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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. 22 fibula or femur samples from 21 children (1-18 years old, mean age: 9.7± 5.8 years old) were compared to 16 fibula samples from 16 elderly patients (50-95 years old, mean age: 76.2 ± 13.5 years old). Stiffness coefficients were evaluated via an ultrasonic method and anisotropy ratios were calculated as the ratio of C_{33}/C_{11}, C_{33}/C_{22} and C_{11}/C_{22}. Stiffness coefficients were highly correlated with age in children ($R>0.56$, $p<0.01$). No significant difference was found between C_{11} and C_{22} for either adult or child bone ($p>0.5$), nor between C_{44} and C_{55} ($p>0.5$). We observe a transverse isotropy with $C_{33} > C_{22} = C_{11} > C_{44} = C_{55} > C_{66}$. For both groups, we found no correlation between age and anisotropy ratios. This study offers the first complete analysis of stiffness coefficients 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.

1. Introduction

Bone is a hierarchical and organized structure with properties varying by successive stages from juvenile to mature state. Numerous studies have aimed to determine the mechanical properties of cortical bone tissue collected from adult human subjects (Bensamoun et al., 2004; Choi et al., 1990;

Cuppone et al., 2004; Grimal et al., 2009; Ho Ba Tho et al., 1991; Keller et al., 1990; Lotz et al., 1991; Reilly et al., 1974; Reilly and Burstein, 1975; Smith and Smith, 1976; Zioupos and Currey, 1998). Ultrasonic waves have frequently been used in the measurement of the elastic properties of adult bone *in vitro* (Ashman et al., 1984; Yoon and Katz, 1976; Rho, 1996; Espinoza Orías et al., 2009; Rudy et al., 2011; Baumann et al., 2012; Bernard et al., 2013). A method based on the measurement of both longitudinal and shear ultrasonic bulk wave velocities (BWV) allows the determination of numerous stiffness coefficients of the elasticity tensor C_{ijkl}, on a single specimen (Ashman et al., 1984; Rho, 1996; Espinoza Orías et al., 2009; Rudy et al., 2011; Baumann et al., 2012; Lang, 1969). Cortical bone is an anisotropic medium due to 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 (Haïat et al., 2009; Neil Dong and Edward Guo, 2004; Rho, 1996; Yoon and Katz, 1976) assume that human cortical bone can be considered as transverse isotropic (five independent elastic coefficients), 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 (Ashman et al., 1984; Hoffmeister et al., 2000; Rho, 1996) (with three perpendicular planes of symmetry), 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. Several papers study mechanical properties of children's bone by uniaxial bending (Currey and Butler, 1975; Jans et al., 1998; Davis et al., 2012; Agnew et al., 2013; Berteau et al., 2013; C. I. Albert et al., 2013; Albert et al., 2014), compression (McPherson et al., 2007; Ohman et al., 2011) or ultrasonic characterization (Berteau et al., 2012, 2013). Some even study mechanical properties at the tissue level by nanoindentation (Fan et al., 2006; Weber et al., 2006; C. Albert et al., 2013; Imbert et al., 2014). However, 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. Due to the limited number of samples available, papers have up to now focused on mechanical properties in only one axis, generally the axial direction. The notion of anisotropy, particularly transverse isotropy or orthotropy, has rarely been investigated. Only one study on this subject reports orthotropy in children's bone before ossification (McPherson and Kriewall, 1980). In our study, children's bone samples were recovered from small chirurgical bone waste, with exclusion criteria; the only way to obtain non-pathologic bone samples from children. Yet this adds a difficulty: the specimens have been cut into very small cubes (~2mm), smaller than those used in a previous study which tested 5mm samples on Resonant Ultrasound Spectroscopy (RUS) and obtained promising results (Bernard et al., 2013).

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_{11}, C_{22}, etc.). To our knowledge, this study is the first to provide numerous stiffness coefficients on non-pathologic pediatric cortical bone. The major aim of the study was to obtain stiffness coefficients of children's cortical bone samples, and to analyze the anisotropic Hooke's law enabling us to explore the anisotropic behavior of child cortical bone. Values from children were compared with those from elderly adult cortical bone samples to evaluate how stiffness evolves with age. To achieve this objective, we required an experimental protocol specifically for measuring ultrasonic parameters with very small samples, both compressional and shear; the protocol needed to be reproducible and robust.

