# Three Quantitative Ultrasound Parameters Reflect Bone Structure

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**Abstract.** We investigated whether quantitative ultrasound (QUS) parameters are associated with bone structure. In an *in vitro* study on 20 cubes of trabecular bone, we measured broadband ultrasound attenuation (BUA) and two newly defined parameters—ultrasound velocity through bone (UVB) and ultrasound attenuation in bone (UAB). Bone mineral density (BMD) was measured by dual X-ray absorptiometry (DXA) and bone structure was assessed by microcomputed tomography ($\mu$CT) with approximately 80 $\mu$m spatial resolution. We found all three QUS parameters to be significantly associated with bone structure independently of BMD. UVB was largely influenced by trabecular separation, UAB by connectivity, and BUA by a combination of both. For a one standard deviation (SD) increase in UVB, a decrease in trabecular separation of 1.2 SD was required compared with a 1.4 SD increase in BMD for the same effect. A 1.0 SD increase in UAB required a reduction in connectivity of 1.4 SD. Multivariate models of QUS versus BMD combined with bone structure parameters showed squared correlation coefficients of $r^2 = 0.70$–$0.85$ for UVB, $r^2 = 0.27$–$0.56$ for UAB, and $r^2 = 0.30$–$0.68$ for BUA compared with $r^2 = 0.18$–$0.58$ for UVB, $r^2 < 0.26$ for UAB and $r^2 < 0.13$ for BUA for models including BMD alone. QUS thus reflects bone structure, and a combined analysis of QUS and BMD will allow for a more comprehensive assessment of skeletal status than either method alone.

**Key words:** Osteoporosis — Ultrasound — Bone densitometry — Bone structure.

Bone densitometry is an established, important method for predicting osteoporotic fracture risk [1–5]. However, the following observations indicate that the predictive accuracy could potentially be further improved by additional assessment of the bone's internal microstructure, and the material properties of the mineralized tissue itself. (1) Mechanical testing on excised trabecular bone showed that although bone mineral density (BMD) explains about 58–93% of the variability in bone strength, this still may leave residual errors in the estimate of bone strength of 35–55% [6–12]. (2) Treatment studies typically show paralleling increases in BMD along with decreases in fracture risk. However, under certain conditions, increased fracture risk has been observed

despite increasing BMD [13] which may be due to uncoupling of improvements in BMD versus bone structure and material properties. (3) Prevalent vertebral fractures have been shown to predict incident fractures independently of the association between BMD and fracture risk [14]. This could be due to alterations of the forces and moments in a spine that entails fractured vertebrae, but it is more likely that skeletal factors such as bone structure and the material properties may contribute to this enhanced fracture risk. (4) Based on engineering principles it is obvious that the strength of an object does not only depend on the amount of material built in but also on the internal structure, the shape, and the mechanical properties of the constituting material(s).

For all of these reasons it would be important to develop methods for noninvasive assessment of bone micro- and macrostructure and of the material properties of the bone matrix which could complement existing bone densitometry techniques.

Quantitative ultrasound (QUS) techniques have recently been introduced as alternative methods free of ionizing radiation for noninvasive assessment of skeletal status in osteoporosis [15–22]. QUS may permit an assessment of bone microstructure which currently is unachievable by bone densitometry techniques. QUS parameters include broadband ultrasound attenuation (BUA), a measure of the frequency dependence of ultrasound attenuation, and speed of sound (SOS), reflecting the transmission velocity of ultrasound passing through soft tissue and bone tissue, generally of the calcaneus. In an earlier study we demonstrated that the correlation between BMD and BUA when measured at the same site is only moderate, with correlation coefficients ranging from $r = 0.58$ to $r = 0.72$ [23]. Similar results have also been observed for site-matched velocity and BMD measurements [24]. For both parameters this leaves about 50% of unexplained variability, demonstrating that ultrasound parameters partly reflect properties that are unrelated to BMD. In another study we have subsequently shown that unlike BMD, BUA depends on trabecular orientation [25]. Similar to bone strength, BUA was found to be larger when measured parallel rather than perpendicular to the principal orientation of the trabeculae.

