Ultrasonic assessment of children's cortical bone anisotropy

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Résumé :

L'os cortical de l'enfant est peu étudié du fait de la rareté des échantillons disponibles. L'une des méthodes les plus prometteuses pour obtenir des informations sur le comportement mécanique de l'os enfant non pathologique repose sur la préparation et la caractérisation ultrasonore d'échantillons de faibles dimensions issus de déchets chirurgicaux. Dans cette étude, des cubes de 2 mm de côté ont été usinés à partir d'échantillons de fibulae enfants et adultes. Les 6 cœfficients de rigidité diagonaux (C_{ii}) *ont été évalués par mesure de vitesse d'ondes de volume de compression et de cisaillement selon les 3 directions de l'espace, à 5 MHz. Le comportement anisotrope des échantillons testés correspond à une isotropie transverse, le plan perpendiculaire à l'axe de l'os pouvant être considéré comme isotrope.*

Abstract :

Child cortical bone tissue is rarely studied because of the difficulty of obtaining samples. Yet the preparation and ultrasonic characterization of the small samples available, while challenging, is one of the most promising ways of obtaining information on the mechanical behavior of non-pathological children's bone. We investigated children's cortical bone obtained from chirurgical waste. Stiffness cœfficients were evaluated via an ultrasonic method. We observe a transverse isotropy with the plane perpendicular to the bone axis as an isotropic plane for adults and children. Stiffness cœfficients were highly correlated with age in children.

Mots clefs : os cortical enfant, ultrasons, anisotropie

1 Introduction

Cortical bone is an anisotropic medium because of its highly oriented, mineralized collagen fibril structure, and the literature on adults contains different assumptions regarding the type of anisotropy of the cortical bone structure. Some authors assume that cortical bone can be considered as transverse isotropic (five independent elastic coefficients)[1, 2, 3], meaning that bone elastic properties are similar in the transverse directions (radial and tangential) but are different in the axial direction. Others have made the more general assumption of orthotropy (with three perpendicular planes of symmetry) [4, 5, 6], where nine elastic coefficients are needed to fully characterize the medium. Little reference data is available on young bone mechanical behavior, especially on children's cortical bone. Most of these studies were conducted on only a few samples, because of the scarcity of specimens for laboratory testing. Moreover, the representativeness of these samples is questionable, since they are largely associated with child pathologies. The notion of anisotropy, particularly transverse isotropy or orthotropy, has rarely been investigated [7]. Here, we report measurements of ultrasonic wave velocities (compressional and shear) in the three orthogonal bone axes (axial, radial and tangential) to obtain the diagonal elements of the stiffness matrix (C_{ii}). To our knowledge, this study is the first to provide numerous stiffness coefficients on non-pathologic pediatric cortical bone. The major objective of this study is to obtain stiffness coefficients of children's cortical bone samples, and to gain insight into the anisotropic Hooke's law. Values from children were compared with those from elderly cortical bone samples.

2 Material and Methods

2.1 Samples preparation

15 fibula and 7 femur samples from 21 children (1-18 years old, mean age: 9.7 ± 5.8 years old) were extracted from surgical waste during lower limb lengthening surgery performed in Marseille, France. Fibula samples were extracted from the lower 1/3 of the bone. The selected population was composed of walking children not on drugs disturbing their bone metabolism. 16 fibula samples from 16 elderly patients (50-95 years old, mean age: 76.2 ± 13.5 years old) were extracted from the same anatomic location, but from cadavers at Inserm U1033 and UMR-T 9406 Ifsttar/UCBL (Lyon, France) bone bank. The fresh material was frozen and stored, the child bone at -80°C and the adult bone at -20°C. The samples were slowly thawed and then cut with a water-cooled low-speed diamond saw (Buehler Isomet 4000, Buehler, Lake Bluff, IL, USA) into cubic parallelepipeds (dimensions: $2 \times 2 \times 2$mm^3; mean= 1.96 ± 0.56mm). The faces of the specimens were oriented according to the radial (axis 1), tangential (axis 2) and axial (axis 3) directions defined by the anatomic shape of the bone diaphysis. The greatest challenge here was the very small size of the surgical waste bone (less than 1 cm in the axial axis), with the radial thickness of the sample imposed by the cortical thickness taken. The second difficulty was cutting samples this small with parallel faces.

This necessitated an enhanced protocol for the cutting. The mass density (ρ, $\mathrm{g/cm^3}$) was measured with a micrometric balance equipped with a density kit (Voyager 610, Ohaus Corporation, FlorhamPark, NJ, USA, measurement uncertainty of 0.001 $\mathrm{g/cm^3}$) and the dimensions were measured with a digital caliper (Absolute digimatik solar, Mitutoyo, Kanagawa, Japan, measurement error of 0.03 mm).

2.2 Ultrasonics measurements

In this study, we considered cortical human bone as an elastic unlimited medium (the wavelength is smaller than the transverse dimension of the sample). The more general assumption of orthotropy is made for the anisotropic behaviour of the samples. Then, the stiffness matrix **C** is defined by 9 independent coefficients. Because of the smallness of the samples, we only measured the velocities of pure compressional and shear waves propagating along the three principal axes, which gave us the diagonal elements of the stiffness matrix $\mathrm{C}_{ii|1\leq i\leq 6}$. Two mountings, one for compressional waves and the other for shear waves, were used. For both compressional and shear waves, we assumed a non-dispersive medium and we determined the wave velocity V using a comparison method.

