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## Children cortical bone characterisation: the ultrasonic issue

J.-P. Berteau<sup>a</sup>, C. Baron<sup>a</sup>, M. Pithioux<sup>a</sup>, P. Chabrand<sup>a</sup> and P. Lasaygues<sup>b</sup>

<sup>a</sup>CNRS - Aix Marseille Univ, 163 avenue de Luminy, 13288 Marseille, France

<sup>b</sup>Laboratoire de Mécanique et d'Acoustique, 31, Chemin Joseph Aiguier - 13402 Marseille  
Cedex 20

jean-philippe.berteau@univmed.fr



For cortical bone, important changes of the elastic properties values have been clearly shown in ageing but not in childhood, furthermore recent works considered osteoporosis as a pediatric disease with geriatric consequences and children are concerned by specific infantile osteo-pathologies. That is why there is a strong interest in the characterisation of the growing process of children bone. However, few mechanical properties of cortical growing bone are available in literature and do not yield to gold standards. Results found in literature for children bone concern specific location (close to cancerous cells) or cadaveric bone. They indicate a lower Young's modulus for children bone compare to mature bone. The goal of this study is to provide elastic properties values for human growing cortical bone. To reach this goal, we have analysed surgery waste (bone transplantation) from long bone (fibula). In a first step, a non destructive method was used to evaluate the velocity of ultrasonic waves from which the acoustic Young's modulus  $E_a$  is calculated using the difference of sound path duration and the mass density. Then, in a second step, a destructive method was used to obtain mechanical Young's modulus  $E_m$  using a 3-point microbending. Heihteen children bone samples were tested acoustically and due to the specific dimensions needed for micro three points bending fourteen samples were tested this way, but for each step, comparisons were made with adult specimens (twelve samples). The children surgery wastes from fibula (4 to 16 year old children) included in this study show an average  $E_a$  and  $\nu_a$  of 15.5 GPa (+/- 3.4) and 0.24 (+/- 0.08) at 10 MHz, and an average  $E_m$  of 9.1 GPa (+/- 3.5).  $E_a$  and  $\nu_a$  are in the same range for children and seniors but a linear correlation between  $E_a$  and  $E_m$  is found only for the fourteen samples of the children group.

## 1 Introduction

The cortical bone constitutes 80% of the human skeleton mass, hence its characterisation represents a main stake particularly for the diagnosis of several pathologies and most of the time on aging pathologies. Indeed, aging adversely affects the elastic properties of human cortical bone throughout life, from childhood to old age. Lot of studies highlighted differences in the mechanical behavior of physiological and osteoporotic adult bone but the evolution in mechanical properties of bone in childhood has been less studied and nowadays there is a tremendous lack of data on human growing bone.

In paediatrics, diagnosis of bone diseases and treatment decision are provided without specific knowledge of bone mechanical properties. The paediatric numerical models used in orthopaedics or in accidentology include adult mechanical parameters or values from a theoretical method displayed by [11]. This latter is based on the hypothesis of a decrease in Young's modulus from adult values to neonate values. But, this theoretical approach have been invalidated for the Young's modulus of the skull bone [14].

To assess the children bone quality, the quantitative ultrasound techniques seem promising as they are non-radiating and non-invasive. Nevertheless, in acoustical imaging, there is no data for children cortical bone to provide quantitative information about the mechanical characteristics of the children tissues analysed. For cortical bone, few studies considered mechanical characteristics of the growth process[6]. Moreover in most of the case they have chosen specific bone, close to cancellers cells, or cadaver fragments [1, 4], and they did not provide mechanical information on physiological bone.

The aim of this study is to give values of elastic properties ( $E$  and  $\nu$ ) of the human growing bone.

In this study, we have used surgery wastes (bone transplantation) coming from fibula (long bone) essentially composed of cortical bone. A two step method has been carried out, first an ultrasonic evaluation which provided the transverse Young's modulus and Poisson's ratio, and second, a three point microbending test which provided longitudinal Young's modulus. These values have been compared to a senior group composed of mature bone **fibula** (+ 75 YO).

## 2 Materials and Methods

### 2.1 Samples

Using a Marseilles hospital population, we studied cortical bone from autotransplants. From non pathological location, the whole autotransplant is extracted at the bottom of the fibula 5 cm up to the ankle. The non-use parts (wastes) are kept and cut using a low speed diamond saw (Isomet 1000, Buehler; Lake Bluff, IL, USA) in order to obtain parallelepipedic samples (plane and parallel surfaces). A total of 30 rectangular samples with parallel edges were cut. Their dimensions were measured with a digital calliper. One of the major difficulty looking at children bone is the small size of the samples: 15 to 35 mm long (bone axis direction), 10 to 20 mm wide and 2 to 3.5 mm thick (transverse directions). They are designated as (F or M for sex)-(age)-(F for fibula)-(number for each piece) and stored at -20°C. Two groups have been studied : 18 samples composed the children group (4-16 year old) and 12 samples constitute the senior group (+75 year old).

