVp, 114 mA, and 300 ms, respectively.

The specimens were compressed to failure under axial force as described elsewhere [29]. Briefly, each specimen was subjected to ten cycles of preconditioning to 400 N; after which, the specimen was loaded to failure in a stepwise fashion in increments of 1 mm applied at a rate of 0.25 mm/s, and held for the length of time required for the load to equilibrate (approximately 20 min) and to complete a μ CT scan (approximately 3 h). The resulting series of μ CT scans were used in a separate investigation on quantitative visualization of vertebral failure [29]. The ultimate force (F_{Ult}) was defined as the maximum force sustained by the vertebra (Fig. 1b).

Average density and the magnitude of the intravertebral heterogeneity in density

Average volumetric bone mineral density (vBMD) and cross-sectional area (CSA) were calculated for the largest elliptical cylinder that would fit within the trabecular centrum (Fig. 1c) [10]. To quantify the intravertebral heterogeneity in density, a 3-D layout of contiguous, 5-mm cubes was defined in order to fill the trabecular centrum (Fig. 1c). On average, this 3-D layout contained 98 cubes (range, 34–160). vBMD was calculated for each cube, and two quantitative measures of the magnitude of the intravertebral heterogeneity in density were computed: the interquartile range (IQR_{BMD}) and quartile coefficient of variation (QCV_{BMD}) of the cube vBMD values [10]. The QCV is calculated as:

$$QCV = \frac{Q_3 - Q_1}{Q_3 + Q_1} \quad (1)$$

where Q_1 and Q_3 are the first and third quartiles, respectively. We note that the quantity $Q_3 - Q_1$ is the interquartile range. For non-normal distributions of data, the quartile coefficient of variation is a better measure of relative dispersion than is the coefficient of variation. All of the above measurements were made on the high-resolution QCT images. Using the μ CT images, an identical set of measurements was made, only with bone volume fraction (BV/TV) and apparent mineral density (ρ_{App} , defined as the average mineral density of the bone and marrow space combined) as the density measures, instead of vBMD.

The low-resolution QCT images were used to estimate the areal BMD that would be measured by lateral dual-energy X-ray absorptiometry (DXA). In this process, a lateral projection of the vertebra was generated, and the average areal BMD (aBMD) for the entire vertebral body, inclusive of cortical shell and endplates (Fig. 1d), was calculated using this projection and the density phantom in the QCT scan. The low-resolution QCT images were also used to obtain an estimate of the axial rigidity (EA). Axial rigidity is a measure of the effective axial stiffness of a structure; E and A represent the stiffness of the material in the structure and the cross-sectional area of the structure, respectively. In each transverse QCT slice, the axial rigidity of that cross section of the vertebra is computed as

$$EA = \sum_{i=1}^N E_i dA \quad (2)$$

where E_i is the estimated Young's modulus of the tissue contained in the i th pixel, dA is the area of one pixel, and N is number of pixels in the cross section [30, 31]. A published relationship between the axial Young's modulus of vertebral trabecular bone and QCT density (ρ_{QCT}) [32] was used to obtain E_i :

$$E_i = -34.7 + 3230\rho_{\text{QCT}} \quad (3)$$

The minimum value of EA over all transverse cross sections (EA_{min}) was identified for each vertebra because the strength of the vertebra has been hypothesized to be correlated with this minimum value [30].

A subset of the total sample ($n=22$ vertebrae) was used to examine whether measures of the magnitude of the intravertebral heterogeneity in density depended on the cube size. A cube layout was defined in each centrum using a different, alternate cube size: 4.38, 5.63, 6.25, 8.13, and 10 mm. The data from these cubes were analyzed in the same manner as the 5-mm cubes.

Spatial distribution of bone density

The spatial distribution of bone density throughout the centrum was first examined by classifying each cube according to its location in each of the three anatomic planes (Fig. 1e): anterior, middle, posterior coronal plane; superior, middle, inferior transverse plane; and left, middle, right sagittal plane. The boundaries between planes divided the centrum into approximate thirds. The spatial distribution of densities among these 27 regions was qualitatively observed to differ among vertebrae primarily within the posterior coronal plane, in the relative magnitudes of anterior to posterior densities, and in the relative magnitudes of densities in the outer regions vs. in the central core of the centrum. Thus, a set of five ratios of densities was calculated for each vertebra: anterior to posterior (A/P), central to outer (C/O), inferior to mid-transverse (I/T), superior to mid-transverse (S/T), and medial to lateral (M/L). Within each of the locations used in one of these ratios (e.g., A, P, C, O, etc.), the median cube density was used in calculation of the ratio.

Intervertebral disc health

Frontal and sagittal radiographic views were constructed from the high-resolution QCT images by generating 2-D projections from the 3-D image data. Disc space narrowing (DSN) in the IVDs adjacent to L1 was scored based on the Lane scoring system [33]. IVDs were also scored according to a new measure, the “apparent loss of disc integrity” (ALDI), that was developed for this study based on the appearance of the IVDs in transverse QCT slices (Fig. 2). As supported by subsequent histological examination, the presence of a dark central region in the QCT image of the IVD indicated a relatively healthy nucleus pulposus, as would be expected based on the high water content of this tissue in the healthy state. Lack of discernibility of the presumptive nucleus pulposus in the image and/or the appearance of

fissures and clefts were taken to indicate degenerative changes. As with DSN, ALDI scores range from 0 to 2, with a higher score indicating increased degenerative changes (Fig. 2).

The subsequent histological examination for comparison to ALDI scores involved first extracting a mid-sagittal section (~7-mm-thick) of the T12–L1 and L1–L2 IVDs, inclusive of endplates and adjacent subchondral bone, using a low-speed saw (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL). This extraction was performed after mechanical testing of the spine segments. After extraction, the specimens were fixed in 10 % paraformaldehyde for 8 days (solution changed every 2 days) and decalcified in Morse's solution (10 % sodium citrate, 20 % formic acid) for 12 days (solution changed daily). The specimens were then progressively dehydrated in ethanol solutions of increasing concentration (up to 70 %) and were embedded in paraffin. Ten-micrometer-thick, sagittal sections were taken on a microtome (Leica Biosystems, Buffalo Grove, IL), mounted on microscope slides, cleared in xylene, rehydrated, and finally stained with Fast Green, Alcian Blue, Safrinin-O, and Tartrazine (FAST) [34].

Statistical analyses

A significance level of 0.05 was used in all statistical analyses. Pairwise Pearson correlation coefficients were calculated for the different measures of bone density and intravertebral heterogeneity in density measured by QCT vs. μ CT.

Linear regression analyses (JMP 9.0, SAS Institute Inc., Cary, NC) were used to determine the dependence of ultimate force on each of the following combinations of explanatory variables: (1) average vBMD $\times$ CSA, (2) average vBMD $\times$ CSA and IQR_{BMD}, (3) average vBMD $\times$ CSA and QCV_{BMD}, (4) aBMD, and (5) EA_{min}. To examine the additional, predictive effect of accounting for intravertebral heterogeneity in density, restricted vs. full *F*-tests were used to compare regression models 1 and 2, and to compare models 1 and 3. *J*-tests [35] were used to rank the five regression models from best to worst predictive performance. The regression models listed above were also investigated with donor age and sex as additional explanatory variables.

A hierarchical cluster analysis was performed on the five density ratios (JMP 9.0). This analysis identifies groups of specimens exhibiting similar density ratios to one another such that the extent of similarity is high within each group and low between groups. The process begins by placing each specimen in its own cluster. Then at each step, by quantifying the similarity between clusters, the two clusters that are closest together are merged into a single cluster. The measure of similarity, or distance, is a single number representing the squared difference between two clusters' mean density ratios, summed over the five density ratios and normalized by the number of specimens in the two clusters (Ward's method). The process continues until all specimens are contained in one cluster. The number of clusters that results in a natural division of specimens into distinct groups, each with a high degree of similarity among group members, is the number of clusters at which further merging of clusters produces a sharp increase in the distance between the two clusters being merged.

The clusters identified from the analysis just described were examined with respect to the density-adjusted bone strength, defined as the residual from the regression of strength

against the product of average vBMD and CSA (i.e., the residual from regression model 1). The clusters were also examined with respect to IQR_{BMD} , QCV_{BMD} , and disc scores. Differences among clusters in each of density-adjusted strength, IQR_{BMD} , and QCV_{BMD} were determined using analysis of variance. Differences among clusters in disc scores were determined using contingency analysis. IQR_{BMD} , QCV_{BMD} , and density-adjusted strength were classified as “high,” “average,” or “low” according to the upper, middle two, and lower quartiles, respectively.

