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Structural and behavioural analysis of children's cortical bones

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1. Introduction

Children's bones are interesting from both the mechanical and the clinical point of view because they frequently show typical bone fractures (greenstick), which is obviously a different fracture mode from that seen in adults (Currey and Butler 1975; Franklyn et al. 2007).

The assessment of the bone structure of children's cortical bones is a major challenge for the biomechanical engineering community in understanding bone behaviour.

This study provides original results on the properties of children's cortical bones and gains insight into the link between clinical values and bone quality.

2. Methods

2.1 Bone specimens

Fourteen fibulas from 12 children (4–16 years old, mean age: 9.5 ± 3.4 years old) were extracted from lengthening lower limb surgery, in the lower one-third of the fibula. The mean of parameters was used in case of multi samples per patient (typically left and right leg). All samples are weighed to obtain their mass (g).

2.2 Bone mass and microarchitecture assessment

First, bone mineral content (BMC, g) and areal bone mineral density (aBMD, g/cm^2) were measured using dual-energy X-ray absorptiometry (DXA; Discovery, Hologic, Waltham, MA, USA) on fresh bone.

Then, the microarchitecture was measured using a μ -CT device (Skyscan 1174, Aartselaar, Belgium) on the samples immersed in 70% alcohol. A nominal isotropic voxel size of $19 \mu\text{m}$ was used (field of view: 25 mm, X-ray source: 50 kV–800 μA , exposure time: 4000 ms, two-frame averaging, 360° rotation, steps of one degree, aluminium filter: 0.5 mm). The reconstructed slice images were processed with the 3D image analysis software (Nrecon, Skyscan, Belgium). The analysis was done with CTAn (SkyScan,

Belgium). After phantom calibration, grey levels of all voxels were converted into density in g/cm^3 .

BMD was expressed as the mean density of each voxel in the volume of interest. Tissue mineral density (TMD) differs from BMD as TMD is calculated from the mean value of the bone tissue voxels only (excluding pores). We applied a threshold set at $0.77 \text{ g}/\text{cm}^2$, to ignore non-bone voxels.

The structural parameters were also calculated using an adapted threshold: number of closed porosity (Po, N) and total percentage (Po, %).

Depending on the calculated parameters, volume of interest was defined by selecting regions of interest (ROI) of each section (cf. Figure 1): the whole bone to evaluate the volumetric BMD, as an equivalent to DXA (vBMD, g/cm^3) and the cortical part to calculate TMD (g/cm^3) and cortical BMD (cort-BMD, g/cm^3).

2.3 Measurement of microhardness

Before hardness testing, the samples were fixed in 70% alcohol, dehydrated in absolute alcohol, embedded in methyl methacrylate, and meticulously surfaced and polished with an alumina suspension ($1 \mu\text{m}$). Microhardness (HV, kg/mm^2) is measured using a Micromet 5104 (Buehler, Lake Bluff, IL, USA) equipped with a Vickers

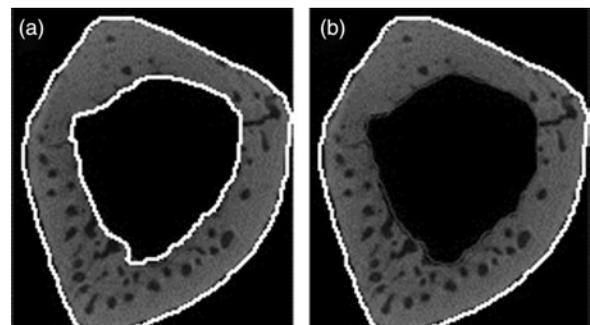


Figure 1. Selection of the ROI for each scanner section (a) ROI for TMD and cort-BMD (b) ROI for vBMD.

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Table 1. Mean values (\pm SD) of samples.

Samples	Mass (g)	DXA		μ CT			HV		
		BMC (g)	aBMD (g/cm ²)	vBMD (g/cm ³)	Cort-BMD (g/cm ³)	TMD (g/cm ³)	Po (N)	Po(tot) (%)	(kg/mm ²)
Mean	1.05	0.55	0.45	0.68	0.87	0.92	6745	8.7	50.4
SD	0.61	0.30	0.12	0.10	0.06	0.05	7477	3.4	2.4

indenter, corresponding to a square-based diamond indenter.

The microhardness tester was equipped with an optical part linked to a digital camera and analyser software (OmniMet HMS v2.31, Buehler, Lake Bluff, IL, USA) to measure both diagonals of the impressions.

Vickers microhardness, defined as the mean pressure that the material will support under load, was computed from:

$$HV = 1854.4 \times P \times d^2$$

where P is the test load (25 g) and d is the mean length of both diagonals in μ m.

For each embedded sample, 20 indents (10 in osteons and 10 in interstitial lamellae) were performed with an applied load of 25 g for 10 s.

2.4 Statistical analyses

Due to the low number of samples, non-parametric tests were used (correlation coefficient of Spearman (r) and Friedman test).

3. Results and discussion

The main results were expressed as mean and standard deviation (SD) in Table 1.

HV between osteons and interstitial bone was found to be significantly different ($HV_{tot} = 50.4 \pm 2.4$, $HV_{osteon} = 48.6 \pm 3.0$ and $HV_{inter} = 52.1 \pm 2.5$ kg/mm²). The interstitial bone was harder than osteonal bone. The same trend was found by Boivin et al. (2008) in an adult cortical bone.

Age was significantly correlated with different parameters reflecting the mineralisation (mass: $r = 0.76$, $p = 0.004$; BMC: $r = 0.68$, $p = 0.015$; aBMD: $r = 0.72$, $p = 0.008$). Age was also linked to Po (N) ($r = 0.82$, $p = 0.001$).

The mass and BMC were linked ($r = 0.97$, $p < 0.001$). This relationship confirmed the feasibility of using DXA on small samples.

aBMD and TMD were linked together ($r = -0.60$, $p = 0.039$) and to the SD of total HV ($r = -0.59$, $p = 0.045$; $r = 0.70$, $p = 0.01$, respectively). Density could be connected with the heterogeneity of the material. TMD was not explained by microhardness, which could highlight that the organic matrix (mainly collagen) also contributes to the tissue hardness.

4. Conclusions

These results are the fruit of a preliminary study. For example, they need further evaluation of matrix parameters which will be done using Fourier transform infrared microspectroscopy in order to characterise the properties of both mineral and collagen (Farlay et al. 2010). Further work needs to be done to validate the results of this study.

Acknowledgements

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