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## **In vitro ultrasonic and mechanic characterization of the modulus of elasticity of children cortical bone**

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Abstract: The assessment of elastic properties in children's cortical bone is a major challenge for biomechanical engineering community, more widely for health care professionals. Even with classical clinical modalities such as X-ray tomography, MRI, and/or echography, inappropriate diagnosis can result from the lack of reference values for children bone. This study provides values for elastic properties of cortical bone in children using ultrasonic and mechanical measurements, and compares them with adult values. 17 fibula samples from 8 children (4 to 16 years old, mean age 10 years old  $\pm$  4.5) were compared to 16 fibulae samples from elderly adults (more than 75 years old). First, the dynamic modulus of elasticity (MOEdyn) and Poisson's ratio ( $\nu$ ) are evaluated via an ultrasonic method. Second, the static modulus of elasticity (MOESta) is estimated from a 3-point microbending test. The mean values of longitudinal and transverse wave velocities measured at 10 MHz for the children's samples are respectively 3.2 mm/ $\mu$ s ( $\pm$  0.5) and 1.8 mm/ $\mu$ s ( $\pm$  0.1); for the elderly adults' samples, velocities are respectively 3.5 mm/ $\mu$ s ( $\pm$  0.2) and 1.9 mm/ $\mu$ s ( $\pm$  0.09). The mean MOEdyn and the mean MOESta for the children's samples are respectively 15.5 GPa ( $\pm$  3.4) and 9.1 GPa ( $\pm$  3.5); for the elderly adults' samples, they are respectively 16.7 GPa ( $\pm$  1.9) and 5.8 GPa ( $\pm$  2.1). MOEdyn,  $\nu$  and MOESta are in the same range for children's and elderly adults' bone without any statistical difference; a ranking correlation between MOEdyn and MOESta is shown for the first time.

1 Title: In vitro ultrasonic and mechanic characterization of the modulus of elasticity of  
2 children cortical bone

3

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14 Abbreviated title: Cortical bone elastic properties in children

15

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1 2. Abstract

2 The assessment of elastic properties in children's cortical bone is a major challenge for  
3 biomechanical engineering community, more widely for health care professionals. Even with  
4 classical clinical modalities such as X-ray tomography, MRI, and/or echography,  
5 inappropriate diagnosis can result from the lack of reference values for children bone. This  
6 study provides values for elastic properties of cortical bone in children using ultrasonic and  
7 mechanical measurements, and compares them with adult values. 17 fibula samples from 8  
8 children (4 to 16 years old, mean age 10 years old +/- 4.5) were compared to 16 fibulae  
9 samples from elderly adults (more than 75 years old). First, the dynamic modulus of elasticity  
10 ( $MOE_{dyn}$ ) and Poisson's ratio ( $\nu$ ) are evaluated via an ultrasonic method. Second, the static  
11 modulus of elasticity ( $MOE_{sta}$ ) is estimated from a 3-point microbending test. The mean  
12 values of longitudinal and transverse wave velocities measured at 10 MHz for the children's  
13 samples are respectively 3.2 mm/ $\mu$ s (+/- 0.5) and 1.8 mm/ $\mu$ s (+/- 0.1); for the elderly adults'  
14 samples, velocities are respectively 3.5 mm/ $\mu$ s (+/- 0.2) and 1.9 mm/ $\mu$ s (+/- 0.09). The mean  
15  $MOE_{dyn}$  and the mean  $MOE_{sta}$  for the children's samples are respectively 15.5 GPa (+/- 3.4)  
16 and 9.1 GPa (+/- 3.5); for the elderly adults' samples, they are respectively 16.7 GPa (+/- 1.9)  
17 and 5.8 GPa (+/- 2.1).  $MOE_{dyn}$ ,  $\nu$  and  $MOE_{sta}$  are in the same range for children's and elderly  
18 adults' bone without any statistical difference; a ranking correlation between  $MOE_{dyn}$  and  
19  $MOE_{sta}$  is shown for the first time.

20

21 Keywords: Ultrasonic wave velocities -Elastic properties-Pediatrics-Cortical bone

### 1 3. Introduction

2           Cortical bone is an organic structure with mineral comprises approximately 80% of the  
3 human skeleton. Pathologies impacting human cortical bone quality include osteoporosis [1]  
4 in adults and osteopenia [2], Crohn´s disease [3] or osteopetrosis [4] in children. In addition to  
5 its cost in terms of health, European estimates predict that the direct cost of osteoporotic  
6 fracture will reach 76.7 billion €by 2050 due to demographic changes, notably the ageing of  
7 European populations [5]. A low bone mass in childhood is now recognized as a high risk  
8 factor for osteoporosis in later life [6] and authors consider the assessment of bone mineral  
9 status in children as a priority [7,8]. The Bone Mineral Density (BMD), which is one of the  
10 most gold standard parameters to assess mineral status, requires in the first intention, the use  
11 of the dual energy X-ray absorptiometry (DXA). The reference study [9] concerns only 7-17-  
12 year-old children, and was conducted with a Hologic DXA scanner (Hologic Inc., Waltham,  
13 MA, USA), ruling out comparison with values obtained via other scanners. However, the  
14 pediatric evaluation of the BMD raises problems of interpretation when the size of the bone  
15 varies related to the statural age of the child. The BMD is not correlated with the bone micro-  
16 architecture, and several studies have shown the ability of ultrasound measurement to assess  
17 the quantity and the quality of the explored bone area (elasticity and structure) [10,11].

18           There is a tremendous lack of data on young bone strength and mechanical behaviors:  
19 several papers [12,13,14] report age-dependence for ultrasonic axial transmission data but, to  
20 our knowledge, the elastic properties of cortical bone in children have been quantitatively  
21 investigated by only two *in vitro* mechanical studies [15,16], both using destructive tests on  
22 dry samples. In both cases, the experimental values for bone in children support the  
23 theoretical optimization hypothesis [17] of an increasing bone modulus of elasticity from  
24 neonate values to adult values, which is currently used in pediatric computational methods.  
25 All this would suggest the likelihood of lower ultrasonic wave velocities and modulus of

1 elasticity in children compared to adults. Yet the findings of a recent study performed by our  
2 team on rib cortical bone from teenagers with scoliosis [18] do not support that hypothesis.  
3 Although our study concerned pathological bone, our conclusion was that the *in vitro*  
4 ultrasonic wave velocities and the MOE values were close to the elderly adult values found in  
5 the current literature. The lack of reference concerning normative pediatric ultrasonic wave  
6 velocities and elastic properties of children's prevents the medical community from using the  
7 diagnosis devices based on analytic model of ultrasound scattering dedicated to adult's  
8 population (Quantitative ultrasound and echography). Consequently, the data collection and  
9 the development of relevant models of bone growth is a critical need to investigate an  
10 effective device of diagnosis and to meet the needs expressed by the medical community.