One specimen was excluded from the study. The sample was misaligned during mechanical testing (male, age 82).

Results

Comparison of QCT and μ CT measures of intravertebral heterogeneity in density

QCT-based measures of the intravertebral heterogeneity in density were correlated to those measured using μ CT. IQR_{BMD} was strongly correlated to IQR_{App} and $IQR_{BV/TV}$ ($R > 0.814$, $p < 0.001$). QCV_{BMD} was correlated to QCV_{App} and $QCV_{BV/TV}$ ($R > 0.504$, $p < 0.005$) (Table 1). Average vBMD was also correlated to average BV/TV and average ρ_{App} ($R > 0.612$, $p < 0.001$; Table 1). For both QCT- and ρ CT-based measurements, the magnitude of the intravertebral heterogeneity in density (IQR and QCV) varied widely among vertebrae (Fig. 3a).

Predictions of vertebral strength

Accounting for the magnitude of the intravertebral heterogeneity in density, as measured by IQR , in addition to mean density, significantly improved strength predictions. Model 2 outperformed model 1 ($R^2 = 0.59$ vs. $R^2 = 0.43$; F -test p -value = 0.018) and was the best performing regression model (Fig. 3b, Table 2). In contrast, model 3, which used QCV_{BMD} as the heterogeneity measure, performed equivalently to model 1 ($R^2 = 0.47$ vs. $R^2 = 0.43$; F -test p -value = 0.237). The model based on aBMD (model 4; $R^2 = 0.22$, $p = 0.030$) was inferior to models 1–3, and EA_{min} (model 5) was not predictive of vertebral strength ($R^2 = 0.01$, $p = 0.730$; Table 2). Notably, the results of model 2 indicated that after adjusting for average vBMD $\times$ CSA, strength was *positively* correlated with IQR_{BMD} , i.e., vertebrae with greater heterogeneity in density were stronger than those with lesser heterogeneity for the same amount of bone.

Accounting for the magnitude of the intravertebral heterogeneity, density also tended to improve the R^2 value when donor age and sex were considered. For example, including IQR_{BMD} in addition to average vBMD $\times$ CSA, age and sex as explanatory variables improved the R^2 value from 0.44 ($p = 0.019$) to 0.59 ($p = 0.005$; F -test $p = 0.027$). In this expanded model, sex and age were not significant variables ($p > 0.783$). Similar results were found for QCV_{BMD} .

The above findings regarding the predictive power of measures of intravertebral heterogeneity were not specific to the 5-mm cube size. Values of IQR_{BMD} computed for the different cube sizes were all correlated with each other ($p < 0.025$ for all cube sizes). Values of QCV_{BMD} were correlated for all cube sizes ($p < 0.049$) except for the 8.13-mm cube size

for which no correlations with the QCV values from other sizes were found ($p>0.065$). Regressions of vertebral strength against IQR_{BMD} in addition to average $vBMD \times CSA$ were significant for all cube sizes ($p<0.004$). However, the added contribution of IQR_{BMD} to the models was only significant for cubes of size 4.38 and 5.63 mm (F -tests: $p<0.044$). For QCV_{BMD} , none of the cube sizes yielded improved predictions of strength as compared to the regression model with only average $vBMD \times CSA$ ($p>0.146$).

Correspondence among spatial distributions of bone density, vertebral strength, the magnitude of the intravertebral heterogeneity in density, and IVD health

Five characteristic spatial distributions were identified by cluster analysis (Fig. 4a–e). No single distribution was associated with high density-adjusted strength or high IQR_{BMD} . Instead, the vertebrae with high density-adjusted strength and/or high IQR_{BMD} were distributed among four of the clusters (clusters 1, 2, 3, 5; Fig. 4a–c, e). The remaining spatial distribution, cluster 4, contained vertebrae with lower density-adjusted strength and lower IQR_{BMD} as compared to clusters 1 and 3 ($p<0.02$), respectively. Cluster 4 was characterized by a high A/P ratio, and over all vertebrae, this ratio was inversely correlated with adjusted strength ($p<0.01$) (Fig. 4f). QCV_{BMD} values were higher in cluster 2 compared to 1 ($p=0.043$). No other differences in QCV_{BMD} were found among clusters ($p=0.216$).