### 2.2 First step : ultrasound measurements

A specific ultrasound frame has been developed to process very small samples. The ultrasonic experimental setup is presented on Figure 1. It provides the evaluation of lon-

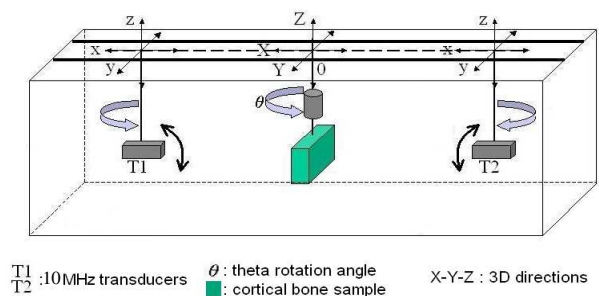


Figure 1: Ultrasonic experimental setup

gitudinal and transverse waves velocities in transverse direction (perpendicular to the bone axis) from the measurement



of the time-of-flight (TOF) before and after the first critical angle. This protocol has been detailed in [3]

The samples prepared for ultrasonic measurements were completely immersed in a temperature-controlled tank filled with distilled, degassed water at 25°C. They are mounted with y-axis corresponding to the axis of the bone (longitudinal direction), and x-axis and z-axis are the radial and circumferential directions (transverse directions). They are moved with 0.1 mm accuracy in x and y-axis. For z-axis, azimuth got 0.01° of accuracy. Two focused broadband transducers at 10 MHz (5 mm diameter, focal length 30 mm ; Imasonic, Besançon, France) operated through each sample. The sample thickness was first calculated using the echo-technique with transducers used as both the transmitter and receiver and then compared to calliper measurements.

Then, the TOF of longitudinal and transverse waves was measured in transmitter-receiver mode on a mechanical support allowing translations in 3D. Given the thickness of the sample and the distance between the transducers, TOF measurement at normal incidence gives the longitudinal wave velocity ( $V_L$ ) and the TOF measurement after the first critical angle gives transverse wave velocity ( $V_T$ ) as done by [13].

Finally, using the Archimedes' principle, a micrometric balance (610 GX0.001G voyager) measured bone mass density,  $\rho$  (each parameter was assessed by performing tested triplicate). Therefore, the transverse Young's modulus  $E_a$  and Poisson's ratio  $\nu_a$  were provided through the following equations:

$$E_a = \rho * \frac{V_T^2 * (3V_L^2 - 4V_T^2)}{(V_L^2 - V_T^2)} \quad (1)$$

$$\nu_a = \frac{V_L^2 - 2V_T^2}{2(V_L^2 - V_T^2)} \quad (2)$$

### 2.3 Second step : 3-point microbending testing

A specific 3-point microbending testing system has been designed for small samples and mounted on an Universal Testing Machine(Instron 5566A, Norwood, MA). To evaluate the cortical bone samples, a span to depth ratio of 10:1 was respected which reduced the number of tested samples to 14 for the children group. The smallest length was 15 mm and the smallest thickness 1.5 mm. A pre-force of 5 N was applied on the sample before testing till rupture. The displacement speed was 0.1 mm/min, close to static testing conditions; the test provided a force/displacement curve for each sample which is transformed in strain/stress curve from which the Young's modulus, noted  $E_m$ , is estimated.

## 3 Results

### 3.1 Ultrasound measurements

Figure 2 reports the values of longitudinal and transverse wave velocities calculated for the 18 children bone samples. The average values of longitudinal velocities are 3225 m/s for the children group and 3499 m/s for the senior group and the average values of transverse velocities are 1827 m/s for the children group and 1930 m/s for the senior group. The deduced transverse Young's modulus and Poisson's ratio,  $E_a$  and  $\nu_a$ , are around 17 GPa and 0.25 respectively, for the two

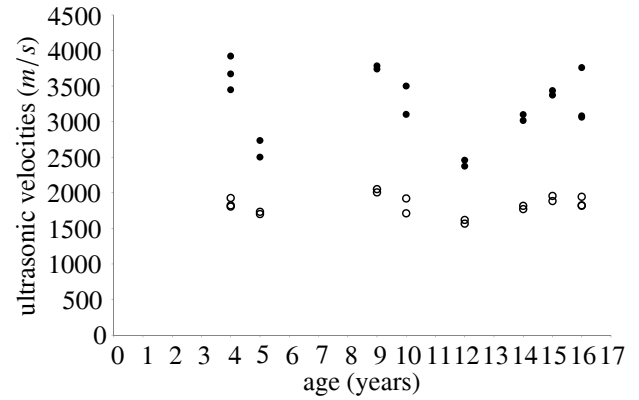


Figure 2: Longitudinal (black circles) and transverse (white circles) waves velocities measured at 10 MHz for children bone samples

age groups (Table 1). The measurement error of ultrasound velocity is estimated to 2.25%.