11         The aim of this study was to obtain ultrasonic wave velocities, dynamic and static  
12 modulus of elasticity, and Poisson's ratio for cortical bone samples from children, and then to  
13 compare these results with elderly adult cortical bone samples. Our two-stage study  
14 proceeded first by performing experimental ultrasound measurements to assess ultrasonic  
15 wave velocities, dynamic modulus of elasticity ( $MOE_{dyn}$ ) and Poisson's ratio ( $\nu$ ) and second,  
16 via 3-point microbending tests, to assess static modulus of elasticity ( $MOE_{sta}$ ).

17

## 18 4. Materials and Methods

19 Figure 1 is a diagrammatic representation of the method used.

### 20 4.1 Samples

21         In accordance with the stipulations of the French ethical committee, we studied  
22 cortical bone samples from Caucasian patients (4 to 16 years old, mean age 10 years old +/-  
23 4.5) of the University Hospital in Marseille who required auto transplant surgery. Surgical  
24 waste bone, largely consisting of cortical bone from fibula diaphysis was studied; the selected  
25 population was composed of walking children not on drugs disturbing their bone metabolism.

1 All auto transplant samples were excised from a non-pathological location in the fibula 5 cm  
2 above the ankle (figure 1). The elderly adult bone fibula samples were extracted from the  
3 same location as for the children's samples, but from cadavers (+ 75 YO) at Inserm U1033  
4 and UMR-T 9406 Ifsttar/UCBL (Lyon, France) bone bank. Samples for study were obtained  
5 by cutting the waste fragments in parallelepipeds (plane and parallel surfaces) using a low-  
6 speed diamond saw (Isomet 1000, Buehler; Lake Bluff, IL, USA). A total of 17 cortical bone  
7 parallelepipeds extracted from 8 children fibula samples were obtained and measured with a  
8 digital caliper. Great care was required due to the small size of the bone samples from the  
9 children: 15 to 35 mm long (bone axis direction), 10 to 20 mm wide and 2 to 3.5 mm deep  
10 (transverse directions). Sixteen elderly adult (+75 years old) bone samples excised from  
11 cadavers were similarly prepared. Each sample was designated (F or M for sex)-(age)-(F for  
12 fibula)-(number for each piece) and stored at -20°C in phosphate buffered saline with less  
13 than 5 freezing cycles.

14

#### 15 4.2. Ultrasonic measurements

16 We used an ultrasonic protocol specifically developed, as detailed in Pithioux et al.  
17 [19] and Loosvelt et Lasaygues [20], to process small and thin samples, and which has been  
18 validated on standard materials and animal and human adult bones. The ultrasonic bench [18]  
19 used consisted of a main arm carrying two linear stages. Each linear stage was carrying the  
20 end-rod transducer, and was moved linearly with increments of hundredths of millimeters.  
21 The parallelepiped bone sample to be tested was placed in the presumed geometrical center of  
22 the bench so that the maximum distance between the transducers and the center was 30 mm.  
23 The surrounding fluid medium was water at a temperature of 18°.

24 The surrounding fluid medium was water at a temperature of 18°. Two focused  
25 broadband transducers (2R, figure2) at 10 MHz, (5 mm diameter, 6 dB-bandwidth ranging

1 from 9 to 13 MHz; Imasonic, Besançon, France), facing each other with their axes aligned,  
2 were used to scan along transverse parallel directions through the sample (perpendicular to  
3 the bone axis). The focal area ( $X_a \times X_L$ , figure2) of the transducer was 3 x 3 mm with a focus  
4 set ( $F$ , figure2) at 30 mm. At this distance, the wave front was assumed to be plane, and the  
5 effect of the secondary lobes can be considered negligible. The wave reached the interface,  
6 also assumed to be plane at this scale, perpendicularly. Because the thickness of the samples  
7 ranged from 2 to 3.5 mm, a nominal frequency of 10 MHz was chosen, making the  
8 wavelength in water ten times greater than the sample thickness. Only propagation processes  
9 were taken into account and the ultrasonic wave attenuation was assumed to be weak.  
10 Longitudinal and transversal waves are excited when the incident sound wave strikes the bone  
11 surface under appropriate incident angles  $\theta_i$  ( $\theta_i \in [0^\circ, 90^\circ]$ ) and therefore only the time-of-  
12 flight (TOF) of the waves was measured.

13 For each sample, thickness  $e$  was first calculated using the pulse-echo technique. TOFs  
14 of the propagating waves were determined in the reflection mode, from the left (respectively  
15 right) transducer to the left (respectively right) interface of the sample. The TOF measured  
16 between transducers without samples was used as reference. All measured thicknesses were  
17 compared to caliper measurements.

18 The velocities  $V_l$ , (respectively  $V_t$ ) of the longitudinal (respectively transverse) waves in the  
19 samples were determined in the transmission mode using the equation (1):

$$20 \quad V_{l,t} = \frac{c_{water}}{\sqrt{1 + c_{water} \frac{\Delta t}{e} (c_{water} \frac{\Delta t}{e} - 2 \cos \theta_i)}} \quad (1)$$

21

22  $c_{water}$  is the ultrasonic wave velocity in water, measured without bone sample, using  
23 the distance between both transducers and the TOF of the wave in between,  $e$  the thickness of  
24 the sample.  $\theta_i$  is the incidence angle and for each incidence angle,  $\Delta t$  is the maximum of the  
25 cross-correlation between the first signal going through the zero stage with the sample, and



1 the reference signal measured earlier without the sample.

