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Composition and microarchitecture of human trabecular bone change with age and differ between anatomical locations

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ABSTRACT

The microarchitecture of trabecular bone adapts to its mechanical loading environment according to Wolff's law and alters with age. Trabecular bone is a metabolically active tissue, thus, its molecular composition and microarchitecture may vary between anatomical locations as a result of the local mechanical loading environment. No comprehensive comparison of composition and microarchitecture of trabecular bone in different anatomical locations has been conducted. Therefore, the objective of this study was to compare the molecular composition and microarchitecture, evaluated with Fourier transform infrared (FTIR) microspectroscopy and micro-computed tomography (μCT), respectively, in the femoral neck, greater trochanter and calcaneus of human cadavers. Specimens were harvested from 20 male human cadavers (aged 17–82 years) with no known metabolic bone diseases. Significant differences were found in composition and microarchitecture of trabecular bone between the anatomical locations. Compositional differences were primarily observed between the calcaneus and the proximal femur sites. Mineralization was higher in the greater trochanter than in the calcaneus (+2%, $p<0.05$) and crystallinity was lowest in the calcaneus (−24%, $p<0.05$ as compared to the femoral neck). Variation in the composition of trabecular bone within different parts of the proximal femur was only minor. Collagen maturity was significantly lower in greater trochanter than in femoral neck (−8%, $p<0.01$) and calcaneus (−5%, $p<0.05$). The greater trochanter possessed a less dense trabecular bone microarchitecture compared to femoral neck or calcaneus. Age related changes were mainly found in the greater trochanter. Significant correlations were found between the composition and microarchitecture of trabecular bone in the greater trochanter and calcaneus, indicating that both composition and microarchitecture alter similarly. This study provides new information about composition and microarchitecture of trabecular bone in different anatomical locations and their alterations with age with respect to the anatomical loading environments.

Introduction

Trabecular bone microarchitecture is adapted to the local mechanical environment according to Wolff's law [1,2]. Moreover, the microarchitecture also changes as the individual ages [3]. Trabecular bone is a metabolically very active tissue, resulting in possible changes in the composition due to loading or aging [4,5]. Hence, the molecular composition and microarchitecture of trabecular bone may vary between different anatomical locations due to different mechanical loading and remodeling rates.

Dual energy X-ray absorptiometry (DXA) provides a measure of the areal bone mineral density (BMD). However, the effects of bone thickness and the degree of mineralization cannot be directly distinguished by DXA. Therefore, BMD measured by DXA alone cannot fully depict or predict bone mechanical properties or fracture risk [6–8]. Other important factors for predicting fracture risk include, e.g. geometry [9,10], micro-damage [11], composition [12,13] and trabecular bone microarchitecture [14,15]. The combined effect of all of these factors defines the bone quality, which depends on the structural and compositional parameters as well as the mechanical properties [16]. Thus, to understand the mechanisms that affect bone strength, it is important to understand both the microarchitecture and the molecular composition.

Micro-computed tomography (μCT) can be used to quantify the microarchitecture of trabecular bone [17–19]. The evaluation of

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three-dimensional morphology of trabecular bone provides an estimation of several indices, such as bone volume fraction (BV/TV), average trabecular thickness (Tb.Th), separation (Tb.Sp) and number (Tb.N) as well as information about the trabecular plate-rod-like shape (structural model index, SMI) and the degree of anisotropy (DA). These indices have been used to examine human trabecular bone in different anatomical locations, e.g. vertebrae, iliac crest, proximal femur and calcaneal bone [3,19–22].

Fourier transform infrared (FTIR) microspectroscopy represents a tool for fast measurements of the spatial molecular composition of bone [4,5,23]. In FTIR transmission spectroscopy, a wide band of infrared (IR) light is guided through the sample. The different molecular bonds absorb IR light of different wavelengths and thus an absorption spectrum is created. Several compositional parameters can be calculated from the bone IR spectra. Mineralization can be evaluated from the mineral/matrix ratio (M/M), whereas carbonate substitution is evaluated from the carbonate/phosphate (C/P) ratio [24,25]. In addition, collagen maturity (C.Mat) [26], and crystallinity [27,28] can be evaluated through second derivative peak fitting.

