Assessment of broadband ultrasound attenuation in the os calcis in vitro


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SUMMARY
1. We have examined the relationship between the attenuation of broadband ultrasound in the os calcis in vitro and its bone mineral density measured by quantitative computed tomography and by physical density.
2. Broadband ultrasound attenuation was found to correlate closely with physical density ($r=0.85$, $P<0.0001$), but the correlation was less than that observed between quantitative computed tomography and physical density ($r=0.92$, $P<0.0001$). Measurements of broadband ultrasound attenuation and quantitative computed tomography were significantly correlated ($r=0.80$, $P<0.0001$).
3. Partial correlation analysis showed a significant relationship between broadband ultrasound attenuation and bone density, but when the effect of physical density was taken into account no significant correlation was found between broadband ultrasound attenuation and quantitative computed tomography ($r=0.08$, not significant).
4. Broadband ultrasound attenuation in three prospective amputees showed a high degree of concordance between measurements in vivo and in vitro, with no interference by surrounding soft tissues.
5. The correlation between physical density and broadband ultrasound attenuation was independent of quantitative computed tomography, suggesting that the technique measures aspects of density which differ from its mineral density. Broadband ultrasound attenuation holds promise as a reproducible, rapid, radiation-free assessment of skeletal status.

Key words: bone density, broadband ultrasound attenuation, os calcis, osteoporosis, quantitative computed tomography.

Abbreviations: BUA, broadband ultrasound attenuation; QCT, quantitative computed tomography; SEE, standard error of the estimate.

INTRODUCTION
The morbidity and mortality associated with post-menopausal osteoporotic fracture is becoming increasingly recognized as a major health care problem in the elderly. Population and prospective studies have shown that the incidence of vertebral and hip fractures is inversely related to bone mass [1–3] and the direct relationship between bone density and skeletal strength is well established in vitro [4] and in vivo [5]. Several techniques have been developed to measure bone mineral content or density non-invasively, including single-photon absorptiometry of the appendicular skeleton [3, 6], dual-photon and X-ray absorptiometry of the whole body or regional sites [7–9], quantitative computed tomography (QCT) [10], Compton scattering and neutron activation analysis. Many of these techniques have sufficient precision and accuracy to be used in the assessment of osteoporosis.

None of these techniques, however, discriminates completely between osteoporotic subjects and normal control subjects, which reflects in part the effect of other extra-skeletal and intra-skeletal factors on fracture risk not captured by these measurements [11–14]. In addition, many of these techniques are not widely available and are not likely to become so as they are time-consuming and expose the patient to radiation. For this reason we wished to explore the value of broadband ultrasound attenuation (BUA) in the os calcis [15], which has been suggested to provide a measure of bone mineral content. The technique has been reported to discriminate between patients with hip fracture and age-matched control subjects more completely than other techniques [16].

In this study, we compared measurements of BUA in the os calcis from post-mortem specimens with bone
mineral density assessed by QCT and by measurements of physical density. In order to assess the effects of soft tissue around and within the heel, we also examined the relationship between BUA measurements obtained in vitro and in vivo.

METHODS

Measurements

Os calcis were obtained at post mortem from 25 fresh cadavers (13 female, 12 male), ranging in age from 47 to 87 years (mean 69.3 years). After defatting and degassing in alcohol, the os calcis were stored in 10% formaldehyde solution. Measurement of BUA was undertaken with the os calcis placed between two 25 mm diameter broadband (1 MHz) ultrasonic transducers (Walker Sonix Ltd, Worcester, MA, U.S.A.), one of which acted as a transmitter and the other as a receiver [16]. The transmitting and receiving transducers were respectively attached to a pulse generator and a computer-controlled frequency analyser (Fig. 1). The os calcis was positioned so that the long axis was perpendicular to the path of the ultrasonic beam and the exact site of measurement was recorded. As ultrasound is markedly attenuated by air, the os calcis, mount and the transducers were submerged in a temperature-controlled water tank. Once the specimen was in position the scan took approximately 7 s to complete. BUA was measured over a range of frequencies (200 kHz to 1 MHz in steps of 16 kHz). The difference between the ultrasonic spectra obtained with and without the os calcis in the scanning path provided the frequency dependency of attenuation in the os calcis. The slope of the linear relationship between BUA and frequency (dB/MHz) was recorded for each os calcis (Fig. 2).

