Determination of trabecular bone tissue elastic properties by comparison of experimental and finite element results

DETERMINATION OF TRABECULAR BONE TISSUE ELASTIC PROPERTIES BY COMPARISON OF EXPERIMENTAL AND FINITE ELEMENT RESULTS

B. VAN RIETBERGEN, J. KABEL*, A. ODGAARD* AND R. HUISKES
Biomechanics Section, Institute of Orthopaedics, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
**Orthopaedic Research Laboratory, University Hospital Aarhus Building 1A 1st floor, Nørrebrogade 44, 8000 Aarhus C, Denmark*

1. Introduction

Trabecular bone, found near the ends of long bones and inside vertebral bodies, is a spongy type of bone with a very complex internal architecture build of rods and plates. Its main function is to distribute compressive loads in the skeleton to carticular surfaces. Due to this load carrying function, its mechanical properties are of great importance for the integrity of bones. In particular in the elderly, the integrity of trabecular bone can be jeopardized due to loss of bone mass or deterioration of its internal architecture as a result of bone diseases (osteoporosis) or adverse bone remodeling effects (for example: after long bedrest). In particular in those situations, an accurate assessment of mechanical properties of trabecular bone is of great importance to diagnose the bone fracture risk, such that preventive measures can be taken in time when needed.

The mechanical properties of trabecular bone are dependent on two factors. First the mechanical properties of the bone tissue itself, which are correlated with the degree of mineralization. The second is morphology which represents the internal arrangement of the trabecular architecture. Trabecular architecture can be represented by two factors. First the volume fraction, which determines the volume of bone tissue per unit of volume. This quality may also be expressed in the total mass per unit of bone volume, which is called the apparent density, and comprises the mineralization as well. Second the directionality of the trabecular anisotropy. Strong dependencies of bone mechanical properties (stiffness and strength) on its apparent density have been demonstrated in a large number of studies (Carter and Hayes, 1977; Rice *et al.*, 1988; Hodgskinson and Currey, 1992). More recent studies have also included a measure of the directionality of the architecture to further improve the predictive value of these relationships (Turner *et al.*, 1990; Goldstein *et al.*, 1993; Goulet *et al.*, 1994). Much uncertainty exist, however, with respect to the mechanical properties of the bone tissue material.

In earlier studies the tissue elastic properties and strength have been measured using standard engineering test methods such as tensile tests, three- or four-point bending tests and buckling tests. Values found for the tissue modulus range from 0.76 to 10 GPa when using tensile tests, from 3.2 to 5.4 GPa for three- or four-point bending tests and

H. Sol and C.W.J. Oomens (eds.), Material Identification Using Mixed Numerical Experimental Methods, 183-192.

from 8.7 to 14 GPa for buckling tests (for an overview see Rho *et al.*,1992). A major problems when using standard engineering tests methods for the determination of bone tissue properties is the small size of trabeculae (thickness: 100-200 micron, length 1-2 mm), resulting in inaccuracies in the displacements measurements and thus in the calculation of moduli. Another problem is the irregular shape of trabeculae, where standard engineering tests require a standardized specimen geometry. To overcome the latter problem, some studies have used machined specimens (Choi *et al.*, 1990; Choi and Goldstein, 1992), but it is unclear to what extent machining artifacts can affect the stiffness of the specimens. A somewhat less common engineering test method that has been used for the measurement of tissue elastic properties is ultrasound measurement. Values found with this method are generally higher than those obtained from the standard tests: 11 to 15 GPa (Ashman and Rho, 1988; Rho *et al.*, 1992). Recently, nanoindentation test have been developed for the measurement of tissue elastic properties (Rho *et al.*, 1997), providing a tissue modulus at the microlevel ranging from 15-20 GPa.

