Quantitative ultrasound yields biomarkers of bone mechanical competence

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Résumé :

Les deux dernières décennies ont vu des changements dans notre capacité à évaluer les propriétés mécaniques multi-échelles du squelette humain. Les méthodes ultrasonores quantitative (QUS) ont joué un rôle crucial dans cette évolution en apportant des solutions techniques en matière d'évaluation mécanique osseuse, allant de l'échelle de l'ostéon jusqu'à l'échelle du millimètre. Ces techniques permettent de caractériser ex vivo l'anisotropie élastique et sont susceptibles de contribuer à faire progresser notre connaissance fondamentale concernant les déterminants de propriétés élastiques de l'os. D'autre part, les techniques QUS appliquées in vivo permettent de caractériser d'importants facteurs de qualité osseuse tels que les propriétés matérielles ou la structure, en vue d'améliorer la prédiction du risque de fracture.

Abstract :

The last two decades have witnessed changes in our ability to assess multi-scale mechanical properties of the human skeleton. Quantitative ultrasound (QUS) has played a critical role in this development, providing gains in bone mechanical status assessment, spanning the scales of bone organization ranging from elementary bone structural units to the mm-scale level. QUS technologies can help in characterizing the anisotropic stiffness ex vivo and are prone to provide answers to some questions that remain open regarding the determinants of bone elastic properties. On the other side, in vivo QUS has potential to assess important bone quality factors such as material properties or structure, and to enhance fracture risk prediction.

Mots clefs: Biomécanique, Ondes guidées, Os, Risque de fracture, Spectroscopie de résonance ultrasonore

Keywords: Biomechanics, Bone, Fracture risk, Guided waves, Resonant ultrasound spectroscopy

1 Introduction

Bone biomechanical competence is determined by a variety of compositional, microstructural and ultra-structural properties of the bone mineralized tissue. Elasticity measurement combined with microstructure assessment can help understanding pathological variations of bone functional properties and guiding diagnosis strategies. In contrast to X-ray based technologies, the interaction of ultrasound waves with bone tissue carries information about elastic and structural properties. Quantitative ultrasound (QUS) has played a crucial role to enhance our ability to assess multi-scale mechanical properties of the human skeleton [1]. Resonance ultrasound spectroscopy (RUS) [2] and scanning acoustic microscopy (SAM) [3] can be used ex vivo to assess the mesoscale stiffness tensor and elastic maps of the tissue matrix at micro-scale resolution, respectively. Ultrasound estimates of elastic properties are suitable as input for numerical computation and numerical bone models (e.g., FEM, FDTD). Nonlinear acoustics methods are sensitive to damage accumulation and investigations are currently ongoing to assess whether the nonlinear response can be used as a marker of bone damage [4,5]. In vivo, quantitative ultrasound (QUS) methods represent powerful alternatives to ionizing X-ray based assessment of fracture risk. New in vivo applicable methods such as guided waves-based axial transmission techniques permit assessment of fracture-relevant properties [6] at fragile anatomical regions, e.g. the distal radius. In this paper results in the main recent research axes developed in our group are highlighted.

2 Anisotropic elasticity of human cortical bone measured ex vivo by resonant ultrasound spectroscopy

There is a strong interest in the characterization of mm-scale bone elastic properties as they can reveal alterations of bone quality at smaller scales. In particular, concurrent assessment of elastic properties and investigations of micro- and nanoscales features such as the lacuno-canalicular network, the plywood-like structure of lamellar tissue, or the distribution of mineral have the potential to unravel relationships of elasticity with disease-related collagen cross-linking, mineral or microstructure alterations [7]. Such studies may expand current understanding and clinical applications in metabolic bone disease.

Resonant Ultrasound Spectroscopy (RUS) is currently developed with the aim to become a routine technique for the accurate assessment of mm-scale anisotropic elastic and viscoelastic properties of mineralized tissues. RUS allows estimating all the terms of the stiffness tensor of an anisotropic material from the measurement of the mechanical resonant behavior of a specimen. It is based on a comparison of measured and model-predicted resonant frequencies. While RUS was developed in the 1990's to measure metals [8], the difficulty raised by the high level of mechanical damping of bone (which causes resonance peaks to overlap) has only been recently overcome by our group [2]. Furthermore, some predicted frequencies cannot be observed and one faces the issue of correctly pairing the predicted and measured frequencies in the definition of a cost function. We proposed to introduce Bayesian statistics into the RUS inversion calculation [9,10]. Our method does not suffer from the drawbacks and limitations associated with the conventional sound velocity approach that has been used to measure bone elasticity by a number of authors. In particular RUS can measure small parallelepiped samples of dimension of a few millimeters. We showed that RUS is suited to the evaluation of stiffness tensors of large collections of specimens by non-expert users [11].

