Spatial variation of acoustic properties is related with mechanical properties of trabecular bone

To cite this article: O Riekkinen et al 2007 Phys. Med. Biol. 52 6961

View the article online for updates and enhancements.
Spatial variation of acoustic properties is related with mechanical properties of trabecular bone

O Riekkinen\textsuperscript{1,2}, M A Hakulinen\textsuperscript{1}, J Töyräs\textsuperscript{3} and J S Jurvelin\textsuperscript{1,2}

\textsuperscript{1} Department of Physics, University of Kuopio, POB 1627, FI-70211 Kuopio, Finland
\textsuperscript{2} Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital and University of Kuopio, POB 1777, FI-70211 Kuopio, Finland
\textsuperscript{3} Department of Clinical Neurophysiology, Kuopio University Hospital and University of Kuopio, POB 1777, FI-70211 Kuopio, Finland

E-mail: Ossi.Riekkinen@uku.fi

Received 30 May 2007, in final form 26 September 2007
Published 12 November 2007
Online at stacks.iop.org/PMB/52/6961

Abstract
In clinical applications, ultrasound parameters are measured as an average value over a region of interest (ROI) or as a value at a single measurement point. Due to natural adaptation to loading conditions, trabecular bone is structurally, compositionally and mechanically heterogeneous and anisotropic. Thus, spatial variation of ultrasound parameters within ROI may contain valuable information on the mechanical integrity of trabecular bone. However, this issue has not been thoroughly investigated. In the present study, we aimed at investigating the significance of the spatial variation of ultrasound parameters for the prediction of mechanical properties of human trabecular bone. For this aim, parametric maps of apparent integrated backscattering (AIB), integrated reflection coefficient (IRC), speed of sound (SOS), average attenuation (AA) and normalized broadband ultrasound attenuation (nBUA) were calculated for femoral and tibial bone cylinders ($n =$ 19–20). Further, the effect of time window length on the AIB, variation of AIB within ROI and association between AIB and bone mechanical properties were characterized. Based on linear correlation analysis, spatial variation of AIB, assessed as standard deviation of measurements within ROI, was a strong predictor of bone ultimate strength ($r = -0.82$, $n =$ 19, $p <$ 0.01). Further, the time window length affected absolute values of AIB and strength of correlation between AIB and bone ultimate strength. Interestingly, linear combination of mean IRC and spatial variation of AIB within ROI was the strongest predictor of bone ultimate strength ($r = 0.92$, $n =$ 19, $p <$ 0.01). In conclusion, our findings suggest that the measurement of two-dimensional parametric maps of ultrasound parameters could yield information on bone status not extractable from single point measurements. This highlights the potential of parametric imaging in osteoporosis diagnostics.
Introduction and literature


Instead of potential fracture sites, current clinical ultrasound measurements are conducted at peripheral sites such as calcaneus. Ultrasound measurements at typical osteoporotic fracture sites, such as proximal femur, could improve ultrasound diagnostics of osteoporosis. Due to high ultrasound attenuation in bone, these measurements are difficult with a through-transmission technique whereas pulse-echo technique may provide a feasible approach for measurements at these sites. With the pulse-echo technique, ultrasound reflection and backscattering can be determined. These parameters are significantly related to mechanical properties, composition and structure of trabecular bone (Wear and Garra 1998, Hakulinen et al 2004, 2005, Hoffmeister et al 2006, Riekkinen et al 2006). As trabecular bone is structurally heterogeneous, depth of analysis, i.e. the length of the applied time window, may significantly affect the determined backscattering parameters. However, this issue has not been extensively investigated.

Most commercially available ultrasound devices measure acoustic properties at single point of calcaneus or by scanning an acoustic map of heel. In previous studies, precision and ability of acoustic mapping to predict BMD have been studied (Fournier et al 1997, Jorgensen and Hassager 1997, Frost et al 2000). Further, the effect of the size and the location of the region of interest (ROI) in a parametric image have been investigated (Damilakis et al 2001, Van den Bergh et al 2001). Recently, an automatic technique for discerning bone pixels from the acoustic map was presented (Padilla et al 2004). However, the variation of ultrasound parameters within ROI as well as relation of spatial variation with the mechanical properties of trabecular bone has not been studied.