Based on the large variation in the results obtained from these studies, it has been questioned if *the* tissue properties of trabecular bone can be defined at all. Bone tissue is a structure at all levels of organization (Reilly and Burstein, 1974) and, consequently, different properties will be found if different test methods are performed at different levels. It has been proposed that each test will measure 'a stiffness' for some level of organization rather than an intrinsic property of bone material (Choi *et al.*,1990). The obvious question then is: how to measure tissue properties that are relevant for the mechanical behavior of trabecular bone at the apparent (global) level?

To answer this question, an indirect way for the determination of tissue material properties has been proposed. With this approach, the results of experimental tests for larger bone specimens are compared with those calculated from computer models, based on the finite element method, that represent the internal architecture of the tested specimens. By adjusting the tissue modulus in the finite element model such that experimental and simulation results coincide, a tissue modulus is found that represents the relevant properties *in situ*. Earlier studies (Williams and Lewis, 1982; Gibson, 1985), have used two-dimensional finite element models and finite element models that represent idealized bone architectures, modeled as a repetitive structure build of unit cells. In general, however, trabecular bone architecture can not be represented as such. More recently, three-dimensional reconstruction techniques in combination with large-scale finite element models have been introduced, that allow for finite element modeling of the trabecular architecture of reconstructed specimens in full detail (Fyhrie *et al.*, 1992; Hollister *et al.*, 1993;Van Rietbergen *et al.*, 1995a). In earlier studies we have demonstrated that, using this indirect approach, it is possible to determine an 'effective' isotropic modulus for the tissue material of a reasonable large specimen (1 cm^3) (Van Rietbergen *et al.*, 1995b).

The purpose of this chapter is to demonstrate that the 'effective' isotropic modulus calculated from this combined experimental – large-scale-FE approach indeed is a relevant modulus in the sense that it can be used to predict the apparent properties of trabecular bone specimens and in the sense that it can be considered as an intrinsic material property of trabecular bone tissue *in situ*, that can be determined in an accurate and reproducible way. In the following, the combined experimental – large-scale FE-

method for the determination of tissue elastic properties will be described first. An assessment of factors that can affect the results will be given. The effects of boundary artifacts in compression tests is further investigated, since this has been described as one of the most common and critical errors. The reproducibility is then determined by calculating the effective modulus for four trabecular bone specimens that originate from the same bone.

2. The Combined Experimental – Numerical Approach using Large-scale FE-models of Trabecular Bone

With the large-scale FE-techniques, the internal architecture of trabecular bone specimens is represented in detail by a large number of elements. The complex internal geometry of these models is based on high-resolution three-dimensional computer reconstruction techniques. Presently, two methods are available for creating such reconstructions. With the first technique, the specimen is embedded in a plastic raisin and placed in an automated microtome to create sequential slices. A digital camera, mounted on top of the microtome, is used to capture the exposed surface after each slice is taken (Odgaard *et al.*, 1994). By stacking the binarized set of images in a computer, a three-dimensional reconstruction is obtained build of voxels (3-D pixels) that can represent either bone or void. The resolution of this technique is on the order of 1/1000 of the specimen size, i.e. 10 microns for a 1 cm specimen. The second technique is a non destructive one that uses sequential cross-sectional images obtained from CT or MRI scanning (Feldkamp *et al.*, 1989; Rüegsegger *et al.*, 1996; Majumdar *et al.* 1997). The resolution of this technique is dependent on the scanning volume. Typically, laboratory micro-CT and micro-MRI scanners can obtain a resolution of 15 microns for a 15 mm specimen size.

These three-dimensional computer reconstructions can be used for the generation of large-scale finite element models by simply converting each voxel that represents bone tissue to an equally sized brick element in a FE-model (Van Rietbergen *et al.*, 1995). Typically, such FE-models will consist of on the order of 1E6 elements for a 10 mm bone specimen. Problems of this size can not be solved with standard FE-solvers. However, by using the special features of these FE-models (all elements have the same size, geometry and orientation) very fast special purpose FE-solvers can be used that can reduce the cpu-time needed for solving to an hour or less when using supercomputers for the calculations (Van Rietbergen *et al.*, 1996a).