2. Methods

2.1. Sample 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 chirurgical waste during lower limb lengthening surgery performed in Marseille, France. 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 (to lessen the impact of collagen degradation, which will be analyzed in a future study) 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: 2x2x2mm^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 (Figure 1).

The greatest challenge here was the very small size of the chirurgical waste bone (less than 1cm in the axial axis), the radial thickness of the sample being imposed by the cortical thickness taken. The second difficulty was cutting samples this small with parallel faces. This necessitated an enhanced mounting protocol for the cutting. The mass density (ρ, 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 g/cm^3) and the dimensions were measured with a digital caliper (Absolute digimatik solar, Mitutoyo, Kanagawa, Japan, measurement error of 0.03 mm).

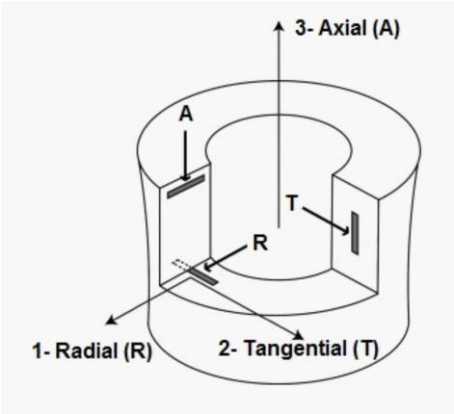


Figure 1: Sketch showing orientation of cortical bone samples prepared to study bone anisotropy. Figure adapted from (Reilly et al., 1974).

2.2. Theoretical approach

In this study, we considered cortical human bone as an elastic unlimited medium (the wavelength is smaller than the transverse dimension of the sample). Human bones are generally considered to be orthotropic (Ashman et al., 1984; Yoon and Katz, 1976).

For generally anisotropic media, Hooke's law is written as follows:

$$\sigma_{ij} = C_{ijkl}\,\varepsilon_{kl} \text{ where i, j, k, l} \in \{1,2,3\} \quad (1)$$

In Equation 1, σ_{ij} denotes the ij component of the stress tensor, ε_{kl} represents the components of the strain infinitesimal tensor and C_{ijkl} is the stiffness tensor. Assuming orthotropic behavior of the bone requires nine independent elastic coefficients of the stiffness tensor which can be expressed in Voigt notation as follows:

$$C_{IJ} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{21} & C_{22} & C_{23} & 0 & 0 & 0 \\ C_{31} & C_{32} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{bmatrix} \quad (2)$$

We calculated the velocities of pure compressional and shear waves propagating along the three principal axes, which gave us the diagonal elements of the stiffness matrix. The relationships between the velocities and elastic coefficients of the material are:

$$\begin{aligned} C_{11} &= \rho V_{11}^2 \\ C_{22} &= \rho V_{22}^2 \\ C_{33} &= \rho V_{33}^2 \\ C_{44} &= \rho V_{23}^2 = \rho V_{32}^2 \\ C_{55} &= \rho V_{13}^2 = \rho V_{31}^2 \\ C_{66} &= \rho V_{12}^2 = \rho V_{21}^2 \end{aligned} \quad (3)$$

V_{ii} : velocity of a compressional wave propagating in the i direction, with particle motion in the i direction;

V_{ij} : velocity of a shear wave propagating in the i direction, with particle motion in the j direction;

Anisotropy was measured as the ratio of elastic constants in the axial/radial (C_{33}/C_{11}), in the axial/tangential (C_{33}/C_{22}) and in the radial/tangential (C_{11}/C_{22}) anatomic specimen axes (Rudy et al., 2011; Baumann et al., 2012).

2.3. Ultrasonic measurements

To find the diagonal elements of the stiffness matrix, the velocities of compressional and shear waves need to be determined. 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 propagating in the x_i direction using a comparison method:

$$V_{ij} = \frac{l_{sample\_i}}{-\Delta t + \frac{l_{ref}}{V_{ref}}}$$

V_{ij} : compressional (i=j) or shear (i≠j) wave velocity;

l_{sample_i} : thickness of the sample in direction x_i;

Δt: time delay between the first arriving signal travelling in the reference medium and the first arriving signal propagating through the bone sample;

l_{ref} : distance between the two transducers in the reference medium;

V_{ref} : ultrasonic wave velocity in the reference medium.