The aim of present study was to compare two-dimensional ultrasound (2.25 MHz) parametric imaging and single point measurements for prediction of bone mechanical properties. Especially, significance of the spatial variation of ultrasound parameters on bone mechanical strength was investigated. In addition, the effect of the time window length (1–5 μs) at ultrasound backscattering was analyzed.

Materials and methods

Sample preparation

Cylindrical trabecular bone samples (diameter = 16 mm, thickness = 8 mm) were drilled from human (n = 12, eleven males and one female, age = 57 ± 18 years) distal femur (n = 10) and proximal tibia (n = 10) (National Authority for Medicolegal Affairs, permission 1781/32/200/01) using a hollow drill bit. The end surfaces of the bone cylinders were cut to be parallel by using a micro grinding system (Macro Exact 310 CP, Exact, Hamburg, Germany). The samples were moistened with phosphate-buffered saline (PBS) during cutting to avoid dehydration. After preparation, the samples were immersed in PBS and stored frozen (−20 °C) until the measurements.
Figure 1. (a) A typical pulse-echo signal from a bone sample with the time window for reflection and backscattering analysis (ROIrec and ROIemb, respectively). (b) and (c) Significant spatial variation of AIB and IRC is apparent at the 2.25 MHz (ROI = 88 mm²). Mean value and standard deviation (SD) of acoustic parameters within the ROI are presented underneath the parametric images.

Ultrasound methods

Acoustic measurements were conducted with an ultrasound system (UltraPAC, Physical Acoustic Co., NJ, USA) consisting of a 500 MHz A/D board and a 0.2–100 MHz pulser-receiver board. The ultrasound system was equipped with a tank and scanning drives. The measurements were conducted in a degassed PBS bath using a pair of focused ultrasound (2.25 MHz, focal depth = 50.3 mm, focus diameter = 1.4 mm) transducers (Panametrics V304, Panametrics Inc., Waltham, MA, USA) adjusted at a distance of 10 cm between transducers. The samples were placed in the focal plane between the transducers and the measurements were conducted at room temperature (~21 °C). The ultrasound system was controlled with a custom measurement program (LabVIEW 6.1, National Instrument, Austin, TX, USA).

Speed of sound (SOS) was determined with the time-of-flight method (Njeh et al 1997), based on the measurement of ultrasound pulse arrival time through water with and without the bone sample. Normalized broadband ultrasound attenuation (nBUA, (Langton et al 1984)) and average attenuation (AA, (Riekkinen et al 2006)) were determined with the substitution method (Langton et al 1984), i.e. using the measurements of ultrasound spectrum with and without the sample. Further, attenuation spectrum was calculated as a logarithm difference of these spectrums. Apparent integrated backscattering (AIB, Cherin et al 1998) and integrated reflection coefficient (IRC, Cherin et al 1998) were determined as a logarithm difference of pulse-echo signal spectrums recorded from the bone and from the stainless-steel plate. Ultrasound signals in both transmission and pulse-echo modes were recorded across a scan area of 16 mm × 16 mm with a pixel size of 0.5 mm × 0.5 mm (figures 1(b) and (c)). nBUA was derived from the linear part of the attenuation spectrum (1–2.8 MHz). Average
attenuations, AIB and IRC, were determined at the effective frequency range (1.53–3.8 MHz, −6 dB) of the transducers. The ultrasound parameters were calculated as mean values within the circular ROI (area 88 mm², figure 1) as well as at a discrete point (0.25 mm²) at the ROI center. The spatial variation of ultrasound parameters within ROI was quantified as standard deviation (SD). Additionally, to investigate the effect of signal windowing, AIB was analyzed using five different time window lengths (tw, figure 1) from 1 to 5 μs with 1 microsecond steps. More detailed descriptions of ultrasound measurements and analyses have been presented in our previous publication (Hakulinen et al 2005).