By choosing appropriate boundary conditions, such large-scale FE-models can be used to simulate (multi-axial) compression test experiments on the bone specimen. The element material properties in these models represent the bone tissue material properties, which are not known. However, by assuming linear elastic, homogenous and isotropic tissue material behavior, the results of the compression test simulation are a linear function of the tissue Young's modulus. By assuming a reasonable value for this modulus first, the (multi-axial) apparent elastic properties of the specimen as a whole calculated from the FE-simulations can be scaled later to obtain the best fit between the results of the simulations and those of the experiments (Van Rietbergen *et al.*, 1995b). The best fit can be defined as the tissue modulus scaling factor s that minimizes an error function:

$$Err=\sqrt{\sum_{i=1}^{3}\left(E_i^{\exp}-sE_i^{FE}\right)^2}, \quad (1)$$

with $E_i^{\exp}$ the three orthogonal elastic moduli measured in the experiment, and E_i^{FE} the same moduli calculated from the FE-analyses. With this error function, the best fitting tissue modulus E^T can be calculated from:

$$E^T=s_{\min}E_0^T=\frac{\sum_{i=1}^{3}\left(E_i^{\exp}E_i^{FE}\right)}{\sum_{i=1}^{3}\left(E_i^{FE}\right)^2}E_0^T, \quad (2)$$

with E_0^T the tissue modulus used for the FE-analyses.
An overview of the voxel conversion method is shown in Fig. 1.

3. Evaluation of factors that can affect the accuracy and reproducibility of the combined method

The accuracy of the tissue modulus determined by comparing the apparent elastic properties measured from multi-axial experiments and from large-scale FE-simulation of these experiments can be affected by several factors which can be classified in factors related to the experiment, factors related to the reconstruction, factors related to the FE-calculations and factors related to the protocol used.

Factors that can affect the accuracy of compression tests have been well

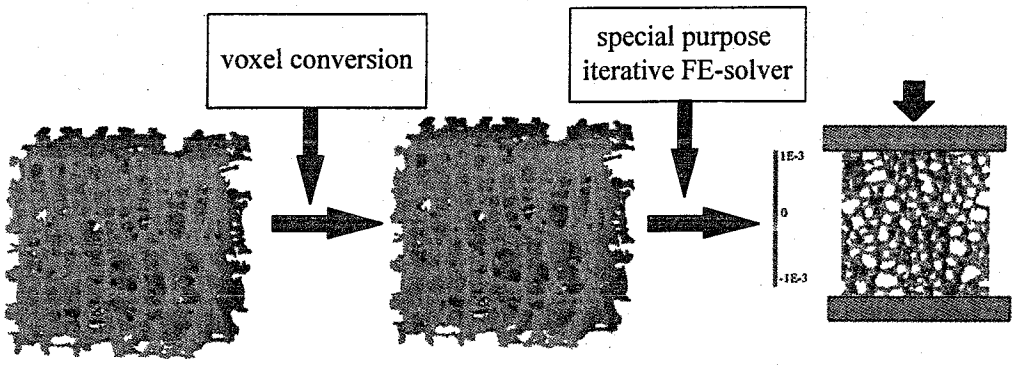

Fig. 1 Summary of the large-scale FE-approach for the calculation of mechanical properties of trabecular bone. A three-dimensional computer reconstruction of a trabecular bone specimen (left) is converted to a FE-model by simply converting all bone voxels in the reconstruction to equally sized brick elements in the FE-model. The resulting FE-model (middle) has exactly the same geometry as the reconstruction it is based on. For a 10 mm specimen, the FE-model consists of on the order of 10^5 to 10^6 elements. To solve these large FE-problems, special-purpose iterative solvers are used. By applying the appropriate boundary conditions, the FE-models can be used to simulate compression test experiments and, after solving the corresponding FE-problem, to calculate the elastic properties of the specimen according to eq. (2).

described (for an overview see Linde, 1993). In these studies, boundary artifacts were identified as one of the most common and critical errors in compression test experiments. Boundary artifacts are created when the bone specimen is excised from its environment. Trabeculae near the sectioned faces of the specimen, which were connected *in situ*, become free-ends, thus forming a region of reduced elastic properties where trabeculae bend and slide. It has been demonstrated that the effect of this artifact can affect the results by as much as 75%, depending on specimen size and geometry and boundary conditions (Keaveny *et al.*, 1993; Odgaard and Linde, 1991). Another factor that can significantly affect the results of compression tests is friction at the bone platen interface. It has been described that, with standard low-friction compression tests, friction can still result in a significant overestimation of the measured Young's modulus (Odgaard and Linde, 1991).