No differences in IVD scores were found among clusters ($p>0.493$). However, clusters 2 and 5 (Fig. 4b, e) did not contain any vertebrae with severe DSN, and the common features of their spatial distributions of density were relatively high C/O and M/L ratios. In contrast to the vertebrae in cluster 2, those in cluster 5 did not have healthy ALDI scores and tended to have higher S/T ($p=0.052$) and I/T ($p=0.052$) ratios. Further, for clusters 2 and 5, vertebrae with high density-adjusted strength tended to have lower DSN score, and/or those with low density-adjusted strength tended to have higher DSN score, relative to the other vertebrae in the same cluster.

Discussion

The intravertebral distribution of bone density is a key determinant of how loads transferred from the IVDs are borne by the vertebra. Thus, investigation of the interplay among the spatial distribution of density within the vertebra, IVD health, and vertebral strength has many implications for understanding the biomechanical mechanisms of vertebral fracture and for noninvasive assessments of vertebral strength and fracture risk. This study has advanced this line finding that the regression model with IQR_{BMD} outperformed the model that accounted only for average $vBMD$ and CSA , demonstrate the potential for using QCT-based measurement of IQR_{BMD} to improve predictions of vertebral strength. A key part of this result was that after adjustment for mean density, vertebral bodies with greater heterogeneity in density (i.e., larger values of IQR_{BMD}) exhibited *higher* vertebral strength under axial compressive loading. Second, we found that high IQR_{BMD} and high density-adjusted strength were not associated with any one particular spatial distribution of density within the vertebra (i.e., one cluster), but rather multiple spatial distributions. Third, we found that for two of the characteristic spatial distributions (clusters 2 and 5), the density-adjusted strength showed some correspondence with DSN. If IVD health is indicative of the

manner in which an applied compressive load is distributed across the endplate [19, 20], then this correspondence suggests that, after adjustment for mean density, the strength of the vertebra may depend on how well matched the locations of high regional densities are to the locations subjected to a greater fraction of the applied load. Together, these results indicate that noninvasive predictions of vertebral strength can benefit from considering the magnitude of the intravertebral heterogeneity in density and that further benefits may come from considering the congruence between the specific spatial distribution of density throughout the vertebral body and the health of the adjacent IVDs.

The primary strength of this study lies in the direct comparison of the spatial distributions of bone density to vertebral strength and IVD health. Moreover, because these comparisons used a measure of vertebral strength that was adjusted for average vBMD and CSA, the analyses could examine the role of the spatial heterogeneity in density within the vertebral body without the confounding influence of differences in average density and bone size. These comparisons enabled us to delve into the intriguing finding that greater IQR_{BMD} was positively correlated with vertebra strength, even after accounting for average vBMD and CSA. Although prior studies have suggested that smaller amounts of heterogeneity might confer higher bone strength [11, 12], larger amounts of heterogeneity could be advantageous if the particular spatial distribution of bone density matches the way that load is distributed throughout the vertebral body. For example, prior measurements have shown that in erect spinal postures, less than half of the total load applied to the vertebral body is distributed over the anterior half, and that this fraction decreases with age [17, 28]. Vertebral bodies with higher density posteriorly than anteriorly would be expected to exhibit higher strength under this type of load distribution; this supposition is supported by the inverse correlation shown in Fig. 4f. In addition, from experimental studies and computer simulations, a prevailing hypothesis has emerged that IVD degeneration results in transfer of more of the applied load to the outer regions of the vertebral body, thus causing resorption in the central and mid-transverse regions [14, 24, 25, 27]. Consistent with this hypothesis, our results show that in the two clusters with an absence of severe DSN (clusters 2 and 5), the vertebrae exhibited relatively high densities in the central and medial regions, and among these vertebrae, those with poor ALDI scores tended to have relatively low densities in the mid-transverse region. It is important to note that different activities and postures, such as forward flexion [17], may change the extent of the correspondence between load distribution and density distribution.

