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Scaling relations between trabecular bone volume fraction and microstructure at different skeletal sites

Christoph Räth [a,1], Thomas Baum [b,*,1], Roberto Monetti [a], Irina Sidorenko [a], Petra Wolf [c], Felix Eckstein [d], Maiko Matsuura [e], Eva-Maria Lochmüller [d], Philippe K. Zysset [f], Ernst J. Rummeny [b], Thomas M. Link [g], Jan S. Bauer [b,h]

[a] Max-Planck-Institut für extraterrestrische Physik, Giessenbachstr. 1, 85748 Garching, Germany
[b] Institut für Radiologie, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 München, Germany
[c] Institut für Medizinische Statistik und Epidemiologie, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 München, Germany
[d] Institute of Anatomy and Musculoskeletal Research, Paracelsus Medical University Salzburg, Strubergasse 21, 5020 Salzburg, Austria
[e] Anatomische Anstalt, Ludwig-Maximilians-Universität München, Pettenkoferstr. 11, 80336 München, Germany
[f] Institute for Surgical Technology and Biomechanics, University of Bern, Stauffacherstr. 78, 3014 Bern, Switzerland
[g] Musculoskeletal and Quantitative Imaging Research Group, Department of Radiology and Biomedical Imaging, University of California San Francisco, 185 Berry Street, Suite 350, San Francisco, CA 94107, USA
[h] Abteilung für Neuroradiologie, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 München, Germany

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ABSTRACT

In this study, we investigated the scaling relations between trabecular bone volume fraction (*BV* / *TV*) and parameters of the trabecular microstructure at different skeletal sites.

Cylindrical bone samples with a diameter of 8 mm were harvested from different skeletal sites of 154 human donors in vitro: 87 from the distal radius, 59/69 from the thoracic/lumbar spine, 51 from the femoral neck, and 83 from the greater trochanter. μCT images were obtained with an isotropic spatial resolution of 26 μm. *BV* / *TV* and trabecular microstructure parameters (*TbN*, *TbTh*, *TbSp*, scaling indices ($<>$ and σ of α and α_z), and Minkowski Functionals (*Surface*, *Curvature*, *Euler*)) were computed for each sample. The regression coefficient β was determined for each skeletal site as the slope of a linear fit in the double-logarithmic representations of the correlations of *BV* / *TV* versus the respective microstructure parameter.

Statistically significant correlation coefficients ranging from $r = 0.36$ to $r = 0.97$ were observed for *BV* / *TV* versus microstructure parameters, except for *Curvature* and *Euler*. The regression coefficients β were 0.19 to 0.23 (*TbN*), 0.21 to 0.30 (*TbTh*), −0.28 to −0.24 (*TbSp*), 0.58 to 0.71 (*Surface*) and 0.12 to 0.16 ($<\alpha>$), 0.07 to 0.11 ($<\alpha_z>$), −0.44 to −0.30 ($\sigma(\alpha)$), and −0.39 to −0.14 ($\sigma(\alpha_z)$) at the different skeletal sites. The 95% confidence intervals of β overlapped for almost all microstructure parameters at the different skeletal sites. The scaling relations were independent of vertebral fracture status and similar for subjects aged 60–69, 70–79, and >79 years.

In conclusion, the bone volume fraction–microstructure scaling relations showed a rather universal character.

Introduction

The German anatomist and surgeon Julius Wolff developed as early as 1892 the theory that bone remodels in response to the mechanical stresses it experiences [1]. He postulated his theory, known as Wolff's law, based on his observations that the trabeculae in the femoral head were aligned with the principal stress lines. Wolff concluded that bone tissue is constantly adapting to the external forces acting on the bone to achieve maximum mechanical competence with minimal bone mass through optimization of bone shape and microstructure.

In the last decades, the molecular mechanism behind bone remodeling with two effector cell types, bone-resorbing osteoclasts and bone-forming osteoblasts, has been identified and extensively studied [2–6]. Molecular biological studies demonstrated the ability of osteocytes to respond to external loading conditions, thus supporting Wolff's theory of bone remodeling [7–11]. Furthermore several computational techniques have been developed to simulate bone remodeling. Based on finite element analysis, computer models simulated the adaptation of the trabecular bone to changes of the external forces acting on the bone [12–17]. Wolff's law was also validated in several in vivo studies: Barak et al. induced changes in joint loading orientation in sheep and observed that the alignment of the trabecular bone structure was adjusted to the changes in peak loading direction [18]. Lambers et al. reported increasing bone formation rate and decreasing bone resorption rate in mouse tail vertebrae as adaptation to cyclic mechanical

* Corresponding author. Fax: +49 89 4140 4834.
E-mail address: thbaum@gmx.de (T. Baum).
[1] Christoph Räth and Thomas Baum equally contributed to this work.

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loading [19]. Nikander et al. measured bone mineral density (BMD) of the femoral neck using Dual Energy X-ray Absorptiometry (DXA) in adult female athletes and found higher BMD values in subjects performing high-impact sports (e.g., volleyball) and odd-impact sports (e.g., soccer) compared to subjects performing low-impact sports (e.g., swimming) [20]. In a similar study population, they determined bone characteristics such as cortical and trabecular density at the humerus, radius, and tibia using peripheral quantitative computed tomography (pQCT) [21]. The skeletal adaptation at the upper and lower extremities were in accordance with the different loading history and once more indicated the validity of Wolff's law of bone remodeling.