In this study, we investigated whether QUS parameters are associated with other parameters of bone structure measured by high resolution microcomputed tomography ($\mu$CT). Besides BUA we defined and studied two new QUS parameters: ultrasound velocity through bone (UVB), a measure of the bone velocity component of SOS, and ultrasound attenuation in bone (UAB), a measure of the mean attenuation of ultrasound in bone (definitions in Methods section). Measurements were carried out *in vitro* on bone specimens, and

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**Table 1.** DXA, QUS, and μCT results—descriptive statistics

| Parameter | Unit | Mean | Min | Max | CV (%) | $CV_{intra}$ | $CV_{inter}$ |
|-----------|------|------|-----|-----|--------|--------------|--------------|
| BMD | $g/cm^2$ | 0.427 | 0.254 | 0.637 | 23.7 | 3.3% | 23.9% |
| BUA | dB/MHz | 60.2 | −9 | 116.5 | 42.0 | 36.6% | 25.9% |
| UVB | m/s | 1922.1 | 1333.7 | 2450.3 | 13.1 | 11.6% | 8.1% |
| UAB | dB | 12.5 | 5.1 | 23.2 | 32.5 | 28.1% | 20.0% |
| BV/TV | % | 32.1 | 16.8 | 45.9 | 22.7 | N/A | N/A |
| BS/TV | 1/mm | 10.8 | 9.3 | 13.1 | 10.2 | N/A | N/A |
| Tb.Th | μm | 188 | 153 | 220 | 10.4 | N/A | N/A |
| Tb.Sp | μm | 441 | 205 | 1144 | 45.8 | N/A | N/A |
| Connectivity | $1/mm^3$ | 3.93 | 0.66 | 11.1 | 65.9 | N/A | N/A |
| MIL | μm | 441 | 315 | 637 | 16.1 | 11.2% | 13.5% |

multivariate models were analyzed to investigate to what extent QUS parameters reflect bone structure independent of bone density.

## Materials and Methods

Twenty specimens of purely trabecular bone were cut from fresh bovine proximal radii. Based on high-resolution radiographs, orientations of the cuts were arranged in a fashion that one of the three principal axes of the cube (the z-axis) was approximately aligned with the trabeculae. The specimens were cut to $1.2 \times 1.2 \times 1.2$ cm$^3$. Bone marrow was extracted and specimens were immersed in water, and put under vacuum to extract air bubbles prior to QUS measurements.

We evaluated three different parameters of QUS. BUA reflects the frequency dependence of ultrasound attenuation [17]. Secondly, we measured the transmission velocity of sound. This measurement is referred to as ultrasound velocity through bone (UVB) to distinguish it from SOS [26]. Whereas SOS measurements include a portion of ultrasonic velocity through soft tissue, UVB solely reflects ultrasound velocity when passing through the largely trabecular calcaneal bone. Third, we measured the average attenuation of ultrasound in the frequency range of 228–577 kHz based on the average absorption at 27 equidistant frequencies within that range, sampled by means of Fourier analysis from the transmitted ultrasound pulse. This parameter is referred to as ultrasound attenuation in bone (UAB). Ultrasound attenuation has been investigated earlier [27], but unlike BUA or SOS it has not been studied extensively. All QUS measurements were carried out on the Walker Sonix UBA 575+ (Walker Sonix Inc., Worcester, MA). In order to achieve good reproducibility and to block ultrasound signals from the waterbath, a special holder was used that has been described in previous studies [25]. Reproducibility errors were defined as root mean square average of standard deviations (SD) of repeated measurements with interim removal and repositioning of the specimens. Each specimen was measured along the three orthogonal principal axes of the cubes.

μCT images were acquired on the μCT system of the Orthopaedic Research Laboratories at the University of Michigan, Ann Arbor [10, 28]. This system enabled us to reconstruct the images of the central 1 cm$^3$ portion of the cubes with approximately 80 μm isotropic image resolution. For calculation of parameters equivalent to those typically used in histomorphometry (including trabecular volume fraction, BV/TV; trabecular surface to volume ratio, BS/TV; trabecular plate thickness, Tb.Th; and trabecular plate separation, Tb.Sp) the number of bone voxels per total number of voxels $P_P$ and the number of intersections between bone and nonbone components per total length of test lines $P_L$ were determined. Perpendicular test lines were used for the calculation of an average $P_L$ from three orthogonal directions [10]. This approach is based on the assumption of a plate model [29]. The Euler-Poincaré number was calculated, and connectivity was defined as Euler number/volume of specimen [30, 31]. For the analysis of anisotropic parameters, the method of direct secants was applied to the full 3-D data set. Mean intercept length (MIL) was evaluated by a stepwise rotated analysis

grid with MIL data then fitted to the equation of an ellipsoid according to the method of Harrigan and Mann [32]. The three-dimensional μCT data allowed for evaluation of all planes rather than just the three orthogonal surface planes [10]. MIL along the x-, y-, and z-axes of the cube were then calculated from the three principal MIL values. Further details on the μCT approach can be obtained from the literature [10, 28, 31]. Associations between QUS parameters and MIL were both calculated between QUS and the MIL component parallel to the ultrasound beam (termed $MIL_{PARA}$) and between QUS and the average of the two MIL components perpendicular to the ultrasound beam ($MIL_{PERP}$). Similarly, we also investigated associations between QUS and the corresponding parallel and perpendicular components of trabecular separation $Tb.Sp_{PARA}$ and $Tb.Sp_{PERP}$ derived from Tb.Sp and MIL.