The ultrasound path through the bone can be considered to be cylindrical. As the measurement site was known, the 25 os calcis were then cored to produce a bicortical cylinder of bone (diameter 25 mm) from the measurement site. Using a specially designed Perspex mount, measurements of BUA were then obtained in each of the core samples (Fig. 1). An estimate of the mean apparent physical density of each core was obtained by weighing in water and in air. The former was carried out by suspending the core by fine wire from an analytical balance and submerging the suspended core fully in water. The loss of weight in water from that of weighing in air provides an estimate of bone volume, and the mean physical density was calculated as the weight (in air) per unit volume [17]. The reproducibility of paired measurements was 2%.

QCT imaging of the os calcis cores was undertaken using a Technicare 2020 CT scanner [18]. The scanning equipment was unchanged throughout the period of time over which all of the 25 os calcis were measured, and the machine was calibrated using a phantom immediately before each measurement in order to minimize scanner drift. Sections (4 mm thickness) were taken every 4 mm at 120 kV and 50 mA. Measurement was confined to within the trabecular bone in each core, and the mean density, expressed in Hounsfield units, was measured in an ovoid section of interest (area 5 cm²) in at least three consecutive slices through each core.

To examine the relationship between BUA measured in vitro and in vivo, BUA was studied in three patients undergoing below-knee amputations for peripheral vascular disease. Measurements in the os calcis were obtained before surgery, immediately after amputation, and after various stages of dissection of the os calcis. Informed consent was obtained before the study.

Statistical analysis

Linear regression analysis was used to compare BUA, QCT and physical density in the os calcis core samples.

![Fig. 1. Diagram of the apparatus for measurement of BUA. The os calcis core was held in a specially designed Perspex mount.](image)

![Fig. 2. Typical traces of BUA in a normal subject (a) and in an osteoporotic patient (b) across the range of frequencies used in this study.](image)
The standard error of the estimate (SEE), expressed as a percentage of the mean value on the y-axis, provides a measure of the scatter of data points around the regression line. Analysis of partial correlation coefficients was used to examine the relationship between two of the three variables (BUA, QCT and physical density) when the effect of the third variable was eliminated. Values within the various groups are expressed as means±SEM, unless otherwise stated, and statistical comparison was made using the paired t-test where appropriate.

RESULTS

The precision of BUA was 2.4% for repeated measurements on a single os calcis and 1.3% in duplicate measurements in a series of specimens. Measurements of BUA in the 25 whole os calcis correlated closely with values obtained in the os calcis cores (r=0.95, P<0.0001), although values in the latter were significantly lower than in the intact os calcis (58.2±4.7 and 62.4±4.4 dB/MHz, respectively; P<0.05). The coefficient of variation of duplicate measurements in a series of core samples was higher (4%) than that observed in the whole os calcis.

There was no significant difference in the mean ages of the male and female cadavers (69.2±2.8 and 69.4±3.4 years, respectively). The mean BUA in the os calcis cores was significantly lower in the female cadavers (42.1±4.0 dB/MHz) than in the males (74.6±5.5 dB/MHz, P<0.0001). BUA decreased with age in the female cadavers but not in the males (1.3% per annum, r=−0.48, P=0.09).

In the 25 cored samples, values of BUA correlated significantly with mean core density measured by weighing in air and water (r=0.85, P<0.0001, Fig. 3). The SEE was 12.5 dB/MHz (21.6% of the mean value). As also shown in Fig. 3, a highly significant correlation was observed between BUA and density measured by QCT (r=0.80, P<0.0001, see 25.9%). The closest correlation was that between QCT and the physical density of the cores (r=0.92, P<0.0001). This also had the lowest percentage SEE (16.9%, Fig. 4). Analysis of partial correlations confirmed a significant relationship between QCT and physical density (r=0.76, P<0.001) and between BUA and physical density (r=0.49, P<0.05). However, when the effect of physical density was taken into account there was no significant correlation between BUA and QCT (r=0.08; Fig. 5).

Table 1 shows the pre- and post-operative values of BUA in the three patients undergoing below-knee amputations. The mean values for BUA in the os calcis pre-operatively and after full dissection, defatting and degassing (82.0 and 81.2 dB/MHz, respectively) suggest that there was little (<1%) BUA by the surrounding soft tissues and marrow fat in vivo.

DISCUSSION

Several studies have attempted to examine the potential of ultrasound for measuring bone density and quality [19-21]. However, these studies examined the relationship between ultrasound velocity and bone elasticity and density, and were confined to measurements in compact cortical bone. Due to intrinsic difficulties in these measurements, the overall precision was in the order of 10-15%, thus limiting its clinical application. Values of elasticity and density were not found to be significantly different in normal and osteoporotic subjects, with less than one-third of osteoporotic females lying below the normal range [22].