### 3.2 Three-point microbending test

Three-point microbending test provides an average longitudinal Young's modulus  $E_m$  of 9.1 GPa (+/- 3.5) for the children group and of 5.8 GPa (+/- 2.1) for the senior group (Table 1). The measurement error of the cell-force is estimated to 0.23.%

	$\rho$ kg/m <sup>3</sup>	$E_a$ (GPa)	$\nu_a$	$E_m$ (GPa)
Children	1.84	15.5 ± 3.4	0.24 ± 0.08	9.1 ± 3.5
Senior	1.73	16.7 ± 1.9	0.27 ± 0.05	5.8 ± 2.1

Table 1: Average values and standard deviation of  $E_a$ ,  $\nu_a$  and  $E_m$  comparing children group samples to senior group samples

## 4 Discussion

Both the values of  $E_a$  and  $E_m$  are consistent with values usually reported in the litterature [7, 10].

The elastic properties of children bone assessed by ultrasonic method are compared to old age bone samples extracted from the same anatomical site (bottom of the fibula, 5 cm up to the ankle). Our results show no statistic difference in transverse Young's modulus and Poisson's ratio between children and old age bone (Student T test  $p > 0.05$ ). The adult bone values found in this study spread in the typical range found in the literature [17, 2]. Concerning the Poisson's ratio, the average values is roughly 0.2-0.3, as usual for cortical bone. Furthermore, mass density of cortical bone from fibula measured according to the Archimedes' principle (1) are in the usual range found in the literature [16]. The values obtained for the mechanical properties are quite similar between children bone samples and old age bone samples. Nevertheless, further experiments are needed to explore the age-related variation in mechanical properties, in particular the age ranges between the children group and the senior group. Indeed, referring to the paper of [8], the speed of



sound (SOS) evaluated at the phalanx is quite the same for children around 10 year old and 70-80 year old people. But Drozdowska and colleagues put on evidence that the SOS at the phalanx increases linearly to a maximum value reached around 25 year old and then the values decrease more slowly up to the 80 year old.

These acoustical measurements on mechanical properties of children bone are looked at in relation to the results obtained from three-point microbending test (Figure 3). It has to be noted that the span to depth ratio of 10:1 is not theoretically sufficient and can induce some errors in the evaluation of  $E_m$  as shown in [15]. Nevertheless, all the samples are in the same span to depth ratio which allows to compare the results obtained for the children group to the results obtained for the senior group. We investigate the correlation between the elasticity in the two directions: longitudinal ( $E_m$ ) and transverse ( $E_a$ ).

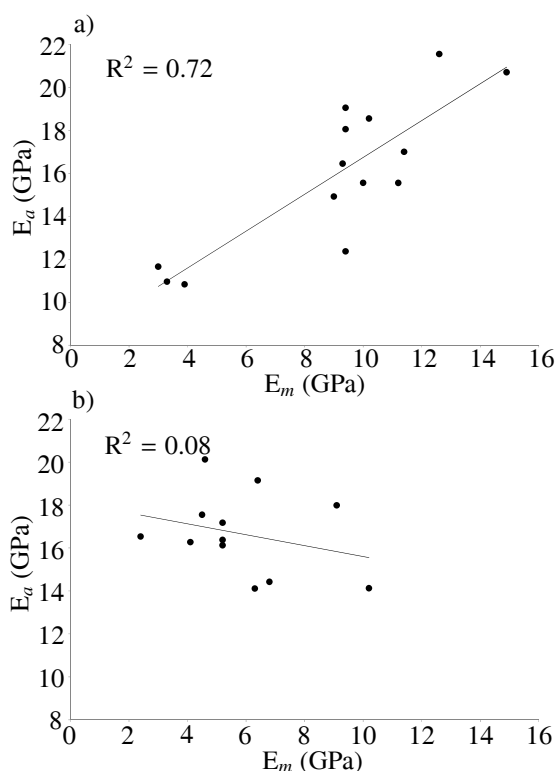


Figure 3: Correlation between Young's modulus measured by 3-point bending test (longitudinal direction) and Young's modulus measured by ultrasound (transverse direction): a)children group; b)senior group

In the literature, some studies on mature bone found low positive or non-significant correlations between elasticity in longitudinal and transverse directions. A recent study (Grimal2009) obtained a marginal negative correlation in agreement with the results we obtained on the senior group (Figure 3 (b)). On the other hand a very different trend appears concerning the 14 children bone samples testing by the two modalities: Figure 3 (a) shows a high positive correlation between  $E_a$  and  $E_m$ . To our knowledge this result is the first one showing a strong link between the elasticity of the longitudinal and transverse directions for human growing bone. Further work is needed to extend these observations to the anisotropy of growing bone which is a major parameter of the mechanical behaviour of children bone but still unknown.

## 5 Conclusion

Even if changes of material properties of cortical bone are more and more studied in ageing, this changes are not clearly shown in childhood. Indeed, some studies compare bone density (Bone Mineral Density : BMD) of children, like in Crohn's disease [9], but not the stiffness, strength or elastic modulus. The few studies considering mechanical characteristics of this process have chosen specific bone, close to cancerous cells [12], or cadaver fragments [5]. One of the goal of this study is to underline that the use of mature bone elastic properties to study growing bone pathologies is unsuitable. As a consequence the work started in this study is particularly relevant to gain insights on the specific mechanical behaviour of children bone.

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