Bone mineral density (BMD) was obtained on all cubes using the Norland XR-26 Dual X-Ray absorptiometry (DXA) scanner. Measurements were obtained in three orthogonal directions employing the high resolution scanning mode.

To examine the associations between QUS and bone structure, univariate and multivariate regression analyses were carried out. Correlations between QUS and bone structural parameters, without and with adjusting for BMD, are expressed by correlation coefficients (r) and partial correlation coefficients ($r_p$), respectively. Corresponding levels of significance are derived by testing that the effect is zero using the F statistic. Optimum multivariate models for QUS parameters were derived by interactive backward and forward stepwise regression analyses. Optimal multivariate models were derived in three steps. First, we investigated associations between mean QUS values (i.e., mUVB, mUAB, mBUA), BMD, and bone structural parameters—those means being obtained by averaging the readings in the three orthogonal projections. Second, we studied associations between (anisotropic) QUS parameters, BMD, and bone structure using the same model for the x-, y-, and z-axes. Third, we repeated the latter analyses but allowed separate models for the three orthogonal directions. Models were optimized with respect to overall model r$^2$, and the level of significance for including a variable into the model was set to $P < 0.10$. To determine which variables were most important with respect to their associations with QUS parameters we calculated their semipartial correlation coefficients ($r_s$). These coefficients reflect the unique contribution of a variable to the overall model and are given by the difference in r$^2$ between a model containing all variables and the model with all variables except for the one assessed. Statistical analyses were carried out using either JMP (SAS Institute Inc., Cary, NC) or SYSTAT (SYSTAT, Inc., Evanston, IL) statistical analysis software.

## Results

Table 1 shows mean, minimum, maximum, and coefficient of variation (CV = SD/Mean × 100) of BMD, QUS, and bone structural parameters. When splitting the coefficient of variation into an intraspecimen variability component and an interspecimen variability component, BMD shows minimal intraspecimen variability, whereas all QUS parameters and

**Table 2.** Univariate associations between QUS parameters and BMD or bone structure[a]

|  | UVB | UAB | BUA |
|---|---|---|---|
| BMD | ↑↑ | (↓) | |
| BV/TV | ↑↑ | ↓ | |
| Connectivity | | ↓↓ | (↓) |
| BS/TV | | (↓) | |
| TB.Th | | (↑) | |
| $MIL_{PARA}$ | ↑↑ | | |
| $MIL_{PERP}$ | | | |
| Tb.Sp | ↓↓ | (↑) | (↓) |
| $Tb.Sp_{PARA}$ | (↓) | (↑) | |
| $Tb.Sp_{PERP}$ | ↓↓ | (↑) | (↓) |

[a] Upward (downward) pointing arrows indicate positive (negative) correlations with levels of significance of $P < 0.001$ for duplicate arrows, $P < 0.01$ for single arrows; arrows in parentheses indicate borderline significant trends with $P < 0.2$ or with $P < 0.01$, but $0.01 < P < 0.2$ once outliers are removed from data set

**Table 3.** Associations of BMD-adjusted QUS parameters with bone structure[a]

|  | UVB | UAB | BUA |
|---|---|---|---|
| BV/TV | (↑) | | |
| Connectivity | | (↓) | |
| BS/TV | | (↓) | |
| TB.Th | | (↑) | |
| $MIL_{PARA}$ | ↑ | (↑) | |
| $MIL_{PERP}$ | ↓ | | |
| Tb.Sp | (↓) | | (↓) |
| $Tb.Sp_{PARA}$ | | | (↓) |
| $Tb.Sp_{PERP}$ | ↓↓ | | (↓) |

[a] See note to Table 2

mean intercept length have about equal or even greater intraspecimen variability than interspecimen variability.

Short-term reproducibility errors were 0.5 dB/MHz (2.4%) for BUA, 5.7 m/s (0.3%) for UVB, 0.18 dB (1.3%) for UAB, and 0.007 g/cm² (1.95%) for BMD.