The accuracy of three-dimensional computer reconstructions of trabecular bone is largely determined by the resolution and the slice thickness of the images. In order to image individual trabeculae, the voxel size must be less than the intertrabecular width i.e. on the order of 200 microns. Depending on the purpose of the reconstruction, a (much) smaller voxel can be needed. For the determination of typical morphological parameters (volume fraction, trabecular number, thickness and spacing) it was found that the resolution should be at least 175 microns (Müller *et al.*, 1996). Another parameter that can have a large effect on the resulting reconstruction is the threshold value, used to generate the binary images from the original gray-level images. In particular when using images with a poor resolution, the choice of this parameter can result in a significant thickening or thinning of trabeculae.

Since the geometry of the large-scale FE-models is generated directly from the three-dimensional computer reconstructions, its accuracy depends on the accuracy of the reconstructions as well. The effect of the resolution and consequent element size on the calculation of the apparent elastic properties of a bone specimen was investigated in an earlier study (Van Rietbergen *et al.*, 1995a). In this study it was found that the maximum difference between a model built of 100 micron elements and one build of 20 micron elements was less than 20%, and that this difference was correlated with small differences in volume fraction between the models. The effect of the threshold value on the elastic properties has not been rigorously established but, since the stiffness of the specimen is largely dependent on the volume fraction, it is expected that small changes in the threshold value and, consequently the small changes in the volume fraction of a specimen can have a significant effect on the calculation of elastic properties. Numerical errors in the stiffness calculation can be introduced with the direct conversion of voxels to brick elements, since with this method the surface of trabeculae is not smooth but represented by a 'jagged' composition of brick elements. It has been shown that errors in the stress/strain calculation near the trabecular surfaces can result (Jacobs *et al.*, 1993; Guldberg and Hollister, 1994). However, since these are local artifacts, these errors do not affect the calculation of the stiffness of the specimen as a whole. Other factors that affect the accuracy of the FE-simulation include the fact that the simulation can not capture non-linear phenomena that play a role in the real experiment. For example: the 'toe' that is usually seen in experimental force-displacement curves due to the fact that at low load magnitudes not all trabeculae are load carrying, is not present in the linear FE-models where care is taken that all trabeculae are load carrying. Finally, the modeling of the tissue material properties as

linear elastic, homogenous and isotropic can cause differences between experimental and FE-results.

Protocol differences can also be a factor that affects the accuracy of the tissue modulus determined. Differences in storage, and test conditions of the specimen, differences in specimen geometry and size and differences in strain-rate used with testing can result in incomparable results. Consequently, results between experiments can only be compared when using the same protocol.

4. The Effect of Boundary Artifacts on the Determination of the Tissue Modulus

Among all factors that can affect the accuracy of the tissue modulus determination as mentioned in the previous paragraph, those related to the compression test boundary artifacts have been reported as the most common and significant. To address the effect of this artifact for the value of the tissue modulus with the specimen size and test protocol used here, we have developed a special compression test, adapted from Linde and Hvid, (1989), in which the effect of boundary artifacts could be largely excluded (Van Rietbergen *et al.*, 1996b). With this compression test, steel discs are glued to the loaded faces of a cubic specimen of trabecular bone, such that the sliding of trabeculae during testing was restrained. After removing the discs with a blade saw, the test can be repeated with discs glued at the other faces to measure the stiffness in all three orthogonal directions. Since the uniaxial stress condition, required for compression test experiments, is not satisfied in this experiments the moduli measured from this experiment can not be directly compared to those obtained from standard compression tests. However, by simulating the special fixed-platen compression tests in the computer, using a large-scale FE-model that represents the tested specimen and the appropriate fixed-platen boundary conditions, a tissue Young's modulus can be calculated in the same way as described for the standard compression test, which is not affected by boundary artifacts. By comparing the tissue modulus thus obtained to that obtained by comparing experimental and simulation results for a standard compression test for the same specimen, it is possible to quantify the effect of boundary artifacts on the tissue modulus determined.

