Journal of Biomechanics ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Letter to the Editor

Letter to the Editor referring to the article "Mineral heterogeneity affects predictions of intratrabecular stress and strain" published in Journal of Biomechanics (volume 44, Issue 3, Pages 402–407)

We read with interest the recent article "Mineral heterogeneity affects predictions of intratrabecular stress and strain" by Renders et al. (2011). Several recent studies have identified the importance of incorporating mineral heterogeneity in tissue level analysis and the paper by Renders et al. adds to this literature. The authors present a method for assigning an elastic modulus to each microFE voxel based on the attenuation value of the corresponding voxel from a microCT scan. The implementation of the method proposed by Renders et al. addresses a critical need many researchers face when modeling tissue level structures; that of how to appropriately assign material properties to microFE models. Such a method could help improve the understanding of the complex relationship between microstructure and material heterogeneity as related to the overall macroscopic level bone strength. Because of the important need and potential widespread adoption of the method proposed by Renders et al. for deriving elastic modulus from microCT scan data, we feel it is necessary to highlight several assumptions that are inherent with this method, which are not explicitly stated in the text. We hope to clearly identify and discuss these assumptions such that other researchers are aware of the potential limitations when implementing a similar procedure.

In the article by Renders et al., the authors relate the elastic modulus of bone tissue to the calcium content. The authors state

> "… the tissue stiffness (E_t^{He}; expressed in GPa) for each element was approximated from the DMB value of the corresponding microCT voxel according to $^{10}\log E_t = -8.58 + 4.05 \log[Ca]$ (Currey, 1999; Mulder et al., 2008), where $[Ca] = (0.4[HA])/2.0$ mg/g as approximately 40% of HA consists of calcium (Roschger et al., 1998) and the specific density of mandibular trabecular bone tissue is 2.0 g/cm^3 (Giesen et al., 2001)."

In the equation used to determine [Ca] (calcium content weight fraction), the authors use the value of the hydroxyapatite density, [HA], obtained from the microCT scan data for each voxel within a given specimen. The authors use a constant relationship of 40% to scale the microCT derived [HA] density to an equivalent calcium density. The equation also requires a value for the bone tissue density, which the authors give as a 2.0 g/cm^3, to convert the microCT derived equivalent calcium density into [Ca] (mgCa/g-dry defatted bone). In the study by Renders et al., the value of bone tissue density is considered a constant and was taken from the literature.

The implementation utilized by Renders et al. to convert the microCT derived HA density to calcium content weight fraction has several issues related to the following inherent assumptions:

(1) Bone tissue density of dry defatted marrow-free bone is constant and has a value of 2.0 g/cm^3.
(2) The values of [HA] density and bone tissue density used in the equation to determine the Ca content weight fraction are independent.

The validity of the first assumption is evaluated with a review of the literature values for bone tissue density, which have been shown to vary substantially. For example, Giesen et al. (2001) report a mean tissue density of 2.146 ± 0.054 g/cm^3 for human mandibular cancellous bone in the axial direction. The range based on two standard deviations on either side of the mean value is 2.038–2.254 g/cm^3. For human cancellous bone taken from the proximal tibia, Ding et al. (1997) report a mean tissue density of 2.20 g/cm^3, with a range based on two standard deviations of 2.06–2.34. Hernandez et al. (2001) calculated the tissue density of vertebral (cancellous) and femoral (cortical) bone samples using ash fraction and found results ranging from 1.634 to 2.26 g/cm^3. Finally, Day (2005) measured the bone tissue density of dried, defatted specimens from cadaveric proximal tibiae taken from 35 donors ranging in age from 38 to 85 years. The lowest mean tissue density value was 2.11; the highest mean tissue density was 2.33. This uncertainty in bone tissue density is magnified when using the equations from Currey and Renders et al. to calculate an elastic modulus, due to the markedly nonlinear relationship. In the example presented by Renders et al., a tissue density of 2.0 g/cm^3 yields an elastic modulus of 9.0 GPa for an [HA] value of 1130 mg/cm^3 (the [HA] value of 791 in Renders et al. is apparently a typographical error). Assuming a 15% greater tissue density (2.3 g/cm^3) yields an elastic modulus of 5.1 GPa. Assuming a 15% smaller tissue density (1.7 g/cm^3) yields an elastic modulus of 17.4 GPa. The value of the bone tissue density used in the equation to covert [HA] density to elastic modulus is obviously very important.

An additional point related to the first assumption is in regard to the appropriateness of using a dry defatted bone tissue density to calculate the calcium content weight fraction. We acknowledge that for a microCT scan voxel of a dry defatted bone specimen, the simple division of HA density (mgHA/cm^3) by the dry defatted bone tissue density (g/cm^3), and then multiplied by the constant 0.4 factor, will yield the appropriate measure of calcium content per grams of dry defatted bone (mgCa/g-dry defatted bone). However, if the specimens are not dry and defatted, additional calculations (not introduced by Renders et al.) must be performed. For example, if the specimen being analyzed is that of wet marrow free bone, the voxel of analyzed bone consists of ash, organics, and water (where previously the dry defatted bone only consisted of ash and organics). As such, the appropriate tissue

0021-9290/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.jbiomech.2011.03.041

density should be that calculated from a wet defatted bone to obtain the calcium content per wet defatted bone (mgCa/g-wet defatted bone). Using a constant weight fraction of 0.828 g-dry defatted bone per g-wet defatted bone (O'Flaherty 1991), an appropriate value for the equation relating Ca content weight fraction to elastic modulus can be attained.

The second assumption that [HA] (mineral density) and bone tissue density are independent is contradictory to what one would expect. The following derivation demonstrates that for a specimen of dry defatted bone, mineral density and tissue density are related through the ratio of ash to organic weight.

Using the following definitions: [HA] is the the mineral density of a bone voxel (hypothetically with no pores) from a microCT scan, rho_t the tissue density for the same voxel, and V the volume of the bone voxel.

From the previous definitions, the mineral and tissue weights for the volume V are

$[HA] \times V$=mineral weight of the bone voxel and

$rho\_t \times V$=tissue weight of the bone voxel.

Using the underlying constituents of dry defatted bone as being comprised of ash and organic components, the mineral and tissue weights can be redefined as

$[HA] \times V$=mineral weight=ash weight;

$rho\_t \times V$=tissue weight=ash+organic weight.

Ash weight appears as a contributor to both [HA] and rho_t. Therefore, only for the case where ash weight=0 (i.e. osteoid) does the original assumption that [HA] and rho_t are independent remains true. In all other situations, [HA] and rho_t will be related through the ratio of ash to organic weight.

In determining the elastic modulus using the approach given by Renders et al., the ratio of hydroxyapatite density to tissue density is critical. Unfortunately, the primary output from a microCT scan is a voxel-based gray scale or attenuation value that is typically converted to HA density, based on the use of a manufacturer-specific calibration curve. The bone tissue density, however, is not directly measured during a microCT scan.