2 When  $\theta_i$  is lower than the first critical angle  $\theta_c$ , we consider that the wave velocity estimated  
3 is the velocity of longitudinal waves ( $V_l$ ) and when  $\theta_i$  is greater than  $\theta_c$ , we consider that the  
4 wave velocity estimated is the velocity of transverse waves ( $V_t$ ). Each parameter was assessed  
5 by performing the tests in triplicate. Given the density ( $\rho$ ), the transverse dynamic modulus of  
6 elasticity  $MOE_{dyn}$  and Poisson's ratio  $\nu$  were calculated using the following equations (2 and  
7 3) [21] [19]:

$$8 \quad MOE_{dyn} = \rho \frac{V_t^2(3V_l^2 - 4V_t^2)}{(V_l^2 - V_t^2)} \quad (2)$$

$$9 \quad \nu = \frac{V_t^2 - 2V_l^2}{2(V_l^2 - V_t^2)} \quad (3)$$

10 The US protocol tested the transversal axis of each bone samples, consequently only the  
11 transversal dynamic modulus of elasticity is calculated here.

#### 12 4.3. Mechanical measurements

13 We designed a 3-point microbending testing system specifically to deal with such  
14 small samples (figure 1), mounted on a Universal Testing Machine (Instron 5566A, Norwood,  
15 MA). To evaluate the cortical bone samples, a span-to-depth ratio of 16:1 is a general rule;  
16 but in small samples it cannot be achieved, the shortest sample tested here was 15 mm to  
17 guarantee that 85- 90% of the flexure of the bone is due to bending [22] leading to a minimum  
18 span-to-depth ratio of 10:1 for all samples. The thinnest sample was 1.5 mm leading to a  
19 mean width-to-thickness ratio around 4, which corresponds to a shear factor of 0.833 [23].  
20 Consequently, the number of samples from children was reduced to 12 and the number of  
21 elderly adult samples to 8. A pre-force of 5 N was applied on the sample before testing until  
22 rupture. The displacement speed was 0.2 mm/min, close to static testing conditions; the test  
23 provided a force/displacement curve for each sample, which was transformed into a  
24 strain/stress curve from which the static modulus of elasticity,  $MOE_{sta}$ , was estimated.

1 The F3P protocol tested the longitudinal axis of each bone samples, consequently only the  
2 longitudinal static modulus of elasticity is calculated here.

#### 3 4.4. Statistical Analysis

4 The Shapiro-Wilk test was performed to evaluate the distribution of results, Student's  
5 t-test was performed for normal distribution and a Spearman correlation was performed for  
6 abnormal distribution. The significance level is  $p < 0.05$ . All data generated by the  
7 experimental setup were analyzed using Excel 2007 and Analyse-it (Microsoft, Redmond,  
8 WA, USA).

9

### 10 5. Results

11 All the experimental results are presented as mean values in SIU (signal interface unit)  
12 with +/- standard deviation in brackets.

13

#### 14 5.1 Ultrasonic measurements

15 Figure 3 shows the longitudinal and transverse wave velocities calculated for the 17  
16 bone samples from children. The mean values of the longitudinal and transverse wave  
17 velocities measured at 10 MHz for the children's bone samples are respectively 3.2 mm/ $\mu$ s  
18 (+/- 0.5) and 1.8 mm/ $\mu$ s (+/- 0.1); for the elderly adults' bone samples, values are respectively  
19 3.5 mm/ $\mu$ s (+/- 0.2) and 1.9 mm/ $\mu$ s (+/- 0.09). The measurement uncertainty for ultrasonic  
20 wave velocity on our bench is estimated at 2.25%. The mean density ( $\rho$ ) for each group is  
21 reported in table 1, as are the deduced dynamic modulus of elasticity ( $MOE_{dyn}$ ) and the  
22 Poisson's ratio ( $\nu$ ).

23

24

25

## 1 5.2 Mechanical measurements

2 The three-point microbending test provided a mean  $MOE_{sta}$  for each group, as shown  
3 in Table 1. The measurement uncertainty for the cell-force is estimated at 0.23%.

4

## 5 5.3 Statistical analysis

6 The ultrasonic measurements show no statistical difference between the bone from  
7 children and from elderly adults with respect to longitudinal wave velocities, transverse wave  
8 velocities,  $\rho$ ,  $MOE_{dyn}$  and  $v$  (Student's t-test,  $p \gg 0.05$ ) (Table1). The mechanical  
9 measurements show no statistical difference between the bone from children and from elderly  
10 adults with respect to mean  $MOE_{sta}$  (Student's t-test,  $p \gg 0.05$ ) (Table1). In the bone from  
11 children, the distribution of  $MOE_{dyn}$  values is normal (Shapiro-Wilk test;  $W = 0.96$ ,  $p = 0.81$ )  
12 and the distribution of the  $MOE_{sta}$  values is abnormal (Shapiro-Wilk test;  $W = 0.88$ ,  $p = 0.08$ ).  
13 The  $MOE_{dyn}$  measured by ultrasound and the  $MOE_{sta}$  measured by 3-point microbending test  
14 for the children are plotted in figure 4 with white circles. A Spearman rank correlation test  
15 between  $MOE_{dyn}$  and  $MOE_{sta}$  for the children's bone shows a positive value ( $R = 0.765$ ,  $p =$   
16  $0.0014$ ).

17

## 18 6. Discussion

19 The assessment of cortical bone mineral status in children via non-radiating and non-  
20 invasive techniques using ultrasound technics may well enhance diagnosis in pediatrics, but  
21 their successful use must rely on gold-standard reference values. The aim of the study was  
22 first to measure ultrasonic wave velocities ( $V_l$  and  $V_t$ ) in children's cortical bone samples  
23 excised from the fibula from which to calculate the elastic properties ( $MOE_{dyn}$ ), then to  
24 experimentally obtain the elastic properties for the same samples via a mechanical test

1 (MOE<sub>sta</sub>), and finally to compare these bone values from children with elderly adult's cortical  
2 bone samples excised from the fibula bone values obtained via the same protocol.

