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● *Original Contribution*

ACOUSTIC PROPERTIES OF TRABECULAR BONE—RELATIONSHIPS TO TISSUE COMPOSITION

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Abstract—In osteoporosis, changes in tissue composition and structure reduce bone strength and expose it to fractures. The current primary diagnostic technique, *i.e.*, dual energy X-ray absorptiometry, measures areal bone mineral density (BMD) but provides no direct information on trabecular structure or organic composition. Although still poorly characterized, ultrasound techniques may bring about information on bone composition and structure. In this study, relationships of 2.25-MHz ultrasound speed, attenuation, reflection and backscattering with composition of human trabecular bone ($n = 26$) were characterized experimentally, as well as by using numerical analyses. We also determined composition of the trabecular sample (fat and water content, bone volume fraction) and that of the calcified matrix (mineral, proteoglycan and collagen content of trabeculae). In experimental analyses, bone volume fraction and mineral content of the calcified matrix were the only determinants of BMD. Further, bone volume fraction served as the strongest determinant of ultrasound parameters ($r = 0.51–0.87$). In numerical simulations, density and mechanical properties of the calcified matrix systematically affected ultrasound speed, attenuation, reflection and backscattering. However, partial correlation coefficients revealed only low associations($|r| \leq 0.4$) between the composition of calcified matrix and ultrasound parameters in experimental measurements. To conclude, the content and structure of calcified matrix, rather than its composition, affect more significantly acoustic properties of healthy trabecular bone. (E-mail: Ossi.Riekkinen@uku.fi)

Key Words: **Trabecular bone, Composition, Osteoporosis, Ultrasound, DXA.**

INTRODUCTION

Early diagnosis of osteoporosis is essential for prevention of prospective fractures. Osteoporosis is defined by the World Health Organization (WHO) as areal bone mineral density (BMD) at or over 2.5 standard deviations (T-score) below the normal peak BMD values of young adults (Kanis 2002). Bone mineral density is determined traditionally with the use of dual energy X-ray absorptiometry (DXA).

Mechanical properties of trabecular bone depend on its trabecular structure, organic composition and mineral density (Njeh et al. 2001; Burr 2002; Mittra et al. 2005). Trabecular bone is composed of calcified matrix and bone marrow. The calcified matrix consists mainly of calcium hydroxyapatite and collagen. Calcium hydroxyapatite is an important determinant of bone stiffness and strength, and collagen contributes primarily to bone toughness and strength (Wang et al. 2001; Burr 2002). In osteoporosis, volume fraction of calcified bone (or BMD) is known to decrease while osteoporotic changes in the calcified matrix properties are not fully understood. In certain bone diseases, such as osteogenesis imperfecta, bone collagen is affected and overall bone strength decreases (Dominguez et al. 2005).

Most clinical quantitative ultrasound (QUS) measurements of bone are conducted on the calcaneus by using a through-transmission technique (Njeh et al. 1997). The most common clinical QUS parameters include speed of sound (SoS) and broadband ultrasound attenuation (BUA). Ultrasound backscattering and reflection measurements have also been introduced for osteoporosis diagnostics (Wear and Garra 1998; Roux et al. 2001; Hakulinen et al. 2004). In principle, reflec-

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tion and backscattering measurements may be conducted using a pulse-echo method at typical fracture sites, *e.g.*, the proximal femur. For through-transmission measurements, such sites are difficult to access because of the extensive ultrasound attenuation. Ultrasound backscattering has been demonstrated to be associated with trabecular structure and to serve as a significant predictor of bone mechanical properties (Hakulinen et al. 2004, 2005, 2006; Chaffai et al. 2002; Wear and Laib 2003; Jenson et al. 2003).

Quantitative ultrasound may provide more diverse information on bone properties than DXA, which gives information on BMD only (Roux et al. 2001; Drozdzowska et al. 2002; Hoffmeister et al. 2002a). QUS detects decollagenization and diagnoses collagen disruption (Ehlers-Danlos syndrome) with increased sensitivity over DXA (Cheng et al. 1999; Hoffmeister et al. 2002a). On the other hand, contradictory findings have been published on the effect of bone marrow content and composition on QUS parameters (Alves et al. 1996; Hoffmeister et al. 2002b; Nicholson and Bouxsein 2002). Because the knowledge on the relationships between QUS parameters and composition of trabecular bone is still limited, we investigated applicability of the pulse-echo and through-transmission techniques to evaluate the composition of the calcified matrix using experimental measurements and numerical simulations.