The approach proposed by Render et al. addresses a critical need for voxel based material property assignment for heterogeneous models. However, the question remains of how appropriate is it to use a constant value of tissue density when the measured [HA] density on a voxel by voxel varies substantially. At this point in time it seems reasonable to conclude that if a constant value of tissue density is used for all voxels, regardless of the local [HA] density, then there is the potential for substantial error in the estimated value of the elastic modulus for some voxels.

Conflict of interest statement

We have no conflicts of interest to report.

References

Currey, J.D., 1999. What determines the bending strength of compact bone? The Journal of Experimental Biology 202, 2495–2503.

Day, J.S., 2005. Bone Quality: The Mechanical Effects of Microarchitecture and Matrix Properties. Ph.D. thesis. Department of Orthopaedics, Erasmus MC, Rotterdam, Netherlands.

Ding, M., Dalstra, M., Danielsen, C.C., Kabel, J., Hvid, I., Linde, F., 1997. Age variations in the properties of human tibial trabecular bone. The Journal of Bone and Joint Surgery, British 79, 995–1002.

Giesen, E.B., Ding, M., Dalstra, M., van Eijden, T.M., 2001. Mechanical properties of cancellous bone in the human mandibular condyle are anisotropic. Journal of Biomechanics 34, 799–803.

Hernandez, C.J., Beaupré, G.S., Keller, T.S., Carter, D.R., 2001. The influence of bone volume fraction and ash fraction on bone strength and modulus. Bone 29, 74–78.

Mulder, L., Koolstra, J.H., den Toonder, J.M.J., van Eijden, T.M.G.J., 2008. Relationship between tissue stiffness and degree of mineralization of developing trabecular bone. Journal of Biomedical Materials Research, Part A 84, 508–515.

O'Flaherty, E.J., 1991. Physiologically based models for bone-seeking elements. I. Rat skeletal and bone growth. Toxicology and Applied Pharmacology 111, 299–312.

Renders, G.A.P., Mulder, L., van Ruijven, L.J., Langenbach, G.E.J., van Eijden, T.M.G.J., 2011. Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics 44, 402–407.

Roschger, P., Fratzl, P., Eschberger, J., Klaushofer, K., 1998. Validation of quantitative backscattered electron imaging for the measurement of mineral density distribution in human bone biopsies. Bone 23, 319–326.

David W. Wagner*, Gary S. Beaupre
Bone & Joint Center of Excellence (153),
VA Palo Alto Health Care System, 3801 Miranda Avenue,
Palo Alto, CA 94304, USA
E-mail address: dwwagner@va51.stanford.edu (D.W. Wagner).

19 March 2011

* Corresponding author.

Journal of Biomechanics ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Letter to the Editor

Response to 'Letter to the Editor referring to the article "Mineral heterogeneity affects predictions of intratrabecular stress and strain" published in Journal of Biomechanics (volume 44, Issue 3, Pages 402–407)'

We thank Drs. David Wagner and Gary Beaupré for their interest in our work and their constructive comments. Generally, we agree completely with their analysis. We realize that, apart from regional variations in HA concentration, more factors determine the elastic modulus of a bone tissue. The presented study can be regarded as a qualitative analysis on how local differences in HA concentration translate into mechanical behavior of whole trabecular elements. To that end we only included local differences in HA concentration, and already these differences demonstrated to have a considerable influence on trabecular, and herewith global mechanical behavior of bone. Wagner and Beaupré emphasize two additional factors that should be taken into account when using microCT-based local HA concentrations to study its quantitative influence on trabecular bone mechanical behavior in finite element analyses. They rightly point out that these should have been discussed. Below are our two additional comments about these factors relating to the presented study.

The first deals with the fact that bone tissue density may differ from 2.0 g/cm^3 (this value suggests constancy while ranges between 1.6 and 2.3 g/cm^3 have been reported). However, the aim of our study was not to determine accurately the magnitudes of stress and strain, but to look at their changing patterns within the trabecular elements. We do not think that the assumption of a tissue density of 2.0 g/cm^3 has affected these qualitative results. Furthermore, our study concerns only trabecular bone of human mandibles. Giesen et al. (2001) found for this type of bone a standard deviation of 2.5% in tissue density. Therefore, contrary to the example in the letter, the effect on tissue stiffness is much less. However, it still causes an uncertainty in the tissue modulus, but only of ± 1 GPa (the range is from 8.21 to 9.94 GPa). From earlier studies we know that the variation in tissue modulus due to the differences in mineral density is approximately 5 times larger. That means that the error introduced by neglecting the variations in the density is an order of magnitude smaller than the variations in HA concentration, for which we are introducing a correction.

Regarding our second additional comment, we agree with the authors that for future quantitative analysis it is also important that the equations, which, in the presented study are used for wet specimens should actually only be applied for dry defatted specimens. And that for wet specimens the equations have to be adjusted for instance in the way suggested by Wagner and Beaupré.

To conclude, we also assume that the mineral density and tissue density are correlated. But since the tissue density varies little in condylar trabecular bone from the mandible we are of the opinion that ignoring this correlation had little effect on the intratrabecular stress and strain patterns. However, it is not difficult to find bone specimens with a much larger variation in mineral densities. For these specimens we also think that this correlation can no longer be neglected to answer certain research questions. The line of thoughts of Wagner and Beaupré as expressed in their letter is an excellent guide on the additional contributors to tissue modulus for finite element analyses.

Conflict of interest statement

We have no conflicts of interest to report.

References

Giesen, E.B., Ding, M., Dalstra, M., van Eijden, T.M., 2001. Mechanical properties of cancellous bone in the human mandibular condyle are anisotropic. Journal of Biomechanics 34, 799–803.

Greetje A.P. Renders*, Lars Mulder,
Leo J. van Ruijven, Geerling E.J. Langenbach
Academic Center for Dentistry Amsterdam, University of Amsterdam and VU University, Functional Anatomy, Gustav Mahlerlaan 3004, 1081 LA Amsterdam, The Netherlands
E-mail address: g.renders@acta.nl (G.A. Renders)

9 April 2011

* Corresponding author.