**DXA measurements and mechanical testing**

Areal bone mineral density (BMD) was determined with a Lunar Prodigy DXA device (GE Medical, Wessling, Germany) using the AP spine mode of the software. To simulate soft tissue, the samples were immersed in a water bath during the measurements. The measurements were conducted in the direction perpendicular to the parallel ends of the samples. The ultimate strength of the samples was determined by means of destructive testing (Zwick 1484, Zwick GmbH & Co. KG, Ulm, Germany). The samples were compressed to 5% strain with the strain rate of $4.5 \times 10^{-3} \text{s}^{-1}$ and the ultimate strength was obtained as the maximum stress. More details on DXA and mechanical testing and data analysis have been presented in our previous publication (Hakulinen et al 2005).

**Statistical analyses**

The normality of distribution of bone ultimate strength, BMD and ultrasound parameters was tested with the Shapiro–Wilk test. The limit for normal distribution was set to be $p > 0.05$. Pearson’s correlation analysis was used for the investigation of linear associations between the ultrasound, BMD and mechanical parameters. For the parameters not normally distributed, Spearman’s correlation analysis was used. The Wilcoxon test for two related samples was used to investigate significance of the differences for ultrasound parameter values determined at a discrete point at the ROI center and as a mean within the ROI. The limit of significance was set to be $p < 0.05$. A stepwise linear regression analysis was used to analyse the relations between the ultimate strength and the linear combinations of ultrasound parameters. SPSS v.11.5 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

**Results**

SOS and AIB but not nBUA, AA or IRC, determined as a mean value within the ROI or as discrete values at the center of ROI, were significantly different ($p = 0.03$, $n = 19–20$, table 1). Linear correlation coefficients between the mean values of ultrasound parameters within the ROI and the ultimate strength were higher than those obtained for the single point measurements (table 2). Based on the stepwise linear regression analysis, the linear combination of mean and SD of AA was a significantly stronger predictor of the ultimate strength than either the mean or SD of AA alone (stepwise linear regression, $r = 0.76$, $p < 0.01$). However, the variation (SD) of ultrasound parameters within ROI, only SD of AIB was a significant predictor of ultimate strength (tables 2 and 3).

The increase of the time window length increased the values of AIB within the ROI and decreased the variation of AIB within the ROI (table 3). Further, the strength of correlation between AIB and ultimate strength was dependent on the window length (table 3). Mean AIB within the ROI predicted ultimate strength strongly only when assessed using a short time
Table 1. Mean values of ultrasound parameters within the ROI and at the discrete measurement point of the ROI center. SOS and AIB (tw = 2 µs) were significantly different (p = 0.03, n = 19–20), whereas no differences were found in nBUA, AA or IRC.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ROI mean</th>
<th>ROI center point</th>
<th>ROI SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOS (m s⁻¹)</td>
<td>2236</td>
<td>2451</td>
<td>359</td>
</tr>
<tr>
<td>nBUA (dB/Hz/cm)</td>
<td>11.0</td>
<td>10.3</td>
<td>3.3</td>
</tr>
<tr>
<td>AA (dB cm⁻¹)</td>
<td>38.1</td>
<td>39.3</td>
<td>4.1</td>
</tr>
<tr>
<td>IRC (dB)</td>
<td>−12.7</td>
<td>−12.2</td>
<td>2.6</td>
</tr>
<tr>
<td>AIB (2 µs tw)</td>
<td>−26.7</td>
<td>−27.8</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 2. Linear correlations between the mean values of ultrasound parameters within the ROI and the ultimate strength showed a trend for higher values as compared to those obtained for a single point. SD of AIB within ROI was a significant predictor of the ultimate strength (n = 19–20).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ROI mean</th>
<th>ROI center point</th>
<th>ROI SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOS</td>
<td>0.78**</td>
<td>0.44a</td>
<td>−0.30a</td>
</tr>
<tr>
<td>nBUA</td>
<td>0.61*</td>
<td>0.50*</td>
<td>0.34</td>
</tr>
<tr>
<td>AA</td>
<td>0.65**</td>
<td>0.57**</td>
<td>−0.32</td>
</tr>
<tr>
<td>IRC</td>
<td>0.87**</td>
<td>0.65**</td>
<td>−0.17</td>
</tr>
<tr>
<td>AIB (2 µs tw)</td>
<td>0.53*</td>
<td>0.41</td>
<td>−0.74**</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01  
a Spearman’s correlation coefficients are calculated for these values as those were not normally distributed.

