Speed of Sound Reflects Young's Modulus as Assessed by Microstructural Finite Element Analysis


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We analyzed the ability of the quantitative ultrasound (QUS) parameter, speed of sound (SOS), and bone mineral density (BMD), as measured by dual-energy X-ray absorptiometry (DXA), to predict Young's modulus, as assessed by microstructural finite element analysis (μFEA) from microcomputed tomography (μCT) reconstructions. With μFEA simulation, all bone elements in the model can be assigned the same isotropic Young’s modulus; therefore, in contrast to mechanical tests, only the trabecular structure plays a role in the determination of the elastic properties of the specimen. SOS, BMD, and μCT measurements were performed in 15 cubes of pure trabecular bovine bone in three orthogonal directions: anteroposterior (AP); mediolateral (ML); and cranio-caudal (CC). The anisotropy of the architecture was determined using mean intercept length (MIL) measurements. SOS, MIL, and Young’s modulus (E) values were significantly different in all three directions (p < 0.001), with the highest values in the CC direction. There was a strong linear relationship between E and SOS in each of the three orthogonal directions, with r² being 0.88, 0.92, and 0.84 (all p < 0.0001) for the CC, ML, and AP directions, respectively. The relationship between E and BMD was less strong, with r² being between 0.66 and 0.85 (all p < 0.0001) in the different directions. There was also a significant, positive correlation between SOS and BMD in each of the three orthogonal directions (r² being 0.77, 0.92, and 0.93 in the CC, ML, and AP directions, respectively; p < 0.0001). After correction for BMD, the correlations between SOS and E in each of the three directions remained highly significant (r² = 0.77, p < 0.0001 for the AP direction; r² = 0.48, p < 0.001 for the CC direction; r² = 0.52, p < 0.005 for the ML direction). After correction for SOS, BMD remained significantly correlated with Young’s modulus in the AP and CC directions (r² = 0.52, p < 0.005; r² = 0.30, p < 0.05, respectively), but the correlation in the ML direction was no longer statistically significant. In a stepwise regression model, E was best predicted by SOS in each of the orthogonal directions. These observations illustrate the ability of the SOS technique to assess the architectural mechanical quality of trabecular bone. (Bone 26: 519–524; 2000) © 2000 by Elsevier Science Inc. All rights reserved.

Key Words: Speed of sound (SOS); Ultrasound; Trabecular bone; Architecture; Microcomputed tomography (μCT); Finite element analysis.

Introduction

Osteoporosis is a skeletal disorder, characterized by a reduction of bone mineral density (BMD) that accompanies architectural deterioration. At present, the clinical assessment of osteoporosis relies mainly on BMD measurements by dual-energy X-ray absorptiometry (DXA) or quantitative computed tomography (QCT). Density is a commonly used predictor of cancellous bone stiffness. However, cancellous bone stiffness is also dependent on the trabecular architecture and the quality of the mineralized tissue.

Quantitative ultrasound (QUS) was developed as a tool for the assessment of cancellous bone mechanical quality. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) are the two most commonly used parameters. Because QUS parameters and BMD show independent associations with hip fracture risk, it is likely that QUS measures other qualities of bone as compared with BMD. Postmortem specimen studies have shown that ultrasound velocity is structure-related, as evidenced by its dependence on the measuring direction in bone cubes.

Furthermore, strong correlations between elastic moduli and ultimate strength, on the one hand, and ultrasound velocity, on the other, have been found in bovine and human trabecular bone samples. Recently, high-resolution microcomputed tomography (μCT) reconstruction of trabecular bone was introduced. The μCT system generates sequential microscopic optical images and it allows a geometric reconstruction of the trabecular architecture of bone specimens by reconstructing the images in a three-dimensional matrix. This represents the geometry of the trabecular architecture. The microarchitecture can be assessed accurately by μCT when its resolution is around 20 μm. Uchiyama et al. showed significant correlations between μCT-assessed microarchitecture and conventional histomorphometry, using a spatial μCT resolution of 26 μm. The voxel matrix can be used as input for microstructural finite element analysis (μFEA) models. These μFEA models can be used to simulate real mechanical tests and to evaluate the elastic properties of trabecular bone specimens.