MATERIALS AND METHODS

Sample preparation

Cylindrical trabecular bone samples (diameter = 16 mm, thickness = 8 mm) were drilled from human (n = 12: 11 males and 1 female; age = 55 ± 19 y) distal femurs (n = 16) and proximal tibias (n = 10) (National Authority for Medicolegal Affairs, permission 1781/32/200/01 for study human samples) using a hollow drill bit (Fig. 1). Cartilage was removed and end surfaces of the bone cylinders were cut to be parallel by using a micro-grinding system (Macro Exact 310 CP, Exact, Hamburg, Germany). To avoid dehydration of the samples, we moistened them with phosphate-buffered saline (PBS) during cutting. After preparation, the fresh samples were immersed in PBS and stored in a freezer (−20°C) until the measurements were taken.

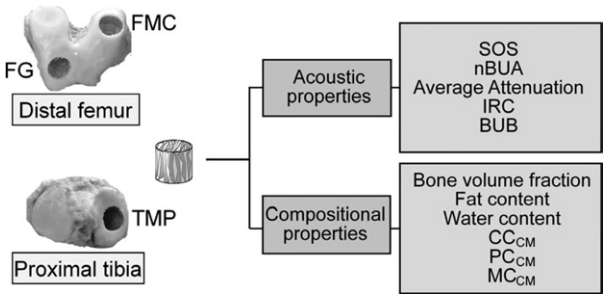

Fig. 1. Anatomical sampling locations of trabecular bone specimens. Five acoustic and six compositional parameters were determined for the samples. CC_{CM}, PC_{CM} and MC_{CM} stand for collagen, proteoglycan and mineral contents of calcified matrix, respectively.

Ultrasound methods

Acoustic measurements were conducted with an ultrasound system (UltraPAC, Physical Acoustic Co., NJ, USA) consisting of a 500-MHz A/D-board, a 0.2 to 100-MHz pulser-receiver board and an immersion tank with scanning drives. The measurements were conducted in degassed PBS bath using a pair of focused ultrasound (2.25 MHz) transducers (Panametrics V304, Panametrics Inc., Waltham, MA, USA) adjusted at a distance of 10 cm between transducers. The sample was placed in the focal plane between the transducers. During measurements, temperature of the PBS bath varied between 20.3 and 23.0°C. The ultrasound system was controlled with a customized program (LabVIEW 6.1, National Instrument, Austin, TX, USA).

Speed of sound, normalized broadband ultrasound attenuation (nBUA), average attenuation (AA), broadband ultrasound backscatter (BUB, Roux et al. 2001) and integrated reflection coefficient (IRC, Cherin et al. 1998) were determined. Normalized broadband ultrasound attenuation was derived from the linear part of the attenuation spectrum (1–2.8 MHz). Average attenuation, BUB and IRC were determined at the effective frequency range (1.53–3.8 MHz, −6 dB) of the transducers. Ultrasound parameters were determined as mean values inside circular region-of-interest (ROI, area 35 mm^2) at the center of the sample. More details on ultrasound transducers, measurements and analyses were presented in our previous paper (Hakulinen et al. 2005).

Analyses of bone composition

First, volumes and weights of the bone cylinders were determined using the Archimedes principle. Subsequently, the samples were freeze-dried (Christ Alpha 1 to 2, B. Braun Biotech International, Melsungen, Germany) for the determination of dry weights. To determine the fat-free weight of the sample, the fat was dissolved in acetone. The acetone was then removed from the samples by drying them at 45°C for 18 h. Finally, the water and fat contents were determined by normalizing water and fat masses with the sample volume. Bone volume fraction (%), *i.e.*, the volume fraction of the calcified matrix within the sample, was determined using a high resolution computed tomography (microCT) (SkyScan 1072, SkyScan, Aartselaar, Belgium) (Hakulinen et al. 2006).