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Accuracy of Elastic Property Measurement in Mandibular Cortical Bone is Improved by Using Cylindrical Specimens

Ultrasonic determination of elastic properties in human craniofacial cortical bone is problematic because of a lack of information about the principal material axes, and because the cortex is often thinner than in long bones. This study investigated solutions that permit reasonable determination of elastic properties in the human mandible. We tested whether ultrasonic velocities could be reliably measured in cylindrical samples of aluminum and mandibular bone, and the effects of reduced specimen thickness. Results indicted that (1) varying shape had minimal effects on ultrasonic velocities or derived elastic properties, and (2) ultrasonic velocities have relatively increased measurement error as propagation distances decreased. The increased error in velocity measurements of mandibular cortical specimens of less than 1.2 mm in thickness should be considered when assessing the reliability of single measurements. [DOI: 10.1115/1.1517567]

Keywords: *Ultrasound, Mandible, Femur, Aluminum, Human*

Introduction

Studies of cortical bone elastic properties using mechanical, ultrasonic and nanoindentation techniques, have shown differences between species, between individuals and within an individual, that relate to bone mineralization, quality and functional adaptation [1,2]. Likewise, studies have suggested that accurate regional elastic properties are essential in understanding the mechanical effects of skeletal pathologies, like osteoporosis or osteopetrosis [3–5]. Elastic properties are also necessary to refine finite element models to better approximate in vivo patterns of stress and strain [6,7]. An understanding of regional and interspecific variations in elastic properties may also be important in elucidating potential cellular and tissue-level mechanisms underlying adaptation of the skeleton.

Measurement of elastic properties in the cranial skeleton is of interest because of the unique function and structure of the various bones of the skull, and the unique clinical problems associated with them. For instance, attempts to understand stress patterns in the facial skeleton using experimental or modeling approaches have been limited by a lack of information on cortical elastic properties [8,9]. Likewise, efforts to understand the biologic and mechanical effects of dental disorders, such as edentulation, which results in a significant loss of skeletal structure and mass, have largely ignored potential changes in elastic properties and their mechanical significance.

The hierarchical structure of cortical bone necessitates a clear definition of scale in studies of elastic properties. Here, we are concerned with tissue-level elastic properties at what Frost [10] has called the middle intermediate organization (MIO). In concert with the overall gross shape of a skeletal organ, this is the level at which cortical bone functions in resisting functional loads. In cortical bone, the MIO includes primary and secondary osteons, resorption spaces, circumferential lamellae, the vascular network, and the innervation. The three dimensional structure of the MIO is often modeled as orthotropic or transversely isotropic. These structural patterns are thought to relate to the organization and orientation of osteons and associated structures of the MIO [11]. Presumably, variations in these structures result in variations in elastic properties at this level.

The pulse transmission ultrasonic technique is most useful for MIO-level study of material properties of the craniofacial skeleton because it is non-destructive, and allows three-dimensional orthotropic characterization within a single *in-vitro* bone organ. The ultrasonic technique also allows examination of regional variations within a bone organ and repeated measures of the same specimen. The majority of published studies have been limited to long bones. Several human and a few animal bones, including the femur, tibia, humerus and phalanx, have been characterized [1,2]. These studies in long bones used 5.0 mm cubes (hexahedrons) or 5.0×5.0×3.0 mm bricks (rectangular parallelepipeds) of bone with two opposing corners trimmed to allow measurement of ultrasonic velocities at a 45° angle to the orthogonal faces [7,11,12]. Kohles [13] has extended this method with higher frequency transducers to measure elastic properties in the cortex of the long bones of rats, where single dimensions of a cortical specimen may be less than 1.0 mm.

This type of specimen preparation is appropriate in the diaphyses of long bones because it is reasonable to assume that the axis of maximum stiffness approximates the longitudinal anatomical axis of a whole bone [14–17]. Preliminary investigations have demonstrated that for craniofacial cortical bone, the orientation of the axis of maximum stiffness is not easily determined from whole bone geometry [18,19]. Therefore, determination of elastic properties in the craniofacial skeleton is difficult with current techniques because (1) the principal axes are not known, (2) the cortex is sometimes quite thin (less than 1.0 mm), and (3) specimen collection is sometimes compromised by constrictive geometries that do not provide a flat cortical surface of sufficient size [18–21]. Because of these problems, few sites in the skull have been adequately characterized [22–25].

Misalignment between the actual principal axes and assumed principal axes generates errors in elastic property calculation. Turner and Cowin [24] theoretically demonstrated that elastic properties measured at 10° ''off-axis'' resulted in differences in elastic moduli of 1.3% and differences in shear moduli of 5.0%.

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At 20° "off-axis," measurement error increased 3-fold to about 4% and 18% respectively. Therefore, regional variations in the orientation of the principal axes within the craniofacial skeleton [16,17], as well as regional variations in postcranial bones like the femur [20], may strongly impact elastic property calculations.

To modify specimen preparation for the craniofacial skeleton, we used a 4.0 mm inner diameter trephine burr to harvest cortical bone with a uniform cylindrical shape [19,26,27]. This technique offered considerably less wastage of cortex than the standard technique of machining brick specimens, and allowed for principal axes determination, as described subsequently. Also, the smaller cortical surface of the cylindrical face (4.0 mm in diameter), compared to a cubic face of 5.0 mm on a side, reduced difficulties associated with complex surface topographies, as most cortical regions of the cranial skeleton offered flat or nearly flat topographies at this specimen size.

This study validates a modification of the pulse transmission ultrasonic technique that addresses the unknown orientations of the axis of maximum stiffness of craniofacial cortical bone, and the more occasional problems associated with a thin cortex and complex cortical topology. In so doing, we present empirical data about ultrasonic velocities in cortical bone from the human mandible, the human femur, and aluminum phantoms, in order to determine the impact of specimen orientation, size, and shape for ultrasonically estimating elastic properties in craniofacial cortical bone.

While the ultrasonic tests used samples from throughout the cortical surface of human mandibles, we also calculated elastic constants for cortical specimens of different shapes from a more limited anatomical region, the anterior portion of the mandible. This region was chosen because the bone is relatively homogeneous in density and yet has a large range of cortical thicknesses ranging from some of the thickest bone in the mandible (anteroinferior border) to some of the thinnest (anterosuperiorly near the alveolar margin) [28]. Likewise, the orientations of the axes of maximum stiffness cannot be easily determined based on gross anatomical shape.

Our objectives were to evaluate the effects of measuring ultrasonic velocity (1) when varying specimen thickness, (2) when varying specimen thickness perpendicular (at right angles) to the direction of ultrasound propagation, and (3) when varying shape, specifically a cylindrical compared to a cubic or brick shape. We further tested specimen shape effects by comparing the elastic properties of cortical bone from the anterior mandible that were calculated from ultrasonic velocities measured on (1) traditional trimmed brick specimens and (2) cylindrical specimens.

