Evaluation of trabecular mechanical and microstructural properties in human calcaneal bone of advanced age using mechanical testing, μCT, and DXA

Erik Mittra\textsuperscript{a}, Clinton Rubin\textsuperscript{b}, Barry Gruber\textsuperscript{c}, Yi-Xian Qin\textsuperscript{b,}\textsuperscript{*}

\textsuperscript{a}Department of Radiology-Nuclear Medicine, Stanford University Medical Center, 300 Pasteur Dr., Rm. H0101, Stanford, CA 94305, USA
\textsuperscript{b}Department of Biomedical Engineering, Stony Brook University, NY, USA
\textsuperscript{c}Department of Internal Medicine, Stony Brook University, NY, USA

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Abstract

Early detection of fracture risk is important for initiating treatment and improving outcomes from both physiologic and pathologic causes of bone loss. While bone mineral density (a quantity measure) has traditionally been used for this purpose, alternative structural imaging parameters (quality measures) are proposed to better predict bone's true mechanical properties. To further elucidate this, trabecular bone from cadaveric human calcanei were used to evaluate the interrelationship of mechanical and structural parameters using mechanical testing, dual energy X-ray absorptiometry (DXA) scanning, and micro computed tomography (μCT) imaging. Directional specific structural properties were assessed in three-dimensional (3-D) and correlated to mechanical testing and DXA. The results demonstrated that μCT-derived indices of bone quality (i.e., volume fraction and structural model index) are better than DXA-derived bone mineral density for the prediction of the mechanical parameters of bone (i.e., elastic modulus, yield stress, and ultimate stress). Diagnostically, this implies that future work on the early prediction of fracture risk should focus as much on bone quality as on quantity. Furthermore, the results of this study show that a loss of bone primarily affects the connectedness and overall number of trabeculae. Ultimate stress, however, is better correlated with trabecular number than thickness. As such, primary prevention of osteoporosis may be more important than later countermeasures for bone loss.

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Keywords: Trabecular bone quality; Fracture; Osteoporosis; Microstructure; Mechanical stress; DXA; Bone strength

1. Introduction

The ability to accurately assess bone quality \textit{in vivo} is essential for improving the diagnostic and therapeutic goals for bone loss from such varied etiologies as osteoporosis, microgravity, bed rest, or stress shielding from an implant. Early diagnosis is particularly important as the effectiveness of treatment diminishes with disease progression, yet patients are rarely symptomatic before considerable bone loss has occurred (Davidson, 2003; Homminga et al., 2004).

As bone strength cannot be directly assessed \textit{in vivo}, indirect parameters provide surrogates for diagnosis. In general, the evaluation of bone status is difficult because the bone changes are initially quite small, may not be highly related to apparent density, and are effectively localized to region (i.e., one femoral head but not the other) and type of bone (i.e., trabecular but not cortical). The current focus of clinical bone evaluation is apparent bone mineral density (BMD) as measured by dual energy X-ray absorptiometry (DXA) (Kanis et al., 2002; Brunader and Shelton, 2002). As it provides only a 2-D image of apparent density, however, its ability to assess early fracture risk is limited. While density (quantity) positively correlates with strength (Keaveny et al., 2001), about 30% of the variability in bone strength remains unexplained.
DXA also cannot differentiate trabecular from cortical bone. This is unfortunate since the effects of bone loss are more prevalent in trabecular bone due to its higher surface area, and because the greater amount of bone mineral content in cortical bone can conceal small changes in trabecular bone (Brismar et al., 2001).

Increasingly, evidence shows that bone quality is important for the evaluation of bone status (Grimm and Williams, 1997; Pothuaud et al., 2002). While the idea of quality is accepted, it remains ambiguous in practice. In essence, quality refers to a combination of bone apparent modulus and strength as well as some quantified measure of the trabecular geometry, stereology, micro-damage, material (tissue) and chemical properties of the bone (Judex et al., 2003).

Other diagnostic modalities (e.g., peripheral DXA, single energy X-ray absorptiometry, radiographic absorptiometry, and single and dual photon absorptiometry) have similar shortcomings as DXA as they are reliant on the same underlying technology. The broadband ultrasound attenuation and ultrasound velocity coefficients of quantitative ultrasound have been significantly correlated to both density and bulk mechanical properties, respectively, but does not directly provide structural or quantity measures and so primarily serves as a screening tool (Faulkner, 2001; Nichols et al., 2000).

