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Longitudinal elastic properties and porosity of cortical bone tissue vary with age in human proximal femur

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ABSTRACT

Tissue level structural and mechanical properties are important determinants of bone strength. As an individual ages, microstructural changes occur in bone, *e.g.*, trabeculae and cortex become thinner and porosity increases. However, it is not known how the elastic properties of bone change during aging. Bone tissue may lose its elasticity and become more brittle and prone to fractures as it ages. In the present study the age-dependent variation in the spatial distributions of microstructural and microelastic properties of the human femoral neck and shaft were evaluated by using acoustic microscopy. Although these properties may not be directly measured *in vivo*, there is a major interest to investigate their relationships with the linear elastic measurements obtained by diagnostic ultrasound at the most severe fracture sites, *e.g.*, the femoral neck. However, before the validity of novel *in vivo* techniques can be established, it is essential to understand the age-dependent variation in tissue elastic properties and porosity at different skeletal sites. A total of 42 transverse cross-sectional bone samples were obtained from the femoral neck (*Fn*) and proximal femoral shaft (*Ps*) of 21 men (mean ± SD age 47.1 ± 17.8, range 17–82 years). Samples were quantitatively imaged using a scanning acoustic microscope (SAM) equipped with a 50 MHz ultrasound transducer. Distributions of the elastic coefficient (c_{33}) of cortical (*Ct*) and trabecular (*Tr*) tissues and microstructure of cortex (cortical thickness *Ct.Th* and porosity *Ct.Po*) were determined. Variations in c_{33} were observed with respect to tissue type ($c_{33Tr} < c_{33Ct}$), location ($c_{33}(Ct.Ps) = 37.7$ GPa $> c_{33}(Ct.Fn) = 35.3$ GPa $> c_{33}(Tr.Ps) = 33.8$ GPa $> c_{33}(Tr.Fn) = 31.9$ GPa), and cadaver age ($R^2 = 0.28$–0.46, $p < 0.05$). Regional variations in porosity were found in the neck (superior 13.1%; inferior 6.1%; anterior 10.1%; posterior 8.6%) and in the shaft (medial 9.5%; lateral 7.7%; anterior 8.6%; posterior 12.0%). In conclusion, significant variations in elastic coefficients were detected between femoral neck and shaft as well as between the quadrants of the cross-sections of neck and shaft. Moreover, an age-related increase in cortical porosity and a stiffening of the bone tissue were observed. These findings may explain in part the increase in susceptibility to suffer low energy fractures during aging and highlight the potential of ultrasound in clinical osteoporosis diagnostics.

Introduction

The risk of sustaining a fracture increases with age [1,2]. In particular, fractures of the proximal femur increase morbidity, shorten life expectancy, and represent an economic burden of the health care system [3,4]. The prevalence of fractures depends on both internal (quality of the musculoskeletal system) and external risk factors (*e.g.*, decreased balance, limited vision or slipperiness of the ground). The external factors are believed to have a minor or even no effect on the mechanical strength of bone tissue, but are related to the number of falls and thereby increasing the number of fractures [5–7]. On the contrary, bone quality, *i.e.*, the sum of factors such as bone geometry, degree of mineralization, microstructure and mechanical (elastic) properties, determine the mechanical strength and fragility of bone [8–10]. As these important properties cannot be tested *in vivo*, there is major interest in their estimation from linear elastic measurements obtained by diagnostic ultrasound at the most severe fracture sites, *e.g.*, the femoral neck. Indeed, there is excellent evidence that quantitative ultrasound is a valid (radiation-free and inexpensive) method for fracture risk assessment [11]. However, before one can establish the validity of these emerging technologies, it is essential to understand the age-dependent variation in tissue elastic properties and porosity at different skeletal sites. Since these properties

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directly influence the tissue acoustic properties, *e.g.*, as measured by non-invasive ultrasound. Furthermore, assessment of bone properties at the tissue level may improve the understanding of the alterations occurring in the tissue due to aging, medication, or pathology, and may further help to clarify more accurately the contribution of each internal factor toward the overall fracture risk.

It is possible to obtain highly localized direct information of tissue elastic and viscoelastic properties by means of nanoindentation [12,13]. However, the technique is not truly capable of assessing the elastic properties at high resolution over a large area such as the cross-section of the femoral neck. Quantitative acoustic microscopy has been widely used for assessing the elastic properties of bone *in vitro* [14–18]. By measuring bone samples over a wide range of frequencies and focal spot sizes, one can obtain both micro- and macro-scale assessments of bone [19,20]. The ultrasound reflection amplitude relates to the average elastic properties of the investigated material at the focal spot. Since structures smaller than the spot size cannot be detected, the transducer must be chosen carefully, *e.g.* when assessing porosity of cortical bone. In scanning acoustic microscopy (SAM) of bone, focal spot diameters in the range of 1 to 150 μm have been applied [20,21]. In human cortical bone, a spatial resolution of 23 μm has been proven to be sufficient to resolve Haversian canals and resorption cavities [20]. Most commonly in SAM, the amplitude of the reflected ultrasound pulse is measured, and used as a measure of the acoustic reflectivity which is determined by the material density and elastic properties in the probing direction [14,15]. The tissue elastic coefficient can be estimated from the acoustic impedance measurements [15]. For these reasons, SAM has been used extensively in the quantitative evaluation of spatial variation in elastic properties and microarchitecture of bone over the whole cross-sectional samples [22–24]. Furthermore, SAM has been used to obtain the material parameters which can be used in computational models of bone tissue mechanics [25].