Materials and Methods

Specimen Preparation

To Study the Effects of Varying Thickness. The effect of varying thickness was evaluated using three materials: solid, extruded aluminum rounds (aluminum 6061-T6511), femoral bone, and mandibular bone. The aluminum phantoms tested the effects of varying thickness on a homogeneous material. Femoral bone specimens were taken from a common orientation in the anterior aspect of a single femur to allow evaluation of an orthotropic material with less material variation than that found in the diverse sample of mandibular specimens. The mandibular bone was our tissue of interest and the cortical specimens had much greater variability in ultrasonic velocities than the aluminum or femoral bone samples.

Aluminum rounds were cut with an Buehler Isomet low speed saw and hand polished to produce metal specimens (N=135) ranging in thickness from 0.2–12.0 mm. These samples had a homogeneous character and a constant diameter of 6.4 mm.

A femoral diaphysis was harvested from a 71 year old embalmed Caucasian adult male cadaver. Documented medical history did not demonstrate a history of bone disease. Although it is well known that embalming will have a small effect on elastic properties of bone in mechanical tests [29], little is known about the effect on ultrasonic velocities. This concern was not considered relevant as elastic properties were not calculated for the femoral specimens, and embalming will not alter the basic orthotropic nature of the material.

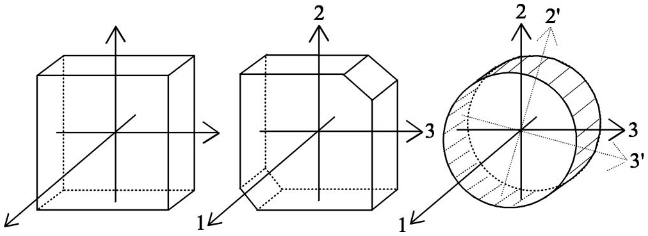

Fig. 1 Coordinate axes of the three different shapes of specimens: brick, trimmed brick and cylindrical. Direction 1 is the specimen thickness. Direction 2 is the assumed axis of minimum velocity (or stiffness) within the plane of the cortical plate and direction 3 is the assumed axis of maximum velocity (or stiffness) within the plane of the cortical plate. The brick specimen (left) was used for aluminum specimens, which are homogeneous and do not have differences in elastic properties by direction. In the trimmed brick mandibular specimens (center), material axes were based on anatomical coordinates. In the cylindrical mandibular specimens, directions 2 and 3 represent material axes based on anatomical coordinates while directions 2′ and 3′ were determined ultrasonically.

Two rough cortical bone strips were removed from the anterior face of the femoral shaft using a 702 dental burr and a handpiece. A Unimat miniature lathe with grinding wheels smoothed the opposing bone faces to produce blocks of equilateral widths of approximately 5.0 mm. Using the Isomet low speed saw, the blocks were then sectioned into bricks (N=97) ranging in thickness from 0.2–12.0 mm. If necessary, opposing surfaces were modified with a grinding wheel to remove any minor inconsistencies between the two faces and to assure parallel faces, which were assessed with calipers and by eye during preparation.

Ten unembalmed frozen dentate mandibles provided cortical cylinders (N=600) with a 4.0 mm diameter and ranging in thickness from 0.6–6.0 mm. Specimens were from different regions throughout the entire cortex of the mandible and exhibited more variation in local cortical topographies and presumed functions than our femoral sample. Regional variation among the mandibular specimens is described elsewhere [28]. Bone cylinders were removed from the facial and lingual cortices with a trephine burr (Nobel-Pharma). A Unimat miniature lathe with grinding wheels removed visible cancellous bone and modified, if necessary, the cortical surfaces to make them parallel. All bone samples were stored in a solution of 95% ethanol and isotonic saline in equal proportions, which maintains ultrasonically determined cortical bone elastic properties over time with minimal change [26,30,31].

To Study the Effects of Varying Shape. This experiment with aluminum bars and rounds assessed the impact of surface shape on measurements of ultrasonic velocities. We used a homogeneous rather than an orthotropic material to eliminate the effects of accurately determining the material axes prior to ultrasonic measurement and so that the effects of surface curvature could be isolated. Three different aluminum shapes were evaluated (Fig. 1). The first group consisted of 36 cylindrical aluminum specimens with diameters from 1.0-12.0 mm at 1.0 mm increments (three specimens per diameter), which were prepared with a Sherline 4400 lathe. The second and third groups included brick (N=36) and trimmed brick (N=30) aluminum samples prepared with a Sherline 2000 mill and ranged in width from 1.0–12.0 mm at 1.0 mm increments (three specimens per width). The trimmed brick group excluded specimens with widths of 1.0 mm or 2.0 mm due

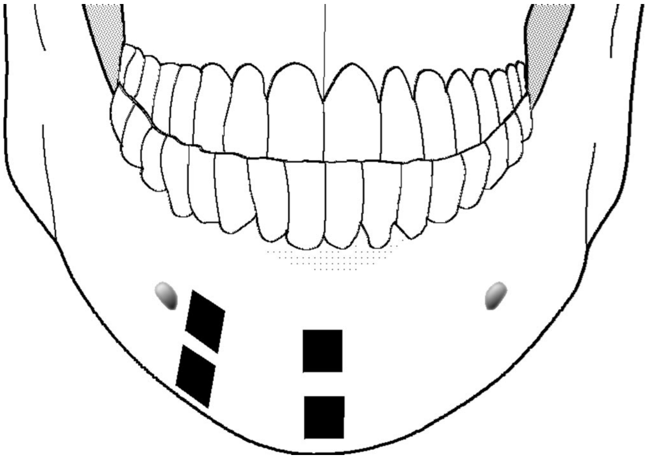

Fig. 2 Anterior mandibular sites for comparison of trimmed brick and cylindrical cortical specimens. These sites were chosen because of similarities between them in elastic properties and density. The data from the four sites were combined for this study.

to difficulties accurately trimming corners in these small metal specimens. The trimmed brick specimens were used to assess whether cubes or bricks with two opposite corners removed would have similar ultrasonic velocities as cylindrical and square samples at different specimen sizes. An Isomet saw cut all specimens at a constant thickness of 3.0 mm.

We also examined the effects of specimen shape on calculated elastic properties from a limited sample of cortical bone specimens from the anterior mandible. Two different mandibular bone shapes were evaluated, cylindrical (N=40) and trimmed brick (N=40) bone samples (Fig. 1). Four anterior mandibular sites from the buccal cortex (Fig. 2), including mid-body and inferior border sites at both the symphysis and just anterior to the mental foramen, were harvested from twenty unembalmed frozen cadaver heads. These four sites were selected because of overall similarity of the elastic moduli among them. Results from these specimens are pooled in this investigation. We have reported the results by site elsewhere [28]. Trimmed brick specimens were from ten mandibles with dental conditions ranging from partially (2 teeth) to fully (14 teeth) dentate, and ranged in thickness from 0.9–4.4 mm with widths ranging from 2.1–7.6 mm. Cylindrical specimens were from the remaining ten mandibles, each with at least 12 teeth, and ranged in thickness from 0.6–6.0 mm with a diameter of 4.0 mm. The principal axis of stiffness was determined for each cylindrical specimen prior to material property analysis, as described in the next section. Specimen thickness was the thickness of the cortical plate.