In an earlier study, a 10 mm specimen taken from the human proximal tibia, was subjected to this special fixed-platen experiment first and a to standard low-friction compression test later (Van Rietbergen *et al.*, 1996b). Due to the fixed boundary conditions, the moduli found in the first experiment (Average= 482 MPa) were higher than those found in the standard test (Average=436 MPa) (Fig. 2). After reconstruction and conversion to a FE-model, both experiments were simulated and the moduli calculated. By fitting the measured and calculated moduli according to eq. (2), a tissue Young's modulus of 14.6 GPa was calculated for the fixed platen experiment and 14.2 GPa for the standard test. With these values, the anisotropic material properties of the specimen as measured in the experiments were well reproduced by the FE-models (Fig. 2), although the fit was somewhat better for the fixed platen experiment (Err= 39.1 MPa) than for the standard test (Err= 74.4 MPa).

Since the difference between the elastic tissue modulus calculated from the special fixed-platen experiment and that calculated from a standard compression test was

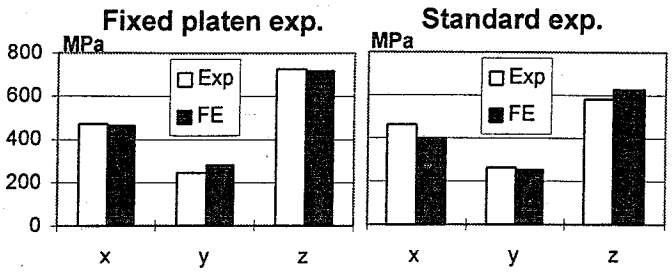

Fig. 2 Apparent Young's moduli measured in the experiments and calculated from the FE-models for the fixed-platen experiment in which the specimen was glued to steel discs (left) and for a standard low-friction compression test (right). The results of the FE-analyses are scaled for the tissue modulus calculated from eq. (2).

less than 3%, it was concluded that, for the specimen size and protocol used here, boundary artifacts would not significantly affect the results of the determined tissue modulus. It is also concluded that the tissue modulus determined for this specimen is relevant in the sense that, in combination with the large-scale FE-models, it can be used to calculate the apparent anisotropic material properties of the bone specimen.

5. Reproducibility: Determination of Tissue Elastic Properties for Four Specimens

To evaluate the reproducibility of the combined experimental–FE approach for the determination of tissue elastic properties, another four specimens were analyzed. All specimens were obtained from one whale vertebral body to ensure that all specimens would have similar tissue properties as much as possible. The specimens were subjected to a standard low-friction compression test while measuring the forces and transversal displacements. From these measurements, three orthogonal moduli and six Poisson's ratios were calculated for each specimen. After testing, three-dimensional computer reconstructions of all specimens were made using the serial sectioning technique (Odgaard *et al.*, 1994). The reconstructions were converted to large-scale FE-models of up to 411,474 elements, and the compression test experiments were simulated with no-friction boundary conditions. Elastic moduli and Poisson's ratio were calculated from the results of the FE-simulations in the same way as done for the real experiments. For each of the four specimens a tissue effective isotropic Young's modulus was calculated that gave the best fit between its three orthogonal moduli determined from the experiment and from the FE-calculations according to eq. (2).