When assessing univariate associations between QUS parameters and BMD or bone structural parameters, several highly significant relationships were observed, both without and with adjusting for BMD (Tables 2 and 3, respectively; significance levels have been set to 0.01 and 0.001 reflecting adjustments for multiple comparisons). These associations were particularly strong for UVB which was associated with decreasing $Tb.Sp_{PERP}$ ($r = -0.58, P < 0.0005$), Tb.Sp ($r = -0.53, P < 0.0005$), and $Tb.Sp_{PARA}$ ($r = -0.37, P < 0.004$) as well as with increasing $MIL_{PARA}$ ($r = 0.45, P < 0.0005$), BV/TV ($r = 0.49, P < 0.0005$), and BMD ($r = 0.42, P < 0.0008$). UAB showed a strong association with decreasing connectivity ($r = -0.43, P < 0.0006$) and weaker, but still significant associations with decreasing BMD ($r = -0.31, P < 0.02$) and BV/TV ($-0.34, P < 0.007$), and increasing Tb.Sp ($r = 0.31, P < 0.02$) and $Tb.Sp_{PARA}$ ($r = 0.32, P < 0.02$). Only borderline significant trends were observed between BUA and decreasing bone structural parameters, including connectivity and trabecular separation. These associations are summarized in Table 2 along with other borderline significant associations among UVB, UAB, and bone structural parameters. For highly significant associations, the relationships between changes in bone structure and corresponding changes in QUS parameters were quantified. A 1.0 SD increase in UVB (equivalent to 252 m/s or 13.1% of the mean UVB of the sample) was associated with a 1.4 SD increase in BMD (equivalent to 0.141 g/cm² or 33%), a 1.3 SD increase in BV/TV (equivalent to 0.092 mm³/mm³ or 28.7%), a 1.3 SD increase in $MIL_{PARA}$ (equivalent to 95 μm or 21.5%), a 1.2 SD decrease in Tb.Sp (equivalent to 245 μm or 55.5%), or a 1.2 SD change in $Tb.Sp_{PERP}$ (equivalent to 237 μm or 47.1%). A 1.0 SD change in UAB (equivalent to 4.1 dB or 32.5% of the mean UAB of the sample) was associated with a 1.4 SD change in connectivity (equivalent to 3.6 l/mm³ or 92.1%).

The anisotropy of bone structural parameters was found to be most closely reflected in the anisotropy of UVB. Intraspecimen variability along the x-, y-, z-axes showed very similar trends for MIL and UVB (Fig. 1). A positive association between MIL and BUA or UAB was observed, but did not reach significance for this sample.

After adjusting for BMD, UVB was still highly significantly associated with decreasing $Tb.Sp_{PERP}$ ($r_p = -0.44, P < 0.0005$), increasing $MIL_{PARA}$ ($r_p = 0.36, P < 0.005$), and decreasing $MIL_{PERP}$ ($r_p = -0.35, P < 0.007$). UAB was significantly associated with decreasing connectivity ($r_p = -0.32, P < 0.02$). Several other borderline significant associations are additionally listed in Table 3.

Using interactive stepwise regression analysis, the following sets of parameters of bone density and structure were found to best predict mBUA, mUVB, and mUAB: (1) mUVB was predicted best by BMD ($r_p = 0.62, P < 0.0005$), connectivity ($r_p = -0.72, P < 0.0005$), and Tb.Sp ($r_p = -0.88, P < 0.0005$) with overall model $r^2 = 0.91, r = 0.95$, RMSE $= 47.7$ m/s, CV $= 2.5\%$; (2) mUAB was predicted best by connectivity alone with model $r^2 = 0.51, r = 0.71$, RMSE $= 1.73$ dB, CV $= 13.9\%$; (3) mBUA was predicted best by BMD ($r_p = -0.49, P < 0.0005$), connectivity ($r_p = -0.80, P < 0.0005$), and Tb.Sp ($r_p = -0.84, P < 0.0005$) with overall model $r^2 = 0.74, r = 0.86$, RMSE $= 8.0$ dB/MHz, CV $= 13.4\%$. Figure 2a shows the respective unique contributions (represented by semipartial correlation coefficients) of BMD, connectivity, and trabecular separation to mUVB, mUAB, and mBUA.