Ultrasonic Velocity Determination for all Specimens. A Max-Cal digital caliper verified the dimensions of each specimen to the nearest 0.1 mm. Material property testing used the pulse transmission ultrasonic technique, performed with a Hewlett-Packard pulse generator, two mounted piezoelectric transducers (2.25 MHz longitudinal, Panametrics V323-SU and 5.0 MHz shear, Panametrics V156-RM) and an oscilloscope (Tektronix TDS 420) [28].

Longitudinal and transverse ultrasonic waves were propagated through specific directions of each specimen (Fig. 1). The thickness experiments assessed the effects of varying specimen thickness on ultrasonic velocities through both the direction of varying thickness and perpendicular to this direction. Thus for the aluminum rounds of varying thicknesses, ultrasonic velocities were measured through either the specimen thickness or diameter. For the femur, velocities were measured through the axis of the specimen through which the thickness varied (parallel to the bone's longitudinal axis), and through both the radial direction (periosteal-endosteal direction), and the circumferential direction. For mandibular samples, velocities were measured through the thickness of the cortical plate (periosteal-endosteal direction), through which cortical thickness varied, and through the direction of maximum and minimum stiffness parallel to the plane of the cortical plate. In the 600 mandibular specimens used for the thickness experiments, we determined the directions of maximum and minimum stiffness by rotating each cylindrical specimen between the longitudinal ultrasonic transducers at 22.5° increments from 0° to 180°. Ultrasonic velocity measurements at these angles showed the axes of maximum (highest velocity) and minimum (lowest velocity) stiffness. These two velocities were at or near 90° to each other confirming orthotropy [28].

For the 40 cylindrical and 40 trimmed brick specimens from the anterior mandible, we measured longitudinal and ultrasonic velocities along the principal axes of the specimens and at 45° to the principal axes, according to the technique of Ashman et al. [11] for determining technical constants from densities and ultrasonic velocities in cortical bone specimens. We determined axes of maximum and minimum velocity (stiffness) within the plane of the cortical plate for the cylindrical specimens by rotating the specimens between longitudinal ultrasonic transducers, as discussed above. We used anatomical structure to determine the principal axes for the trimmed brick specimens. The axis of maximum stiffness was oriented parallel to the inferior border of the mandible and the axis of minimum stiffness was 90° to this axis and oriented inferosuperiorly or circumferentially.

Ultrasonic velocities were calculated by dividing the distance of wave transmission (specimen thickness, width, or diameter) by the apparent time delay minus the constant system time delay. Ultrasonic wavelengths were calculated by dividing average velocity by the transducer frequency. Wavelength was indicated on the figures by a vertical line to assess whether specimen dimensions of less than wavelength in the various considered directions had any impact on the measured velocities. Specimen dimensions of less than wavelength may produce bar wave velocities, which are less than the expected bulk wave velocities. However, the effect of specimen shape on bar wave production is not clear. Typically, bar waves are produced in long thin specimens where the transverse dimensions are less than wavelength [7,12,13]. For cranial bone, cortical thickness may be less than wavelength for the thinnest specimens if the transducers are in the 2.25–5.0 MHz range. Thus, the data allowed a test for this effect.

Elastic technical constants and other material properties are presented only for the mandibular specimens. Moist weights were obtained on a Mettler PM460 analytical balance, and submerged weights obtained with the Mettler suspension gig, reading to the nearest 0.01 g. Apparent density calculations were based on Archimede's principle of buoyancy [11]. For comparison, densities were determined for the femoral specimens. Using Excel spreadsheets with Mathcad macros, elastic moduli and shear moduli were calculated from the each specimen's dimensions, ultrasonic velocities, and apparent density [11].

Analysis. Data was stored in Microsoft EXCEL spreadsheets and analyzed using the Minitab statistical analysis program. Graphs of time versus distance and velocity versus distance were plotted for each material and transducer combination, and correlation coefficients were calculated where appropriate. Coefficients of variation (CV), which is the standard deviation of the residuals for propagation time expressed as a percentage of the mean propagation time, were calculated for each regression line. Differences were tested (1) among the velocities in the aluminum samples by shape and size through the use of two-way analysis of variance, (2) between the elastic properties for the cylindrical and trimmed brick mandibular bone samples with one-way analysis of variance, and (3) between mandibular and femoral longitudinal ultrasonic velocities by two-way analysis of variance (bone and direction). Posthoc Tukey tests were used to compare individual groups were appropriate.

Results

Effects of Varying Propagation Distance. Longitudinal and transverse ultrasonic time delays in all materials were highly correlated with propagation distance at $R>0.96$ (Fig. 3). As indicated by the larger CVs, the cortical mandibular sample had relatively greater scatter or heterogeneity, that coincided with the diverse provenance of these specimens from 10 cadavers at multiple sites throughout the mandible.

Longitudinal and transverse ultrasonic velocities in all materials were constant throughout the varying propagation distances (Fig. 4). However, variation in the calculated velocities increased at the smallest propagation distances and this variation was the largest at propagation distances of less than 1.0 mm. Overall, more variation at small propagation distances in aluminum and femoral bone was found with the 2.25 MHz longitudinal wave transducers than with the 5.0 MHz transverse wave transducers. The relative increase in scatter appeared less for mandibular cortical bone, but more small specimens as well as the smallest specimens were in the aluminum and femoral bone samples.

Effects of Varying Specimen Thickness at Right Angles to the Direction of Ultrasound Propagation. Longitudinal and transverse ultrasonic velocities in all materials were constant throughout most of the range of specimen thicknesses (Fig. 5). The most prominent deviation was the reduction in velocities in the aluminum specimens when specimen thickness perpendicular to the direction of ultrasonic wave propagation was less than 1.5 mm. A similar deviation was not found for transverse waves in aluminum. In femoral specimens, a slight decline in longitudinal and transverse velocities was found in specimens less than 0.5 mm in thickness. In mandibular specimens, a clear decline was not evident due to the greater variability, although the smallest specimens (0.6–1.2 mm) had velocities less than the mean (Fig. 5).

Effects of Varying Aluminum Shapes. Longitudinal and transverse time delays for brick, trimmed brick or cylindrical aluminum specimens were highly correlated with varying propagation distances or widths of the specimens ($R=0.999$) (Fig. 6). The slopes of the lines for each of the three shapes passed through the origin and were not significantly different from each other. The plot of data (Fig. 6) revealed overlap of the data points and lines such that each group could not be distinguished.

Velocities showed differences by specimen width (or propagation distance) (Fig. 6), which were significant as assessed with analysis of variance ($P<0.05$). Posthoc Tukey tests revealed that these differences were primarily due to the decrease in longitudinal velocities for specimens of 1.0 and 2.0 mm in cross-section, and an increase in transverse velocities for specimens of 1.0 mm dimension. When these smaller specimens were removed there were no significant differences between shapes and sizes (Table 1, Fig. 6).