Few diagnostic modalities have the ability to evaluate both the quantity and quality of trabecular bone equally well, except perhaps the newer generation of high-resolution computed tomography (CT) and magnetic resonance imaging (MRI) scanners (Genant et al., 1999). The CT-based scanners include peripheral QCT (clinical) and micro CT (μCT) (research), while the MR-based modalities include general MRI scanners (clinical) and high-resolution MRI (research). Considering only the higher-resolution research scanners, the CT-based machines have better resolution (e.g., 20 × 20 × 20 μm$^3$ voxel size for μCT), but are limited by their high radiation dose and small allowable sample size. MR-based scanners have lower resolution (156 × 156 × 300 μm$^3$ voxel size for hrMRI), but are not irradiating and could therefore more easily be used in vivo (Brismar et al., 2001).

μCT, which has a limit of approximately 10 μm, provides the best resolution short of using a synchrotron light source (1 μm limit) and has therefore become the imaging modality of choice in research. Though not used clinically yet, μCT has been adapted for in vivo use in humans (Kohlbrener and Ruegsegger, 2000). Therefore, understanding the clinical applicability of these very high-resolution μCT images is of value.

The objective of this study is to understand the relative effects of bone quantity and structure on apparent mechanical properties, i.e., elastic modulus, yield strength, and ultimate strength. The following two aims are pursued. First, a comprehensive analysis of all the μCT indices is undertaken (and compared with DXA) to understand which indices best predict trabecular bone strength. Second, the mechanical implications of architecture versus quantity provided by these indices is considered in terms of their clinical importance.

### 2. Methods

The trabecular bone samples for this study were obtained from the calcanei of 19 human cadavers (12 right, 7 left). The age and sex of two cadavers was unknown. The remaining 17 include seven males and 10 females with a mean age of 84 years (range: 66–97 years).

#### 2.1. DXA

After being harvested from the whole foot, but prior to any further manipulations, the intact calcanei were scanned in a DXA machine (Hologic QDR 4500A) with lumbar spine settings in array mode (100 and 140 kVp, 2.5 mA average). BMD was measured for the whole bone, as well as a region of interest (ROI) corresponding to the area in the posterior section from where the cylindrical bone would be subsequently harvested. The correlations in this paper were done only with the BMD from the posterior ROI. As the calcaneus is not scanned clinically, reference ranges are unavailable. Because of this, the experimental bones could not be classified as normal, osteopenic, or osteoporotic based on T and Z scores. However, given the advanced age of all the samples, they are likely to be at least osteopenic if not osteoporotic.

#### 2.2. Bone harvesting

Specimens of trabecular bone were harvested from the posterior region of each calcaneus using a low-speed diamond blade saw (Microslice, Metals Research Limited, Cambridge, England) with continuous water irrigation. All harvesting was performed by one person and standard visual guidelines were used. The bones were extracted as cylinders, 14 mm in diameter and up to 20 mm long (in the mediolateral direction) as depicted in Fig. 1a. The mediolateral dimension varied somewhat as the calcanei varied in width.

After harvesting, the specimens were stored in equal amounts normal saline and 70% ethanol at 4 °C. This storage method was chosen based upon the previous work of Ashman and colleagues in which they harvested cancellous bone specimens for mechanical testing and ultrasound studies (Ashman et al., 1984, 1985, 1987, 1989). Additional information related to our storage protocol and its methodological implications can be found on the supplemental website.

#### 2.3. Materials testing

Direct force–displacement testing was used to determine the Young's (elastic) modulus and strength of the bulk specimens. Using a mechanical testing machine (MTS Systems Corporation, PA), the bone cubes were compressed in the mediolateral direction using displacement control. To overcome slight deviations from surface parallelism, a smoothly curved nail head was placed above the bone specimen such that the force would be distributed evenly to the bone (Fig. 1b). An upper limit of 300 N of force—determined by prior loading of non-experimental but otherwise identical bone samples—was established to prevent the plastic yielding of any specimens. The loading rate was approximately 1000 μm/s. Prior to data collection, several preconditioning cycles of 1% strain was used to overcome edge effects from the harvesting process. This preconditioning consisted of at least five cycles, and was stopped once the preload stabilized at around 10 N or reached eight cycles (whichever came first). Subsequently, three experimental compressions of 1% strain were done and the final result was taken to be the average of these three values.