For all specimens the three Young's moduli as measured in the experiments were accurately reproduced by the FE-models. Errors calculated from eq. (1) ranged from 3.2 MPa to 46 MPa for an average modulus of 262 MPa and 307 MPa respectively. Very similar values were found for the tissue modulus of the four specimens, ranging from 5.01 GPa to 5.71 GPa. When plotting the Young's moduli and Poisson's ratios measured in the experiment versus those calculated from the FE-models scaled for the best fitting tissue modulus, it can be seen that there is an excellent correlation between the moduli, and a

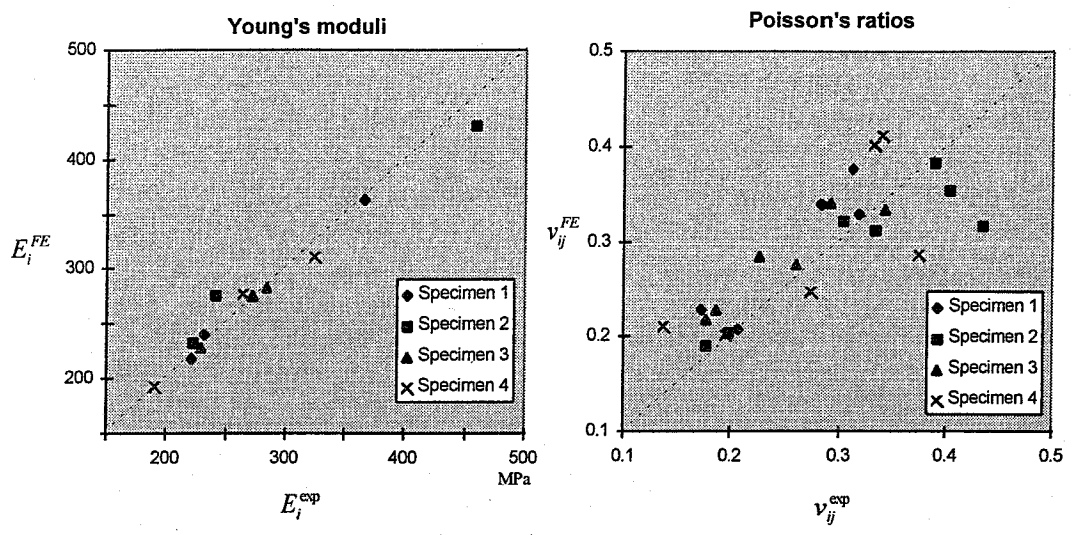

Fig. 3 Young's moduli (left) and Poisson's ratios (right) of the four specimens measured in the experiment versus those calculated from the FE-analyses

good correlation between Poisson's ratios (Fig. 3). The somewhat lesser correlation for the Poisson's ratios is probably due to the larger errors in the measurements of transversal displacement compared to those of the longitudinal displacements in the experiments and due to small differences between these measurements in the experiment and in the FE-model.

Since the variation in the tissue modulus determined for four similar specimen was only 13%, it was concluded that the calculated tissue modulus represents an inherent material property of the trabecular tissue that can be determined in an accurate and reproducible way. Part of the remaining variation in the tissue modulus can be due to variation in the tissue material properties of the specimens. It is possible that the variation is further reduced if more information about the tissue properties is available (in particular the tissue mineralization).

6. Discussion

For all specimens investigated, the anisotropic material properties are well represented by the FE-model with an isotropic 'effective' tissue modulus. It was thus concluded that the 'effective' tissue modulus is a relevant parameter in the sense that, in combination with these large-scale FE-models, it can be used to calculate the anisotropic material properties of trabecular bone. It was also found that the effective tissue moduli determined for specimens that originate from the same bone vary only within a narrow range, and thus, that the tissue modulus is relevant in the sense that it is accurate and reproducible.

The tissue modulus found for the human specimen (14.6 GPa) is much larger than the average value found for the whale bone specimens (5.6 GPa). These differences can not be explained by differences in the level of testing, since the same test method was used in both cases and since both types of bone have similar morphological properties.