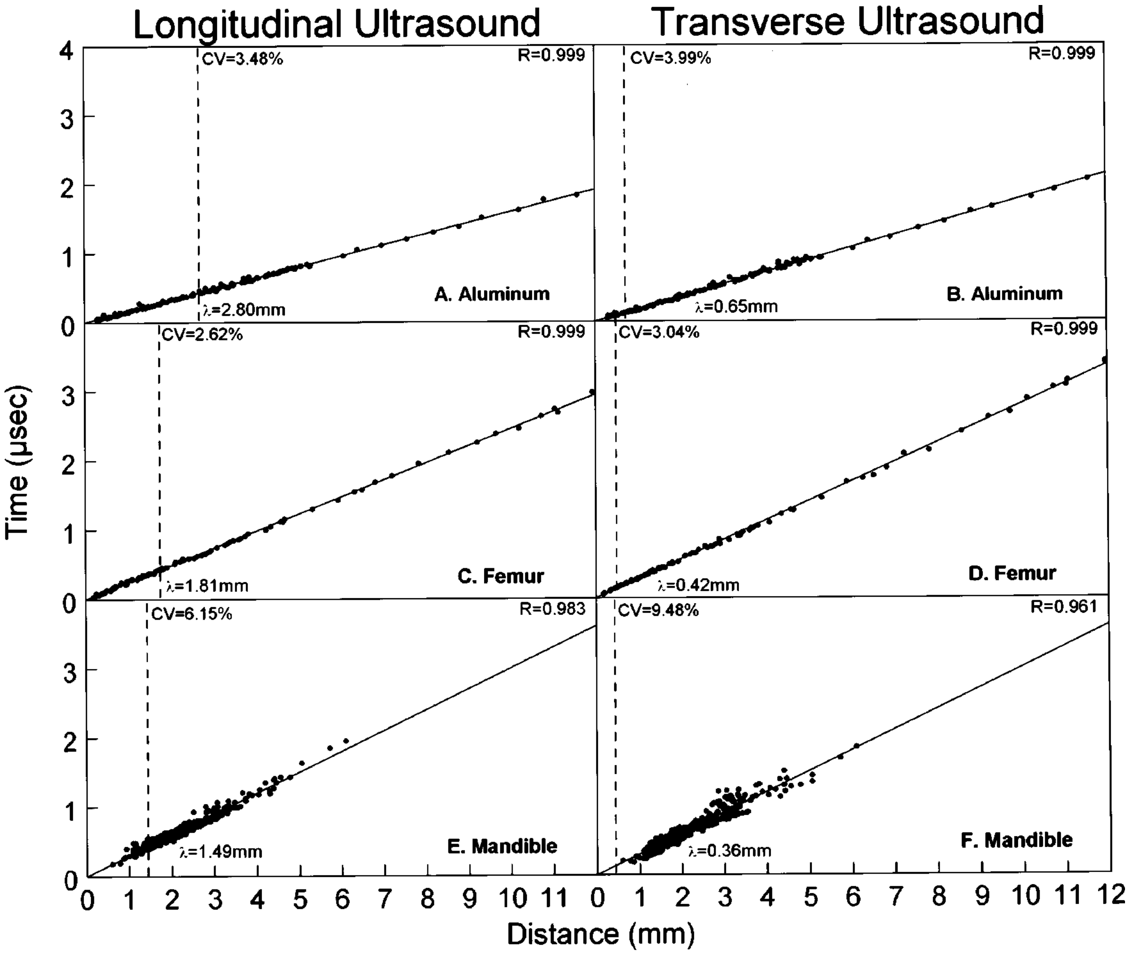

Fig. 3 Longitudinal and transverse wave propagation times versus distances for aluminum (A&B), femoral bone (C&D), and human mandibular bone (E&F). R values and coefficients of variation (CV) are presented on the graph. Mean wavelength is indicated by a dashed vertical line.

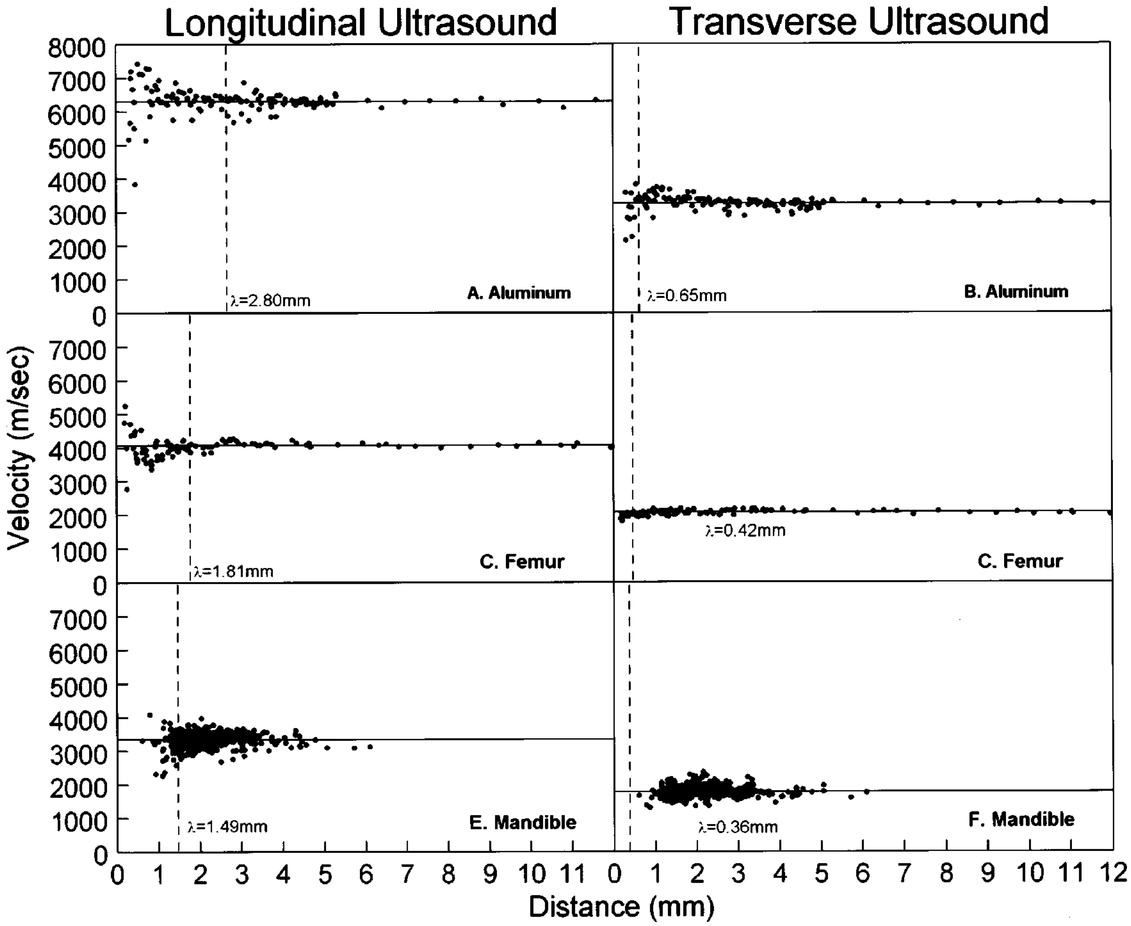

Fig. 4 Longitudinal and transverse ultrasonic velocities through varying specimen thicknesses for aluminum (A&B), femoral bone (C&D), and human mandibular bone (E&F). Samples correspond to those illustrated in Fig. 3. Mean wavelength is indicated by a dashed vertical line. Mean velocity is indicated by the solid horizontal line.

