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Technical Note

Age-related changes of vertical and horizontal lumbar vertebral trabecular 3D bone microstructure is different in women and men

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ABSTRACT

The study presents a 3D method for subdividing a trabecular network into horizontal and vertical oriented bone. This method was used to investigate the age related changes of the bone volume fraction and thickness of horizontal and vertical trabeculae in human lumbar vertebral bone estimated with unbiased 3D methods in women and men over a large age-range.

The study comprised second lumbar vertebral body bone samples from 40 women (aged 21.7–96.4 years, median 56.6 years) and 39 men (aged 22.6–94.6 years, median 55.6 years). The bone samples were μCT scanned and the 3D microstructure was quantified. A voxel based algorithm inspecting the local neighborhood is presented and used to segment the trabecular network into horizontal and vertical oriented bone.

For both women and men BV/TV decreased significantly with age, Tb.Th* was independent of age, while SMI increased significantly with age. Vertical (BV.vert/TV) and horizontal (BV.horz/TV) bone volume fraction decreased significantly with age for both sexes. BV.vert/TV decreased significantly faster with age for women than for men. Vertical (Tb.Th*.vert) and horizontal (Tb.Th*.horz) trabecular thickness were independent of age, while Tb.Th*.horz/Tb.Th*.vert decreased significantly with age for both sexes. Additionally, the 95th percentile of the trabecular thickness distribution increased significantly with age for vertical trabeculae in women, whereas it was independent of age in men.

In conclusion, we have shown that vertical and horizontal oriented bone density decreases with age in both women and men, and that vertical oriented bone is lost more quickly in women than in men. Furthermore, vertical and horizontal trabecular thickness were independent of age, whereas the horizontal to vertical trabecular thickness ratio decreased significantly with age indicating a relatively more pronounced thinning of horizontal trabeculae. Finally, the age-related loss of trabecular elements appeared to result in a compensatory hypertrophy of vertical trabeculae in women, but not in men.

Introduction

During aging, human vertebral bone is lost resulting in weaker bone and thereby a higher fracture risk. It has been shown that bone density is a major determinant of vertebral bone strength [1,2], but it has also been suggested that the microstructure of the bone tissue plays a role for the bone strength [3–6]. Hence, Hui et al. showed that even for constant bone mass, fracture risk increases with age [7]. Thus, bone fracture strength is not only dependent on bone density, but also on bone microstructure, micro damage accumulation, and mineralization [8]. The conventional view is that a compressive load on a vertebral body is mainly carried by the vertical trabeculae, whereas the horizontal trabeculae serve to prevent buckling of the vertical trabeculae [9–12]. This view is reinforced by finite element analyses of human vertebral bone specimens, which demonstrates that vertical trabeculae are more highly strained than horizontal trabeculae under normal compressive loading [13–16]. Furthermore, Fields et al. hypothesized that vertebral bone strength is better explained by the bone volume fraction of the vertical trabeculae alone, than by the bone volume fraction of all trabeculae [15]. In a subsequent study, Fields et al. found that the vertical trabeculae played a particular important role for the compressive bone strength of vertebrae with low bone density [17]. Consequently, it is important to quantify the age-related changes in trabecular thickness as well as bone volume fraction for horizontal and vertical trabeculae separately.

It has, for a long time, been debated whether the removal of vertical trabeculae with age would lead to a higher compressive load on the remaining trabeculae, and thereby to a compensatory thickening of these [12,18–22]. Thus, in 1983 Parfitt et al. suggested that, when trabeculae are removed during aging, the remaining trabeculae would be more widely separated and therefore might undergo compensatory thickening [23]. Furthermore, in a 1999 perspective article Harold Frost invited other researchers to provide proof that vertical trabeculae can strengthen and 'thicken' in adults under increased mechanical

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loading [18]. We have previously investigated this using 2D histomorphometry on human vertebral bone, but were unable to find evidence of a compensatory thickening of vertebral trabeculae with age [24]. However, the 2D analysis was limited by the use of the parallel plate model for determination of the trabecular thickness. The development of micro computed tomography (μCT) scanners has now made it feasible to investigate the microstructure of the 3D trabecular network in human vertebrae using model assumption free methods for determination of the trabecular thickness.

Therefore, the aim of the present study was to present a 3D method for segmenting the trabecular network into horizontal and vertical oriented bone. Furthermore, we applied the method to 79 human vertebral bone specimens and tested the hypothesis that the age related changes of the bone volume fraction and thickness of horizontal and vertical trabeculae in human vertebral bone do not differ in women and men. Finally, we investigated whether vertical vertebral trabeculae can thicken with age.

Materials and methods

Bone specimens

Second lumbar vertebral bodies from 40 women (aged 21.7–96.4 years, median 56.6 years) and 39 men (aged 22.6–94.6 years, median 55.6 years) were analyzed. The subjects were chosen so as to provide an even age and sex distribution in the 20–90 years age range. All individuals were Caucasian. Vertebral bodies with fractures identified from lateral-projection X-ray images were excluded from further investigations as previously reported [1]. Samples from individuals with known cancer or drug abuse were excluded from the study at the time of autopsy. Subsequently, a thorough review of hospital records and autopsy information available was used to exclude individuals with cancer (discovered at autopsy), metabolic disease, severe liver or kidney disease, medication affecting bone metabolism, or periods of more than 2 weeks of immobilization prior to death as previously described [25].