2.3.1. Compressional wave velocity measurement

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 samples (V_{ref}). The bone sample to be tested was then placed over a gelatin block (agar) to keep it aligned between the transducers (Figure 2).

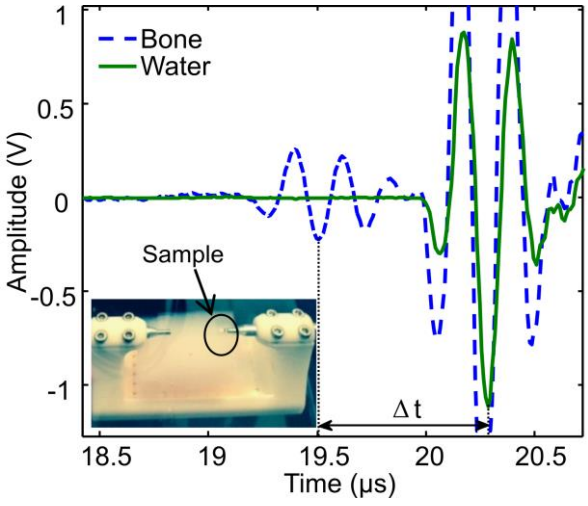


Figure 2: Picture of the experimental set-up (left), and example of a reference signal (water) and ultrasonic wave through the bone sample.

The entire protocol was validated on bovine bone samples. We obtained $V_{11} = 3375 \pm 65$ m/s, $V_{22} = 3637 \pm 91$ m/s and $V_{33} = 3999 \pm 31$ m/s, in agreement with the literature (Lees et al., 1979; Lipson and Katz, 1984; Lasaygues and Pithioux, 2002).

2.3.2. Shear wave velocity measurement

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 (Figure 3).

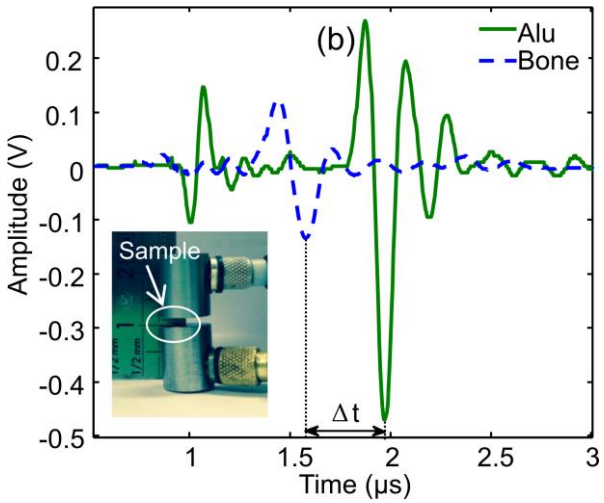


Figure 3: Picture of the experimental set-up (left), and example of a reference signal (aluminum sample) and ultrasonic wave through the bone sample.

2.4. 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 coefficients.

3. Results

Raw data are presented. All the values of ultrasonic wave velocities are given in Table 1, with mean and standard deviation for each group. The relationships established above between the velocities and the stiffness coefficients of the material gave the C_{ii} coefficients summarized in Table 2. The mean values of elastic coefficients from our study are also compared with values from the literature (Table 2).

The elastic coefficients for adult fibulae are quite similar to those from the literature for femur and tibiae evaluated with ultrasonic methods (Ashman et al., 1984; Hoffmeister et al., 2000). Values from the children's bone, especially the femur, are lower than those from the adults. Due to the mean age gap of the two groups (resp. 12.9 ± 3.3 y.o for fibula and 3.6 ± 5.3 y.o for femur), we cannot compare fibula and femur values in these children. A significant correlation was found in the children's bone between all the stiffness coefficients and age ($R>0.56$, $p<0.01$). Moreover, the stiffness coefficients are all correlated ($R>0.55$, $p<0.01$). In the elderly adult bone, we only found a negative correlation between C_{33} and age ($R=-0.63$, $p<0.01$).