0021-9290/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2011.04.013

Journal of Biomechanics ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Mineral heterogeneity affects predictions of intratrabecular stress and strain

G.A.P. Renders [a,b,*], L. Mulder [c], L.J. van Ruijven [a,b], G.E.J. Langenbach [a,b], T.M.G.J. van Eijden [a,1]

[a] Department of Functional Anatomy, Academic Center for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Gustav Mahlerlaan 3004, Room 12N13, 1081 LA, Amsterdam, The Netherlands
[b] Research Institute MOVE, Amsterdam, The Netherlands
[c] Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

ARTICLE INFO

Article history:
Accepted 11 October 2010

Keywords:
Biomechanics
Degree of mineralization
Finite element model
Mechanical loading
MicroCT

ABSTRACT

Knowledge of the influence of mineral variations (i.e., mineral heterogeneity) on biomechanical bone behavior at the trabecular level is limited. The aim of this study is to investigate how this material property affects the intratrabecular distributions of stress and strain in human adult trabecular bone. Two different sets of finite element (FE) models of trabecular samples were constructed; tissue stiffness was either scaled to the local degree of mineralization of bone as measured with microCT (heterogeneous) or tissue stiffness was assumed to be homogeneous. The influence of intratrabecular mineral heterogeneity was analyzed by comparing both models. Interesting effects were seen regarding intratrabecular stress and strain distributions. In the homogeneous model, the highest stresses were found at the surface with a significant decrease towards the core. Higher superficial stresses could indicate a higher predicted fracture risk in the trabeculae. In the heterogeneous model this pattern was different. A significant increase in stress with increasing distance from the trabecular surface was found followed by a significant decrease towards the core. This suggests trabecular bending during a compression. In both models a decrease in strain values from surface to core was predicted, which is consistent with trabecular bending. When mineral heterogeneity was taken into account, the predicted intratrabecular patterns of stress and strain are more consistent with the expected biomechanical behavior as based on mineral variations in trabeculae. Our findings indicate that mineral heterogeneity should not be neglected when performing biomechanical studies on topics such as the (long-term or dose dependent) effects of antiresorptive treatments.

1. Introduction

The biomechanical behavior of bone depends not only on its microarchitecture but also on its mineral content (Currey, 1984; Hodgskinson et al., 1989; Zysset et al., 1999). In trabecular bone, e.g., microarchitecture is correlated to the apparent stiffness, i.e., Young's modulus of bone tissue (Liu et al., 2006). However, we recently have shown that variations in the degree of mineralization of bone (DMB) could explain up to 29% of the variance in the apparent stiffness, emphasizing the importance of this material property (Renders et al., 2008). Knowledge of the biomechanical influence of DMB at the trabecular level is still limited and needs to be expanded.

As a result of the continuous bone remodeling process, trabecular bone tissue is composed of so-called bone packets, each with its own mineral content corresponding to its tissue age. A highly specific distribution of the mineral content throughout trabecular bone has been found with Fourier transform infrared microspectroscopy (Paschalis et al., 1997) and back-scattered electron microscopy (Boyde et al., 1993; Ciarelli et al., 2003; Roschger et al., 2003; Fratzl et al., 2004). At the trabecular surface, the site where remodeling predominantly occurs, relatively younger tissue with a low DMB is found. Whereas, the relatively older and more mineralized tissue is found with increasing distance from the trabecular surfaces (Roschger et al., 2003). Although accurate, these methods are limited by their inability to measure the mineral distribution in three dimensions. This problem can be overcome with micro-computed tomography (microCT) that considers the three-dimensional structure and thus the true spatial distribution of DMB (Nuzzo et al., 2002; Mulder et al., 2004). Indeed, several microCT studies have confirmed that mineral heterogeneity exists at the trabecular tissue level in both developing bone (Mulder et al.,

* Corresponding author at: Department of Functional Anatomy, Academic Center for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Gustav Mahlerlaan 3004, Room 12N13, 1081 LA, Amsterdam, The Netherlands. Tel.: +31 20 59 80 887.
E-mail addresses: g.renders@acta.nl (G.A.P. Renders), L.Mulder@tue.nl (L. Mulder), l.v.ruijven@acta.nl (L.J. van Ruijven), g.langenbach@acta.nl (G.E.J. Langenbach).
[1] Passed away.

0021-9290/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2010.10.004

2006) and adult bone (Van der Linden et al., 2001; Jaasma et al., 2002; Bourne and Van der Meulen, 2004; Renders et al., 2006; Van Ruijven et al., 2007).

The intratrabecular mineral distribution in bone tissue is nearly constant in healthy adult individuals and is independent of age, gender, ethnic origin, and skeletal site (Roschger et al., 2001; Ruffoni et al., 2007). Roschger et al. stated that the remarkable small biological variance in mineral distribution could suggest the existence of an evolutionary optimum with respect to the biomechanical performance of bone trabeculae. Any deviations from a normal mineral distribution due to either disease and/or treatment might therefore be of significant biological and clinical relevance (Roschger et al., 2006). Nano-indentation studies have determined a close relationship between variations in tissue stiffness and the corresponding local mineralization (Rho et al., 1997; Zysset et al., 1999; Roy et al., 1999; Hoffler et al., 2000; Mulder et al., 2008). Consequently, a change in mineral heterogeneity caused by unusually high or low levels of bone remodeling may be expected to have considerable effects on trabecular bone biomechanics due to this close relationship (Currey, 1984; Roschger et al., 1998; Fratzl et al., 2004; Easley et al., 2010).

MicroCT-based finite element (micro-FE) analysis enables predictions and visualization of the biomechanical consequences of variations in bone structure and mineralization (Van Rietbergen et al., 1995; Van Ruijven et al., 2007; Mulder et al., 2008; Renders et al., 2008). Nowadays, micro-FE analysis has become a standard tool to examine trabecular bone's mechanical properties (Verhulp et al., 2008). It allows for simulations of a variety of load cases and can predict local stress and strain patterns at the trabecular level. To neglect a material property, such as mineral heterogeneity, in biomechanical simulations could have a significant influence on the predicted outcome. For example, in newborn porcine trabecular bone the incorporation of mineral heterogeneity in FE analysis tends to decrease the predicted average von Mises equivalent stress and to raise the average equivalent strain (Mulder et al., 2007). Moreover, predicted intratrabecular stress and strain patterns were affected. Whether such an influence is similar in adult human bone is yet to be determined. Compared to newborn bone, adult bone has had time to develop into a more defined structure due to an overall loading pattern, which results in differences in structure (e.g., trabecular thickness: Mulder et al., 2005, 2006; Renders et al., 2008). Such structural differences might influence the outcome of our FE study compared to the previous work.

The aim of our study was to investigate how specimen-specific mineral distributions can affect predicted intratrabecular distributions of stress and strain in human adult trabecular bone in FE models. This was achieved by comparing two sets of FE simulations in which mineral heterogeneity was either included or neglected. In the latter, a homogeneous tissue stiffness was used assuming a homogeneous mineral distribution. This means that the mineral content near the trabecular core will be relatively underestimated, whereas in the most superficial region it will be overestimated. According to Currey (1984), a relatively higher mineral content leads to higher stress and lower strain values. Hence, we expected that in human trabecular bone both the predicted outcome of average stress and strain values and their intratrabecular patterns will be affected when the mineral heterogeneity in FE analysis is neglected.