Consequently, these differences must be due to differences in the mineral content and microstructure of the bone tissue. As indicated for the whale bone specimens, differences in tissue mineralization can explain a part of the variation in the tissue modulus. It has been proposed, however, that tissue density alone might not be a sufficient estimator for modulus when comparing structural different bone, but that microstructural variations should be accounted for (Choi and Goldstein, 1992). To determine a realistic range for the variation of tissue moduli between species, individuals and locations, a much larger and more diverse set of specimens will be needed.

When considering the microarchitecture of trabecular tissue material, the question may arise how it is possible that such good results are obtained when using models with isotropic tissue properties. The tissue material consists of a plywood-like lamellar organization of a collagen-crystal composite, with lamellae aligned with the trabeculae. Consequently, it is unlikely that the tissue material properties are isotropic. However, since most trabeculae are loaded in bending or compression, the longitudinal modulus of the trabeculae largely determines the mechanical behavior of the tissue *in situ*. It is thus likely that the 'effective' isotropic tissue modulus found with the methods described in this chapter in fact represents the longitudinal stiffness of trabeculae, whereas their stiffness in transversal directions can be less.

An important consequence of the finding that the anisotropic material properties are well predicted from FE-models with isotropic tissue properties is that the anisotropy of trabecular bone must be purely due to its architecture. This implies that relationships to predict the anisotropic material properties of trabecular bone from measurements of its density and its morphology only can exist.

Acknowledgments This project was sponsored by The Netherlands Foundation for Research (NWO/Medical Sciences), by the National Computing Facilities Foundation (NCF), by the Danish Rheumatism Association (233-588) and by the Danish Research Councils (5210563-1 and 9313411).

References

Ashman R.B. and Rho, J.Y. (1988) Elastic modulus of trabecular bone material, *J. Biomech.*, **21**, 177-181

Carter D. R. and Hayes, W.C. (1977) The behavior of bone as a two-phase porous structure, *J. Bone Jt. Surg.* **59-A**, 954-962

Choi, K., Kuhn, J.L., Ciarelli, M.J. and Goldstein, S.A. (1990) The elastic moduli of human subchondral, trabecular and cortical bone tissue and the size-dependency of cortical bone modulus, *J. Biomech.*, **23**, 1103-1113

Choi, K. and Goldstein, S.A. (1992) A comparison of the fatigue behavior of human trabecular and cortical bone tissue, *J. Biomech.*, **25**, 1371-1381

Feldkamp, L.A., Goldstein, S.A., Parfitt, A.M., Jesion, G. and Kleerekoper, M. (1989) The direct examination of three dimensional bone architecture in vitro by computed tomography, *J. Bone Min. Res.*, **4**, 3-11

Fyhrie, D.P., Hamid, M.S., Kuo, R.F. and Lang, S.M. (1992) Direct three-dimensional finite element analysis of human vertebral cancellous bone, *Proc. 38th Meeting ORS*, p. 551

Gibson L.J. (1985) The mechanical behavior of cancellous bone, *J. Biomech.*, **18**, 317-328

Goldstein S.A., Goulet, R. and McCubbrey, D. (1993) Measurement and significance of three dimensional architecture to the mechanical integrity of trabecular bone, *Calcif. Tiss. Int.* **53**, S127-133

Goulet, R.W., Goldstein, S.A., Ciarelli, M.J., Kuhn, J.L., Brown, M.B. and Feldkamp, L.A. (1994) The relationship between the structural and orthogonal compressive properties of trabecular bone, *J. Biomech.*, **27**, 375-389

Guldberg, R.E. and Hollister, S.J. (1994) Finite Element solution errors associated with digital image-based mesh generation, *ASME-BED*, **28**, 147-148

Hodgskinson, R. and Currey, J.D. (1992) Young's modulus, density and material properties in cancellous bone over a large density range, *J. of Mat. Science: Materials in Medicine*, **3**, p. 377-381

Hollister, S.J., Brennan, J.M. and Kikuchi, N. (1994) A homogenization sampling procedure for calculating trabecular bone effective stiffness and tissue level stress, *J. Biomech.*, **27** 433-444