Table 3. The effect of time window length (tw) on the values and variation (SD) of AIB within ROI. Mean value of AIB within ROI is significantly correlated with the ultimate strength only using a short time window. SD of AIB within ROI predicts ultimate strength significantly also when determined by using long time windows (n = 19).

<table>
<thead>
<tr>
<th>Time window length (µs)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIB values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROI mean (dB)</td>
<td>−27.5</td>
<td>−26.7</td>
<td>−26.1</td>
<td>−26.0</td>
<td>−25.9</td>
</tr>
<tr>
<td>ROI SD (dB)</td>
<td>4.4</td>
<td>3.3</td>
<td>2.7</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Linear correlation between the ultimate strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROI mean of AIB</td>
<td>0.62**</td>
<td>0.53*</td>
<td>0.39</td>
<td>0.35</td>
<td>0.37</td>
</tr>
<tr>
<td>ROI SD of AIB</td>
<td>−0.57*</td>
<td>−0.74**</td>
<td>−0.54**</td>
<td>−0.82**</td>
<td>−0.80**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01  
a Spearman’s correlation coefficients are calculated for these values as those were not normally distributed.

window (tw = 1 µs, r = 0.62). However, the strongest association (r = −0.82, p < 0.01) between SD of AIB within ROI and ultimate strength was revealed with the long time windows (tw = 4 µs). IRC is a parameter quantifying ultrasound reflection at the bone surface, while AIB is a parameter calculated to determine the amount of ultrasound backscattering inside the bone. By using the stepwise linear regression analysis, we investigated the potential of combined ultrasound parameters to predict bone ultimate strength. Interestingly, the combination of pulse-echo ultrasound parameters, mean IRC and SD of AIB within the ROI, served as a particularly strong predictor (r = 0.92, p < 0.01) of the bone ultimate strength (figure 2).
Discussion and summary

In the present study, differences in values of ultrasound parameters, determined by the point measurement or from parametric images, were investigated. Further, the effect of the time window length on calculated values of AIB was analyzed. Finally, the linear associations between the bone ultimate strength and ultrasound parameters were investigated. Mean SOS and AIB within the ROI or at a discrete measurement point at the ROI center were significantly different. The linear correlations between the mean values of ultrasound parameters within the ROI and the ultimate strength showed higher values as compared to those obtained for a single point. These findings may be explained by the high variation of ultrasound parameter values within the ROI that diminishes the value of point measurements and emphasizes potential of parametric imaging. As the spatial variation of ultrasound parameters within e.g. human proximal femur has been reported earlier (Padilla et al 2004), the detected variation is not surprising. The variation in acoustic properties may be explained by the structural characteristics of trabecular bone and the relatively small focus size of the transducer, a diameter of 1.4 mm (−6 dB) for ultrasound beam at focus. The more focused the beam is, the more it is affected by spatial variation in trabecular structure, density and mechanical properties. As the mean trabecular separation of the samples was between 0.3 and 0.8 mm, the variation in pore size and porosity can significantly contribute to detected spatial variation in ultrasound parameter values.