2. Materials and methods

2.1. Bone samples

This study was performed using nine right condyles of dentate mandibles obtained from embalmed human cadavers (mean age: 68 ± 14 yr). All cadavers were stored in a mixture of glycerol, alcohol, and phenol. Only male specimens were used to rule out any influence of postmenopausal osteoporosis. There were no macroscopic signs of temporomandibular disorders. The use of these human specimens conforms to a written protocol that was reviewed and approved by the Department of Anatomy and Embryology of the Academic Medical Center of the University of Amsterdam.

2.2. MicroCT

A μCT 40 system (Scanco Medical AG, Brüttisellen, Switzerland) was used to obtain 3D reconstructions from the trabecular bone of the condylar specimens. The microCT was equipped with an aluminum filter and a correction algorithm that compensates for the effects of beam hardening, thereby reducing the estimated error of the measured linear attenuation to 10% or less (Mulder et al., 2004). Within all nine condyles, four cubic volumes of interest ($3 \times 3 \times 3$ mm^3) were selected giving a total of 36 trabecular samples (Renders et al., 2008). Information on the scan and reconstruction procedures is specified in previous papers (Renders et al., 2006, 2008).

2.3. Finite element analysis

All 36 trabecular samples (nine condyles; four regions per condyle) were transformed into FE models conforming to our previous FE study (Renders et al., 2008), using the 3D microCT reconstructions in which the voxels were directly converted into 8-node brick elements. Two sets of FE models of each trabecular sample were created by including or neglecting the measured mineral heterogeneity (i.e., DMB; Fig. 1A and B). Two sets were defined as heterogeneous and homogeneous. In the heterogeneous FE model the tissue stiffness (E_t^{He}; expressed in GPa) for each element was approximated from the DMB value of the corresponding microCT voxel according to $^{10}\log E_t = -8.58 + 4.05 \log[Ca]$ (Currey, 1999; Mulder et al., 2008), where $[Ca] = (0.4[HA])/2.0$ mg/g as approximately 40% of HA consists of calcium (Roschger et al., 1998) and the specific density of mandibular trabecular bone tissue is 2.0 g/cm^3 (Giesen et al., 2001). This resulted in an average of $E_t^{He} = 11.4 \pm 2.6$ GPa and a range of 4.7–16.7 GPa. In the homogeneous FE model an artificially standardized E_t^{Ho} (9.0 GPa) was empirically chosen (irrespective of the corresponding microCT attenuation), so that the average E_t of both models approximately coincided (Renders et al., 2008). By back-calculation of Currey's (1999) equation, E_t^{Ho} corresponded to an artificial DMB value of 791 mg HA/cm^3.

The models were solved using a linear elastic material model and the elements were considered isotropic with Poisson's ratio of 0.3 (Kabel et al., 1999; Van Ruijven et al., 2003). Uniaxial compression in the supero-inferior direction was simulated by applying a uniform strain (1%) on the superior surface of the specimen cubes. Displacement of nodes at the inferior surface was suppressed (Fig. 1C). The supero-inferior compressions were assumed to correspond most closely to the average joint loading direction (Koolstra, 2002). From the stress and strain tensors, the average von Mises equivalent stress and equivalent strain were calculated. 3D distributions of the von Mises equivalent stress and equivalent strain in the trabeculae were studied by applying a peeling algorithm written in Matlab 7.0.4 (Mulder et al., 2005; Renders et al., 2006: Fig. 1D).

2.4. Statistics

Since no significant differences were found between the four trabecular subregions, all parameters were averaged per condyle for further analysis ($N=9$). A paired-samples t-test was applied for analysis of average stress and strain values *between* both models. Furthermore, a general linear model for repeated measures was used to analyze the peeling algorithm data: per layer *between* both models and the intratrabecular differences *within* both models (including all 36 trabecular specimens). In addition, the Bonferroni correction was applied to adjust for multiple comparisons. From frequency distributions the average von Mises equivalent stress, average equivalent strain and their standard deviation were determined. SPSS 16.0.2 software (SPSS Inc.) was used to perform the statistical analyses. A p-value < 0.05 was considered statistically significant. To avoid the influence of partial volume effects and resolving power, we did not include the two most superficial bone layers in the calculation of average values and in any statistical analysis.

3. Results

When mineral heterogeneity was disregarded, the average von Mises equivalent stress was approximately 3.2 times higher ($stress^{Ho}$: 3.80 ± 0.71 MPa; $stress^{He}$: 1.20 ± 0.37 MPa; $p < 0.001$). Thus, in the homogeneous model a higher stress was needed to obtain a similar strain. In both models, an interesting effect was seen regarding the intratrabecular stress distribution. In the homogeneous model, the highest stresses were found at the surface with a significant decrease towards the core ($p < 0.001$). In the heterogeneous model this pattern was different. Between 0.008 and 0.032 mm from the trabecular surface the predicted stress

Please cite this article as: Renders, G.A.P., et al., Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics (2010), doi:10.1016/j.jbiomech.2010.10.004