Both displacement and force was digitized using MTS BasicTestware software. Force–displacement was converted to an analogous stress–strain...
curve by dividing force by the cross-sectional area, and displacement by length (each sample was measured independently to reduce the geometrical error inherent in harvesting). After scanning in the μCT, the bones were tested to failure in the mediolateral direction. The material properties studied include modulus, yield strength (calculated using the 0.2% strain offset method), and ultimate strength.

2.4. Micro computed tomography

After the initial materials testing, the bones were scanned in a μCT machine (μCT-20, Scanco Corp.) with a voxel resolution of 34 μm in all axes. A sample image from the μCT machine is shown in Fig. 1c of a bone cube with dimensions of 1 × 1 × 1 cm³. The μCT properties analyzed (using built-in code) include bone volume over total volume (BV/TV), mean intercept length (MIL), degree of anisotropy (DA), structural model index (SMI), trabecular separation (TbSp), trabecular number (TbN), trabecular thickness (TbTh), and connectivity density (ConnD). Brief definitions of these microstructural indices are given in Table 1. In addition, the MIL analysis ultimately gives the anisotropy tensor from which the principal trabecular axis (PTA) (the highest ranked eigenvalue) can be derived (Cowin, 1985). The complete explanation of the various μCT indices and their respective derivation/calculation is found in several sources (Fajardo and Muller, 2008).

Table 1

<table>
<thead>
<tr>
<th>Indices</th>
<th>Abbrev.</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Bone mineral density</td>
<td>BMD</td>
<td>2-D derivation of mineral density (g/cm²) as derived by DEXA</td>
</tr>
<tr>
<td>Bone volume fraction</td>
<td>BV/TV</td>
<td>Relative percentage of bone within 3-D ROI</td>
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<tr>
<td>Connectivity density</td>
<td>ConnD</td>
<td>Quantification of relative connectedness of one trabeculae to the next</td>
</tr>
<tr>
<td>Structural model index</td>
<td>SMI</td>
<td>Quantification of relative shape of trabeculae from rod-like to plate-like</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>TbN</td>
<td>Quantification of relative number of individual trabeculae within 3-D ROI</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>TbTh</td>
<td>Quantification of relative thickness of individual trabeculae within 3-D ROI</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>TbSp</td>
<td>Quantification of relative spacing between individual trabeculae within 3-D ROI</td>
</tr>
<tr>
<td>Mean intercept length</td>
<td>MIL</td>
<td>Quantification of the three-dimensional anisotropy of a 3-D ROI. These numbers provides eigenvectors based on the fabric tensor that defines the principal direction of the ROI.</td>
</tr>
<tr>
<td>Degree of anisotropy</td>
<td>DA</td>
<td>Ratio of the largest to the smallest MIL value. It provides another way to quantify the relative anisotropy of the ROI.</td>
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</tbody>
</table>

Fig. 1. (a) A sagittal view of a calcaneus with the lateral cortical shell removed, with lines indicating the location and orientation of trabecular specimen harvesting (either cube or cylinder); (b) a schematic close-up of the bone cube placement within the MTS machine, showing the nail head (black) above the bone; (c) a sample image of a trabecular bone cube from the Scanco μCT-20.
2001; Hildebrand et al., 1999; Hildebrand and Ruegsegger, 1997a, b) and so is not repeated here.

It should be noted that the 'quantity' data that μCT provides is different than that from DXA. μCT gives density as BVF (i.e., bone volume/total volume (BV/TV)) while DXA gives a value for mineral density (g/cm²). However, recent studies (Dalstra et al., 2001) have shown the ability to use the greyscale images from μCT to derive the bone mineral content as well. Our thresholding methodology was based on converging the analyzed image (using different thresholds) to the original digital (greyscale) image of the trabecular bone.