Variation of AIB within the ROI was a strong predictor of bone ultimate strength. Notably, the linear association between the variation of AIB and ultimate strength was negative. Backscattering is related to scatterer size i.e. to the thickness of trabeculae or to size of the pores (Chaffai et al 2002). With small pore sizes, e.g. in compact bone, bone is acoustically more homogenous diminishing the variation of AIB. With greater pore sizes, e.g. in osteoporotic trabecular bone, trabecular bone is acoustically more heterogeneous, increasing the variation in AIB.

The association between AIB and ultimate strength was dependent on the length of the time window in ultrasound analysis. Mean AIB within ROI predicted ultimate strength significantly only with a short time window ($r = 0.62$, $tw = 1 \mu s$) while variation of AIB within the ROI was a significant predictor also with longer time windows ($r = -0.82$, $tw = 4 \mu s$). Previously, Hoffmeister et al (2002a) found weak negative and positive correlations ($r = -0.35$–$0.50$) between AIB and BMD in human trabecular bone in vitro. In bovine
trabecular bone, Hoffmeister et al (2000) reported a weak negative association between AIB and apparent density ($r = -0.34$ to $-0.51$). Both studies (Hoffmeister et al 2000, 2002a) were conducted using an ultrasound transducer with a 2.25 MHz center frequency. In their analyses, a 4 $\mu$s time window length was applied. Recently, Hoffmeister et al (2006) reported a strong negative correlation ($r = -0.90$) between AIB and BMD, measured using a 5.0 MHz center frequency and a 4 $\mu$s time window length. In the present study, positive linear correlation between AIB and ultimate strength may be explained by the smaller effect of ultrasound attenuation. As ultrasound backscattering arises from the deeper bone structures with the long time windows, the backscattered sound is more attenuated. Further, ultrasound attenuation increases as a function of frequency. Thus, attenuation effect on AIB is higher at 5.0 MHz than at 2.25 MHz. This may explain the negative association between AIB and BMD reported by Hoffmeister et al (2006) as well as the positive correlation between AIB and ultimate strength in the present study. In human trabecular bone, the true backscattering (broadband ultrasound backscattering, BUB), compensated by attenuation, is similar as measured with 2.25 or 5.0 MHz (Hakulinen et al 2005, Riekkinen et al 2006). Our results suggest that the association between AIB and mechanical properties is positive when low frequencies and short time windows (in the present study, $f = 2.25$ MHz and $tw = 1$ $\mu$s) are used. Due to the attenuation effect, increases in frequency and time window length diminish the strength of correlation between AIB and mechanical properties.

In conclusion, the absolute AIB values and their ability to predict bone mechanical properties depend on both ultrasound frequency and time window length. Further, our findings suggest that the measurement of two-dimensional parametric ultrasound maps could yield information on bone status not extractable from single point measurements. This highlights the diagnostic potential of parametric imaging. DXA, the gold standard technique in osteoporosis diagnostics, provides information on density only. The present ultrasound technique could provide diagnostically valuable information on the status of trabecular bone. As the future bone fractures can only be moderately predicted by bone density alone, any method providing information on other qualities (e.g. structure) can have a major clinical significance.

Acknowledgments

Financial support from the National Technology Agency (TEKES, projects 40464/05 and 70015/05), Finland and Kuopio University Hospital, Kuopio, Finland (EVO, project 5213) is acknowledged.

References

Hakulinen M A et al 2005 Prediction of density and mechanical properties of human trabecular bone in vitro by using ultrasound transmission and backscattering measurements at 0.2–6.7 MHz frequency range Phys. Med. Biol. 50 1–14
Jorgensen H L and Hassager C 1997 Improved reproducibility of broadband ultrasound attenuation of the os calcis by using a specific region of interest Bone 21 109–12
Njeh C F, Boivin C M and Langton C M 1997 The role of ultrasound in the assessment of osteoporosis: a review Osteoporos. Int. 7 7–22
Padilla F et al 2004 In vitro ultrasound measurement at the human femur Calcif. Tissue Int. 75 421–30