For predicting the three individual orthogonal x-, y-, z-components of UVB, UAB, and BUA from one common model for each of the QUS parameters, the following combinations of predictors proved to yield optimal results: (1) UVB predicted best by $MIL_{PARA}$ ($r_p = 0.66, P < 0.0001$), $MIL_{PERP}$ ($r_p = -0.57, P < 0.0001$), $Tb.Sp_{PARA}$ ($r_p = -0.47, P < 0.0002$), $Tb.Sp_{PERP}$ ($r_p = 0.28, P < 0.04$), and at a borderline significant level connectivity ($r_p = -0.25, P < 0.07$), with overall model $r^2 = 0.70, r = 0.83$, RMSE $= 145.2$ m/s, CV $= 7.6\%$; (2) UAB predicted best by connectivity ($r_p = -0.33, P < 0.02$), $MIL_{PERP}$ ($r_p = -0.31, P < 0.03$), $MIL_{PARA}$ ($r_p = 0.29, P < 0.04$), and at borderline significant levels $Tb.Sp_{PERP}$ ($r_p = 0.26, P < 0.06$), $Tb.Sp_{PARA}$ ($r_p = -0.25, P < 0.07$), with model $r^2 = 0.27, r = 0.52$, RMSE $= 3.6$ dB, CV $= 29.1\%$; (3) BUA predicted best by connectivity ($r_p = -0.47, P < 0.0002$), and $Tb.Sp_{PERP}$ ($r_p = -0.47, P < 0.0002$) with overall model $r^2 = 0.25, r = 0.50$, RMSE $= 22.2$ dB/MHz, CV $= 37.0\%$.

To characterize which variables were most important, Figure 2b displays semipartial correlation coefficients for these models. For simplification, parallel and perpendicular components of MIL and Tb.Sp are pooled.

Allowing separate models for each x-, y-, z-component improved the overall model $r^2$: (1) the x-, y-, z-components of UVB were predicted with $r^2 = 0.85, r^2 = 0.86$, and $r^2 = 0.86$, respectively; (2) the x-, y-, z-components of UAB were predicted with $r^2 = 0.52, r^2 = 0.54$, and $r^2 = 0.61$, respectively; (3) the x-, y-, z-components of BUA were predicted
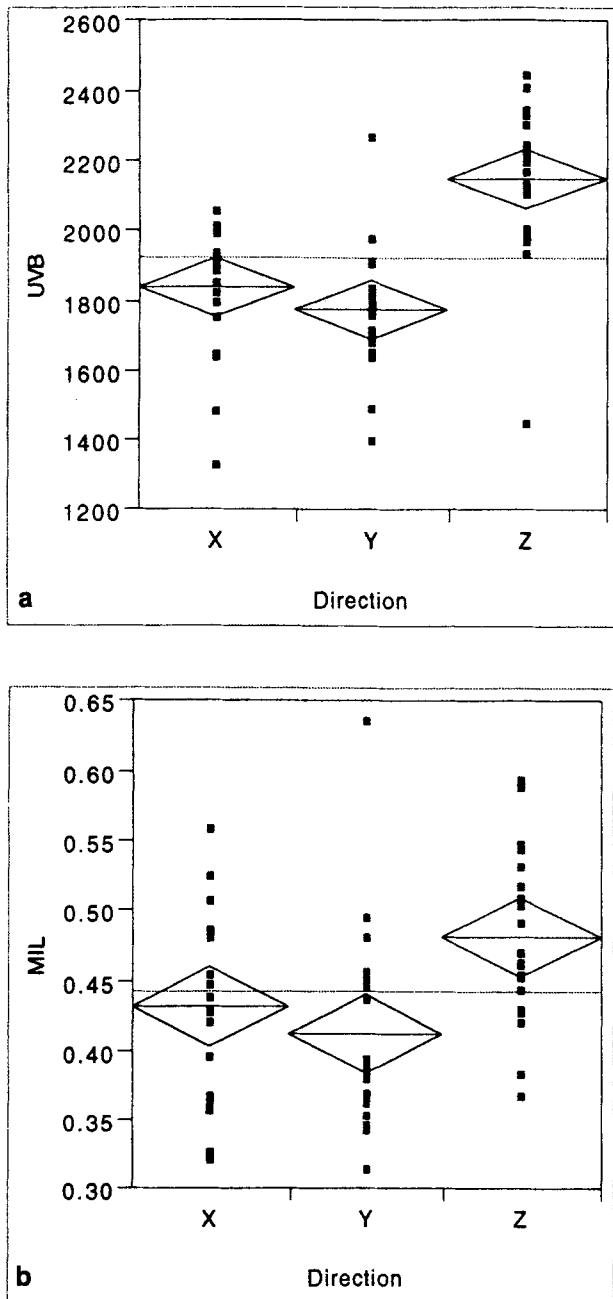
Fig. 1. Anisotropy of (a) ultrasound velocity through bone (UVB in m/s) and (b) mean intercept length (MIL in mm) demonstrate similar directional dependencies. Diamonds represent means with 95% confidence intervals.

the plane perpendicular to the ultrasound beam. BMD was positively correlated with UVB. Connectivity showed a negative association with all three QUS parameters.