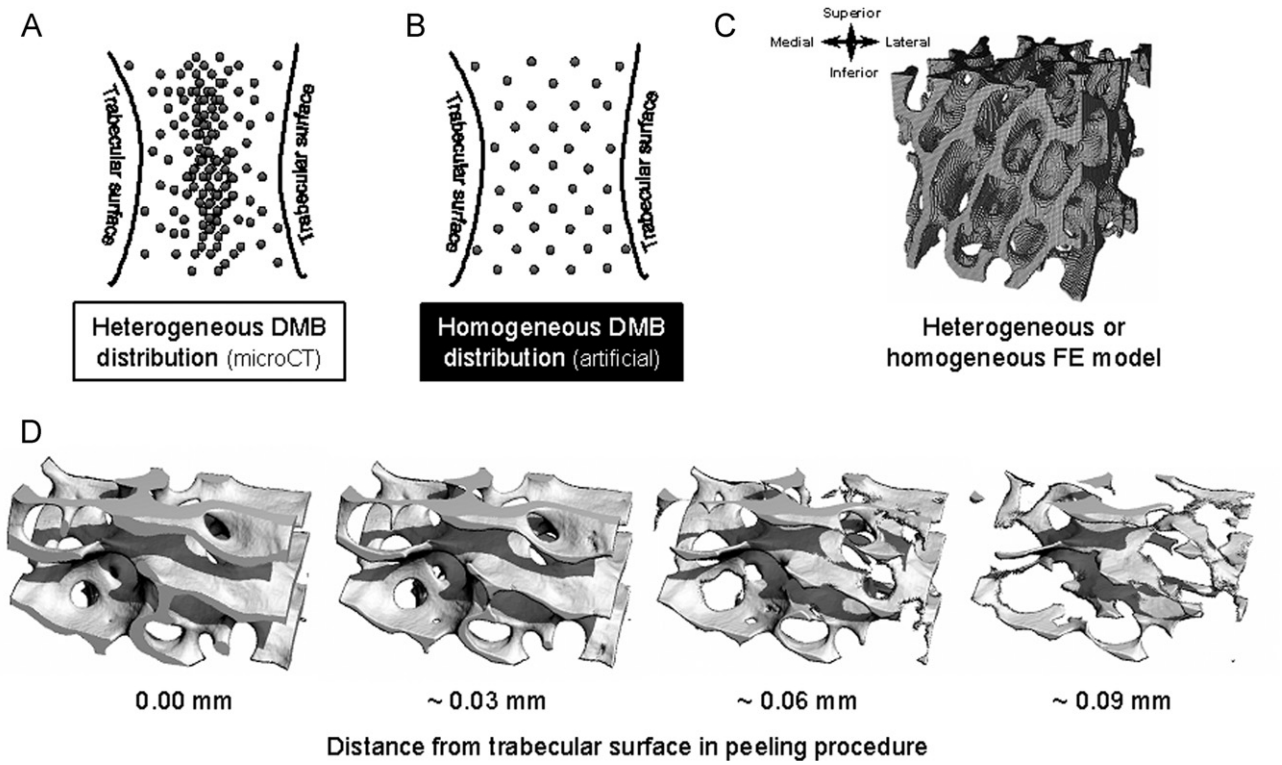

Fig. 1. Graphic representation of material property implementation, FE model boundary conditions, and peeling procedure. A microCT-based heterogeneous mineral distribution (A) or an artificially homogeneous mineral distribution (B) was used to implement tissue stiffness in two different sets of FE models. (C) FE models were based on the 3D microCT reconstructions, ensuring the correct incorporation of the microarchitecture. The boundary conditions for all FE models ($3 \times 3 \times 3$ mm^3, $n=36$) were according to our previous study (Renders et al., 2008). The direction of loading was supero-inferior. (D) Peeling procedure was used to determine intratrabecular stress and strain distributions (Mulder et al., 2005; Renders et al., 2006): Layers of bone-containing voxels were consecutively peeled off from trabecular surface to core (average layer thickness: 7–8 μm). In a small section of trabecular bone several steps of the peeling procedure are depicted with 0–0.09 mm distance from the trabecular surface.

values increased significantly ($p < 0.001$). A maximum was reached at 0.040 mm from the surface. Deeper towards the trabecular core the predicted stress significantly decreased ($p < 0.005$; Fig. 2A). When both FE models were compared, the overall predicted intratrabecular stresses were higher when the mineral distribution was neglected (Fig. 3: upper plot, solid line; $p < 0.001$). However, when a scaling procedure was applied to the homogeneous model (that resulted in the same apparent stiffness as that of the matching heterogeneous model; Renders et al., 2008) it resulted in an overall decrease in intratrabecular stress values. Nevertheless, the pattern remained the same with the highest stress at the trabecular core and a significant decrease towards the core (Fig. 3: upper plot, dotted line). Compared to the heterogeneous model, significant higher stress values were found at the surface ($p < 0.005$) and significant lower stress towards the core ($p < 0.001$).

In retrospect, the validity of our choice for E_t^{Ho} expressed itself in the fact that the average equivalent strain in both models was not significantly different (microstrain^{Ho}: 768 ± 113; microstrain^{He}: 749 ± 142). Logically this should be the case because a set strain (1%) in both models was applied. At the trabecular level, in both models a significant decrease in strain values with distance from the trabecular surface was observed (Fig. 2B: $p < 0.001$). However, statistical analysis (general linear model for repeated measures) of the difference in predicted strain per layer *between* the models showed a significant difference between both patterns, except between 0.024 and 0.040 mm (Figs. 2B and 3: lower plot, $p < 0.001$). The effect of incorporating mineral heterogeneity as a material property in FE analysis was also visualized (Fig. 3: middle and right panel). For instance, regions of lower stress predictions were found in the heterogeneous model compared to the corresponding regions in the homogeneous model (Fig. 3: white bordered selections).

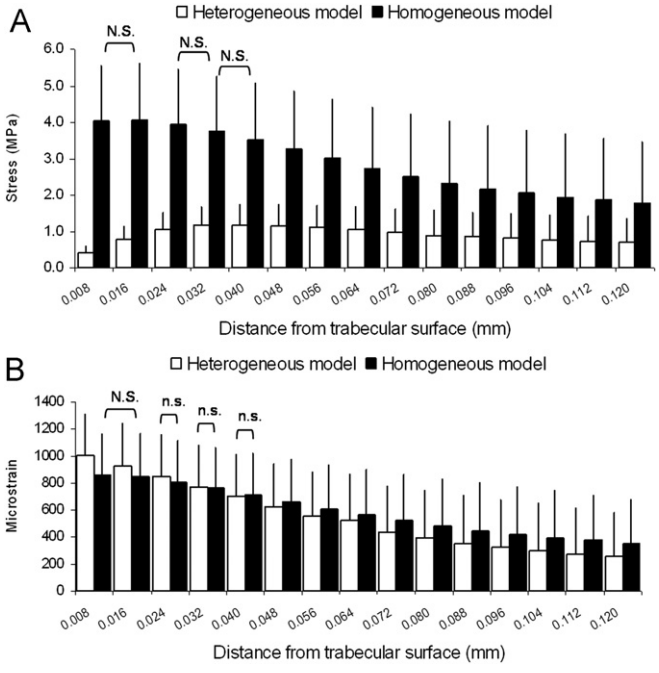

Fig. 2. Intratrabecular distributions of average stress and strain estimations. These distributions were determined by the stepwise peeling procedure. The *x*-axis represents the approximate distance from the trabecular surface. (A) Intratrabecular distribution of the von Mises equivalent stress in both FE models. Note the difference between the patterns. (B) Intratrabecular distribution of the equivalent microstrain in both FE models. Standard deviations are a measure for inter-individual variation ($N=9$). N.S.: No significant difference *within* FE model. n.s.: No significant difference *between* FE models.