The femoral neck has to withstand continuous high compressive and shear forces. During standing these are roughly 1×BW (body weight), but they can be higher during physical activities (Fig. 1A) [29]. The greater trochanter is mainly subjected to tensile forces from the muscles (e.g. gluteus maximus, medius and minimus) [29] (Fig. 1A), whereas in the calcaneus tensile forces act through the Achilles tendon, plantar fascia and plantar ligaments [30] (Fig. 1B). Since the weight is divided between the heel and forefoot, the calcaneus does not experience as high static compressive loading as the femoral neck. However, the calcaneus has to withstand impact loading during e.g. walking and running, which may be up to 10×BW [30]. Bone adaptation is driven by dynamic loading [31] which typically increases bone strength through increased cross-sectional area of bone [32,33]. However, it is possible that concomitant changes in composition occur during adaptation, e.g., the mineral/matrix ratio increases without an increase in cross-sectional area [34]. However, these changes in bone quality have been investigated much more rarely. Hence, trabecular bone adapts to loading environments through modeling and remodeling, which alter its microarchitecture. Moreover, the molecular composition is also altered, at least temporarily, during the process of remodeling [28,35]. Thus, local compositional and structural evaluations can provide important information about normal development of trabecular bone and its adaptation to different loading environments.

Currently, data on the variation in the composition of trabecular bone at several anatomic sites within the same donor is limited [36]. Specifically, the composition at the femoral neck, greater trochanter and calcaneus within the same donor has not been studied. Thus, in the present study, we quantified and compared the molecular composition and microarchitecture of trabecular bone in the femoral neck, greater trochanter and calcaneus in human donors with healthy bones. Additionally, changes in and relationships between molecular composition and microarchitecture of trabecular bone with age during adult life were investigated.

Materials and methods

Experimental overview

Trabecular bone samples were harvested from the femoral neck, greater trochanter and calcaneus of male human cadavers (age range 17–82 years, $n=20$) with permission from the National Authority for Medicolegal Affairs (TEO, 5783/04/044/07) (Fig. 1). Medical records were available and none of the cadavers had any known metabolic bone diseases. First, DXA measurements were conducted on the intact proximal femur and calcaneus. Thereafter, two trabecular bone samples (diameter 10 mm, length 10–15 mm) were obtained from each location using a coring tool. The first sample was dehydrated with ascending series of ethanol solutions and embedded in Technovit (EXAKT Technovit 7200 VLC, Heraeus Kulzer GmbH, Germany). Subsequently, 3 μm thick sections were prepared for the FTIR measurements and the sections were placed on ZnSe windows. The second sample was stored frozen (−20 °C) in phosphate buffered saline (PBS) until imaging with micro-computed tomography.

Dual energy X-ray absorptiometry (DXA)

The bone mineral density (BMD) of the proximal femur and the calcaneus was determined with DXA (Lunar Prodigy Advance, GE Healthcare, Madison, USA), using the clinical hip measurement protocol. The soft tissue was simulated by immersing the bones into 10 l of phosphate buffered saline (PBS) in a plastic container. The samples were positioned according to the *in vivo* anatomy and all measurements were repeated twice. From the DXA images, the BMD was determined from the femoral neck, and from custom regions of analyses from the greater trochanter and the calcaneus (Fig. 2A). The regions of interest were chosen to represent the areas where the bone samples for FTIR and μCT were taken.

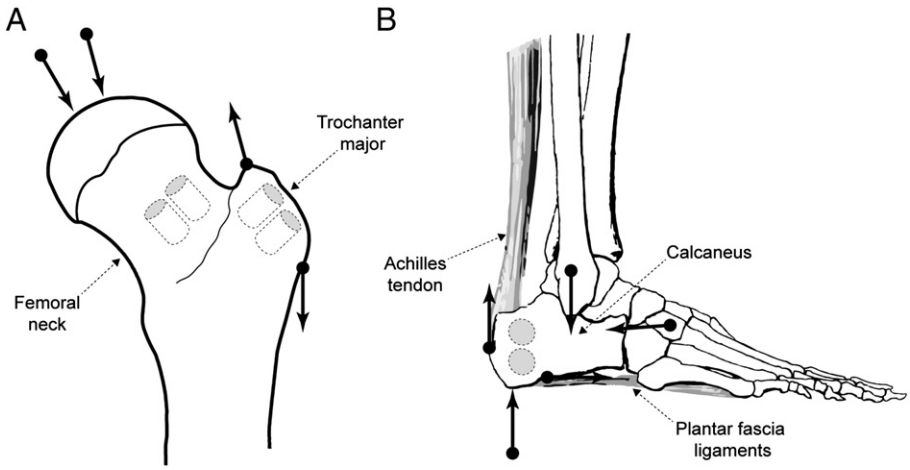

Fig. 1. Schematic figures of A) the proximal femur and B) the foot. The solid arrows indicate the directions of major forces influencing the different parts of the bones. Additionally, the locations of the harvested samples in the femoral neck, greater trochanter and calcaneus are indicated.