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 $C_{33} > C_{22} = C_{11} > C_{44} = C_{55} > C_{66}$. In both groups, we found no correlation between age and anisotropy ratios.

Figure 4 shows the evolution of stiffness coefficients with age, revealing that stiffness coefficients increase in growing bone. Moreover, the effect of main direction is observed, with the axial stiffness coefficient (C_{33}) 1/3 above radial and tangential values (respectively C_{11} and C_{22}).

Figure 5 illustrates the evolution of the axial stiffness coefficient (C_{33}) with age. Depending on age range, the linear interpolation slope changes from positive to negative. A Spearman correlation was found between age and C_{33}; 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 ($R=-0.634$, $p=0.08$).

Figure 6 represents the evolution of anisotropy ratios with age. In both groups, we found no correlation between age and anisotropy ratios.

212 **Table 1. Ultrasonic wave velocities (compressional and shear) for all directions**

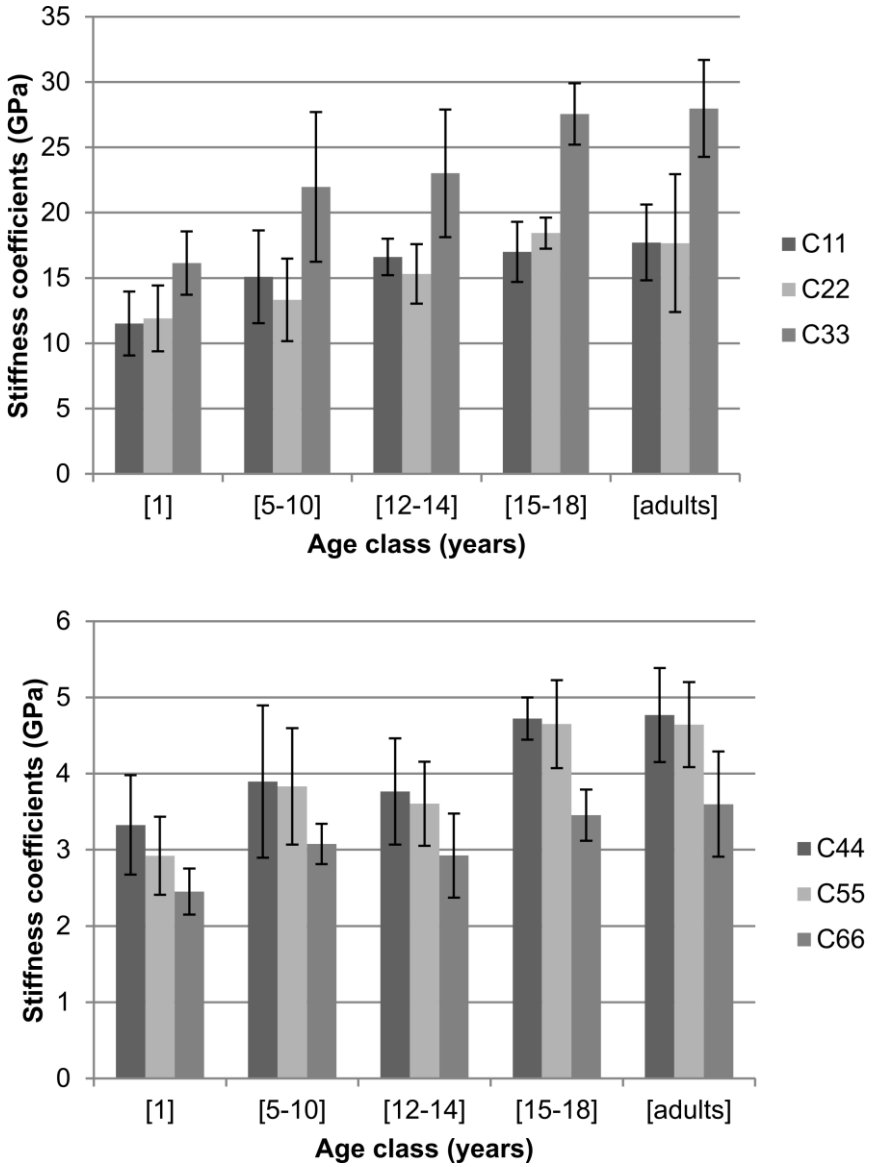
Samples	Age	Mass density	V_{11}	V_{22}	V_{33}	V_{31}	V_{32}	V_{13}	V_{12}	V_{23}	V_{21}
	years	kg/m^3	m/s	m/s	m/s	m/s	m/s	m/s	m/s	m/s	m/s
fibula 1	6	1873	2924	2912	3632	1506	1553	1446	1279	1535	1340
fibula 2*	10	1864	2972	2537	3596	1603	1701	1525	1306	1598	1378
fibula 3	10	1398	2406	2449	2920	1309	1347	1435	1339	1248	1460
fibula 4	10	1768	3170	3137	3994	1621	1608	1628	1354	1613	1379
fibula 5	10	1690	3181	2836	3358	1462	1409	1459	1361	1501	1202
fibula 6	12	1735	3033	3069	3873	1615	1591	1549	1355	1547	1431
fibula 7	13	1664	3053	3155	3491	1351	1514	1471	1319	1526	1243
fibula 8	14	1598	3194	2728	3320	1314	1348	1462	1165	1280	1199
fibula 9	14	1790	3228	3031	3964	1520	1616	1352	1478	1444	1269
fibula 10	15	1848	2985	3183	3918	1608	1599	1628	1369	1664	1295
fibula 11	15	1798	3099	3166	3666	1548	1551	1540	1347	1584	1365
fibula 12	16	1882	3199	3100	4057	1662	1612	1737	1436	1557	1329
fibula 13	17	1617	3455	3566	4012	1525	1616	1583	1364	1666	1396
fibula 14	18	1764	3071	3103	3918	1641	1684	1489	1440	1651	1351