The vertebral bodies from 24 of the women and 24 of the men have previously been investigated using 2D histomorphometry [24,26]. The investigation presented here is a subset of a larger study called the "Danish in Vitro Bone Study" (DAVIBO) [1,25,27]. The collection of the material and the study design were approved by the local ethical committee.

Specimen preparation

The bone specimens had previously been prepared for histomorphometry [28]: The vertebral bodies were cleaned of soft connective tissue and halved along the anterior–posterior axis using a diamond parallel precision saw (Exakt, Apparatebau, Otto Herrmann, Norderstedt, Germany). An approximately 9-mm-thick frontal bone specimen was sawed from the center of one half of the corpus and embedded undecalcified in methyl methacrylate (Technovit 9100, Heraeus Kulzer, Wehrheim/Ts., Germany). Subsequently, the tissue blocks were trimmed using the diamond saw to remove all excessive methyl methacrylate to facilitate μCT scanning.

Micro computed tomography (μCT)

The bone specimens were placed in a μCT scanner (μCT35, Scanco Medical AG, Brüttisellen, Switzerland) so that they were aligned along the *x*-axis of the scanner and so the vertebral endplates were parallel with the *xy*-plane. The specimens were scanned in high-resolution mode (1000 projections per 180°) with an isotropic voxel size of 18.5 μm, an X-ray tube voltage of 70 kVp, an X-ray tube current of 114 μA, and an integration time of 800 ms. The reconstructed μCT data was exported to a PC running Linux (OpenSUSE 11.2, http://www.opensuse.org), where the trabecular bone volume of interest (VOI) was interactively delineated as closely as possible to either the cortical bone or the sawing planes using custom-made software written by one of the authors (JST) [29]. The resulting image masks were transferred back to the μCT scanner and imported into IPL (version 5.11, Scanco Medical). The 3D data sets were low-pass filtered using a Gaussian filter ($\sigma = 0.8$, support $= 1$) in order to remove noise and were subsequently segmented with a fixed threshold filter. The minimum point between the marrow and the bone peak in the attenuation histogram was automatically determined using IPL for 25 vertebral bone specimens, and the median of these thresholds (450.7 mg HA/cm^3) was used for all segmentations.

Standard quantification of the microstructure of the trabecular bone network was performed using the software provided with the μCT scanner (version 6.0, Scanco Medical). Microstructural measures included bone volume per total volume (BV/TV), trabecular thickness (parallel plate model: Tb.Th [30] and directly estimated: Tb.Th* [31]), trabecular number (Tb.N*) [32], connectivity density (CD) [33], and structural model index (SMI) [34]. The computation of these structural measures has previously been described in detail [35]. In addition, the degree of anisotropy (DA) was also computed [36].

Quality assurance was performed by weekly (density) and monthly (geometry) scans of the solid-state calibration phantom provided with the scanner.

Trabecular orientation

The voxels of the trabecular bone network was classified as either horizontal or vertical by inspecting the orientation of the bone tissue surrounding the voxel. The method is an extension of our previously presented 2D method into 3D [24].

Consider a voxel characterized by its Cartesian coordinates (x_0, y_0, z_0). Let $l^*(\theta, \varphi)$ denote the Euclidian length from the voxel at (x_0, y_0, z_0) to either the bone-marrow boundary or the VOI boundary, where θ is the polar angle and φ is the azimuthal angle (Fig. 1). Let

$$h(x_0,y_0,z_0) = \sum_{j=0}^{N-1}\sum_{i=\frac{N}{8}}^{\frac{3N}{8}-1} l^*\left(2\pi\frac{i}{N}, 2\pi\frac{j}{N}\right) \quad (1)$$

$$v(x_0,y_0,z_0) = \sum_{j=0}^{N-1}\sum_{i=0}^{\frac{N}{8}-1} l^*\left(2\pi\frac{i}{N}, 2\pi\frac{j}{N}\right) + \sum_{j=0}^{N-1}\sum_{i=\frac{3N}{8}}^{N-1} l^*\left(2\pi\frac{i}{N}, 2\pi\frac{j}{N}\right) \quad (2)$$

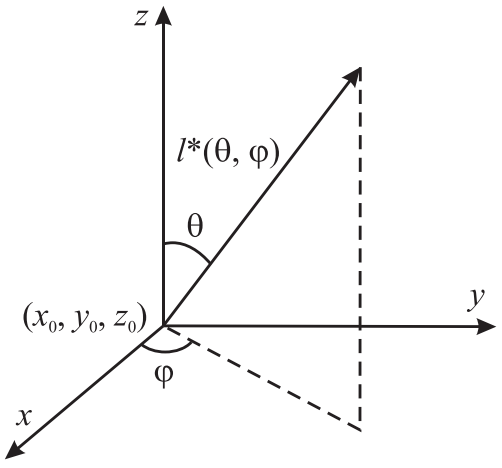

Fig. 1. Coordinate system with the origin placed in the voxel under inspection (x_0, y_0, z_0) used for the calculations. The Euclidian length from the origin to either the bone-marrow boundary or the VOI boundary is denoted $l^*(\theta, \varphi)$, where θ is the polar angle and φ is the azimuthal angle.