2.5. Statistics

The data were analyzed using Analyze-it version 1.67. Comparisons of like indices were analyzed with a one-way ANOVA with Tukey posthoc pairwise comparisons. Unrelated indices were compared with multiple linear regression and Pearson’s product moment correlation coefficient. In all cases, the significance level was set at \( p < 0.05 \).

Table 2

Average values for select μCT, DEXA, and mechanical indices

<table>
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<tr>
<th>n</th>
<th>BV/TV</th>
<th>BMD (g/cm²)</th>
<th>Elastic modulus (MPa)</th>
<th>Yield stress (MPa)</th>
<th>Ultimate stress (MPa)</th>
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<tr>
<td>19</td>
<td>0.14 ± 0.05</td>
<td>0.40 ± 0.16</td>
<td>70 ± 59</td>
<td>1.8 ± 1</td>
<td>1.9 ± 1</td>
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Table 3

\( r^2 \) values (with signs) between all mechanical and microstructural indices analyzed, as well as age

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<th>YS</th>
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<th>BV/TV</th>
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<th>SMI</th>
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Note: \( r^2 \) values without associated \( p \)-values indicate \( p < 0.001 \) (regular font), or \( p > 0.05 \) (italics font).

3. Results

The average values for BVF, modulus, and strength are shown in Table 2 and are consistent with the literature (Keaveny et al., 2001).

Table 3 lists the coefficients of determination between all the variables studied. Note that where \( p \)-values are not given in the following text, \( p < 0.001 \). First, with regard to the experimentally derived mechanical parameters, the elastic modulus \( (E) \) correlates well with both yield stress \( (\sigma_y) \) \( (r^2 = 0.72) \) and ultimate stress \( (\sigma_u) \) \( (r^2 = 0.69) \). Also, \( \sigma_y \) and \( \sigma_u \) are strongly correlated \( (r^2 = 0.98) \). These correlations are expected and provide an intrinsic validity to our mechanical testing results.

Age did not correlate significantly with any index except for (DXA derived) BMD, and even so relatively poorly \( (r^2 = 0.28, p = 0.0276) \). BMD, however, correlated significantly with all indices except the DA \( (r^2 = 0.20, p = 0.0551) \), and especially well with BVF \( (BV/TV) (r^2 = 0.74) \) and SMI \( (r^2 = 0.64) \).

Of the μCT-derived structural parameters, BV/TV and SMI consistently correlated better than other structural parameters (or BMD) with respect to all mechanical indices, including the most clinically important one of ultimate stress \( (\sigma_u) \). As might be expected, then, BV/TV and SMI were highly correlated with each other.
They both correlated strongly with $s_y$ ($r^2 = 0.77$) and $s_u$ ($r^2 = 0.76$), and less so with $E$ ($r^2 = 0.42$). The correlation coefficient of BMD with these same mechanical parameters were much lower: $s_y$ ($r^2 = 0.49$) and $s_u$ ($r^2 = 0.48$), and $E$ ($r^2 = 0.25$).

Considering the other structural indices, TbN correlated well with TbSp ($r^2 = 0.96$), but not significantly with TbTh ($r^2 = 0.00$, $p = 0.9907$). ConnD correlated well with both TbN ($r^2 = 0.90$) and TbSp ($r^2 = 0.86$) and not significantly with TbTh ($r^2 = 0.04$, $p = 0.44$). The DA correlated best with SMI and poorly with BV/TV, MIL 1, and MIL 3.

Correlation of the three MIL values (a quantification of the 3-D anisotropy of the trabeculae) is most notable for the observation that the principal (MIL 1) and tertiary (MIL 3) indices are similarly and quite well correlated with ultimate strength ($r^2 \approx 0.63$), as well as BMD ($r^2 \approx 0.70$), BV/TV ($r^2 \approx 0.87$), and SMI ($r^2 \approx 0.83$). As would be expected, they correlate well together ($r^2 = 0.92$). However, MIL 2 correlates very poorly with most indices except BMD ($r^2 = 0.42$).