Earlier studies using SAM have demonstrated the feasibility of this technique in the assessment of both the microstructural and the tissue elastic properties in fresh and embedded bone samples [16,18,20,21,24,26]. For example, variations of acoustic impedance and porosity have been analyzed in the human distal radius [27]. Furthermore, in previous studies the variation of bone acoustic impedance, elastic properties and their directional dependencies (anisotropy) have been investigated in the femoral shaft of human donors [14,17,18,26,28]. However, previously no systematic investigation has been conducted in to the variations in elastic properties and porosity in the proximal femur with respect to age. Furthermore, the relationship between tissue elastic properties and porosity has not been examined using bones harvested from the most severe fracture site [27]. The present study aims to fill this gap in our knowledge. We collected data on the regional distribution in the acoustic impedance in tissue along the cross-section of the femoral neck and shaft and investigated the variation of tissue acoustic impedance values with age. Furthermore, microstructure and cortical porosity were analyzed to determine possible variations with respect to aging and between skeletal sites. This is essential information if one wishes to estimate diagnostic determinants of bone fractures in elderly.

Materials and methods

Twenty-one human proximal femurs were obtained from male cadavers ($n=21$, aged 47.1 ± 17.8 years, range 17–82 years) at Kuopio University Hospital. Ethical approval for collection of samples was granted by the National Authority for Medicolegal Affairs (permission number: 5783/04/044/07). The cadavers were classified into three groups, young (<40 years, $n=8$), middle aged (40–60 years, $n=9$) and old (>60 years, $n=4$). The cadavers had no pre-existing conditions that might have affected bone metabolism (Table 1).

After soft tissue removal, the proximal femurs were stored in a freezer (−20 °C) until extracting the test samples. It has been reported that freezing and thawing do not affect significantly the elastic properties of bone tissue [29–31].

Table 1
Basic anthropometric data of the cadavers.

Age [years]	Age group	Height [cm]	Weight [kg]	BMI [kg/m^2]
17	1	178	74	23.4
22	1	186	106	30.6
26	1	175	60	19.6
30	1	184	105	31.0
32	1	171	69	23.6
34	1	187	102	29.2
37	1	177	74	23.6
39	1	185	84	24.5
43	2	171	98	33.5
44	2	179	96	30.0
46	2	185	85	24.8
49	2	178	85	26.8
51	2	185	108	31.6
52	2	180	136	42.0
54	2	176	73	23.6
58	2	169	96	33.6
59	2	175	73	23.8
63	3	170	68	23.5
74	3	166	64	23.2
78	3	177	72	23.0
82	3	165	53	19.5
47.1 ± 17.8	–	177.1 ± 6.7	84.8 ± 20.0	26.9 ± 5.5

Transversal cross-sections were cut from the femoral neck ($n=21$) and shaft ($n=21$) with a band saw (KT-210, Koneteollisuus Oy, Helsinki, Finland). A minor cut was made on the medial bone surface to enable correct orientation of the sample during the measurements. Sections of the shaft were cut perpendicular to the long axis of bone, below the trochanter minor. Sections from the middle of the neck were cut perpendicular to the long axis of the neck (Fig. 1). Samples were dehydrated in ethanol and embedded in polymethylmethacrylate (PMMA) according to standard protocols [14,19]. In order to obtain smooth planar sample surfaces, the samples were ground with a plane grinder (EXAKT 400CS, Exakt Apparatebau, Norderstedt, Germany) with successively decreasing grain size (ISO/FEPA grit: P500, P800, P1000, P1200 and P4000, Hermes Abrasives Ltd., Virginia Beach, VA and Struers A/S, Ballerup, Denmark). The final polishing was performed with cloths containing 6 μm and 1 μm diamond particle suspensions in ethylene glycol (Phoenix 4000, Buehler Ltd., Lake Bluff, IL.). Between the polishing and measurements, an ultrasound cleaner and a soft paint brush were used to remove debris and diamond suspension from the specimen.

Scanning acoustic microscopy

A custom built quantitative scanning acoustic microscope was used [32]. Briefly, the SAM consisted of a three-axis high-precision scanning stage, a 200 MHz pulser–receiver (Panametrics 5900PR, Panametrics-NDT, Waltham, MA), and a 400 MS/s 12-bit A/D-board (CompuScope 12400, GaGe, Lockport, IL). All components were controlled by custom-made software (SAM200Ex, Q-BAM, Halle, Germany).

Measurements were conducted in a temperature controlled tank containing 25 °C distilled and degassed water using a 50 MHz spherical focused transducer (V605/60°, −6 dB bandwidth 26–64 MHz, Valpey Fisher, Hopkinton, USA) with a focal length of 5.2 mm and beam diameter at focus of 23 μm [14,20,32].

The incidence angle between the ultrasound pulse and the water-bone interface was manually adjusted by using a custom made tilting table. In order to ensure the perpendicular alignment and focusing of the transducer on the bone surface, a scout scan was conducted over the bone area before the actual measurements. After adjusting the time of flight between the bone surface and the transducer to be constant within the region of interest (ROI), optimal perpendicularity between the incident ultrasound pulse and the water-bone interface