Just as congruence between the spatial distribution of density and the way in which the IVDs distribute the load across the vertebral endplates supports the role of bone adaptation in producing the observed clusters, a lack of such congruence may jeopardize vertebral strength. For example, the combination of healthy IVDs and a spatial distribution of density characterized by low density in the central and mid-transverse regions of the vertebral body has been suggested to be particularly prone to vertebral fracture [25]. Our results also indicate that the opposite spatial distribution (high density in the central and mid-transverse regions), when combined with moderate DSN, tended to be associated with low density-adjusted strength. This notion of incongruence may be relevant to the conflicting reports that exist as to whether IVD degeneration is a risk factor for, unassociated with, or protective against vertebral fracture [36–38]. Continued examination of associations among spatial

distributions of density, IVD degeneration, and vertebral strength may help resolve this conflict.

Several limitations of this study should be mentioned. First, the QCT scans performed here would subject patients to higher radiation dose as compared to DXA. However, given that QCT is now a standard research tool in large-scale studies of the epidemiology of, and interventions for, osteoporosis, the methods used herein could be applied to these patient cohorts in both cross-sectional and longitudinal investigations to provide a powerful examination of the correspondence among spatial heterogeneity in density, IVD degeneration, and vertebral strength. Second, due to the size restrictions of the μ CT scanner and the need to have μ CT scans to validate the QCT-based measurements of heterogeneity, the posterior elements were removed prior to testing, thus rendering the applied loading somewhat non-physiologic. Future studies using newer μ CT systems that can accommodate larger specimens are needed to examine the effect of load transmission through the facet joints on the results presented here. Another consequence of removing the posterior elements was that simulating anterior–posterior DXA measurements would be much less physiologic than simulating the less commonly used lateral DXA measurements. However, recent studies have shown that aBMD measured by lateral DXA is more strongly correlated with vertebral strength than PA DXA [8, 39], which suggests that the regression models with IQR_{BMD} would also outperform a model using aBMD measured by PA DXA. Third, the applied loading was stepwise, and it is possible that the failure mechanisms differed as compared to the case of application of a steadily increasing load until failure. No experimental method for visualizing failure mechanisms for the latter type of loading has been reported; however, the ultimate loads recorded here (2.2 ± 0.5 kN) are in agreement with previously published data for continuous loading of lumbar vertebral segments [40]. This type of agreement has also been shown for stepwise vs. continuous loading of trabecular bone [41]. Fourth, although the ALDI scores were assigned based on the consensus among four graders, this new scoring system has not yet been fully validated with respect to inter- and intra-observer reliability. Finally, there is some subjectivity in the choice of the number of clusters in hierarchical cluster analysis. Had we chosen four rather than five clusters, then clusters 3 and 4 would have been combined, and the four clusters would not differ among each other with respect to density-adjusted strength or IQR_{BMD} ($p > 0.31$).

The outcomes of this study extend prior investigations on the biomechanical implications of intravertebral heterogeneity in density. Many of the present results, such as the effect of cube size on the measures of heterogeneity and the relatively poor predictive performance of axial rigidity, are in agreement with our previous results using μ CT [10]. The only substantive disagreement with those earlier, μ CT-based results is that those results showed that both measures of the magnitude of the intravertebral heterogeneity in density, IQR and QCV, improved predictions of vertebral strength. An important, additional point of agreement is that both of these studies have found that the IQR is positively correlated with vertebral strength—even after accounting for average density and CSA—as a result of the presence of a larger number of cubes of high density in the vertebrae with large IQR (note the positive skewness in the high-IQR distribution shown in Fig. 3a). That no single spatial distribution of density within the vertebra (Fig. 4) was associated with high density-adjusted

strength suggests that there are multiple combinations of locations of these high-density cubes that can be beneficial to bone strength. Robust identification of these combinations, which will require consideration of multiple loading conditions and IVD health, stands to contribute substantially to current understanding of the pathogenesis of vertebral fractures and to development of more powerful predictors of bone strength and fracture risk.

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Fig. 1.

a Experimental setup for mechanical testing; **b** representative load–displacement curve; **c** representative, transverse QCT cross-section showing the locations of the elliptical cylinder and one layer of the 5-mm cubes; **d** lateral projection of a QCT image stack showing the region used for estimating aBMD as would be measured by lateral DXA; **e** partitioning of the vertebral body into 27 regions created by dividing the vertebral body in thirds along each anatomic direction, and subsequent pooling of these regions for the purpose of computing ratios of regional densities (*gray shading* is for visualization only and does not indicate systematic regional differences in densities)

Fig. 2.