Previous studies reported partly remarkable differences in bone volume fraction (bone volume divided by total volume, *BV* / *TV*) and trabecular bone structure parameters in human bone samples harvested from different skeletal sites [22–25]. However, it remains to be investigated whether site dependent alterations in bone volume fraction are proportional to the corresponding alterations in trabecular microstructure. To give an example, an increase in *BV* / *TV* may be associated with a proportional increase in anisotropy, both in the femur and spine, although absolute values and relationship between the parameters are quite diverse at different skeletal sites. These changes can easily be determined as scaling relations between bone volume fraction and microstructure parameters. Therefore, the purpose of this study was to investigate the scaling relations between bone volume fraction and microstructure at different skeletal sites. We hypothesized that rather universal scaling relations between bone volume fraction and microstructure can be found at the different anatomical sites. Furthermore, we investigated possible bone stability and age-related effects on these scaling relations.

Materials and methods

Bone samples

Three hundred forty-nine bone samples from different skeletal sites were harvested from formalin-fixed human donors. The donors had dedicated their body for educational and research purposes to the local Institute of Anatomy prior to death in compliance with local institutional and legislative requirements. The donor group consisted of 84 males and 70 females with a mean age ± standard deviation (SD) of 80 ± 10 years (median: 81 years; range: 52 to 102 years). Mean body mass index (BMI) amounted to 21.1 ± 4.3 kg/m^2. Aside from osteoporosis, all pathological bone changes like bone metastases, hematological, or metabolic bone disorders were the exclusion criteria for the study. To this end, biopsies were taken from the iliac crest of all donors and were examined histologically.

Anterior–posterior (ap) and lateral radiographs of the thoracic and lumbar spine were obtained to assess the vertebral fracture status of the donors. Osteoporotic vertebral fractures were diagnosed in 69 subjects by a radiologist according to the spinal fracture index previously described by Genant et al. [26].

The cylindrical bone samples with a diameter of 8 mm and a length of 14 mm were harvested using diamond drills (Salzmann, Munich, Germany) under water irrigation from the following skeletal sites: distal radius, thoracic vertebra 10 (T10), lumbar vertebra 2 (L2), femoral neck, and greater trochanter. Radiographs from the radii, vertebrae, and femur were used to exclude those with previous fractures. Radius and femur samples were harvested from the left side. In case of previous fracture or total hip replacement the contralateral side was used. The samples were retrieved as reported previously [22,23,27]. In the distal radius, each sample was harvested at the distal metaphysis, perpendicular to the long axis of the shaft. The distal end of the section was located 2 mm proximal from the wrist joint cavity and the sample was obtained in the center of the section. In T10 and L2, each sample was harvested in the superior–inferior direction at 50% of the medio-lateral length of the vertebra and at the transition of the anterior third to the posterior two thirds of the anterior–posterior length. In the femoral neck, the orientation of the trabeculae was determined using an anterior–posterior contact radiograph. Then, a 14 mm plane parallel section was obtained using a high precision band saw (EXAKT Trennschleifsystem, Otto Herrmann, Norderstedt, Germany). The section was obtained in the middle of the femoral neck, perpendicular to the primary trabecular orientation. The cylindrical sample was retrieved from the main trabecular bundle within the section. In the greater trochanter, a 14 mm section was harvested in a direction perpendicular to the direction of a fall on the greater trochanter (10° adduction, 15° internal rotation). This section was radiographed and the cylindrical sample was harvested from the dense central region of the section, perpendicular to the slice and parallel with the impact direction during a fall on the side.

Due to previous fractures, bilateral total hip replacement, and damage during retrieval, bone samples could not be harvested from all skeletal sites in each donor. The final sample size consisted of 87 from the distal radius, 59 from T10, 69 from L2, 51 from the femoral neck, and 83 from the greater trochanter. The samples were stored in a solution of 5% buffered formalin until μCT imaging.

μCT imaging

Three-dimensional (3D) μCT images with an isotropic spatial resolution of 26 μm were obtained from the central 6 mm of each sample using a μCT 20 scanner (Scanco Medical, Bassersdorf, Switzerland). With "medium" scan mode and at an integration time of 100 ms, the scan time per sample amounted to 4.1 h.

μCT image analysis

The gray-value images were segmented using a low-pass filter by convolving the images with a Gaussian kernel with standard deviation of 0.8 and support of 1 to remove noise and a fixed global threshold equal to 22% of the maximal grey value to extract the mineralized bone phase.

Bone volume fraction and three additional morphometric parameters were calculated in analogy to standard histomorphometry using the mean intercept length method [28]: Bone volume divided by total volume (*BV* / *TV*, bone volume fraction), trabecular number (*TbN*), trabecular thickness (*TbTh*), and trabecular separation (*TbSp*).

Minkowski Functionals (MFs) are nonlinear topological parameters, which can be applied to multidimensional objects to characterize the composition of their components [29]. In 3D, the four MFs, namely, *Volume* (*V*), *Surface* (*S*), *Curvature* (*C*), and *Euler* characteristic (*E*), can be used to characterize one object. MFs have been used for the characterization of the trabecular bone architecture [30,31]. In the binarized μCT images, each voxel has six faces, eight vertices, and twelve edges. A bone voxel has an open face/vertex/edge when it is not shared by an adjoining bone voxel. The MFs can be calculated in the binarized μCT images by using the following equations:

$$V = n_b, \quad (1)$$

equivalent to *BV* / *TV*,

$$S = -6n_b + 2n_f, \quad (2)$$

$$C = \frac{1}{2}\left(3n_b - 2n_f + n_e\right), \quad (3)$$

$$E = -n_b + n_f - n_e + n_v, \quad (4)$$

with n_b: number of bone voxels, n_f: number of open faces, n_v: number of open vertices, and n_e: number of open edges.

The scaling index method (SIM) allows the estimation of the local scaling properties of an arbitrary-dimensional point distribution. The SIM has been used in previous studies for trabecular bone structure analysis [30–33]. The binarized μCT images can be interpreted as 3D