Please cite this article as: Renders, G.A.P., et al., Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics (2010), doi:10.1016/j.jbiomech.2010.10.004

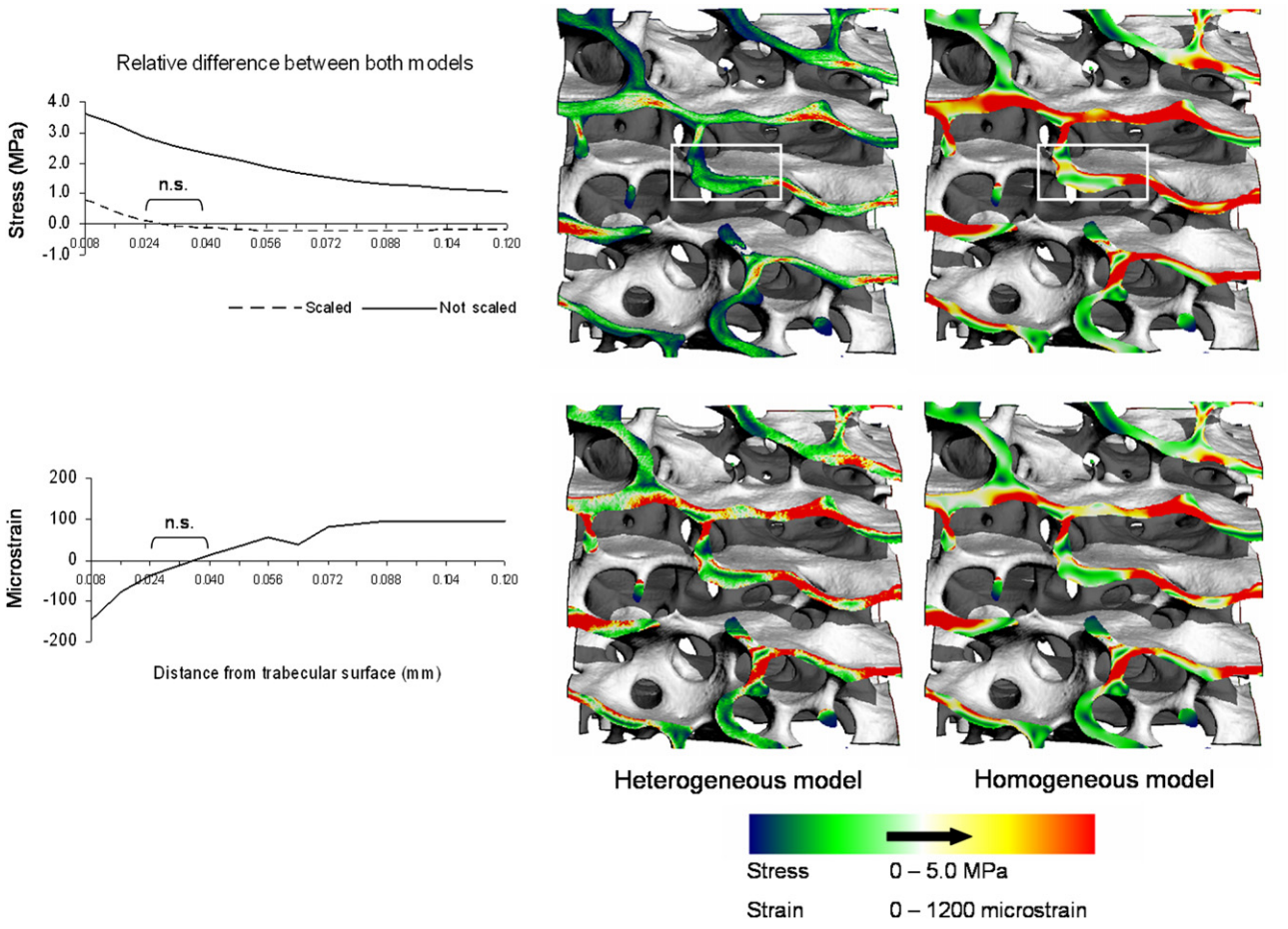

Fig. 3. Left panel: plots of the relative difference of intratrabecular stress/strain patterns between both models (i.e., homogeneous minus heterogeneous). Dotted line in the upper plot is the stress pattern after a scaling procedure was applied (scaling factor $=E_t^{He}/E_t^{Ho}$). n.s.: No significant difference *between* FE models. Middle and right panel: visualization of intratrabecular stress and strain distributions estimated with FE analysis (color scale increasing stress/strain from blue to red, and only visible at the cut plane). Predictions of stress and strain with the heterogeneous FE model in a trabecular cube can be seen in the middle panel. The right panel represents predictions of stress and strain patterns in the same trabecular cube but in the homogeneous FE model (i.e., mineral heterogeneity neglected).

4. Discussion

The aim of this study was to investigate the effect of bone's mineral heterogeneity on predicted intratrabecular stress and strain distributions. This was determined by microCT-based finite element (FE) analysis. We expected that disregarding mineral heterogeneity in FE analysis would affect the predicted average values and patterns of intratrabecular stress and strain. This is likely due to the relative overestimation of the degree of mineralization (DMB) at the trabecular surface compared to the relative underestimation of this parameter at the core in a homogeneous FE model.

When mineral heterogeneity was neglected, an average stress value 3.2 times higher was found in trabecular bone under uniaxial compression. In addition, significantly higher intratrabecular stress values were predicted. These results, however, crucially depend on the choice of E_t^{Ho} in our study setup. When the predicted stresses calculated with the homogeneous model were scaled, so E_t^{Ho} matched the averages measured in the heterogeneous model, no significant difference was found. Nevertheless, the explicit difference in intratrabecular stress patterns between both models remained unchanged. The trabecular stress pattern in the heterogeneous model, with the lowest stress at the trabecular surface, corresponds to the profile described by Mulder et al. (2007). The highest stress values were found at a small distance from the trabecular surface, suggesting that the trabecular elements are bent during compression. Without scaling, an overestimation of the overall intratrabecular stress was predicted using the homogeneous model, whereas Mulder et al. noted an underestimation. Concerning the predicted average strain, the trabecular elements showed the same tendency as the intratrabecular profile in newborn porcine trabecular bone (Mulder et al., 2007). The larger strains found at the trabecular surface indicate that numerous trabeculae undergo bending deformation. Thus, only a small discrepancy in predicted stress between both studies exists. This indicates that the influence of mineral heterogeneity in newborn porcine trabecular bone is approximately similar to that in adult human trabecular bone.

Before elaborating more on the results of our study, a remark has to be made with respect to our method. Despite the excellent reputation of morphological analysis by microCT, a question of its ability to accurately measure mineral distributions remains. One of the causal factors for this discussion is the beam hardening effect, which is the process of increasing the average energy level of an X-ray beam by filtering out low-energy photons. To overcome this problem a beam hardening correction algorithm is used leaving only some artefacts in the trabeculae on the outside of a sample (Mulder et al., 2004). In theory the degree of mineralization on the outside of the sample is somewhat higher than the actual mineral values. As a consequence, the

Please cite this article as: Renders, G.A.P., et al., Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics (2010), doi:10.1016/j.jbiomech.2010.10.004

intratrabecular mineral distribution we found would be more distinct. Additionally, stress and strain patterns should also be clearer and more significant.

Mineral heterogeneity plays an important role in biological bone behavior (Currey, 1984). The presence of minerals (i.e., hydroxyapatite) gives bone tissue the ability to produce considerable resistance to deformation under loading (Martin, 2007). Thus, the relatively high amount of minerals found in the trabecular core is important when the trabeculae are loaded in compression or subjected to bending. Therefore it contributes to the rigidity of the bone (i.e., low strains, high stress; Currey, 1984). On the other hand, relatively less mineralized bone tissue – as observed at the trabecular surface – is more compliant (low stress) and demonstrates a greater ability to undergo larger strains (Rubin and Lanyon, 1985; Turner and Pavalko, 1998; Ciarelli et al., 2003). This bone behavior is consistent with the predicted behavior of the trabeculae in our heterogeneous FE model and thus confirms our findings, although we are obliged to mention that there is a lack of experimental validation in this study. Care should be taken when interpreting the results presented and therefore, only suggestions of possible biomechanical consequences and significance are made.