ALDI scoring system: shown at the *left* in each of the three rows is a transverse QCT slice of the IVD. The ALDI score is shown at the *top left*, and *below the image* is a description of the features of the appearance of the IVD in the QCT image that merit that score (*AF* annulus fibrosus; *NP* nucleus pulposus). Scoring is based primarily on the appearance of the NP and AF and secondarily on the presence or absence of osteophytes. *Yellow, orange, and red* regions are portions of the endplates and calcification within the IVD; no osteophytes were present in the QCT images of the mid-sections of these three IVDs. Shown at the *top* and *bottom right* in each row are a corresponding optical image and histological section (FAST staining), respectively, of a sagittal cross section of the IVD. The QCT image was acquired before mechanical testing, and the optical images and histological sections were obtained after mechanical testing

Fig. 3.

a Representative histograms and box-and-whisker plots showing the distribution of cube BMD values (normalized by the total number of values, to give relative frequency) for the cubes in two vertebrae, one with low IQR_{BMD} and another with high IQR_{BMD} : In each box-and-whisker plot, the *left side of the box* is positioned at the 25th percentile, the *right side* is positioned at the 75th percentile, and the *solid and dashed lines* within the box represent the median and mean of the values, respectively. The lower “whisker” extends down to either the smallest value or to the 25th percentile minus 1.5 times the interquartile range, whichever produces a shorter whisker. If the latter produces a shorter whisker, then the lower values are marked as individual points. The upper “whisker” extends up to either the largest value or to the 75th percentile plus 1.5 times the interquartile range, whichever produces a shorter whisker. If the latter produces a shorter whisker, then the higher values are marked as individual points. For example, for the low IQR_{BMD} specimen, the left whisker extends down to the smallest value, and the right whisker extends up to the 75th percentile plus 1.5 times the interquartile range. **b** Measured ultimate force vs. the ultimate force predicted from average $vBMD \times CSA$ and IQR_{BMD} (model 3). The regression result for the model without IQR_{BMD} (model 1) is shown for comparison

Fig. 4.

a–e Five characteristic, spatial distributions of density within the vertebral body, shown for the L1 vertebrae: *thicker lines* are for vertebrae with high density-adjusted strength. *Line color* denotes average ALDI score for the two adjacent IVDs (blue “inverted letter V” = 0, green “letter X” = 1, red “circle” = 2). *Line style* denotes average DSN score (solid = 0, dashed = 1, dotted = 2); **f** A/P density ratio is negatively correlated with density-adjusted strength ($p < 0.014$). Symbols show ALDI and DSN scores, each averaged over the two adjacent IVDs of inquiry in several ways. First, the strong correlations between QCT- and μ CT-based measures of the magnitude of the intravertebral heterogeneity in density, as well as the

Table 1Correlations between density measures calculated from QCT and μ CT images

QCT measure	μ CT measure	Correlation coefficient	<i>p</i> -value
IQR _{BMD}	IQR _{BV/TV}	0.814	<0.001
IQR _{BMD}	IQR _{App}	0.833	<0.001
QCV _{BMD}	QCV _{BV/TV}	0.504	0.005
QCV _{BMD}	QCV _{App}	0.725	<0.001
Average vBMD	Average BV/TV	0.612	<0.001
Average vBMD	Average ρ_{App}	0.656	<0.001

Table 2

Regressions of vertebral strength against measures of average density with and without IQR_{BMD}, aBMD, and EA_{min} ($n=29$)

Model	Explanatory variable(s)	R^2	p -value	F -test p -value ^a	Rank
1	Average vBMD×CSA	0.43	0.001 [*]		2
2	Average vBMD×CSA, IQR _{BMD}	0.59	0.001 [*]	0.018 ⁺	1
3	Average vBMD×CSA, QCV _{BMD}	0.47	0.003 [*]	0.237	2
4	aBMD	0.22	0.03 [*]		3
5	EA _{min}	0.01	0.73		N/A

The last column shows the rank based on F -tests (rank 1 is the best model; rank 3 is the worst model)

N/A not applicable, since the regression was not significant

^{*} $p<0.05$ (regression is significant);

⁺ $p<0.05$ (F -test indicates an improvement in the R^2 value when IQR_{BMD} is added to regression model 1)

^aThe R^2 value when IQR_{BMD} or QCV_{BMD} are added to regression model 1