In the results of mechanical tests, the effects of architectural
qualities (which depend on morphometric parameters, such as connectivity, trabecular number and width, and bone volume fraction) and of mineralized tissue qualities (which depend on degree of mineralization, lamellar arrangement, microcracks, and resorption lacunae) on specimen stiffness cannot be discriminated. However, with μCT simulation, all bone elements in the model can be assigned the same isotropic Young’s modulus, as it has been found that the tissue anisotropy has a negligible effect on the apparent Young’s modulus. In this way, only the architecture plays a role in the determination of the specimen stiffness. The association of QUS parameters with μCT-based morphometric parameters has been investigated previously, but not the association of QUS parameters with Young’s modulus as determined by the μFEA model.

The objective of this study was to compare the abilities of BMD, as measured by DXA, and the QUS parameter, SOS, to predict the Young’s modulus, as assessed by μFEA from μCT reconstructions. For this purpose, BMD measurements were performed on bovine specimens, and, in addition, the SOS was measured in three directions. The specimens were μCT-scanned, and Young’s moduli were determined in three directions with μFEA.

Materials and Methods

Sample Preparation

Seven proximal and eight distal bovine femora were obtained freshly from a local butcher and kept frozen at −35°C until required for testing. After thawing, 15 cubes of pure trabecular bone measuring 25 × 25 × 25 mm were prepared using a high precision diamond saw. Care was taken to produce parallel surfaces. The edges of the samples were approximately in line with the anteroposterior (AP), mediolateral (ML), and craniocaudal (CC) axes of the bones from which they were obtained. Because defatting has no measurable effect on ultrasound velocity, bone marrow was not removed. During preparation the samples were kept moist. Prior to ultrasound measurements, the specimens were thoroughly degassed underwater in a vacuum desiccator, removing air bubbles trapped within the intertrabecular spaces. For BMD and ultrasound measurements, the whole cubes were used. For μCT measurements, a cylindrical core with a diameter of 15.5 mm and a length of 25 mm was removed in the CC direction from each cube, using a high-precision drill. The AP and ML directions were marked on the cylinders with waterproof ink.

Ultrasound Measurements

SOS measurements were conducted using the ultrasound bone imaging scanner UBIS 3000 (DMS, Montpellier, France). This system uses a pair of focused broadband 0.5 MHz transducers, 29 mm in diameter, mounted coaxially. The resolution at the focal zone is approximately 4–5 mm. A scan of 60 × 60 mm in steps of 1 mm is performed to obtain an image

The measurements were performed underwater at a stable temperature of 30°C. A solution for reducing air bubbles and foaming reactions in the water, delivered by the manufacturer, was added. The bone samples were placed in a Perspex holder for easy and accurate positioning. Each specimen was measured along the three orthogonal axes of the cubes and the measurements were repeated three times with interim repositioning. SOS was calculated using a circular region of interest with a diameter of 15 mm placed within the center of the sample. To avoid measurement artifacts at the edge of the sample, this was the largest diameter used for measurements of the whole specimen.

For comparison with the small volume of interest used for determination of μCT parameters, a subregion with a diameter of 7 mm was also analyzed. Because there was no significant difference between SOS in the 7 and 15 mm regions, only the latter results are reported in this study.

BMD Measurements

BMD measurements were performed in a water tank. The specimens were measured along the three orthogonal axes of the cubes, using the high-resolution scan mode of the Hologic QDR 1000 densitometer. The area of bone analyzed was the total area of the specimen (6.25 cm²). We also analyzed a subregion of 7 × 7 mm in the center of the sample, for optimal comparison with the volume of interest selected for determination of the μCT parameters. There was no significant difference between BMD in the two regions, so only the total area BMD is reported in this study.