Of interest is how this work might be applied more broadly and, particularly, how these results can be applied to, for example, bisphosphonate-treated bone. FE analysis can provide the assessment of biomechanical effects of antiresorptive treatments when mineral heterogeneity is included. Bisphosphonate treatment down-regulates bone turnover, which increases the homogeneity of bone mineral distribution and also the average mineralization (Fratzl et al., 2007). This increase has been suggested as an important contributor to increased brittleness of bone tissue (Mashiba et al., 2001; Bourrin et al., 2002; Day et al., 2004; Misof et al., 2005; Gourion-Arsiquaud et al., 2010). For example, due to the increasing mineral homogeneity during treatment, trabecular stress patterns might shift from those found in our heterogeneous model to those predicted with the homogeneous model. The increased stress values at the trabecular surface could then indicate a significantly higher fracture risk. Therefore, in the future, it is important to consider the bone mineral homogeneity caused by bisphosphonate treatment more carefully (Roschger et al., 1997; Pérez-López, 2004), especially, as a new relevant bone quality factor when analyzing and predicting the effects of therapy duration or dose on bone properties in relation to changes in mineral heterogeneity (Roschger et al., 2003).

To summarize, in adult human trabecular bone mineral heterogeneity affects the predicted outcome of intratrabecular stress and strain patterns as determined with FE analysis. The stress and strain values predicted with the FE analyses in this study may not reflect the actual measured mechanical properties. Still, our findings do indicate that mineral heterogeneity should not be neglected in biomechanical studies on interesting topics such as the (long-term or dose dependent) effects of antiresorptive treatments. Disregarding this parameter could have a major impact on the outcome of future fracture-risk FE studies.

Conflict of interest statement

None declared.

Acknowledgements

Appreciation goes to Peter Brugman for technical assistance and Irene Aartman for statistical advice. We thank Jan Harm Koolstra, Vincent Everts, Nina Scheres, and Wilco de Jong for their comments on the manuscript. This work was funded by the Netherlands Organization for Scientific Research (NWO, Grant number 021.001.050) and sponsored by the National Computing Facilities Foundation (NCF).

References

Bourne, B.C., Van der Meulen, M.C.H., 2004. Finite element models predict cancellous apparent modulus when tissue modulus is scaled from specimen CT-attenuation. Journal of Biomechanics 37, 613–621.

Bourrin, S., Ammann, P., Bonjour, J.P., Rizzoli, R., 2002. Recovery of proximal tibia bone mineral density and strength, but not cancellous bone architecture, after long-term bisphosphonate or selective estrogen receptor modulator therapy in aged rats. Bone 30, 195–200.

Boyde, A., Elliot, J.C., Jones, S.J., 1993. Stereology and histogram analysis of backscattered electron images: age changes in bone. Bone 14, 205–210.

Ciarelli, T.E., Fyhrie, D.P., Parfitt, A.M., 2003. Effects of vertebral bone fragility and bone formation rate on the mineralization levels of cancellous bone from white females. Bone 32, 311–315.

Currey, J.D., 1984. Effects of differences in mineralization on the mechanical properties of bone. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 304, 509–518.

Currey, J.D., 1999. What determines the bending strength of compact bone. The Journal of Experimental Biology 202, 2495–2503.

Day, J.S., Ding, M., Bednarz, P., Van der Linden, J.C., Mashiba, T., Hirano, T., Johnston, C.C., Burr, D.B., Hvid, I., Sumner, D.R., Weinans, H., 2004. Bisphosphonate treatment affects trabecular bone apparent modulus through micro-architecture rather than matrix properties. Journal of Orthopaedic Research 22, 465–471.

Easley, S.K., Jekir, M.G., Burghardt, A., Li, M., Keaveny, T.M., 2010. Contribution of the intra-specimen variations in tissue mineralization to PTH- and raloxifene-induced changes in stiffness of rat vertebrae. Bone 46, 1162–1169.

Fratzl, P., Gupta, H.S., Paschalis, E.P., Roschger, P., 2004. Structure and mechanical quality of the collagen-mineral nano-composite in bone. Journal of Materials Chemistry 14, 2115–2123.

Fratzl, P., Roschger, P., Fratzl-Zelman, N., Paschalis, E.P., Phipps, R., Klaushofer, K., 2007. Evidence that treatment with risedronate in women with postmenopausal osteoporosis affects bone mineralization and bone volume. Calcified Tissue International 81, 73–80.

Giesen, E.B.W., Ding, M., Dalstra, M., Van Eijden, T.M.G.J., 2001. Mechanical properties of cancellous bone in the human mandibular condyle are anisotropic. Journal of Biomechanics 34, 799–803.

Gourion-Arsiquaud, S., Allen, M.R., Burr, D.B., Vashishth, D., Tang, S.Y., Boskey, A.L., 2010. Bisphosphonate treatment modifies canine bone mineral and matrix properties and their heterogeneity. Bone 46, 666–672.

Hodgskinson, R., Currey, J.D., Evans, G.P., 1989. Hardness, an indicator of the mechanical competence of cancellous bone. Journal of Orthopaedic Research 7, 754–758.

Hoffler, C.E., Moore, K.E., Kozloff, K., Zysset, P.K., Brown, M.B., Goldstein, S.A., 2000. Heterogeneity of bone lamellar-level elastic moduli. Bone 26, 603–609.

Jaasma, M.J., Bayraktar, H.H., Niebur, G.L., Keaveny, T.M., 2002. Biomechanical effects of intraspecimen variations in tissue modulus for trabecular bone. Journal of Biomechanics 35, 237–246.

Kabel, J., Van Rietbergen, B., Dalstra, M., Odgaard, A., Huiskes, R., 1999. The role of an effective isotropic tissue modulus in the elastic properties of cancellous bone. Journal of Biomechanics 32, 673–680.

Koolstra, J.H., 2002. Dynamics of the human masticatory system. Critical Reviews in Oral Biology and Medicine 13, 366–376.

Liu, X.S., Sajda, P., Saha, P.K., Wehrli, F.W., Guo, X.E., 2006. Quantification of the roles of trabecular microarchitecture and trabecular type in determining the elastic modulus of human trabecular bone. Journal of Bone and Mineral Research 21, 1608–1617.

Martin, R.B., 2007. The importance of mechanical loading in bone biology and medicine. Journal of Musculoskeletal and Neuronal Interaction 7, 48–53.