A recent study has been conducted on 59 rectangular parallelepiped specimens (typical size 2x3x4 mm^3) prepared from 19 human tibial diaphyseal cross-sections (donors ages 70-94). RUS vibration

spectra were obtained for all specimens in a frequency band (typically 150-500 kHz) containing the first ~30 expected resonant modes. The spectra were fitted to Lorentzian functions to retrieve the resonant frequencies. The latter are inputs of an inverse problem that consists in determining 5 stiffness coefficients (transverse isotropy) together with the optimal pairing of measured and predicted frequencies. The problem is solved without user interaction in a Bayesian framework using an *a priori* knowledge of cortical bone properties from a previous study on human femur bone [11].
The stiffness tensor was successfully obtained for 52 specimens. The method failed for 7 specimens, possibly due to insufficient quality of specimen geometry. Stiffness coefficients measured with RUS are (in GPa): C_{11}=14.8 ± 3.4, C_{33}=26.6 ± 3.5, C_{13}=8.3 ± 1.1, C_{44}=5.5 ± 1.05, C_{66}=3.65 ± 0.9. The Poisson's ratios measured with RUS are ν_{12}=0.40 ± 0.04, ν_{13}=0.19 ± 0.02, ν_{31}=0.39 ± 0.08. Good agreement was obtained with existing data in literature for other skeleton sites. All elastic coefficients (except C_{12}) were highly correlated to apparent mass density [11]. The elasticity database obtained in this study for human tibia, a clinically relevant site for *in vivo* assessment of bone, is unique. RUS successfully assessed elastic anisotropy of human tibial cortical bone despite the small size of the specimens.

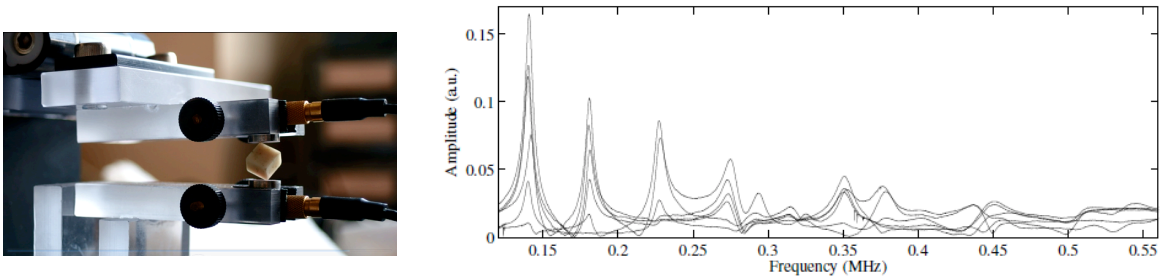

Fig. 1 RUS measurement of a bone specimen placed between two piezoelectric transducers (left). Typical set of spectra (right). The relative amplitudes of the resonant modes vary as the specimen is rotated, allowing one to detect more resonant frequencies than from a single spectrum [11].

3 In vivo estimation of fracture-relevant properties using axial transmission measurements

Osteoporotic fracture risk remains difficult to assess [12]. Cortical bone, known for its key role in the mechanical stability, is the subject of extensive research [13]. New areas of research are directed towards developing novel QUS approaches for *in vivo* cortical bone characterization [1]. A step forward has been made recently with reports showing that cortical bone behaves as a waveguide for ultrasound [14]. Measurements of the dispersion relationships of guided modes (representing the frequency-dependent wave numbers of guided modes propagating in the waveguide) using the so-called axial transmission technique, together with appropriate waveguide modeling have the potential for providing estimates of strength-related bone properties such as cortical thickness (Ct.Th) and stiffness [6]. Stiffness is largely determined by cortical porosity (Ct.Por) [15]. The cortical thinning and the increase of porosity observed with aging or induced by osteoporosis [16] change the propagation of guided waves. Thus, QUS guided waves (GW)-based technologies would be expected to provide estimates of strength-related bone factors that cannot easily be captured by X-ray technologies.

In a typical axial transmission measurement configuration, a 1-MHz ultrasonic transducers array is used to record the propagation of guided waves transmitted in the cortical shell along the main direction of a long bone (*e.g.*, radius or tibia). The full time responses of the waveguide for all possible pairs of transmitter-receiver are recorded and then processed using a 2-D time-space Fourier transform associated with a singular value decomposition-based denoising step, yielding a representation in the *k*

(wavenumber)-f (frequency) space, whose maxima correspond to the wave numbers of the guided modes [17]. The post-processing relies on finding the optimal pairing of measured and predicted wavenumbers *k(f)* by adjusting the thickness and porosity of a 2-D free transverse isotropic plate homogenized waveguide model [6].