Jacobs, C.R., Mandell, J.A. and Beaupré, G.S. (1993) A comparative study of automatic Finite Element mesh generation techniques in orthopaedic biomechanics, *ASME-BED*, **24**, 512-514

Keaveny, T.M., Borchers, R.E., Gibson, L.J. and Hayes, W.C. (1993) Theoretical analysis of the experimental artifact in trabecular bone compressive modulus, *J. Biomech.*, **26**, 599-607

Linde, F. (1993) Elastic and viscoelastic properties of trabecular bone by a compression testing approach. Laegeforeningens Forlag, Kobenhavn

Linde, F. and Hvid, I. (1989) The effect of constraint on the mechanical behaviour of trabecular bone specimens, *J. Biomech.*, **22**, 485-490

Majumdar , S., Newitt, D.C., Link, T.M., Augat, P., Lin, J.C., Ouyang, X. Lang, T. Lotz, J. and Genant, H.K. (1997) Three dimensional assessment of trabecular architecture using magnetic resonance imaging, *Proc. 43rd Meeting ORS*, p. 209

Müller, R., Koller, B., Hildebrand, T. Laib, A., Gianolini, S. and Rüegsegger, P. (1996) Resolution dependency of microstructural properties of cancellous bone based on three-dimensional μ-tomography, *Technol. Health-Care*, **4**, 113-119

Odgaard, A., Andersen, K., Ullerup, R., Frich, L.H. and Melsen, F. (1994) Three-dimensional reconstruction of entire vertebral bodies, *Bone*, **15**, 335-342

Reilly D.T. and Burstein, A.H. (1974) The mechanical properties of cortical bone, *J. Bone Jt. Surg.*, **56A**, 1001-1022.

Rho, J.Y., Ashman, R.B. and Turner, C.H. (1993) Young's modulus of trabecular and cortical bone material: ultrasound and microtensile measurements, *J. Biomech.*, **26**, 111-119

Rho, J.Y., Roy, M.E., Tsui, T.Y. and Pharr, G.M. (1997) Young's modulus and hardness of trabecular and cortical bone in the various directions determined by nanoindentation, *Proc. 43rd Ann. Meeting ORS*, p. 811

Rice, J.C., Cowin, S.C. and Bowman, J.A. (1988) On the dependence of the elasticity and strength of cancellous bone on apparent density, *J. Biomech.*, **21**, p. 155-168

Rüegsegger, P., Koller, B. and Müller, R. (1996) A microtomographic system for the nondestructive evaluation of bone architecture, *Calc. Tiss. Int.* **58**, 24-29

Turner C.H., Cowin, S.C., Rho, J.Y., Ashman, R.B. and Rice, J.C. (1990) The fabric dependence of the orthotropic elastic constants of cancellous bone. *J. Biomech.* **23**, 549-561

Van Rietbergen, B., Weinans, H., Huiskes, R. and Odgaard, A. (1995a) A new method to determine trabecular bone elastic properties and loading using micromechanical Finite-Element models, *J.Biomech.*, **28**, 69-81

Van Rietbergen, B., Huiskes, R., Weinans, H., Odgaard, A. and Kabel, J. (1995b) The role of trabecular architecture in the anisotropic mechanical properties of bone, in: *Bone structure and remodeling* (Ed. by Odgaard, A. and Weinans, H.), World Scientific, Singapore

Van Rietbergen, B. Weinans, H., Polman, B.J.W., and Huiskes, R. (1996a) Computational strategies for iterative solutions of large FEM applications employing voxel data, *Int. J. Num. Meth. in Eng.*, **39**, 2743-2767

Van Rietbergen, B., Odgaard, A. Kabel, J. and Huiskes, R. (1996b) Determination of tissue elastic properties by comparison of experimental and Finite Element results, *Proc. 10th Meeting ESB*, p. 327

Williams J.L. and Lewis J.L. (1982) Properties and an anisotropic model of cancellous bone from the proximal tibia epiphysis, *J. Biomech. Eng.*, **104**, 50-56