Mean	**12.9**	**1734**	**2930**	**2903**	**1466**	**1507**	**1475**	**1324**	**1500**	**1303**	**1466**
SD	**3.3**	**182**	**292**	**279**	**132**	**121**	**120**	**99**	**116**	**89**	**132**
femur 1	1	1498	2491	2532	2960	1307	1362	1343	1272	1440	1383
femur 2	1	1712	2791	2798	3146	1287	1494	1404	1113	1464	1224
femur 3	1	1365	2515	2616	3206	1314	1319	1217	1209	1393	1250
femur 4	1	1873	2751	2858	3132	1403	1479	1314	1213	1505	1255
femur 5	1	1688	2678	2654	3287	1398	1383	1375	1220	1400	1154
femur 6	5	1798	2892	2658	3642	1298	1341	1477	1475	1378	1147
femur 7	15	2197	2439	2883	3613	1500	1520	1552	1397	1503	1315
Mean	**3.57**	**1733**	**2651**	**2714**	**3284**	**1358**	**1414**	**1383**	**1271**	**1440**	**1247**
SD	**5.26**	**268**	**172**	**133**	**255**	**79**	**81**	**109**	**124**	**52**	**84**
Adult 1	67	1748	4258	3190	3382	1599	1707	1645	1385	1618	1399
Adult 2	80	1761	4028	3390	3556	1586	1626	1636	1454	1621	1431
Adult 3	95	1623	3862	2672	3465	1728	1684	1676	1298	1545	1344
Adult 4	68	1664	4402	3367	3514	1727	1732	1679	1426	1705	1421
Adult 5	87	1573	3770	2755	2195	2218	1680	1464	1238	1550	1507
Adult 6	83	1798	3818	3412	3169	1575	1658	1690	1386	1600	1412
Adult 7	78	1647	3965	3248	2442	1699	1564	1684	1609	1579	1490
Adult 8	73	1831	3819	3051	2014	1840	1667	1598	1234	1675	1532
Adult 9	73	1855	4011	3332	3375	1660	1627	1661	1394	1662	1404
Adult 10	77	2230	3830	3020	3128	1473	1492	1728	1788	1476	1359

Adult 11	89	1577	3867	3299	3280	1620	1637	1582	1374	1679	1346
Adult 12	50	1775	4071	3414	3508	1645	1713	1714	1398	1691	1381
Adult 13	76	1882	4192	3016	3447	1529	1720	1665	1464	1669	1497
Adult 14	56	1914	4093	3206	3454	1583	1693	1472	1439	1718	1635
Adult 15	91	1498	3906	3166	3068	1368	1602	1617	1434	1559	1328
Adult 16	57	1623	4015	3250	3191	1504	1675	1653	1502	1575	1398
Mean	**76.2**	**1750**	**3174**	**3137**	**3994**	**1647**	**1655**	**1635**	**1426**	**1620**	**1430**
SD	**13.5**	**177**	**223**	**486**	**178**	**189**	**63**	**76**	**134**	**69**	**82**

* Mean value of two fibulae samples from the same child.