μCT Measurements and μFEA Simulation

For μCT measurements, a μCT 20 (Scanco Medical, Bassersdorf, Switzerland) was used. All samples were positioned in a cylindrical Perspex sample holder with a 15.5 mm inner diameter. After a scout view, a total of 600 microtomographic slices with a resolution of 17 μm were acquired. Measurements were stored in three-dimensional-image arrays. For subsequent analysis, a volume of interest (VOI) of 6.8 × 6.8 × 6.8 mm (400 × 400 × 400 voxels) was selected in the center of the specimen to avoid the influence of preparation artifacts at the surface. For optimal segmentation of bone and marrow, a thresholding optimization procedure was used before analyses of the bone samples. Morphometrical indices can be determined from the microtomographic reconstructions. The anisotropy of specimen architecture was determined using mean intercept length (MIL) measurements. MIL determines the average distance between bone and marrow interfaces and is measured by tracing test lines in different directions in the VOI examined. From this measurement, a MIL tensor was calculated, giving the anisotropy in each of the three orthogonal directions. The bone voxels in the μCT-based voxel matrix were converted to eight-node brick elements. But first, the number of voxels was reduced by grouping 4 × 4 × 4 voxels; a new voxel was assumed to be bone if more than half of its original voxels represent bone. The finite element representation of the specimen was detailed enough to obtain accurate values for the stiffness characteristics. All elements were assigned an arbitrarily chosen isotropic Young’s modulus of 1 GPa, as it has been found that tissue anisotropy has a negligible effect on the apparent Young’s modulus. These studies showed close agreement between the experimentally determined Young’s modulus and the Young’s modulus as calculated from microfinite element models.

The finite element models were subjected to six different mechanical tests (three compression tests and three shear tests), and the local strains in the architecture were determined. From the calculated strains the complete stiffness characteristics of the specimen were determined. In this finite element model, only the architecture plays a role in the determination of the specimen stiffness, because all elements were assigned the same Young’s modulus.

Statistics

The data exhibited a normal distribution. Analysis of variance (ANOVA) with repeated measures was used to determine if any
significant differences occurred among the three orthogonal directions. Simple linear regression was used to determine the associations between ultrasonic and densitometric parameters, on the one hand, and Young’s moduli, on the other. Bonferroni’s correction for multiple comparison procedures was used and two-tailed \( p < 0.001 \) was taken as the level of significance. Partial correlation coefficients were calculated to adjust for multiple variables. Multiple stepwise regression was used to test whether combinations of parameters improved the predictive ability. Values for \( r^2 \) refer to the adjusted coefficient of determination. Data are presented as mean ± SD.

**Results**

**SOS, BMD, MIL, and Young’s Modulus in the Orthogonal Directions**

The mean values (± SD) for SOS, BMD, MIL, and Young’s modulus (\( E \)) in the three orthogonal directions are reported in Table 1. SOS, MIL, and \( E \) values were, in all three directions, significantly different \( (p < 0.001) \), with the highest values in the CC direction. BMD was not different in the three directions.

<table>
<thead>
<tr>
<th>Direction</th>
<th>CC</th>
<th>ML</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOS (m/sec)</td>
<td>1976 ± 182(^{a,b})</td>
<td>1740 ± 115(^{c})</td>
<td>1857 ± 163</td>
</tr>
<tr>
<td>BMD (g/cm(^2))</td>
<td>0.74 ± 0.15</td>
<td>0.74 ± 0.15</td>
<td>0.73 ± 0.14</td>
</tr>
<tr>
<td>MIL</td>
<td>0.70 ± 0.20(^{a,b})</td>
<td>0.51 ± 0.13(^{c})</td>
<td>0.60 ± 0.13</td>
</tr>
<tr>
<td>( E ) (MPa)</td>
<td>123.9 ± 43.3(^{a,b})</td>
<td>74.7 ± 35.9(^{c})</td>
<td>97.7 ± 42.6</td>
</tr>
</tbody>
</table>

\(^{a}\) \( p < 0.001 \) vs. AP direction.
\(^{b}\) \( p < 0.001 \) vs. ML direction.

**Relationship Between SOS, BMD, MIL, and Young’s Modulus in Each Orthogonal Direction**

Linear regression models were used to examine the relationship between Young’s moduli, on the one hand, and SOS, BMD, and MIL, on the other. There was a strong relationship between Young’s modulus and SOS in each of the three orthogonal directions, with \( r^2 \) being 0.88, 0.92, and 0.84 (all \( p < 0.0001 \)) for the CC, ML, and AP directions, respectively. The relationship between Young’s modulus and BMD was less strong, with \( r^2 \) being between 0.66 and 0.85 (all \( p < 0.0001 \)) in the different directions. The regression equation for each orthogonal direction is given in Table 2. There was also a significant, positive correlation between SOS and BMD in each of the three axes \( (r^2 = 0.81, 0.42, \text{and} 0.92 \) in the CC, ML, and AP directions, respectively; \( p < 0.0001 \)) (data not shown in Table 2).