Mashiba, T., Turner, C.H., Hirano, T., Forwood, M.R., Jacob, D.S., Johnston, C.C., Burr, D.B., 2001. Effects of high-dose etidronate treatment on microdamage accumulation and biomechanical properties in beagle bone before occurrence of spontaneous fractures. Bone 29, 271–278.

Misof, B.M., Roschger, P., Baldini, T., Raggio, C.L., Zraick, V., Root, L., Boskey, A.L., Klaushofer, K., Fratzl, P., Camacho, N.P., 2005. Differential effects of alendronate treatment on bone from growing osteogenesis imperfecta and wild-type mouse. Bone 36, 150–158.

Mulder, L., Koolstra, J.H., Van Eijden, T.M.G.J., 2004. Accuracy of microCT in the quantitative determination of the degree of distribution of mineralization in developing bone. Acta Radiologica 45, 769–777.

Mulder, L., Koolstra, J.H., Weijs, W.A., Van Eijden, T.M.G.J., 2005. Architecture and mineralization of developing trabecular bone in the pig mandibular condyle. The Anatomical Record 285A, 659–667.

Mulder, L., Koolstra, J.H., De Jonge, H.W., Van Eijden, T.M.G.J., 2006. Architecture and mineralization of developing cortical and trabecular bone in the mandible. Anatomy and Embryology 211, 71–78.

Please cite this article as: Renders, G.A.P., et al., Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics (2010), doi:10.1016/j.jbiomech.2010.10.004

Mulder, L., Van Ruijven, L.J., Koolstra, J.H., Van Eijden, T.M.G.J., 2007. The influence of mineralization on intratrabecular stress and strain distribution in developing trabecular bone. Annals of Biomedical Engineering 35, 1668–1677.

Mulder, L., Koolstra, J.H., Den Toonder, J.M., Van Eijden, T.M.G.J., 2008. Relationship between tissue stiffness and degree of mineralization of developing trabecular bone. Journal of Biomedical Materials Research: Part A 84, 508–515.

Nuzzo, S., Lafage-Proust, M.H., Martin-Badosa, E., Boivin, G., Thomas, T., Alexandre, C., Peyrin, F., 2002. Synchrontron radiation microtomography allows the analysis of three-dimensional microarchitecture and degree of mineralization of human iliac crest biopsy specimens: effects of etidronate treatment. Journal of Bone and Mineral Research 17, 1372–1382.

Paschalis, E.P., Betts, F., DiCarlo, E., Mendelsohn, R., Boskey, A.L., 1997. FTIR microspectroscopic analysis of normal human cortical and trabecular bone. Calcified Tissue International 61, 480–486.

Pérez-López, F.R., 2004. Postmenopausal osteoporosis and alendronate. Maturitas 48, 179–192.

Renders, G.A.P., Mulder, L., Van Ruijven, L.J., Van Eijden, T.M.G.J., 2006. Degree and distribution of mineralization in the human mandibular condyle. Calcified Tissue International 79, 190–196.

Renders, G.A.P., Mulder, L., Langenbach, G.E.J., Van Ruijven, L.J., Van Eijden, T.M.G.J., 2008. Biomechanical effect of mineral heterogeneity in trabecular bone. Journal of Biomechanics 41, 2793–2798.

Rho, J.Y., Tsui, T.Y., Pharr, G.M., 1997. Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation. Biomaterials 18, 1325–1330.

Roschger, P., Fratzl, P., Klaushofer, K., Rodan, G., 1997. Mineralization of cancellous bone after alendronate and sodium fluoride treatment: a quantitative back-scattered electron imaging study on minipig ribs. Bone 20, 393–397.

Roschger, P., Fratzl, P., Eschberger, J., Klaushofer, K., 1998. Validation of quantitative backscattered electron imaging for the measurement of mineral density distribution in human bone biopsies. Bone 23, 319–326.

Roschger, P., Rinnerthaler, S., Yates, J., Rodan, G.A., Fratzl, P., Klaushofer, K., 2001. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. Bone 29, 185–191.

Roschger, P., Gupta, H.S., Berzlanovich, A., Ittner, G., Dempster, D.W., Fratzl, P., Cosman, F., Parisien, M., Lindsay, R., Nieves, J.W., Klaushofer, K., 2003. Constant mineralization density distribution in cancellous human bone. Bone 32, 316–323.

Roschger, P., Paschalis, E.P., Fratzl, P., Klaushofer, K., 2006. Bone mineralization density distribution in health and disease. Bone 42, 456–466.

Roy, M.E., Rho, J., Tsui, T.Y., Evans, N.D., Pharr, G.M., 1999. Mechanical and morphological variation of the human lumbar vertebral cortical and trabecular bone. Journal of Biomedical Materials Research 44, 191–197.

Rubin, C.T., Lanyon, L.E., 1985. Regulation of bone mass by mechanical strain magnitude. Calcified Tissue International 37, 411–417.

Ruffoni, D., Fratzl, P., Roschger, P., Klaushofer, K., Weinkamer, R., 2007. The bone mineralization density distribution as a fingerprint of the mineralization process. Bone 40, 1308–1319.

Turner, C.H., Pavalko, F.M., 1998. Mechanotransduction and functional response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation. Journal of Orthopaedic Science 3, 346–355.

Van der Linden, J.C., Birkenhäger-Frenkel, D.H., Verhaar, J.A.N., Weinans, H., 2001. Trabecular bone's mechanical properties are affected by its non-uniform mineral distribution. Journal of Biomechanics 34, 1573–1580.

Van Rietbergen, B., Weinans, H., Huiskes, R., Odgaard, A., 1995. A new method to determine trabecular bone elastic properties and loading using micromechanical finite-element models. Journal of Biomechanics 28, 69–81.

Van Ruijven, L.J., Giesen, E.B.W., Farella, M., Van Eijden, T.M.G.J., 2003. Prediction of mechanical properties of the cancellous bone of the mandibular condyle. Journal of Dental Research 82, 819–823.

Van Ruijven, L.J., Mulder, L., Van Eijden, T.M.G.J., 2007. Variations in mineralization affect the stress and strain distributions in cortical and trabecular bone. Journal of Biomechanics 40, 1211–1218.

Verhulp, E., Van Rietbergen, B., Muller, R., Huiskes, R., 2008. Micro-finite element simulation of trabecular-bone post-yield behaviour—effects of material model, element size and type. Computer Methods in Biomechanics and Biomedical Engineering 11, 389–395.

Zysset, P.K., Guo, X.E., Hoffler, C.E., Moore, K.E., Goldstein, S.A., 1999. Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur. Journal of Biomechanics 32, 1005–1012.

Please cite this article as: Renders, G.A.P., et al., Mineral heterogeneity affects predictions of intratrabecular stress and strain. Journal of Biomechanics (2010), doi:10.1016/j.jbiomech.2010.10.004