Table 2. Average stiffness coefficients (SD)

	Children (n=14)	Children (n=7)	Adults (n=16)	Hoffmeister (Hoffmeister et al., 2000)	Ashman (Ashman et al., 1984)
	fibula (GPa)	femur (GPa)	fibula (GPa)	tibia (GPa)	femur (GPa)
C_{11}	16.5 (2.70)	12.2 (2.42)	17.7 (2.89)	19.5 (2.0)	18.0 (1.60)
C_{22}	15.8 (3.24)	12.9 (3.15)	17.7 (5.27)	20.1 (1.9)	20.2 (1.79)
C_{33}	24.0 (5.15)	19.0 (5.50)	28.0 (3.71)	30.9 (2.1)	27.6 (1.74)
C_{44}	4.17 (0.800)	3.57 (0.833)	4.69 (0.518)	5.72 (0.49)	6.23 (0.479)
C_{55}	4.05 (0.746)	3.31 (0.921)	4.72 (0.579)	5.17 (0.57)	5.61 (0.398)
C_{66}	3.13 (0.373)	2.77 (0.656)	3.60 (0.690)	4.05 (0.54)	4.52 (0.371)



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217 Figure 4: Comparison of the mean (± standard deviation) of the stiffness coefficients with age class

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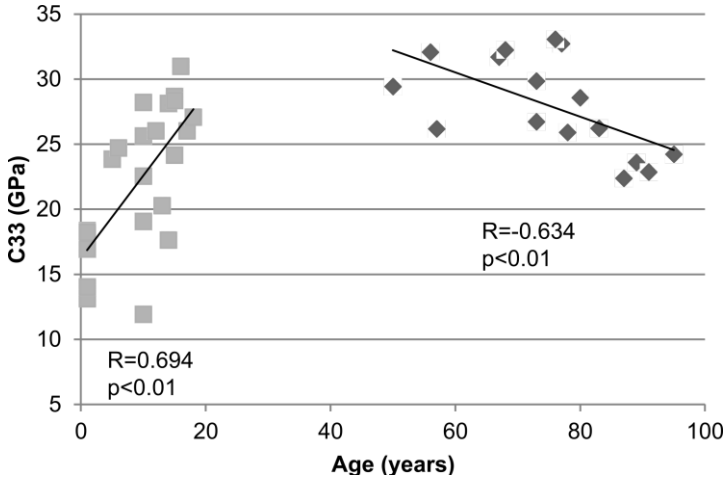

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220 Figure 5: Axial stiffness coefficient measured on children's bone samples (squares) and elderly adults'

221 bone samples (diamonds).