Both SOS and BMD were independent predictors of Young’s modulus. After correction for BMD, the correlations between SOS and \( E \) in each of the three directions remained highly significant \( (r^2 = 0.77, p < 0.0001 \) for the AP direction; \( r^2 = 0.48, p < 0.001 \) for the CC direction; \( r^2 = 0.52, p < 0.005 \) for the ML direction). After correction for SOS, BMD remained significantly correlated with Young’s modulus in the AP and CC directions \( (r^2 = 0.52, p < 0.005; r^2 = 0.30, p < 0.05, \text{respectively}) \), but the correlation in the ML direction was no longer statistically significant. When using stepwise regression models, Young’s modulus was best predicted by SOS in each of the orthogonal directions. When using both SOS and BMD as predictors for Young’s modulus, \( r^2 \) was improved by 0–0.08 when compared with SOS as single predictor (Table 2). In the ML direction, the addition of BMD to SOS did not improve the predictive value of Young’s modulus.

SOS was significantly and negatively correlated with MIL in

**Table 2. Summary of the linear regression equation and coefficient using speed of sound (SOS) and bone mineral density (BMD) as predictors for \( E \) in the three orthogonal directions**

<table>
<thead>
<tr>
<th>Direction</th>
<th>Intercept</th>
<th>Coefficient</th>
<th>( t ) ratio, ( p ) value</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC direction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOS</td>
<td>(-317.8)</td>
<td>0.23</td>
<td>9.93, &lt;0.0001</td>
<td>0.88</td>
</tr>
<tr>
<td>BMD</td>
<td>(-68.1)</td>
<td>260.9</td>
<td>8.46, &lt;0.0001</td>
<td>0.85</td>
</tr>
<tr>
<td>SOS and BMD</td>
<td>(-233.6)</td>
<td>0.14 and 115.1</td>
<td>3.22 and 2.29, &lt;0.01 and &lt;0.05</td>
<td>0.92</td>
</tr>
<tr>
<td>ML direction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOS</td>
<td>(-444.9)</td>
<td>0.30</td>
<td>11.9, &lt;0.0001</td>
<td>0.92</td>
</tr>
<tr>
<td>BMD</td>
<td>(-88.7)</td>
<td>220.3</td>
<td>7.90, &lt;0.0001</td>
<td>0.83</td>
</tr>
<tr>
<td>SOS and BMD</td>
<td>(-421.5)</td>
<td>0.22 and 17.0</td>
<td>3.58 and 0.22, &lt;0.01 and n.s.</td>
<td>0.92</td>
</tr>
<tr>
<td>AP direction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOS</td>
<td>(-349.1)</td>
<td>0.24</td>
<td>8.30, &lt;0.0001</td>
<td>0.84</td>
</tr>
<tr>
<td>BMD</td>
<td>(-75.5)</td>
<td>237.8</td>
<td>4.97, &lt;0.0001</td>
<td>0.66</td>
</tr>
<tr>
<td>SOS and BMD</td>
<td>(-312.0)</td>
<td>0.18 and 110.4</td>
<td>6.54 and 3.64, &lt;0.001 and &lt;0.01</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Figure 1.** Relationship between MIL and SOS in three orthogonal directions for each individual bone cube. Circles: cranio-caudal direction; squares: mediolateral direction; triangles: anteroposterior direction.