3         The ultrasonic method results for the children (Figure 3) indicate that  $V_1$  is in the lower  
4 range of the acoustic experimental values generally found for adult cortical bone (between 2.7  
5 and 3.8 mm/ $\mu$ s [24] and between 3.5 and 3.9 mm/ $\mu$ s [25]). Similarly, the mean MOE<sub>dyn</sub> and  
6 the mean  $v$  (Table 1) are in the lower range of the acoustic experimental values generally  
7 found for adult cortical bone. MOE<sub>dyn</sub> values in the literature generally range between 18.5  
8 GPa and 33.1 GPa [25], most being roughly 20 GPa [21,26], but these previous studies  
9 concerned femur bone and to our knowledge no values concerning the fibula are available.  
10 The  $v$  values generally range between 0.22 and 0.42 [27]. Nevertheless, our results are close  
11 to the ultrasonic wave velocities already obtained with cortical bone from rib cortical samples  
12 of scoliotic teenagers (15 and 17 years old) using the same ultrasonic bench (respectively 3.2  
13 mm/ $\mu$ s and 1.7 mm/ $\mu$ s for  $V_1$  and  $V_t$ ) [18]. Similarly, the mean MOE<sub>dyn</sub> and the mean  $v$  found  
14 in that study (respectively 14.9 GPa and 0.26) are in the same range as ours. The elasticity of  
15 cortical bone is largely dependent on its mineral constituents [28], and major changes in  
16 elasticity properties and mineral quality of physiological cortical bone have been quantified  
17 in ageing [29], or in adulthood [30]. However, little data is available on bone quality in  
18 childhood, so our findings here make a useful contribution to the literature.

19         When we compared the ultrasonic measurement results for the children with those for the  
20 elderly adults (+75YO), we found no statistical difference. This finding contradicts the  
21 theoretical optimization hypothesis [17] of stiffer bones in adults compared to children, which  
22 is currently used in computational models. Interestingly, Drozdowska et al. [31] in their *in*  
23 *vivo* study found the speed of sound (SOS) evaluated at the phalanx roughly the same for  
24 children around 10 years old and people aged 70-80. The authors conclude that the SOS at the  
25 phalanx increases linearly to a maximum value reached at around 25 years old, and then the

1 values decrease more slowly until the age of 80. Given the age of our population (4-16 YO for  
2 children and +75 YO for elderly adults), our *in vitro* results support their *in vivo* evaluation.  
3 Moreover, similarly to Drozdowska et al., an *in vivo* study [32] using peripheral quantitative  
4 computed tomography showed that bone mineral mass in a 1-mm-thick slice of the cortical  
5 bone cross-section of the proximal radius increased from childhood (6 years old) to adulthood  
6 (up to 40 years old). Clearly, the impact of age on bone mineral status could be explored more  
7 thoroughly if samples from younger adults were included. However, for an *in vitro* study on  
8 fresh bone, it is extremely difficult to obtain a wide range of donor ages. Furthermore, one  
9 limitation exists in the preparation process even if all the samples have been prepared in the  
10 same way, the children samples were fresh and the elderly population samples came from  
11 cadavers, however to our knowledge and up to date, no impact of that difference has been  
12 shown on ultrasound propagation. The main difference lies in the time of freezing which is  
13 hard to set with bone from several origins, but frozen bones can safely be used for mechanical  
14 testing, at least for storage periods of up to one year [33] which is consistent with our process.

15 One of the critical points concerning acoustical measurements is the sensitivity of  $V_1$   
16 and  $V_t$  to the ratio  $e/\Delta t$  (equation 1). In figure 5, we plot the longitudinal wave velocity  
17 measured in bone at normal incidence as a function of  $e/\Delta t$  for a given wave propagation  
18 velocity in water  $C_{\text{water}}=1.48 \text{ mm}/\mu\text{s}$ . The black crosses represent the theoretical  $V_1$  values  
19 obtained from equation (1) for the experimental values of  $e/\Delta t$ . It is noteworthy that in a range  
20 between 1 and 2  $\text{mm}/\mu\text{s}$  the sensitivity of  $V_1$  is very high, whereas it becomes more acceptable  
21 over 2  $\text{mm}/\mu\text{s}$ . The critical value corresponds to  $C_{\text{water}}$ , and if we look at the accuracy of  $V_1$   
22 estimation versus the error in  $e/\Delta t$  for different  $e/\Delta t$  ratios between 2 and 5  $\text{mm}/\mu\text{s}$   
23 (corresponding to experimental range), it appears that the sensitivity of  $V_1$  estimation  
24 decreases when the ratio increases. For example, an error of 10% in the estimation of a ratio  
25 around 2 induces an error of 16% in the estimation of  $V_1$ ; and an error of 6% in the estimation

1 of a ratio around 5 induces an error of 7% in the estimation of  $V_1$ . This example highlights one  
2 of the limitations of the ultrasonic method, based on the pulse-echo mode. Research is  
3 underway to tackle these limitations, and new ultrasonic measurement approaches and novel  
4 signal-processing methods are currently being investigated [20]. Theory suggests another  
5 issue concerning acoustical measurement, which is that SOS is influenced by the elasticity of  
6 bone as well as bone mass density; but, measured according to the Archimedes' principle here  
7 (Table 1), the bone mass density ( $\rho$ ) of cortical bone from fibula is in the usual range of  
8 literature values.

9 The mechanical measurements performed here found the mean  $MOE_{sta}$  of the  
10 children's bone samples to be in the typical range of human cortical bone values obtained  
11 from three-point bending tests (between 8.6 (+/-1,5) GPa [34] and 12.5 GPa [35]).  
12 Concerning the comparison with the first study of children bone [15], using samples extracted  
13 from the mid-shaft of the femur (eighteen subjects with age range: 2 to 48 years old), it  
14 showed that the bone specimens taken from children were weaker and less stiff than those  
15 taken from adults. The second [16], with samples extracted from the top part of the femur  
16 diaphysis (12 children from 4 to 15 years old and 12 adults from 22 to 61), showed that bone  
17 from children and adults differed in cortical strength and stiffness, depending on ash density,  
18 although the compressive yield strain was the same. Both studies found a mechanical  
19 difference between children's and adults' bone with regard to stiffness, but the findings of  
20 both should be taken with caution. The first tested bone from cadavers, with insufficient  
21 samples to provide statistics on differences between children and adults for the elasticity  
22 parameters. The second studied bone samples close to cancer locations, which cannot be  
23 considered physiological tissue. Here, we didn't find any statistic difference concerning static  
24 modulus of elasticity but the span-to- depth ratio of 10:1 is not theoretically adequate to  
25 assess the MOE of bone material. Indeed, the contribution of shear deformation cannot be