This approach has been validated on bone-mimicking phantoms and on *ex vivo* human radius specimens (Fig. 2 [6]). It has then been used for *in vivo* estimates of Ct.Th and Ct.Por of the proximal radius in a cohort of 14 healthy subjects (24 - 58 years old). Ultrasound estimates of Ct.Th were validated by comparison with site matched reference values derived from X-ray high-resolution peripheral quantitative computed tomography (HR-pQCT) [18]. Results showed a good agreement between US-derived Ct.Th (3.4 ± 0.4mm) and the pQCT reference values (3,3 ± 0.3, RMSE = 0.15 mm). This approach is currently applied for a pilot clinical study (hôpital Cochin, Paris) in order to evaluate if a new GW-based prototype device (Azalée, Paris) can discriminate between non-traumatic-fractured (F) and non-fractured (NF) postmenopausal women [19]. According to the homogenized model [20], the estimated cortical porosity values, falling in the range 5 – 20%, and thicknesses, ranging form 1.4 to 3.8 mm, are in agreement with known physiological values of cortical porosities [15] and thicknesses [21].

While this *in vivo* pilot study provides evidence of the feasibility of bone strength-related factors characterization, several limitations are currently under investigation. First, estimated porosity must be validated with independent measurements using high-resolution X-ray imaging technologies. However, for safety consideration, such examinations cannot be performed *in vivo*. A currently ongoing ex vivo study will complement the present results. Second, the theoretical model considered here does not account for the full complexity of bone structures: it does not take into account the presence of overlaying soft tissues between the probe and the bone, tubular shape of long bones, irregular geometry and heterogeneity of material properties. More sophisticated models must be investigated. For example, preliminary observations suggest that a bi-layers model might fit the experimental data better than the free plate model [22].

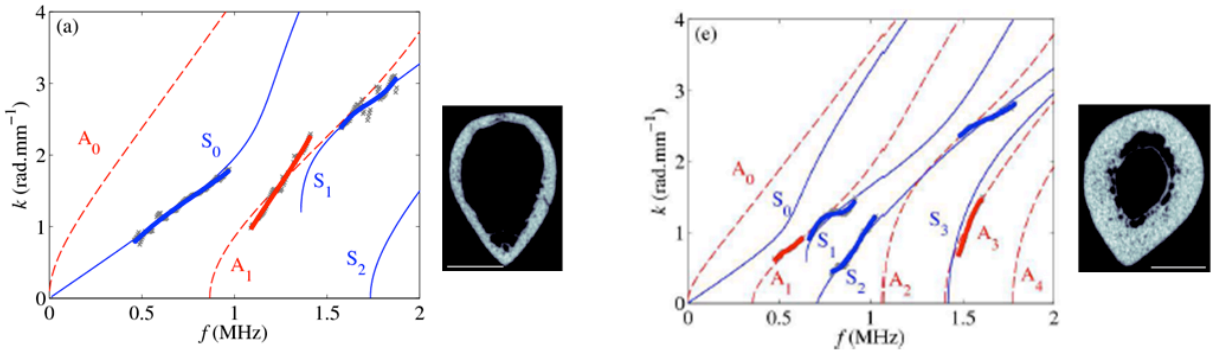

Fig.2. Illustrative examples of the best fit between measured frequency-dependent wavenumber *k(f)* (dots) and theoretical dispersion curves (continuous and dotted lines) for a thin (left) and a thick (right) human radius specimen [6]. Antisymmetric and symmetric guided modes are displayed in red and blue color, respectively.

4 Conclusion

Elastic properties of bone are nowadays widely used in fundamental studies, in conjunction with numerical models, to investigate the structure-function relationships, and in clinical applications to predict fracture risk or to monitor fracture healing. Although still at an early stage of development,

QUS methods currently represent a promising approach for *ex vivo* bone elastic properties characterization and for *in vivo* non-invasive assessment of fragility components, providing prospects for improved assessment of fracture risk. As extensive as they are, these gains still constitute a prelude to what is to come, given the incessant developments of better instrumentation and signal processing techniques.

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References

[1] K. Raum, Q. Grimal, P. Varga, R. Barkmann, C.C. Glüer, P. Laugier, Ultrasound to assess bone quality, Current Osteoporosis Reports 12(2014),154-62.

[2] S. Bernard, Q. Grimal, P. Laugier, Accurate measurement of cortical bone elasticity tensor with resonant ultrasound spectroscopy, J Mech Behav Biomed 18C(2012)12-19

[3] K. Raum, Microelastic imaging of bone, IEEE Trans Ultrason Ferroelectr Freq Control 55 (2008)1417-31

[4] S. Haupert, S. Guerard, D. Mitton, F. Peyrin, P. Laugier, Non destructive Characterization of cortical bone micro-damage by nonlinear Resonant ultrasound spectroscopy, PloS One, 9(2014) e83599

[5] S. Haupert, S. Guerard, D. Mitton, F. Peyrin, P. Laugier, Quantification of nonlinear elasticity for the evaluation of submillimeter crack length in cortical bone, J Mech Behavior Biomed Mater, in press.