Ultrasonic Velocities and Material Properties in Brick and Cylindrical Mandibular Specimens. Longitudinal ultrasonic velocities were significantly different only between cylindrical specimens in the direction of maximum stiffness and trimmed brick specimens along the long axis of the mandible in the presumed direction of maximum stiffness (Table 2). No significant differences were found for transverse velocities. For calculated technical constants, only the shear modulus in the plane of the cortical plate showed a significant difference between shapes (Table 3). However, the absolute difference between the mean longitudinal velocities ($<3\%$) or between the mean shear moduli ($<7\%$) for these two shapes was small, and both shapes had material properties comparable to published data (Table 3).

In the cylindrical specimens, the average orientation of maximum stiffness deviated from the longitudinal anatomical axis of the mandible but this deviation was not large. Overall, the mean axis of maximum stiffness was 14° ($\mathrm{SD}=24.7°$) clockwise from the occlusal plane and was oriented slightly from the inferoanterior to the superoposterior.

Comparisons of Longitudinal Ultrasonic Velocities in Mandibular and Femoral Specimens. Longitudinal ultrasonic velocities through the thickness of the cortical plate were significantly higher in the femoral sample than in the total mandibular sample (Table 4). These were no significant differences between the mandible and femur for either the minimum or the maximum velocity in the plane of the cortical plate.

In the mandible, a comparison of velocities among the three orientations showed significant differences between each direction. Maximum velocities in the plane of the cortical plate were larger than minimum velocities in this plane, which in turn were larger than velocities through the thickness of the cortical plate (tangentially). In the femur, the average maximum velocity in the plane of the cortical plate was significantly larger than the average velocities in the other two directions, which did not differ significantly from each other.

Velocities were more variable in the mandibular samples as indicated by the higher standard deviations than in the femoral samples. This variation was paralleled by greater variability in mandibular densities. In the mandible, densities ranged from 1.51–2.18 grams/cm^3 ($\mathrm{mean}=1.94, \mathrm{SD}=0.11$), while in the femur, densities ranged from 2.05–2.07 grams/cm^3 ($\mathrm{mean}=2.06, \mathrm{SD}=0.01$).

Discussion

Effects of Varying Shapes on Material Properties. One of the more critical aspects of using the pulse transmission technique to estimate orthotropic material properties in cortical bone at an MIO level is a reasonable determination of the principal axes. Yoon and Katz [32], using the pulse transmission technique with multiple cubic specimens, demonstrate an angular dependence of material properties for different regions of the human femoral bone. They verify that the principal material axis of the femur is near the long axis and suggest that the arrangement of collagen fibers reinforces this orientation. Later, using the pulse-echo technique, this angular dependence is shown to change between normal, osteoporotic, and osteopetrotic bone [30]. When using reflec-

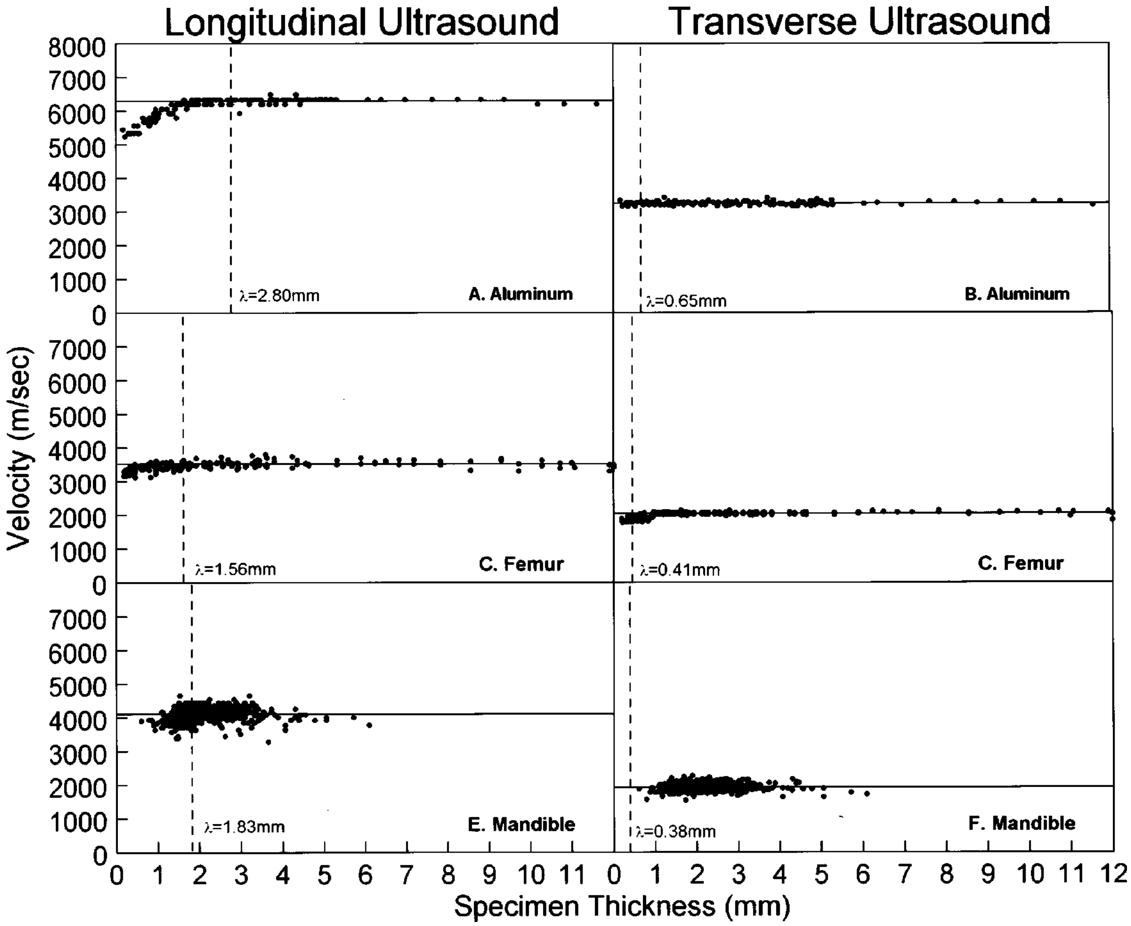

Fig. 5 Longitudinal and transverse ultrasonic velocities perpendicular to the direction of varying specimen thicknesses for aluminum (A&B), femoral bone (C&D), and human mandibular bone (E&F). Samples correspond to those illustrated in Figures 3&4 but velocities are measured along different axes. Mean wavelength is indicated by a dashed vertical line. Mean velocity is indicated by the solid horizontal line.

tive ultrasound, the periodic behavior of cortical specimens is also readily apparent and determined from the two orthogonal faces of the specimen. Antich et al. [21] demonstrate this behavior with cylindrical specimens of seven metals, acetal plastic, acrylic resin, and cubic specimens of human femoral cortex. Mehta et al. [27] demonstrate that the angular dependence of material properties is due to organic matrix organization as well as the mineral components. In sum, these studies suggest material orthotropy in cortical bone at an MIO level and the importance of defining material axes as part of quantifying elastic properties. Information on the orientation of material axes is especially important for studies of regional differences and to approach questions relating bone function to bone adaptation and growth.