Analogously to Figure 2a and b, unique contributions of bone density and structural parameters are represented in Figure 2c.

In all of the models described above we consistently found BMD to account for only a portion of the overall variability of QUS parameters: 18–58% of the variability of UVB, and less than 26 and 13% of the variability of UAB and BUA, respectively. Including anisotropic parameters of bone structure allowed for explanation of 70–85% of the variability of UVB, 27–56% of the variability of UAB and 30–68% of the variability of BUA, depending on whether separate models were allowed for each of the three orthogonal directions (Fig. 3a–c).

## Discussion

The assessment of quantitative ultrasound techniques in the field of osteoporosis originally focused on BUA measurements [16, 17]. More recently, different velocity measurements have been introduced [15, 18, 19, 26], with SOS representing the principal clinical measurement mode for the calcaneal measurement site. Velocity measurements have also been used for the assessment of the elasticity of cortical bone [33–35]. In those studies, a close association between elastic modulus measurements carried out by mechanical testing and those based on ultrasound measurements was observed [35]. BUA techniques had not been applied in those areas. There is very limited information in the literature on why BUA, i.e., a parameter reflecting the frequency dependence of ultrasound attenuation, was originally preferred over a direct assessment of ultrasound attenuation per se [17]. Evans and Tavakoli [27], in fact, reported that the correlation with BMD was somewhat better for ultrasound attenuation than for BUA. On the other hand, the criterion of good correlation with BMD might not be the most appropriate one, particularly when searching for techniques that could complement rather than replace bone densitometry to improve fracture risk prediction. Techniques that would only partially be associated with bone density, but also with other parameters that may impact on bone strength, such as bone structure or the material properties of the bone matrix, might be better suited for this purpose. It is for these reasons that we additionally investigated ultrasound attenuation directly. UAB data are available from the raw data on the Walker Sonix device. Assuming a linear relationship between attenuation and the frequency of ultrasound, the two parameters selected in this study, i.e., BUA (the slope of attenuation versus frequency) and UAB (the mean attenuation of ultrasound across the selected frequency range) should characterize ultrasound attenuation in trabecular bone fairly comprehensively—at least for the frequency range available on current QUS devices.

Our results show that all three QUS parameters reflect aspects of bone structure, each of them in a different fashion: UVB is largely influenced by trabecular separation, UAB by connectivity, and BUA by a combination of both. Having three partially independent QUS parameters in addition to bone density yields a more powerful "tool set" for assessing bone structure *in vivo* compared with approaches that would only yield a single parameter in addition to BMD.