[6] J. Foiret, J.G. Minonzio, C. Chappard, M. Talmant, and P. Laugier, Combined estimation of thickness and velocities using ultrasound guided waves: A Pioneering study on in vitro cortical bone samples, IEEE Trans Ultrason Ferroelectr Freq Control 61(2014) 1478-88

[7] M. Granke, A. Gourrier, F. Rupin, K. Raum, F. Peyrin, A. Saïed, M. Burghammer, P. Laugier. Microfibril orientation dominates the microelastic properties of bone tissue at the lamellar length scale, PloS One 8(2013) e58043

[8] Migliori, J.L. Sarrao, W.M. Visscher, T. M. Bell, M. Lei, Z. Fisk, and R. G. Leisure, Resonant ultrasound spectroscopic techniques for measurement of the elastic moduli of solids, Physica B 183 (1993)

[9] S. Bernard, Q. Grimal, P. Laugier, Resonant ultrasound spectroscopy for viscoelastic characterization of anisotropic attenuative solid materials, J Acoust Soc Am 135(2014) 2601-13

[10] S. Bernard, G. Marrelec, P. Laugier, Q. Grimal, Bayesian normal modes identification and estimation of elastic coefficients in resonant ultrasound spectroscopy, Inverse Problems, in press

[11] S. Bernard, J. Schneider, P. Varga, P. Laugier, K. Raum, Q. Grimal, Elasticity-density and viscoelasticity-density relationships at the tibia mid-diaphysis assessed from resonant ultrasound spectroscopy measurements, Biomech Model Mechanobiol, in press

[12] K. Briot, S. Paternotte, S. Kolta, R. Eastell, D. Felsenberg, D. M. Reid, C. C. Glüer, and C. Roux, “FRAX: Prediction of Major Osteoporotic Fractures in Women from the General Population: The OPUS Study,” Plos One 8, e83436 (2013).

[13] T. Chevalley, J. P. Bonjour, B. Van Rietbergen, S. Ferrari, and R. Rizzoli, "Fracture history of healthy premenopausal women is associated with a reduction of cortical microstructural components at the distal radius," Bone 55, 377–383 (2013).

[14] P. Moilanen, Ultrasonic guided waves in bone, IEEE Trans Ultrason Ferroelectr Freq Control 55 (2008) 1277-1286

[15] M. Granke, Q. Grimal, A. Saïed, P. Nauleau, F. Peyrin, P. Laugier, Changes in porosity are the major determinant of the variations of cortical bone elasticity at the millimeter scale in aged women, Bone, 49(2011) 1020-6

[16] R. M. D. Zebaze, A. Ghasem-Zadeh, A. Bohte, S. Iuliano-Burns, M. Mirams, R. I. Price, E. J. Mackie, and E. Seeman, "Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: A cross-sectional study," Lancet 375, 1729–1736 (2010).

[17] J.G. Minonzio, M. Talmant, P. Laugier, Guided waves analysis using multi-emitters and multi-receivers arrays : decomposition of the transmission matrix, J Acoust Soc Am, 127(2010) 2913-19.

[18] Q. Vallet, J.-G. Minonzio, C. Chappard, M. Talmant, and Pascal Laugier, "In vivo measurements of guided wave at the forearm," IEEE International Ultrasonics Symposium, September 3-6, 2014, Chicago, 931 – 934.

[19] Q. Vallet, J.-G. Minonzio, N. Bochud, A. Etcheto, K. Briot, S. Kolta, C. Roux, and P. Laugier, "Guided wave-based ultrasound biomarkers of cortical bone discriminate fractured from non-fractured post-menopausal women," ASBMR 2015 Annual Meeting – Seattle, WA, USA – Oct 9-12, 2015.

[20] W. J. Parnell and Q. Grimal, "The influence of mesoscale porosity on cortical bone anisotropy. Investigations via asymptotic homogenization," Journal of the Royal Society Interface 6, 97-109, (2009).

[21] V. Kilappa, P. Moilanen, L. Xu, P.H. Nicholson, J. Timonen, S. Cheng, "Low-frequency axial ultrasound velocity correlates with bone mineral density and cortical thickness in the radius and tibia in pre- and postmenopausal women," Ost. Int. 22, 1103-1113 (2011).

[22] N. Bochud, J.G. Minonzio, Q. Vallet, P. Laugier, An anisotropic bi-layered model to estimate cortical bone properties from guided-wave measurement., IEEE International Ultrasonics Symposium –Taipei, Taiwan, 21 Oct - 24 Oct 2015.