Even though cortical bone is suggested to be best modeled as an orthotropic composite at the MIO level [11,13,15,22,26] few studies confirm the orientation of the material axes or have investigated the impact of inadequate predictions of these axes on the calculation of material properties. Carter [23], in an unpublished dissertation, approaches this problem by using cortical bone from 4 edentulous or partially dentate mandibles. He identifies the axis of maximum stiffness by measuring ultrasonic velocities around the perimeter of "coin-shaped" specimens in a method similar to that described here. A comparison of the elastic properties of 37 "coin-shaped" bone specimens and 39 conventional brick specimens shows similar values. However, no statistical tests were done and the samples were diverse, coming from throughout the mandibular body and ramus. Carter concludes that this technique could be implemented in the study of pelvic or skull bones. Our pilot studies in the mandible [18,19] also suggest that comparable elastic properties to those of conventional specimens [22] can be measured using cylindrical specimens.

In this study, the maximum difference between trimmed brick and cylindrical mandibular bone specimens was 1.7% for elastic moduli and 6.5% for shear moduli. There were two potential sources for this difference. First, bone was collected from different sets of cadavers, with each set varying in the completeness of the dentition. Of more relevance to the current investigation, the orientation of the average axes of maximum and minimum velocity (stiffness) in the plane of the cortical plate varied 14° between the two shapes, since the axis of maximum stiffness for the brick specimens was assumed to be parallel to the long axis of the mandible but was determined ultrasonically for cylindrical specimens.

Our results showed a small but statistically significant increase in longitudinal velocity along the axis of maximum stiffness when velocity was measured in the appropriate orientation. We suspect that the lack of a greater absolute difference and the lack of significant differences between shapes in other longitudinal and transverse velocities within the plane of the cortical plate relates to the small average angular differences between the two sets of mandibular samples and the variability inherent to such cross-sectional samples.

The differences in elastic properties between our brick and cylindrical specimens correspond with the findings of Turner and Cowin [20]. In a theoretical analysis, they predict errors in elastic moduli of 1.3% and shear moduli of 5.0% when velocity is mea-

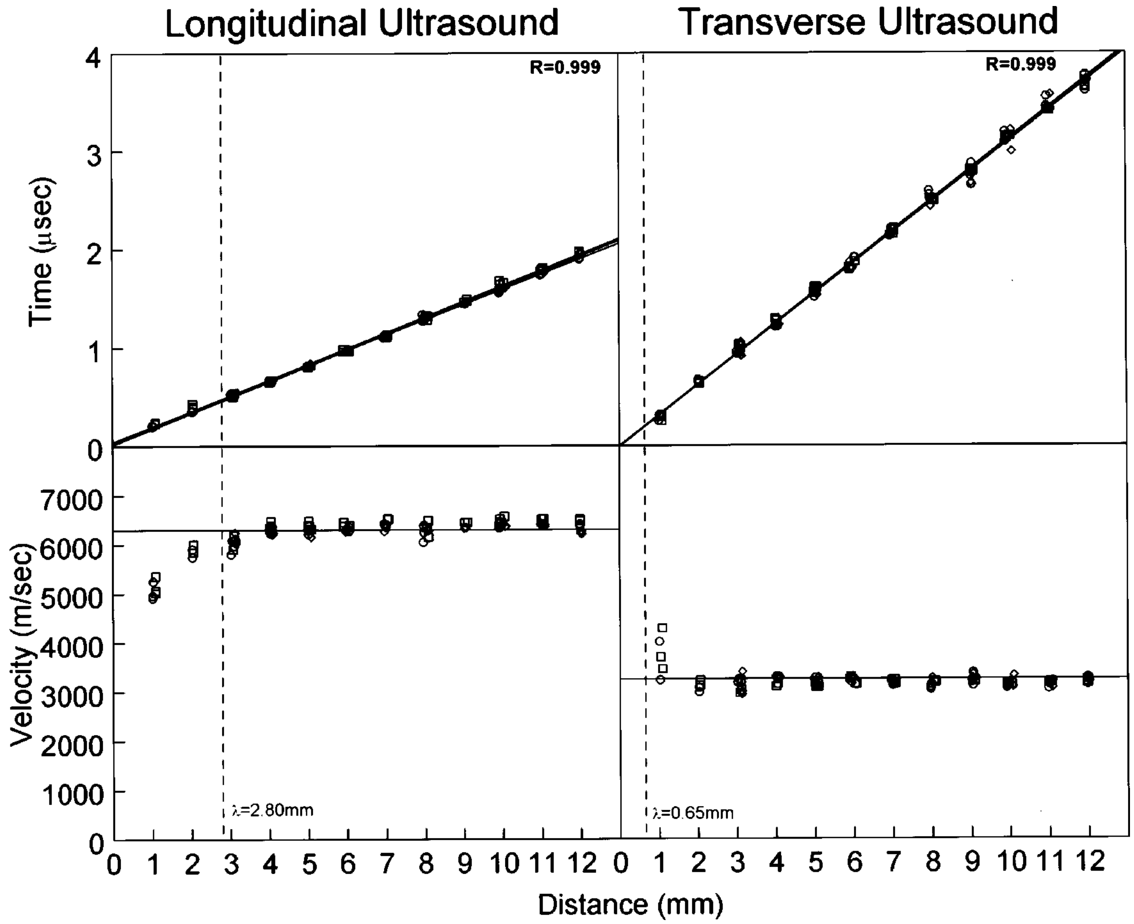

Fig. 6 **Longitudinal and transverse propagation times versus distance (upper figures) and corresponding ultrasonic velocities (lower figures) through brick, trimmed brick and cylindrical aluminum specimens of varying widths (propagation distances). Symbols represent brick ( ), trimmed brick (△) and cylindrical (□) specimens, although the data points overlap to such a degree that it is difficult to discriminate individual shapes. Changing the shape of the specimen did not affect either the longitudinal or transverse velocities, although velocities were reduced in the smallest specimens.**

sured at 10° "off-axis." This error increases to 4% and 18% respectively when velocity is measured at 20° "off-axis." We found no statistically significant differences between the elastic moduli of the two mandibular samples of varying shape. However, this is not surprising given the small differences predicted by Turner and Cowin [20] and the variability in our samples. However, our finding of a significant difference between the two shapes in the average shear modulus in the plane of the cortical plate validates the theoretical prediction.