1 neglected, and consequently the  $MOE_{sta}$  evaluated in this study may be influenced by the  
2 dimensions of the samples and may lead to an underestimation of the MOE of cortical bone.  
3 However, a recent study [36] aiming at design and validate bending test method for  
4 characterization of miniature pediatric cortical bone specimens showed that a span to depth  
5 aspect ratio (5 : 6) provided reasonable results for both Young's modulus and flexural  
6 strength in bovine bone; this aspect ratio is not consistent with the general rule admitted.  
7 Nevertheless, since all the samples tested here showed the same span-to-depth ratio, we are  
8 able to compare results for the children with those for the elderly adults. Comparison of the  
9 children's  $MOE_{sta}$  with the elderly adults'  $MOE_{sta}$  obtained via mechanical tests shows no  
10 statistical difference in the Student T-test (Table 1); this finding contrasts with the literature  
11 [15,16]. One explanation of this absence of statistical difference may be the nature of the bone  
12 tested. Fibula ossification commences in the lower end in the second year; *in vivo*, DeSouza et  
13 al. [37] and El Haj et al. [38] pointed out the key role of mechanical stimulation in healing,  
14 remodeling and regeneration of bone. Even though the fibula bears relatively little weight in  
15 comparison with the tibia, walking may well stimulate the mineralization process of the  
16 bottom part of the fibula more strongly than other bones in the skeleton. However, the fibula  
17 being the preferred location for cortical bone auto transplants, we were unable to obtain other  
18 fresh bone.

19         Ultrasound offers two advantages over static measurements: i) it is a non-destructive  
20 tool; ii) it can be performed *in vivo*. However, the literature on biological tissue  
21 characterization shows that values obtained for static and dynamic elastic moduli differ. The  
22 values of the modulus of elasticity obtained through the ultrasonic method are usually higher  
23 than those found with static deflection. This difference exists not only for bone but also for  
24 biological material like wood. In wood, Halabe et al. [39] explained the difference between  
25 the two moduli of elasticity as follows: wood is a viscous, elastic and highly impact-absorbent

1 material, so its behavior depends on the duration of the excitation: the shorter the excitation is  
2 (ultrasound excitation), the stiffer the material appears. Here, due to the small size of our  
3 samples and the difficulty of obtaining usable surgical waste, we could only assess transversal  
4 elasticity (perpendicularly to bone axis) via acoustical measurements and longitudinal  
5 elasticity via mechanical measurements. However, adult cortical and trabecular bone are  
6 orthotropic [40], so it might be assumed that children's cortical bone is also an anisotropic  
7 tissue. Consequently, future studies on children's bone should also explore the other axes of  
8 the bone for a more thorough comparison of static and dynamic MOE. Nevertheless, the  
9 values for both  $MOE_{dyn}$  and  $MOE_{sta}$  reported here (Table 1) are consistent with those usually  
10 reported in the literature [41], and a Spearman ranking correlation between the two moduli of  
11 elasticity is obtained (Figure 4). To our knowledge, the correlation obtained here is the first to  
12 establish a strong link between dynamic and static moduli for human cortical bone in  
13 children, or even for human cortical bone in general. In a recent study [41] of women's  
14 cortical bone, the authors obtained only a marginal negative correlation between  $MOE_{dyn}$   
15 (ultrasonic measurement with guided waves) and  $MOE_{sta}$  (three-point bending). Investigating  
16 a linear correlation between  $MOE_{dyn}$  and  $MOE_{sta}$  and exploring the hypothesis of anisotropy  
17 of children's bone will require further experiments with more and thinner samples, a  
18 challenging undertaking.

19 In conclusion, this study contributes a new set of ultrasonic wave velocities and  
20 elasticity values for children's cortical bone. Furthermore, for the first time it provides a  
21 ranking correlation between children's cortical bone elasticity values obtained using two  
22 different approaches (acoustical and mechanical measurements). Finally, the comparison  
23 performed here with elderly adults' bone does not support the theoretical optimization  
24 hypothesis [17] of an increasing bone modulus of elasticity values from neonate to adult  
25 which is currently used in pediatric computational methods.



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10

11 8. Conflict of Interest statement

12 There is no conflict of interest.

13

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- 9
- 10

1 9. Tables, figure legends

	$\rho$ (kg/m <sup>3</sup> )	MOE <sub>dyn</sub> (GPa)	$\nu$	MOE <sub>sta</sub> (GPa)
Children	1.84 ± 1.12	15.5 ± 3.4	0.24 ± 0.08	9.1 ± 3.5
Elderly adults	1.73 ± 0.08	16.7 ± 1.9	0.27 ± 0.05	5.8 ± 2.1
Student T-test	P>>0.05	P>>0.05	P>>0.05	P>>0.05

2 Table 1: Mean values (+/- SD) of density  $\rho$ , MOE<sub>sta</sub>,  $\nu$  and MOE<sub>dyn</sub> for the children's (mean  
3 age : 10 years old +/- 4.5) and the elderly adults' bone samples (+ 75 years old)

4

5 Figure 1: a. samples preparation: the waste fragment from auto transplant which was selected  
6 to prepare one of the children's cortical bone samples tested, b. plan of the special ultrasonic  
7 bench to assess acoustic elastic properties, in a water tank the bone sample is located between  
8 transducers ( T1 and T2), all of them mounted on 3D rotational axis c. picture of one sample  
9 during mechanical measurements via the three-point microbending setup.

10 Figure2 : Aperture of the 10MHz-transducer (Imasonic®); R and H are respectively the radius  
11 and the length of the transducer; ; F is the focusing distance; Xa and XL are respectively the  
12 focal length and the slice thickness.