The measurement of BUA in cancellous bone in vivo was first reported by Barger [23]. More recently, the tech-
nique was found to show complete separation between normal elderly control subjects and patients with hip fracture [16]. Although this discrimination may have been due in part to immobilization of the patients with fracture, it does suggest that the technique is capable of providing measurements of clinical relevance.

In this study, we have shown that the reproducibility of BUA in the os calcis in vitro compares favourably with that of more established methods for assessing bone mineral density. The decrease in precision (4%) when utilizing core samples of the os calcis probably reflects difficulties in repositioning of the cores on the mount before scanning. The reproducibility using the whole os calcis is comparable with that obtained in vivo by Langton et al. [16]. The high concordance between values of BUA in vivo and that in the fully dissected os calcis, indicate that the measurement is not significantly affected by the presence of surrounding soft tissues or bone marrow fat. This contrasts with single- and dual-photon absorptiometry and QCT [24, 25].

The correlations observed between BUA, QCT and physical density (Figs. 2 and 3) suggest that BUA, like QCT, is in part dependent on bone density. However, the partial correlation of BUA with density is less than that of QCT with density \( r = 0.49 \) and \( r = 0.76, \) respectively) and no significant correlation was noted between BUA and QCT when the effects of mean physical density were accounted for \( r = 0.08. \) This suggests that BUA captures some elements which contribute to apparent density independent of its bone mineral density. This provides some evidence for the notion [15, 23] that BUA, unlike QCT, may depend in part on the scattering and reflection of ultrasound by the trabecular elements of cancellous bone. Indeed, the degree of attenuation in the os calcis is known to be much greater \( (30 \text{ dB MHz}^{-1} \text{ cm}^{-1}) \) than that in purely cortical bone \( (3 \text{ dB MHz}^{-1} \text{ cm}^{-1}) \) [26, 27].

The competence of the trabecular framework is an important factor in the development of osteoporotic fracture [13, 28–31]. For this reason BUA may provide additional information of value to that obtained by other non-invasive methods. Since the os calcis or patella are the most accessible sites for this technique, it would be of interest to know whether changes in trabecular architecture in the os calcis induced by osteoporosis paralleled

![Fig. 4](image-url)  
**Fig. 4.** Relationship between bone mineral density measured by QCT and mean physical density of the os calcis cores. \( r = 0.92, P < 0.0001. \)

![Fig. 5](image-url)  
**Fig. 5.** Total correlation coefficients (within the triangle) and partial correlation coefficients (outside the triangle) for the three variables. Statistical significance: \*\( P < 0.05, \) \**\( P < 0.001. \)

### Table 1. Values for the slope of BUA at various stages of dissection of the os calcis in three patients undergoing amputation

<table>
<thead>
<tr>
<th>Slope of BUA (dB/MHz)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before amputation</td>
<td>87.5</td>
<td>78.5</td>
<td>890.1</td>
<td>982.0 ± 2.8</td>
</tr>
<tr>
<td>20 min after amputation</td>
<td>88.5</td>
<td>78.0</td>
<td>79.0</td>
<td>82.2</td>
</tr>
<tr>
<td>Skinned</td>
<td>88.5</td>
<td>78.5</td>
<td>79.0</td>
<td>82.2</td>
</tr>
<tr>
<td>Fasciectomy</td>
<td>88.0</td>
<td>74.0</td>
<td>80.6</td>
<td>81.6 ± 2.9</td>
</tr>
<tr>
<td>Isolated os calcis</td>
<td>87.0</td>
<td>77.0</td>
<td>80.8</td>
<td></td>
</tr>
<tr>
<td>Degassed os calcis</td>
<td>87.0 ± 0.5</td>
<td>77.3 ± 1.6</td>
<td>80.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>87.9 ± 0.5</td>
<td>77.3 ± 1.6</td>
<td>80.3 ± 0.4</td>
<td>81.6 ± 2.9</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.6%</td>
<td>2.0%</td>
<td>0.5%</td>
<td></td>
</tr>
</tbody>
</table>

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alterations at other trabecular sites, particularly at the spine and femoral neck. Further study is required to examine the relationship of BUA in the os calcis to measurements of bone mineral at other skeletal sites and its relationship to fracture risk. Whereas the clinical significance of this aspect of BUA awaits further evaluation, we conclude that BUA in vitro and in vivo provides a rapid and reproducible index of skeletal status, which has promise as a non-invasive, radiation-free method for the evaluation of osteoporosis.

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