These 3-D MIL values were also used to calculate the PTA of a subset of 7 of these calcanei of advanced age. The PTA was calculated as the direction associated with the largest eigenvalue of the fabric tensor (derived from MIL analysis of the $\mu$CT image). Due to methodological limitations, this vector could only be calculated with respect to the longitudinal axis of the calcaneus and could not be further evaluated with respect to its mediolateral deviation. As shown in Fig. 3, the PTA lies $23^\circ \pm 16^\circ$ superior to the longitudinal axis. This is similar to the value reported in the literature of $19^\circ \pm 7^\circ$ (Biewener et al., 1996) for healthy bone.

4. Discussion

To elucidate the interrelationship between trabecular mechanical and microstructural properties is important for two principal reasons—to better understand the mechanical consequences of trabecular remodeling, and for the potential improvement of diagnostic measures of bone loss. The results of this study show that non-invasively assessing these parameters of quality can provide a significant predictive ability beyond just the measurement of apparent density.

Of the mechanical parameters analyzed, ultimate strength ($\sigma_u$) is the most relevant clinically and thus the most useful to predict as it represents the point of trabecular fracture. As such, of all the data in Table 3, perhaps the most important are the correlations of ultimate strength to the various indices of bone quality and quantity. As shown in Fig. 3, the most notable finding is that five different purely imaging based microstructural
parameters (BV/TV, ConnD, SMI, TbSp, and TbN) better predicted the point of fracture than did the BMD derived from DXA. That BV/TV (the 3-D μCT surrogate for apparent bone density) performed well is not surprising, but the fact that SMI (a purely architectural index) performed equally well is more so. This is of diagnostic interest since it implies that the focus on bone density for analysis of bone viability may be too limited and that trabecular architecture (i.e., quality) may be equally or more useful for the prediction of bone status. Additionally, it is not surprising that DXA also performs more poorly than μCT indices with respect to yield strength ($\sigma_y$) (BMD: $r^2 = 0.49$, BV/TV: $r^2 = 0.77$) and elastic modulus ($E$) (BMD: $r^2 = 0.25$, BV/TV: $r^2 = 0.42$). These observed differences are likely due to the shortcomings of DXA with respect to its inability to differentiate trabecular from cortical bone, it’s 2-D representation of a 3-D structure, and it’s focus only on apparent BMD rather than indices of bone quality.

The strong correlation between BV/TV and ConnD ($r^2 = 0.71$) is understandable given the close relationship between bone volume and connectivity. Similarly, the even stronger correlation between BV/TV and SMI ($r^2 = 0.86$) implies a close connection between these indices. Most obviously, this suggests that the shape of individual trabeculae is sensitive to the amount of bone present. That is, a loss of bone causes trabeculae to become rod-like while a net increase in bone volume causes them to become plate-like. However, the algorithm for SMI may be sensitive to BV/TV such that it is better able to calculate this index for trabeculae when the bone volume is high and miss thinner plate-like trabeculae when the overall BVF is low.

With regard to the trabecular indices, the strongest correlations are between TbN with TbSp ($r^2 = 0.96$) and connectivity density ($r^2 = 0.90$). The correlation between TbSp and connectivity density is also high ($r^2 = 0.86$). These three trabecular indices were also significantly correlated to ultimate strength ($r^2 \geq 0.61$), and performed better than BMD ($r^2 = 0.48$). Their interconnectedness makes sense in that as trabeculae are lost, their connectivity decreases and the separation between them increases. Interestingly, TbTh, was not significantly correlated to any of the other trabecular indices or ultimate strength. It was significantly correlated, at a low level, only to SMI and BV/TV. Given that these calcanei are of advanced age, this shows that bone loss which ultimately leads to fragility fractures occurs primarily through the loss of trabeculae (with concomitant loss of connectivity and increasing intertrabecular space), but not as a result of trabecular thinning. This is an initially surprising result given the proposed physiology of bone loss occurring via increased osteoclastic activity relative to new bone formation. However, this discrepancy may be explained in that the bone loss leading to fragility fractures occurs in a specific orientation such that the trabeculae parallel to the loading axis are preserved, while the bone is lost in other orthogonal planes. This would lead to an overall reduction in TbN and connectivity, but given that trabecular struts in line with the loading axis are preserved, would confound the thickness index.