13 Figure 3: Longitudinal (black crosses) and transverse (black dots) wave velocities measured at  
14 10 MHz for children's bone samples

15 Figure 4: MOE<sub>dyn</sub> measured by ultrasound and MOE<sub>sta</sub> measured by three-point bending test  
16 on children's bone (white circles)

17 Figure 5: longitudinal wave velocity measured in bone at normal incidence as a function of  
18  $e/\Delta t$  for a given wave propagation velocity in water  $C_{water}=1.48$  mm/ $\mu$ s. The black crosses  
19 represent the theoretical  $V_1$  values obtained from equation (1) for the experimental values of  
20  $e/\Delta t$ .

Figure 1  
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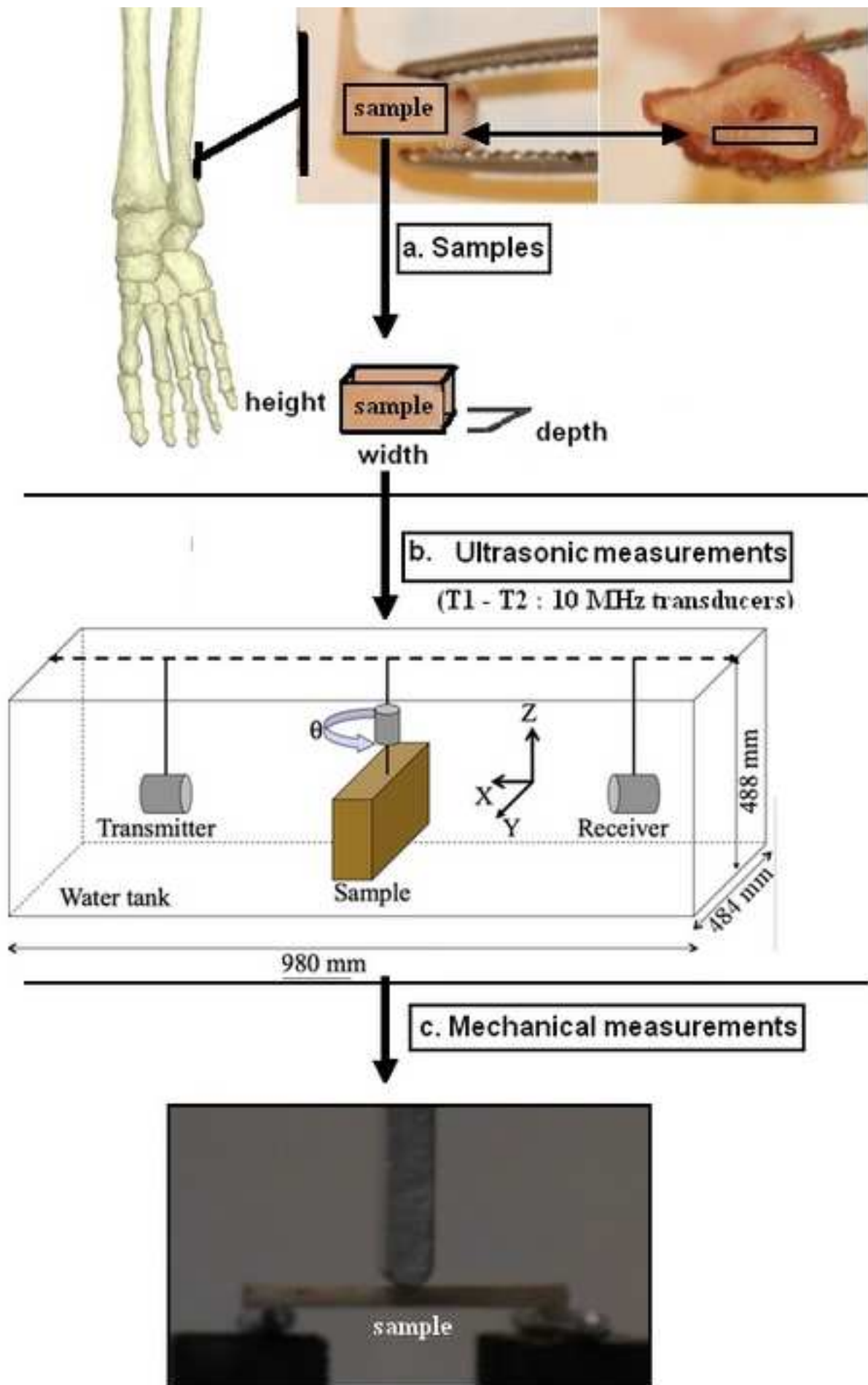




Figure 2  
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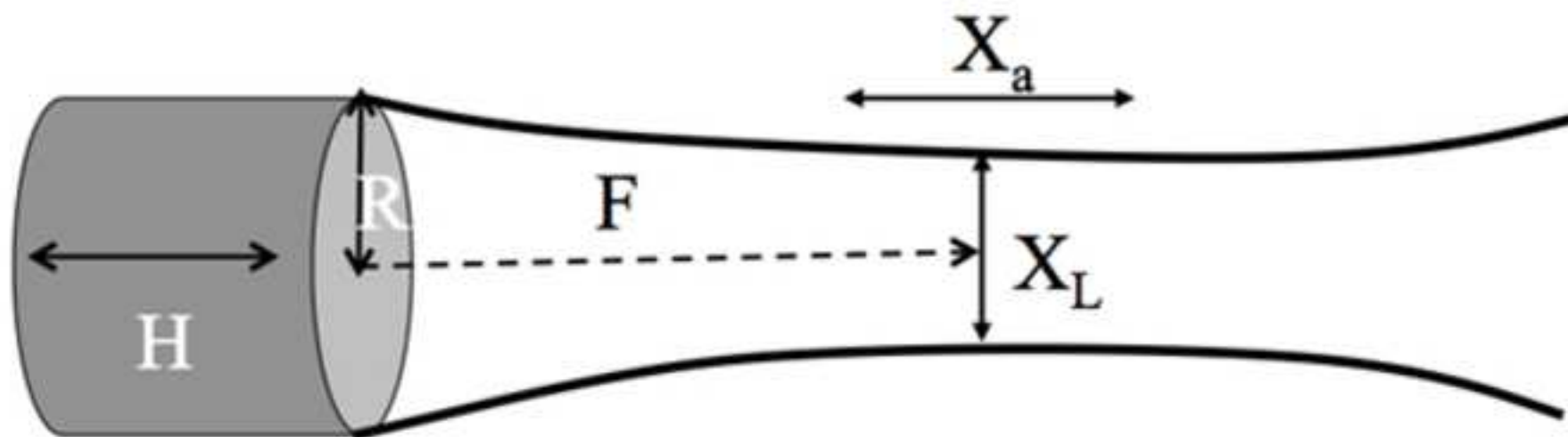