**Table 1.** Mean values (± SD) for speed of sound (SOS), bone mineral density (BMD), mean intercept length (MIL), and Young’s modulus (\( E \)) in the three orthogonal directions, anteroposterior (AP), mediolateral (ML), and cranio-caudal (CC)

**Table 2.** Summary of the linear regression equation and coefficient using speed of sound (SOS) and bone mineral density (BMD) as predictors for \( E \) in the three orthogonal directions.
between 0.79 and 0.83 have been reported. In these studies, with MIL in AP and ML directions:

In the two directions perpendicular to SOS (SOS in CC direction found significant correlations between SOS and MIL measured in each of the three directions (CC: \( r^2 = 0.74; \) ML: \( r^2 = 0.49; \) AP: \( r^2 = 0.70; \) all \( p < 0.001 \)). After correction for BMD, SOS was no longer significantly correlated with MIL. However, in all but one, we found a positive relationship between MIL and SOS when analyzing the three orthogonal directions in each individual bone specimen (Figure 1). In a multiple regression model, MIL did not contribute to the prediction of \( E \) in the models that included SOS or BMD or both SOS and BMD (data not shown).

When the data from the three different directions are combined into a linear regression model, the predictive value of SOS for Young’s modulus is better than that of BMD, with the \( r^2 \) values being 89% and 60%, respectively (the regression lines are depicted in Figure 2).

### Discussion

We observed a directional dependence of the \( \mu \)FEA-estimated Young’s modulus, MIL, and SOS in trabecular bovine bone cubes. The mechanical properties of cancellous bone vary with direction as a consequence of adaptation to the applied load. The highest values for Young’s modulus were found in the principal-weight-bearing CC direction. SOS also varied with direction, with values being highest in the CC direction. Other studies also reported similar results.

In the present study, SOS accounted for 84%–92% of the variance in \( \mu \)FEA-estimated Young’s modulus in each of the three perpendicular directions, and for 89% when the directions were pooled. In previous studies, the associations between stiffness and ultrasound velocity in human bone specimens were reported as \( r \) values between 0.44 and 0.77, and \( r^2 \) values between 0.41 and 0.72. In the bovine model, \( r^2 \) values of between 0.79 and 0.83 have been reported. In these studies, stiffness was assessed by mechanical testing and not by \( \mu \)FEA.

These studies differed also with respect to location and type of trabecular bone used (human calcaneal, lumbar spine or femoral neck bone samples, bovine femoral bone samples, or both human and bovine samples), the preparation and size of the bone samples, and the ultrasound system used, so it is difficult to compare the results with our study. Nevertheless, it seems evident that the associations between SOS and stiffness, estimated by \( \mu \)FEA in the present study, are stronger than the associations reported in the studies in which stiffness was assessed by mechanical testing.

The results of compression tests and those of the \( \mu \)FEA analyses show good agreement (\( r^2 = 92\% \)), indicating that the FE approach can provide information similar to mechanical tests. However, in a number of studies, inaccuracies in compression test experiments and their possible consequences for the apparent mechanical properties measured have been reported. It must be considered that the Young’s modulus can be underestimated by 20%–45%. The application of \( \mu \)FEA models to simulate compression tests can be used to reduce these errors. This may explain the higher association between SOS and \( E \) in the present study. Furthermore, in the results of mechanical tests, the effects of architectural and mineralized-tissue qualities on specimen stiffness cannot be discriminated. With \( \mu \)FEA, an effective tissue modulus is assigned to the mineralized tissue, so only the architecture plays a role in such analysis. This gives the opportunity to assess the relation between BMD and SOS, on the one hand, and the architectural mechanical quality of these bone samples, on the other. The strong association between SOS and stiffness found in this study emphasizes the ability of SOS to assess the architectural mechanical quality of trabecular bone.

BMD accounted for 66%–85% of the variance in Young’s modulus in the three directions and for 60% when the directions were pooled. These associations are similar to those reported by others. Because density is a scalar quantity, which cannot account for anisotropy, different relationships between Young’s modulus and SOS, on the one hand, and BMD, on the other, are bound to be found in the different directions. This also explains the weaker association between BMD and Young’s modulus when the directions were pooled.

Because SOS was significantly correlated with BMD, stepwise regression models were used to examine the relationship between Young’s modulus, on the one hand, and SOS and BMD,
on the other. In these models, SOS was the strongest independent predictor of Young’s modulus in each of the three directions, and the addition of BMD to SOS improved the prediction of the µFEA-estimated Young’s modulus by only 0%–8%. These observations illustrate the ability of the SOS technique to assess architectural mechanical quality of trabecular bone and also its potential for noninvasive clinical assessment of bone quality.

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References