This hypothesis is supported by the analysis of the PTA of these calcanei. The PTA ($23 \pm 16^\circ$ superior to the longitudinal axis) in human calcanei of advanced age is in-line with the calculated principal strain axis ($19 \pm 7^\circ$ (Biewener et al., 1996)) in the calcaneus of healthy bone. This shows that reorientation of the primary trabecular axis does not occur with loss of bone, suggesting that trabeculae longitudinal to the loading axis are preserved. Also, by looking at the anisotropy (MIL) data, it can be seen that the principal (MIL 1) and tertiary (MIL 3) anisotropy indices are the best correlated with both mechanical and structural indices. This fits the expectation that the more extreme axes are better correlated with the mechanical and structural parameters they give rise to.

Of note, the DA index (DA; ratio of the largest to smallest MIL value) is significantly and positively correlated with BV/TV in this study suggesting that as bone volume decreases, the bone becomes less anisotropic. Even if true, this finding is mutually exclusive from the fact that the PTA is retained with bone loss, but would imply that the PTA is not preferentially retained (i.e., that bone loss occurs less in the loading axis). However, this finding is not in line with other reports in which osteopenic bone has a greater DA than normal bone (Newitt et al., 2002), and may also be related to the fact that the DA is underestimated when calculated by MIL (Badiei et al., 2006). As such, the value of DA itself is somewhat unclear in this study.

The MIL analysis above is supported by a similar analysis of mechanically augmented sheep bones from a related project (Mitra et al., 2005), which in turn is based on the prior work of Rubin et al. (2002). In comparing these studies (representing opposite ends of the bone density spectrum), the MIL correlations were stronger in the aged human than the mechanically augmented sheep bones, suggesting that these axes become more pronounced with bone loss. That is, trabecular bone seems to become more anisotropic as bone volume is lost. The remaining trabeculae likely persist in the original loading axes to maximize the force that can be carried by the weakened bone. This is likely why the PTA of the calcaneal samples from this study do not significantly differ from the principal strain axis of healthy calcaneal bone.

It is also interesting that in mechanically augmented sheep bone, ConnD correlated poorly with TbN ($r^2 = 0.14$) and TbSp ($r^2 = 0.01$), but slightly better with TbTh ($r^2 = 0.26$). The opposite is true for the human calcaneal samples from this study. Taken together, this implies that an increase in bone through therapeutics (mechanical augmentation) results in a thickening of existing trabeculae, but does not increase the number of trabeculae. Bone loss during disease, however, causes a reduction in the number of trabeculae, but does not cause such a clear decrease in thickness of existing trabeculae.
Mechanistically, this indicates that an increase in bone volume results in an increase in trabecular diameter (and a concurrent decrease in the space between trabeculae) but does not cause an increase in trabecular number or connectivity. Mechanical countermeasures for bone loss can increase the size of existing trabeculae but do not lead to the formation of new trabeculae. However, ultimate strength is correlated better with TbN ($r^2 = 0.61$) than with TbTh ($r^2 = 0.21$) suggesting that the maintenance of trabeculae is more important for the strength of the bone than is a subsequent increase in the thickness of remaining trabeculae. These conclusions would support the idea that prevention of bone loss may be more important than the subsequent treatment for it. However, it is acknowledged that these findings are based on a limited number of studies, and are therefore preliminary in nature.

While this discussion on the analyzed trabecular indices is insightful for understanding the mechanical alterations leading to fragility fractures, their correlations (with the exception of ConnD) are not as robust for the prediction of ultimate strength as is BV/TV or SMI. Most significant, then, for the future research and development of diagnostic modalities for osteoporosis and fracture-risk assessment, is the conclusion that 3-D BVF or even purely structural indices such as SMI are at least, if not better, at predicting the mechanical properties of bone. While DXA is a valid and ubiquitous modality, the inclusion of additional 3-D indices such as SMI are at least, if not better, at predicting the mechanical properties of bone. While DXA is a valid and ubiquitous modality, the inclusion of additional 3-D parameters of bone, ideally while excluding confounding cortical density information, would be a worthwhile adjunct for improving the predictive ability of future fracture risk. With the advent of in vivo μCT machines (from the efforts of several manufacturers), this goal is certainly within reach and should be implemented.

**Conflict of interest**

The authors of this manuscript (listed below) have no conflicts of interest or other disclosures.

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**Appendix A. Supporting Information**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbiomech.2007.09.003.

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