The use of cylindrical specimens is critical in the analysis of material properties of craniofacial cortical bone because this technique does not assume prior knowledge of material orientation in the plane of the cortical plate. We were concerned that the curvature of a cylindrical surface by altering the area of contact between the transducers and the test specimen might affect the ultrasonic propagation. Further, it was not clear whether the variation in shape itself, especially in small specimens, might not also have an effect. The mandibular studies suggest that these problems are not a concern. However, a more direct test in a homogenous material with less potential interspecimen and interdirectional variability would provide further information. We tested the impact of shape variation in a sample of homogenous aluminum phantoms. At each width, the brick, trimmed brick and cylindrical aluminum specimens did not differ, alleviating our concerns about the effects of specimen shape. However, size was a factor as specimens of 1.0 and 2.0 mm cross-sectional dimension had reduced longitudinal velocities, and specimens of 1.0 mm dimension had increased transverse velocities. If the aluminum shapes were confined to widths of 3.0 mm or greater (longitudinal) or 2.0 or greater (transverse), there were no significant differences in velocities. Effects on other ultrasonic properties, such as attenuation, are possible but were not investigated.

There are several possible explanations for the differences in samples of the small specimens. The most important is likely to be

Table 1 **Average velocities (m/sec) of aluminum specimens of varying shape**

	Brick		Trimmed brick		Cylindrical			
	Mean	SD	Mean	SD	Mean	SD	F	P
Longitudinal:	6188	93	6207	62	6206	92	0.2	NS
Transverse:	3207	68	3241	97	3213	81	1.4	NS

Table 2 Average velocities (m/sec) of mandibular specimens of varying shape from the anterior mandible

	Trimmed brick		Cylindrical			
Velocity	Mean	SD	Mean	SD	F	P
Longitudinal: v_{11}	3.02	0.19	3.08	0.15	2.7	NS
Longitudinal: v_{22}	3.32	0.16	3.35	0.13	1.1	NS
Longitudinal: v_{33}	4.02	0.17	4.13	0.22	5.5	0.02
Transverse: v_{12}	1.70	0.08	1.70	0.15	0.1	NS
Transverse: v_{13}	1.75	0.11	1.74	0.13	0.1	NS
Transverse: v_{21}	1.41	0.09	1.41	0.11	0.1	NS
Transverse: v_{31}	1.54	0.07	1.52	0.09	0.4	NS
Transverse: v_{23}	1.86	0.06	1.87	0.11	1.7	NS
Transverse: v_{32}	1.90	0.07	1.89	0.11	0.2	NS

Maximum (v_{33}) and minimum (v_{22}) longitudinal velocities parallel to the plane of the cortical plate vary in orientation in the cylindrical specimens. These orientations were determined ultrasonically as explained in the text. In the trimmed brick specimens, v_{22} and v_{33} were assumed to be aligned with anatomical axes so that v_{22} was oriented circumferentially and v_{33} was along the long axis of the mandible. Tangential velocity (v_{11}) was through the thickness of the cortical plate (cortical thickness) in all specimens. Directions of transverse velocities are indicated by two subscripts. The first subscript indicates the direction of wave propagation and the second subscript indicates the direction of wave oscillation. As for longitudinal waves, 1 is the direction of maximum velocity in the plane of the cortical plate, 2 is the direction of minimum velocity in the plane of the cortical plate, and 3 is tangential in the direction through the width of the cortical plate.

the greater difficulty in measuring these specimens and thus increased error. This is probably the reason for the difference in the transverse velocities for the 1.0 mm specimens. It is possible that the difference in the longitudinal velocities represents the effects of a conversion from bulk to bar waves, as these specimens were longer (3.0 mm) than their width (1.0 or 2.0 mm), and their width was less than wavelength. But unlike a traditional bar wave test, ultrasonic propagation was through the cross-section of the specimens rather than along its length. But it is interesting to note that a similar reduction in longitudinal velocity was found in aluminum specimens in our experiments in which thickness was varying in cylindrical specimens of standard diameter (see Discussion below and Fig. 5).

Table 3 Average elastic and shear moduli (in GPa) of mandibular specimens of varying shape

	Dechow et al., 1992		Trimmed brick		Cylindrical			
	Mean	SD	Mean	SD	Mean	SD	F	P
E_1	11.3	1.3	11.7	1.5	11.5	1.5	0.2	NS
E_2	14.9	2.1	15.2	2.0	15.3	2.0	0.1	NS
E_3	20.5	4.6	20.8	3.1	20.5	4.2	0.1	NS
G_{12}	4.1	0.4	4.3	0.6	4.5	0.6	2.7	NS
G_{31}	4.9	0.5	5.1	0.5	5.0	0.6	1.1	NS
G_{32}	6.2	0.5	6.4	0.6	6.8	0.9	4.1	0.05

For elastic moduli, subscripts indicate directions as in Table 2. For shear moduli, the subscripts indicate the plane described by the two directions.

Table 4 Average longitudinal velocities (m/sec) of mandibular and femoral specimens

	Mandible		Femur		
Velocity	Mean	SD	Mean	SD	Sig
v_{11}	3089	175	3513	100	**
v_{22}	3457	172	3512	100	-
v_{33}	4107	186	4073	105	-
Sig. v_{11} vs. v_{22}	**		-		
Sig. v_{11} vs. v_{33}	**		**		
Sig. v_{22} vs. v_{33}	**		**		

Maximum (v_{33}) and minimum (v_{22}) longitudinal velocities parallel to the plane of the cortical plate vary in orientation in the mandibular specimens. These orientations were determined ultrasonically as explained in the text. In the femoral specimens, v_{22} and v_{33} were assumed to be aligned with anatomical axes so that v_{22} was oriented circumferentially and v_{33} was along the long axis of the femur. Tangential velocity (v_{11}) was through the thickness of the cortical plate (cortical thickness) in all specimens.** indicates significant difference with posthoc Tukey test at $P<0.05$.

Effects of Varying Thickness

Effects Parallel to Varying Thickness. Velocities were stable for all materials throughout most of the range of the thicknesses, demonstrating that accurate measurements can be made from most specimens despite variations in size (Fig. 4). However, increased variability in velocity was found in thinner specimens. This increase is due to the effects of measurement errors on ratios at small specimen sizes. In our aluminum and femoral samples, residuals of time about the regression line of wave propagation time versus distance (Fig. 3), were similar throughout the size (propagation distance) range of the samples. This error is proportionately larger for the smallest velocities (ratio of distance/time) resulting in larger errors in calculating velocities in smaller specimens.

Most of our samples showed a large increase in error near 1.0 mm propagation distance. This distance did not have any consistent relationship to wavelength, suggesting that this factor is unimportant for measuring velocities through thin specimens. The increase in error increases exponentially as propagation distances decrease, and could be modeled individually for each material and type of wave, which would statistically describe the variations in error illustrated in Fig. 4.

Kohles et al. [13] extends the techniques of Ashman et al. [11] for use in rat long bones. They use methods similar to those described here to test the reliability of ultrasonic velocity measurements in small specimens. They show strong correlations between propagation time and distance for bone and several homogenous polymeric materials down to propagation distances of 0.4 mm (in rat bone). They do not show plots of velocity by propagation distance, so it is not possible to detect an increase in error for small specimen sizes as in our study, but we suspect that a similar tendency would be found. Comparing our CVs with theirs shows that our aluminum sample has more error than their polymeric samples as our values of 3.48% (longitudinal) and 3.99% (transverse) are larger than their values, which range from 0.44% to 1.99%. Because of the homogeneity of aluminum, we were initially surprised at the greater error. However, repeated measurements gave similar results, and closer examination of our specimens revealed more surface flaws, compared to our femoral specimens, although both were cut in a similar manner on a Buehler slow speed saw, and the aluminum samples were also polished to reduce surface imperfection.