The specimens were purposely selected to show substan-

with $r^2 = 0.67$, $r^2 = 0.49$, and $r^2 = 0.87$, respectively. Correlation coefficients given above reflect models with BMD, connectivity, $Tb.Sp_{PARA}$ $Tb.Sp_{PERP}$, $MIL_{PARA}$, and $MIL_{PERP}$ included. However, models containing different sets of variables often produced similarly high correlation coefficients indicating that the sample size of this study was too limited to investigate separate x-, y-, and z-models. Still, qualitatively a number of consistent trends could be observed. UVB was associated with decreasing Tb.Sp, and this decrease appeared to be mostly due to decreased Tb.Sp in
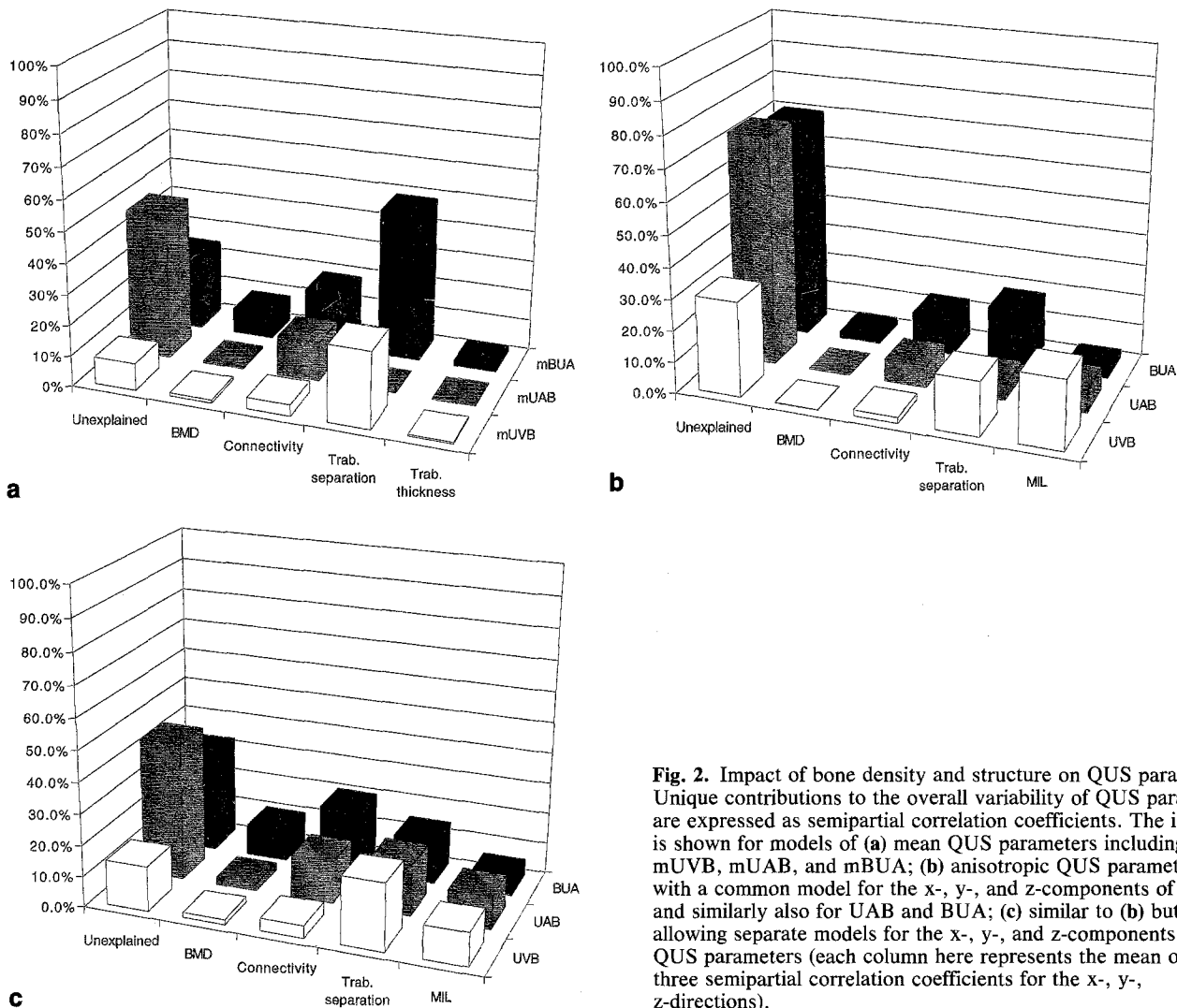
**a**



**b**



**c**

Fig. 2. Impact of bone density and structure on QUS parameters. Unique contributions to the overall variability of QUS parameters are expressed as semipartial correlation coefficients. The impact is shown for models of (a) mean QUS parameters including mUVB, mUAB, and mBUA; (b) anisotropic QUS parameters with a common model for the x-, y-, and z-components of UVB and similarly also for UAB and BUA; (c) similar to (b) but allowing separate models for the x-, y-, and z-components of the QUS parameters (each column here represents the mean of the three semipartial correlation coefficients for the x-, y-, z-directions).

tial anisotropy, with trabeculae principally oriented parallel to the z-axis of the cubes. This sampling scheme thus led to intraspecimen variability that was partly as large as interspecimen variability. As BMD demonstrates hardly any anisotropy, it is not surprising to find very low correlations between anisotropic QUS parameters and isotropic BMD in our sample. Still, the lack of a significant univariate association between BMD and BUA, along with a negative correlation once adjusted for bone structural parameters, is unexpected. Further studies in independent samples are required to investigate whether this represents a meaningful or a spurious finding. For UVB, on the other hand, the observed relationships appear to be more consistent. We found strikingly similar trends in the x, y, z anisotropy for this parameter and MIL (a measure of trabecular thickness, Fig. 1). It is interesting to note that associations with bone structural parameters measured along the direction of the ultrasound beam typically showed correlations of the opposite sign when related to structural parameters measured in a plane perpendicular to the beam.