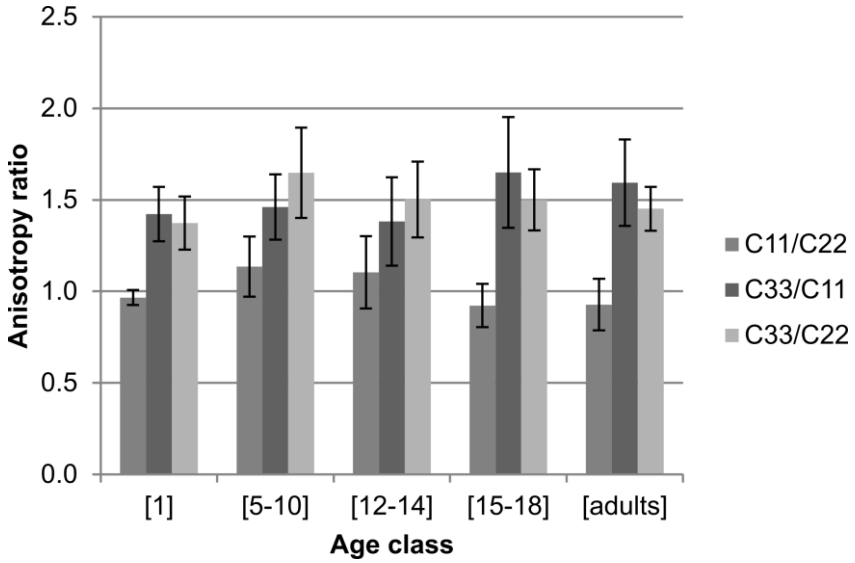

Figure 6: Representation of the mean (± standard deviation) of anisotropy ratios by age class.

4. Discussion

The first aim of the study was to determine and to compare stiffness coefficients in children's and elderly adults' cortical bone samples. The method we used is based on measuring both compressional and shear ultrasonic bulk wave velocities (BWV) propagating along various directions of a bone specimen (Lang, 1969). 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 (~5 mm). 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 (Ashman et al., 1984). In this study, samples were machined from fibulae whose cortical thickness was below 3mm. By improving the cutting process so as to avoid any lack of parallelism, we finally obtained specimens of approximately 2x2x2 mm^3. For both compressional and shear wave velocity measurements, we used a frequency of 5 MHz to achieve a wavelength greater than the typical size of 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 technically possible with our specimen size. This prevented conversion of the elastic stiffness coefficients into engineering coefficients (Young's moduli, shear moduli and Poisson's ratios).

The longitudinal stiffness coefficients (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 (Ashman et al., 1984; Bernard et al., 2013; Espinoza Orías et al., 2009; Hoffmeister et al., 2000). However, these values were for femur or tibia bone; to our knowledge, no value for the fibula is 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 coefficients increase with age up to puberty, when they appear to reach adult values (Figure 4). The evolution of C_{33} with age shows a linear regression by age group, positive in the children and negative in the adults (Figure 5). This trend is similar to the evolution of the bone mineral density with age (Bonjour, 1998; Boot et al., 2010). An *in vivo* study by Drozdzowska et al. (Drozdzowska and Pluskiewicz, 2003) assessed the speed of sound (SOS) at the hand phalanx in a population of people aged from 7 to 80. The authors conclude that the SOS increases linearly to a maximum reached at around 25-30 years old, after which values decrease more slowly up to the age of 80. These data differ from ours because the *in vivo* approach introduces the effect of soft tissue and bone geometry. Moreover, the study was performed on the hand phalanx, which is not mechanically stressed. Nevertheless, even with the gap in age coverage between our studies (our population being children of 1-18 and adults of 50-95), our *in vitro* results exhibit the same trend as their *in vivo* study.

The second 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, and both fibula and femur, at the location tested. Several studies point to the fact that ultrasonic wave velocity measurement relies on anatomical location. It has been suggested that ultrasonic wave velocity depends on the circumferential location (Bensamoun et al., 2004; Rho, 1996). Rudy et al. showed that anisotropy depends on the location along the bone: their tissue specimens, pooled from multiple donors, exhibited orthotropy at all locations along the femoral diaphysis and transverse isotropy at mid-diaphysis (Rudy et al., 2011). In this study, samples were extracted from the lower 1/3 of the bone, which does not explain the transverse isotropy.

Anisotropy in cortical bone can be explained by multiple factors. Bone material properties depend on microscopic-scale components such as hydroxyapatite crystals and collagen (Hasegawa et al., 1994; Burr, 2002; Currey, 2003; Follet, 2004; Boivin et al., 2008), and their layout, as confirmed experimentally in a study showing that ultrasonic velocity is influenced by changes in organic matrix (Mehta et al., 1998). Katz et al. argued that orthotropic versus transversely isotropic symmetry was dependent on whether the tissue exhibited a predominately laminar or Haversian microstructure, respectively (Katz et al., 1984). According to Baumann et al., transverse isotropy is governed primarily by apatite crystal orientations while orthotropy is governed primarily by intracortical porosity (Baumann et al., 2012). While our study did not investigate any of these factors, further exploration would enrich our knowledge of the anisotropy of bone.

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 coefficients with age. Moreover, it offers the first complete analysis of stiffness coefficients 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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