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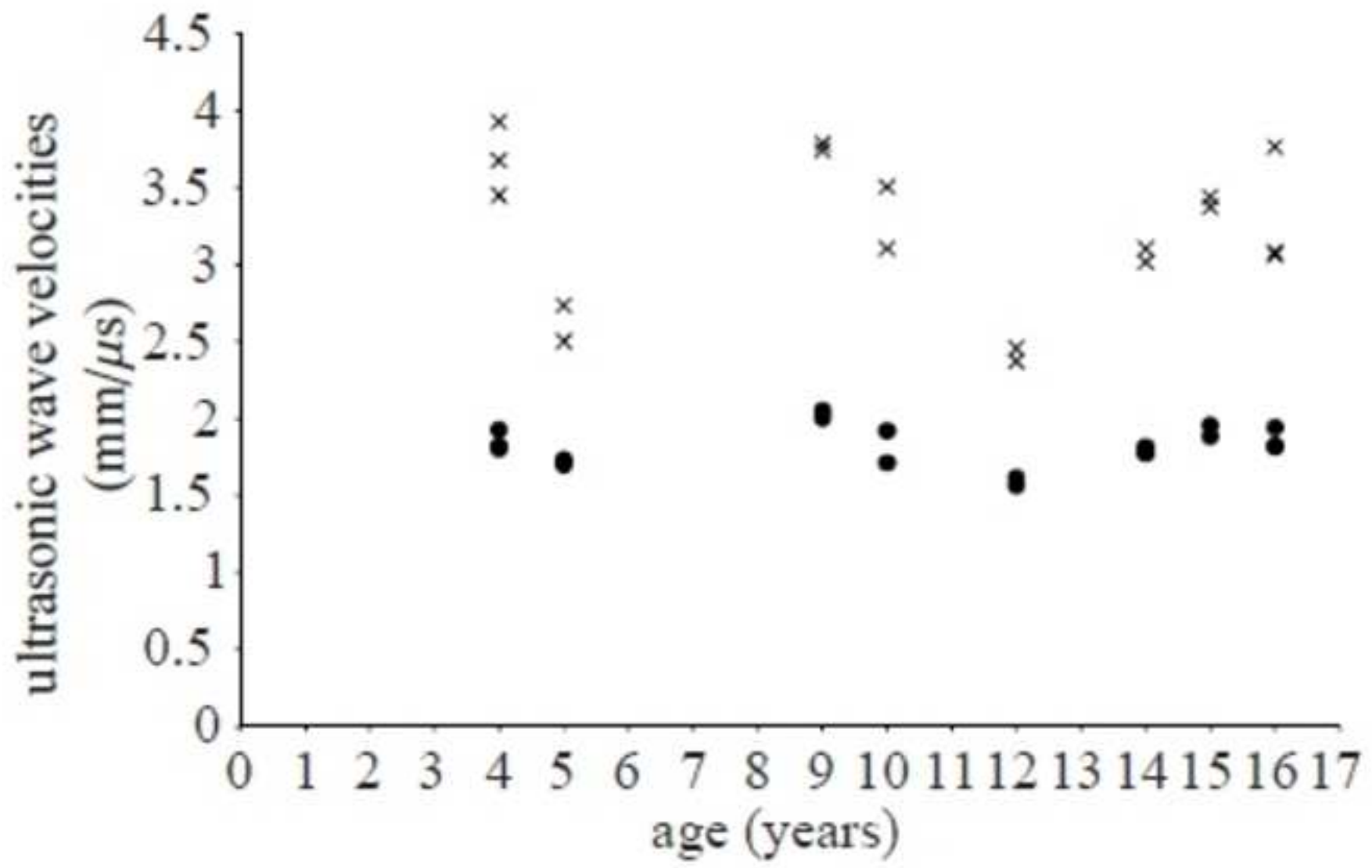