Our study has limitations. With 20 specimens and thus 60 independent measurements along all three axes, the sample size of our study is still fairly small. The associations of

BMD and structural parameters with the mean values of the QUS parameters appeared to be relatively robust. Subsampling and outlier removal did not affect the significance of the strongest observed associations. As we tested a large number of hypotheses on a relatively small sample, adjustments for multiple comparisons may be appropriate, although this remains controversial [36]. In any case, even if $P$-values were set to 0.01 or 0.001, several associations remained significant (Tables 2, 3). The findings were more complex for models that explained, in addition, the anisotropy of the QUS parameters. Selecting a common model for all three directions yielded somewhat lower overall model correlation coefficients compared with models that were specific for each axis. However, our specimens were not perfectly aligned with any of the three principal symmetry axes, and thus structural parameters along the beam direction and perpendicular to it will contribute in different ways [37]. Also, simple linear models such as those employed in this study may not be sufficient. When modeling, for example, elasticity as a function of bone structural parameters, terms of higher order, as well as interaction terms are typically required [38]. However, the limited sample size in our study did not allow us to pursue testing of more complex
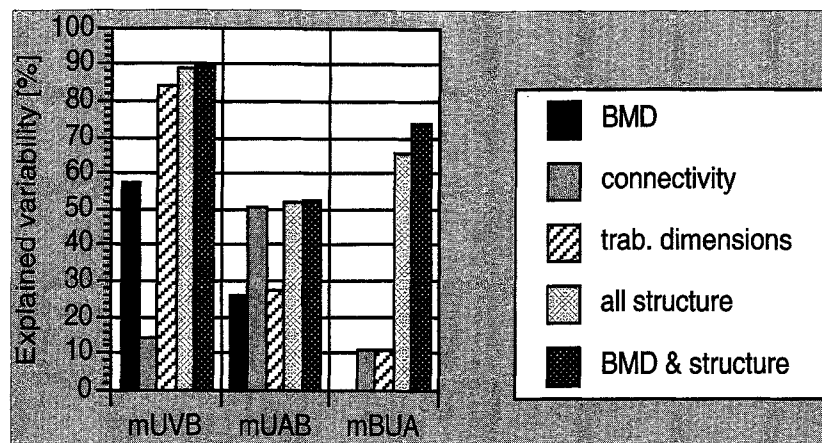
**a**
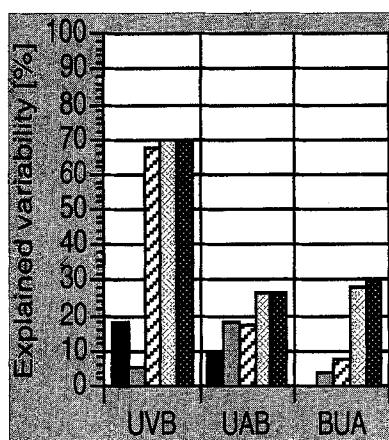


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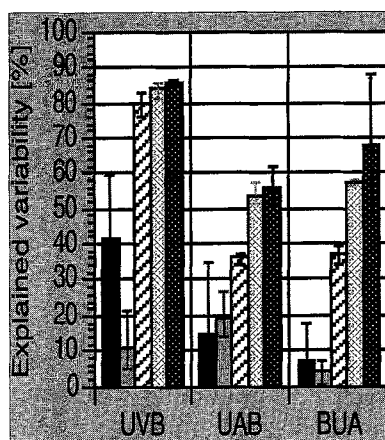


**c**

**Fig. 3.** Variability of QUS parameters that can be explained alone by either BMD, connectivity, or trabecular dimensions (including Tb.Sp$_{PAPA}$, Tb.Sp$_{PERP}$, MIL$_{PARA}$, MIL$_{PERP}$), a combination of both structural parameter groups but excluding BMD, and a combination of bone density and structural parameters. Similar to Figure 2, results are presented for (**a**) mean QUS parameters, (**b**) anisotropic QUS parameters allowing just one common model for all three directions of each of the QUS parameters, (**c**) allowing separate models for each axis (the error bars indicate the range of r$^2$ across the x-, y-, and z-directions).

models in a meaningful fashion. More extensive studies on larger sample sizes and also with greater variability in the structural and density parameters, preferably carried out with human specimens, are warranted. In these studies, attempts should be made to combine several QUS parameters to improve the accuracy of estimates of bone structural parameters. Promising results on combining BUA and ultrasound transmission velocity for estimating Young's modulus have recently been presented [39]. Averaging of normalized BUA and SOS parameters as performed by the Lunar Achilles system, may prove to be useful. However, it may take a set of several more sophisticated algorithms to obtain accurate estimates of the various bone structural parameters.

In conclusion, we have demonstrated several significant associations of bone structure with QUS parameters independent of BMD. These findings strongly suggest that ultrasound evaluations may be appropriate for complementing bone densitometry in the assessment of the integrity of trabecular bone. Cross-sectional studies in patients have recently also shown that ultrasound measurements, specifically BUA, are associated with vertebral fracture prevalence independent of BMD [40]. The findings from our *in vitro* study and the results from investigations *in vivo* suggest that ultrasound carried out in conjunction with bone densitometry will yield a more comprehensive assessment of skeletal status and provide better fracture risk prediction than either method alone.

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