- Compressional wave velocity
  The ultrasonic bench consisted of two transducers (VP1093, center frequency 5MHz, CTS Valpey Corporation, Hopkinton, MA) facing each other with their axes aligned and operating in transmission mode. The whole device was immersed in water. First, a reference measurement was made in water without sample. The bone sample to be tested was then placed over a gelatin block (agar) to keep it aligned between the transducers. The entire protocol was validated on bovine bone samples. We obtained $\mathrm{V}_{radial} = 3375 \pm 65$ m/s, $\mathrm{V}_{tangential} = 3637 \pm 91$ m/s and $\mathrm{V}_{axial} = 3999 \pm 31$ m/s, in agreement with the literature.

- Shear wave velocity
  Measurements were made with two transverse wave transducers (Panametrics V156, 5MHz, Inc., Waltham, MA) facing each other with their axes aligned and operating in transmission mode. First, a reference measurement was made in a 5 mm thick aluminum sample. The bone samples to be tested were then placed in contact between the transducers.

2.3 Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS Statistics 22, IBM, USA). The Shapiro–Wilk test was used to evaluate the normality of the distribution. A Pearson correlation was performed for normal distribution and a Spearman correlation was performed for non-normal distribution. The significance level is $p < 0.05$.

The Wilcoxon rank-sum test was used to determine the difference between C_{11} and C_{22} and between C_{44} and C_{55}.

3. Results

Mean values calculated for C_{ii} are presented in Table I for the fibula samples. The elastic coefficients for adult fibulae are quite similar to those from the literature for femur and tibiae. Values from the children's bone, are lower than those from the adults.

	Child (GPa)	Adult (GPa)
C_{11}	16.5±2.7	17.7±2.9
C_{22}	15.85 ±3.24	17.7±5.3
C_{33}	24.0±5.15	28.0±3.7
C_{44}	4.17±0.8	4.7±0.5
C_{55}	4.05±0.75	4.7±0.6
C_{66}	3.1±0.37	3.6±0.7

Table 1: Mean values of the diagonal stiffness coefficients (fibula samples).

For fibula samples, no significant difference was found between C_{11} and C_{22} and between C_{44} and C_{55}, for either adult or child bone ($p > 0.5$), which confirms transverse isotropy with

$$C_{33} > C_{22} = C_{11} > C_{44} = C_{55} > C_{66}.$$

A significant correlation was found in the children's bone (fibula and femur samples) between all the stiffness coefficients and age ($R > 0.56$, $p < 0.01$). In the elderly adult bone, we only found a negative correlation between C_{33} and age ($R = -0.63$, $p < 0.01$). Depending on age range, the linear interpolation slope changes from positive to negative. In the children's bone, we obtained a positive value ($R = 0.694$, $p < 0.01$) whereas in the elderly adult bone, we obtained a negative value ($R = -0.634$, $p = 0.08$).

3. Discussion

The method we used is based on measuring compressional and shear ultrasonic bulk wave velocities (BWV) propagating along various directions of a bone specimen [8]. While this method is widely used, it has major drawbacks related to specimen size and geometry. With a range frequency of 1-2.5 MHz, the specimen must typically be larger than a few millimeters. This is because measured wave velocities must be linked to bulk waves, which propagate when the wavelength is smaller than the dimension of the specimen [4]. In this study, samples were machined from fibulae whose cortical thickness was below 3 mm. By improving the cutting process so as to avoid any lack of parallelism, we finally obtained specimens of approximately $2 \times 2 \times 2$ mm^3. For both compressional and shear wave velocity measurements, we used a frequency of 5 MHz to achieve a wavelength greater than bone tissue heterogeneities ($<$ a few hundred microns) and smaller than the specimen dimensions. Another limitation of this study was that only elastic constants for the main diagonal of the stiffness tensor could be evaluated. It takes one or several 45° oblique cuts to retrieve all non-diagonal terms of the stiffness tensor, which was not possible with our specimen size. The longitudinal stiffness cœfficients (C_{11}, C_{22} and C_{33}) generally found for adult cortical bone with the ultrasonic method range between 16.8 GPa and 31.7 GPa [4, 6, 9]. However, these values were for femur or tibia bone; to our knowledge, no values for the fibula are available. These results on adult fibulae therefore contribute a new batch of data and allow us to compare adults' and children's values for the same bone from the same anatomic location. The results on children's bone enrich the literature concerning the mechanical properties of children's bone. Our findings show that stiffness cœfficients increase with age up to puberty, when they appear to reach adult values. The evolution of C_{33} with age shows a linear regression by age group, positive in the children and negative in the adults in accordance with an *in vivo* study by Drozdzowska et al. [10]. The aim of this study was to analyze the anisotropic behavior of our samples. The results for all specimens show transverse isotropy for both adult and child bone at the location tested. Anisotropy in cortical bone can be explained by multiple factors. Bone material properties depend on microscopic-scale components such as hydroxyapatite crystals and collagen [11, 12, 13, 14], and their layout, as confirmed experimentally in a study showing that ultrasonic velocity is influenced by changes in organic matrix [15]. Katz et al. [16] argued that orthotropic versus transversely isotropic symmetry was dependent on whether the tissue exhibited a predominately laminar or Haversian microstructure, respectively. According to Baumann et al. [17], transverse isotropy is governed primarily by apatite crystal orientations while orthotropy is governed primarily by intracortical porosity. While our study did not investigate any of these factors, further exploration would enrich our knowledge of the anisotropy of bone.

4. Conclusion

In conclusion, this study contributes a new set of ultrasonic wave velocities and elasticity values for children's cortical bone, providing insights into the evolution of stiffness cœfficients with age. Moreover, it offers the first complete analysis of stiffness cœfficients in the three orthogonal bone axes in children, giving some indication of how bone anisotropy is related to age. Future perspectives include studying the effect of the structure and composition of bone on its mechanical behavior.

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