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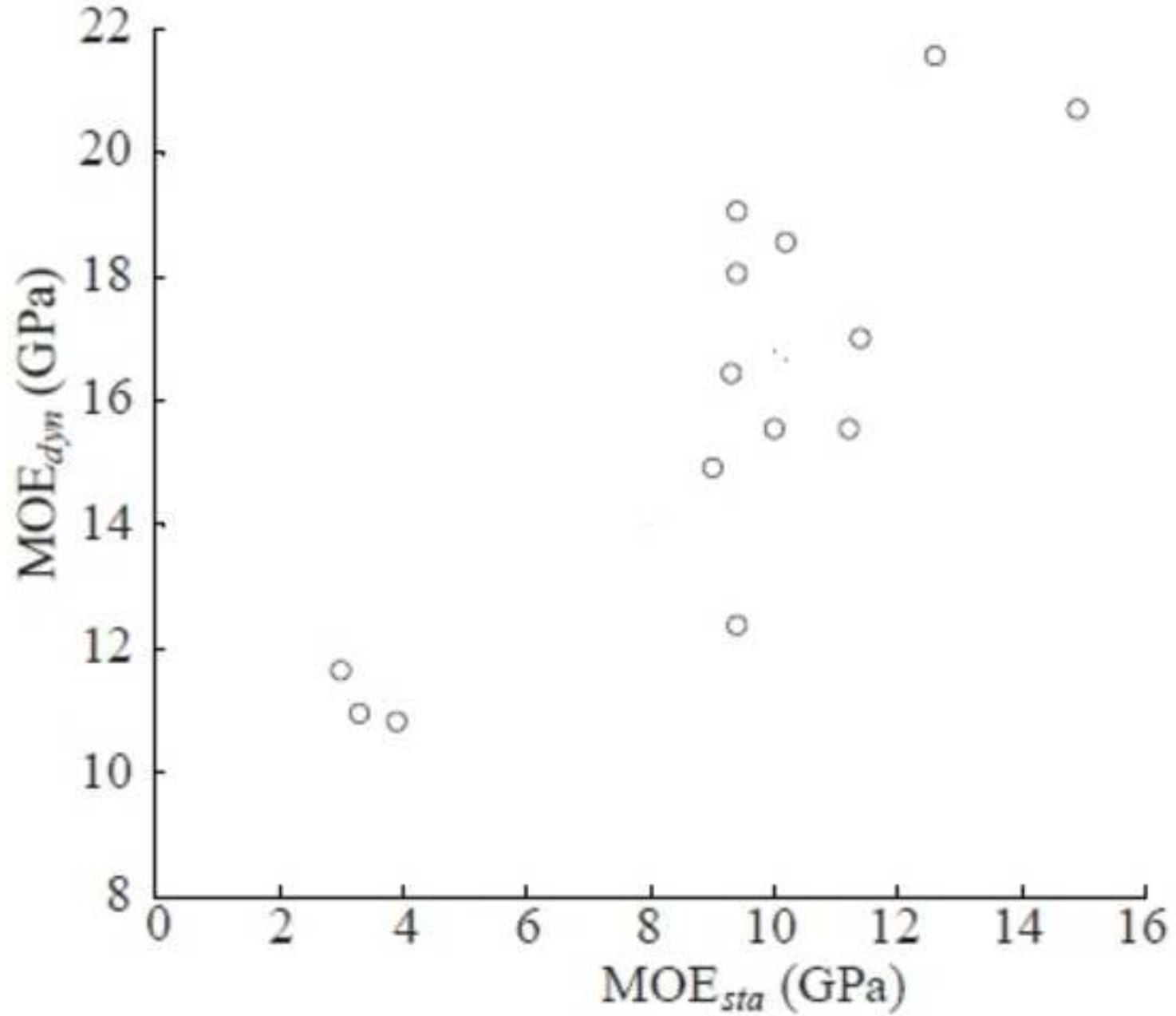
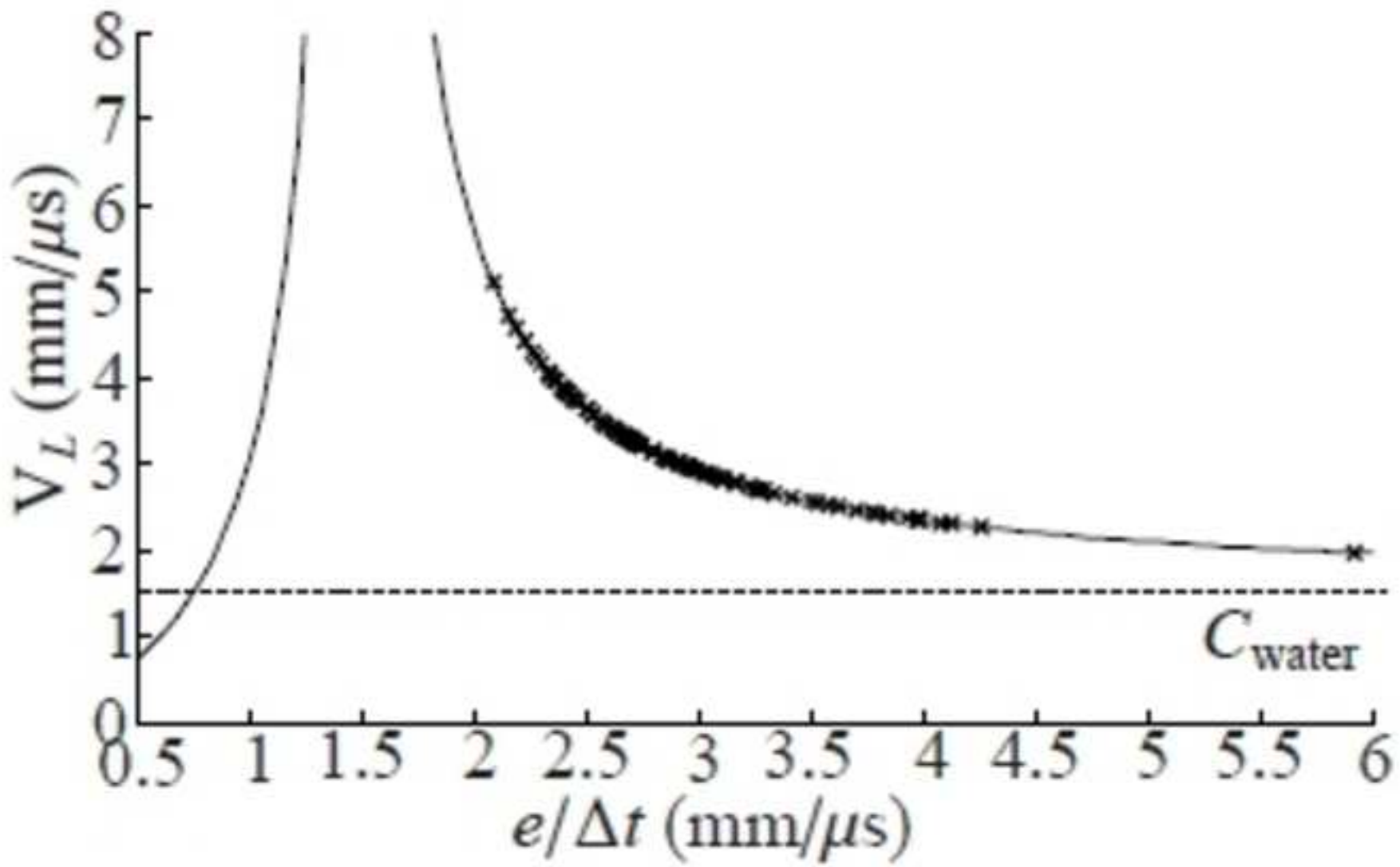


Figure 5  
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## Highlights

- The mean values of longitudinal and transverse wave velocities for children's bone are respectively  $3.2 \text{ mm}/\mu\text{s}$  ( $\pm 0.5$ ) and  $1.8 \text{ mm}/\mu\text{s}$  ( $\pm 0.1$ ).
- Dynamic moduli of elasticity are in the same range for children's and elderly adults' bone without any statistical difference.
- A ranking correlation between dynamic modulus of elasticity and static modulus of elasticity is shown for children cortical bone samples.