The CV of our human femoral sample (longitudinal: 2.62%; transverse: 3.04%) is lower than that found by Kohles et al. [13] in rat femur (longitudinal: 2.91%; transverse: 6.65%), indicating less error. This difference may be because our bone was taken from a single human femur, while the rat bone was from 10 individuals. The CVs of our mandibular sample (longitudinal: 6.15%; transverse: 9.48%) are larger than in the human or rat femurs indicating the greater variation among the diverse anatomical locations in the 10 human mandibles [28].

Effects Perpendicular to Varying Thickness. A potential problem with the measurement of ultrasonic velocities in cortical bone specimens is the possibility that cortical thickness is less than wavelength. If so, measurements of velocities within the plane of the cortical plate (perpendicular to cortical thickness) may be reduced in magnitude as the longitudinal ultrasonic wave transitions from a bulk wave to a bar wave. The computational techniques to derive the orthotropic technical constants require bulk wave velocities [11]. Bulk wave propagation refers to the situation of wave motion in an infinite medium where the wave is not affected by the edges of the specimen. Bar or guided wave propagation refers to the situation in which the ultrasonic wavelength is much larger than the cross-sectional dimension and the longitudinal and transverse waves can be coupled, attenuated and dispersed at the edges of the specimen. It is well known that ultrasonic velocities measured in bar wave propagation are smaller than those measured with bulk wave propagation [12,33]. However, the nature of the transition has not been explored in most materials, except to report maximum velocity differences between pure bulk and pure bar wave propagation.

The bulk wave to bar wave transition and its proximity to a cross-sectional dimension equivalent to wavelength may vary in different materials. Also, a typical brick (or cylindrical) cortical bone specimen is not shaped like a specimen prepared for measurement of its bar wave velocity. Bone specimens are thin in one dimension, their cortical thickness, while specimens prepared for bar wave measurement are long and narrow with both cross-sectional dimensions several times smaller than wavelength. Kohles et al. [13] avoids a potential problem with thin specimens by using higher frequency transducers to assure a smaller wavelength. We were interested in approaching the problem directly to see if we would see changes in velocity below wavelength when using the thin brick and cylindrical shapes. Our longitudinal transducer produced wavelengths larger than our thinnest specimens in all materials.

The apparent velocity should begin to decline below the cross-section dimension corresponding to wavelength [7,11,13]. Ashman [12] and Ashman et al. [11] show declines of 17.0% for plexiglass, 19.6% for aluminum, and 32.3% for bovine femur. Turner and Burr [34] report an increase of 17% for bone going from bar to bulk wave propagation, citing Ashman et al. [11] as a source. But this value was notably smaller than any reported in Ashman's studies for bone, although close to the value for plexiglass.

Ashman [12] and Ashman et al. [11] find a velocity of 6320 m/sec for aluminum that begins to decline at a specimen thickness of 10–15 mm, and reaches a stable bar wave of 5083 m/sec at 2–3 mm using a 2.25 MHz transducer. Our results show a stable velocity around 6200 m/sec that begins to decline at 15 mm and reaches a velocity near 5200 m/sec around 2 mm. The small differences between these experiments may relate to differences in the type of aluminum, which was not reported. Another consideration is the small sample size, in which specimens ranged from 0.5–10 mm in cross-sectional dimension. Our larger sample size defines the curve with greater precision.

In any case, our results show an apparent bulk to bar wave transition in our cylindrical aluminum samples as the cross-sectional thickness of the sample declines. However, it is important to note that this decline does not begin until the cross-section is 54% of average wavelength.

For bovine femur, Ashman [12] measured an initial velocity decrease from 3300 m/s to 3000 m/s within what he called the bulk wave region, followed by a more rapid decrease of 32% to 2200 m/s for bar wave propagation. Ashman's data did not show transitional data between the initial 10% velocity decline and a stable bar wave. The low sample size of 27 specimens does not allow easy determination of the initial point of decline which could occur anywhere between 10–20 mm. The low end of the curve is only defined by 5 specimens between 5–10 mm thick. Our results supported, but also added further details to Ashman's findings for human femoral bone. Our larger data set showed only a slight decline between 7–15 mm thickness from 3500 m/sec to 3450 mm/sec. Between 3–7 mm, velocity declined to about 3200 m/sec. Similar to aluminum, these results indicate that velocities do not have a significant decline until the cross-sectional dimension is about one half of wavelength.

As noted previously, it was difficult to note a trend for decreased longitudinal velocities for our mandibular specimens at small cross-sectional dimensions because of the greater inherent variability. If half of wavelength is used as a reasonable criterion for the lower end of bulk wave velocities, this would include mandibular specimens of less than 0.915 mm in thickness. Out of our total mandibular sample of 600, only four specimens were this thin.

Calculating Velocities and Elastic Properties. For purposes of calculating technical constants for mandibular specimens, the increased variability of velocity measurements of thin specimens in the direction of cortical thickness was of greater concern than the effects of a transition to bar waves for measuring longitudinal velocities perpendicular to specimen thickness. Our results suggest that all velocity measurements through the cortical plate, regardless of transducer frequency or wave type, should be excluded for specimens less than 1.2 mm in thickness because of the increased measurement error. When calculating technical constants, these velocities might be estimated based on velocities measured at similar sites where the cortex is thicker, if the sample only has a small proportion of such thin samples. For instance, in our mandibular sample of 600 specimens, those thinner than 1.2 mm were found at a scattering of sites. Among our 10 mandibles with 62 sites on each, only 12 sites had specimens thinner than 1.2 mm. Most of these sites had a single specimen in that range. Similar analysis is needed to assess the validity of such a substitution for investigations of other bones or skeletal regions.

The impact of error on mean velocities and calculated mean technical constants might also be decreased by larger sample sizes. However, reliable measurement of individual thin samples of less than 1.0 mm in cross-section by the technique of Ashman et al. [11] is problematic.

Elastic properties determined from cylindrical specimens are consistent with findings from brick-shaped specimens. However, the cylindrical specimens allow the additional advantage of determining the material axes within the plane of the cortical plate. This added information may result in more accurately estimated elastic properties compared to aligning specimens by anatomical landmarks, especially if such landmarks have large deviations from the actual material axes in cortical bone.

Although this research improves techniques for measuring elastic properties in cortical bone, other problems remain. For example, an additional assumption is that bone is organized orthotropically relative to the circumferential and transverse axes. Tests of this assumption may be worthwhile in regions of the skeleton where forces are consistently oriented at some angle to the cortical plate, such as in parts of the nuchal region of the skull, where the attached musculature is positioned at about 45° to the cortical surface and provides the only mechanism of loading.

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