Composition of Human Trabecular Bone Changes with Age and Differs between Anatomical Locations: Evaluation with FTIR

INTRODUCTION:
Bone structure and composition adapt to its mechanical loading environment [1] and show variations with age [2,3]. Trabecular bone is highly metabolically active, and is expected to change its composition of the organic and inorganic matrices during remodeling. Hence, the molecular composition of trabecular bone may vary between different anatomical locations due to different mechanical loading and remodeling rates. Fourier transform infrared (FTIR) microspectroscopy provides a tool for fast measurements of the molecular composition of bone [2-6].

Our aim was to compare the molecular composition of human trabecular bone from three anatomical locations with different mechanical loading environment. Moreover, the changes in molecular composition of bone with age during adult life were investigated.

METHODS:
Trabecular bone samples from the femoral neck, trochanter major and calcaneus of male human cadavers with varying age (26-82 year old, n = 20) were harvested with permission from the National Authority for Medicolegal Affairs (TEO, 5783/04/044/07). None of the cadavers had any known metabolic bone diseases. The samples (diameter 10 mm, length 10-15 mm) were obtained with a coring tool, dehydrated in alcohol and embedded in Technovit (Exakt Technovit 7200 VLC, Heraeus Kulzer GmbH, Germany). Subsequently, 3 µm thick sections were prepared for FTIR measurements.

Areal measurements of three different trabeculae from each sample were performed with FTIR (Perkin Elmer Spotlight 300, Waltham, MA) using a pixel resolution of 6.25 µm, spectral resolution of 4 cm⁻¹ and 8 repeated scans. The background scan was performed on a clean BaF₂ window using the same parameters as above, but with an average of 75 scans. The bone spectra were normalized against the Technovit spectrum [7], and the Technovit spectrum was mathematically subtracted from the bone spectra [8]. From the bone spectra, mineral/matrix (M/M), carbonate/matrix (C/M) and carbonate/phosphate (C/P) ratios were determined after linearly correcting the baseline of each peak [2]. Collagen cross-linking ratio (XLR) and crystallinity (perfection of hydroxylapatite crystals) were determined through peak fitting from the ratio of the underlying peaks at 1660 cm⁻¹ and 1060 cm⁻¹, and 1030 cm⁻¹ and 1020 cm⁻¹ [2,9], respectively. All IR spectra were analyzed with custom made Matlab scripts (v. 7.6.0, The MathWorks, Inc., MA).

Wilcoxon signed ranks test was used to test statistical differences between the compositional parameters from the three anatomical locations. Pearson’s correlation test was used to evaluate age-dependency and associations between the same parameters measured in different anatomical locations. All statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, IL).

RESULTS:
The M/M and C/M ratios of both the trochanter major and the calcaneus increased significantly with age (Fig 1A-B). However, the composition in the femoral neck did not change with age. The C/P ratio did not correlate with age in any anatomical location.
The M/M ratio correlated significantly between the trochanter major and the calcaneus (Fig 1C). The C/M ratio of the trochanter major and femoral neck correlated significantly with the C/M ratio of the calcaneus (Fig 1D). Hence, the variation between cadavers was often reflected similarly in several parameters over the anatomical locations. When comparing the different anatomical locations, the M/M ratio was significantly higher (p < 0.05) in the trochanter major than in the calcaneus (Fig 1E). The XLR was significantly lower in the trochanter major compared to the femoral neck (p < 0.01) and calcaneus (p < 0.05) (Fig 1F). The crystallinity was significantly higher (p < 0.01) in the femoral neck than in the calcaneus (Fig 1G).

DISCUSSION:
The composition of the trochanter major and calcaneus changed more gradually throughout life compared to the composition of the femoral neck (Fig 1A-B). No significant correlations between the age and any compositional parameters were observed in the femoral neck. However, micro-CT analyses from these locations showed that the bone structural parameters changed more with age in the femoral neck than in the trochanter major or the calcaneus [10]. The differences in the mechanical loading between the anatomical locations most likely affect both the structure and the molecular composition of the trabecular bone. The femoral neck withstands compressive forces, the trochanter major tensile forces originating from the muscle contraction and the calcaneus impact loads, e.g. during running and jumping. Consistently, as revealed in the present study, the composition and structure differ in these anatomical locations. The M/M and C/M ratios correlate between trochanter major and calcaneus, suggesting similarities between those bones. High impact loading in the calcaneus may inflict micro-cracks, increase the bone remodeling and affect the local bone composition [11]. Increased remodeling and turnover can be assumed to decrease crystallinity. The higher M/M ratio in the trochanter major, compared to the calcaneus, may relate to low forces exposed to the trochanter major, leading to different bone turnover and remodeling rates. XLR was also lower in the trochanter major than in the femoral neck or calcaneus. Collagen cross-links are known to control the mechanical strength of bone [12].

SIGNIFICANCE:
The molecular composition of trabecular bone differs between anatomical locations undergoing different mechanical loading. Moreover, the effect of aging seems differ between the locations.

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REFERENCES: