J Musculoskelet Neuronal Interact 2005; 5(4):310-312

Review Article

Hylonome

Longitudinal micro-CT scans to evaluate bone architecture

J.H. Waarsing, J.S. Day , H. Weinans

Orthopaedic Research Lab, Erasmus University Medical Center, Rotterdam, The Netherlands

Keywords: Micro-CT, Bone Architecture, Trabeculae, *In vivo*, Animal Experiment

For a long time bone mass or BMD has been considered as the main predictor for fracture risk in the clinic. In laboratory experiments bone mass or bone volume fraction is a reasonable good predictor of cancellous bone mechanical properties i.e., stiffness and strength. Obviously, since bone volume fraction is a scalar property it cannot predict all cancellous bone mechanical properties in highly anisotropic regions. It has become clear that bone mass or BMD has strong shortcomings for the prediction of fracture risk. The new paradigm is to measure bone quality as a surrogate for fracture risk. However, there is no clear definition of bone quality. Many architectural parameters have been measured in cancellous bone and have been tested on their ability to predict fracture risk *independent* of bone mass. For most of those measures of bone architecture it is not clear what the relation is with mechanical properties or fracture risk. Trabecular anisotropy seems to be the most promising parameter so far that might be useful as an independent contributor to fracture risk[1,2,3], likely due to a relatively weak structure in the direction of the infrequent off-axis loading direction[4].

The measurement of bone architecture has a long history with a gold standard based on histology. Stereological methods were applied to estimate the three-dimensional architecture from the two-dimensional histological slices. In addition to the morphological descriptions and estimates of the three-dimensional architecture, histology offers a number of other important measures such as the unmineralized osteoid surface, cell counts of osteoblasts and osteoclasts and most important a measure of bone formation rate through use of fluorescent labels incorporated in the mineralizing front. These latter advantages have resulted in the technique still being in widespread use, even though it has big disadvantages like biased two-dimensional (indirect) measuring of bone morphometry parameters and labor intensiveness. Around 1990 a high-resolution version of the clinical CT scanner was introduced in the research laboratory[5], and has become an important modality for bone morphometry measurements. The apparatus yields true 3-dimensional data sets of bone biopsies and whole bones of small animals like the mouse and rat. Voxel sizes can be as small as 5 microns or less. The advantages of this modality above histological sections are the unbiased 3-D morphometric measures of bone architecture, and that the samples can be imaged non-destructively so the bones are available for other measuring techniques such as e.g., failure tests.

The popularity of the device led to a wild growth of new parameters that claim to measure important aspects of bone architecture, ranging from accepted measures like volume fraction and trabecular thickness to more exotic measures like Gaussian curvature and fractal dimensions. It is not clear which of these parameters are useful and what they tell us about bone structure or quality. A standardization of these measures would be useful, just like the standardization in parameters led by Parfitt et al.,[6] following a similar explosion of 2-D measures in the early days of bone histomorphometry.

A common factor for both micro-CT and histology is that all studies using these techniques have a cross-sectional nature, due to the invasiveness or destructiveness. Although very interesting results have been obtained by these methods which have increased our knowledge about bone and bone remodeling to a great extent, many questions remain open, in particular where it concerns adaptation of the trabecular structure due to aging, drug treatment or mechanical loading regimens. To answer these questions more subtle measurements would be needed, a subtlety that can probably only be reached by imaging studies using a longitudinal study set-up. High-resolution *in vivo* micro-CT, similar to clinical CT, would be the most obvious option. However, the high radiation dose that is necessary to obtain usable images has hampered this development. Technological innovations that improved the sensitivity of CCD cameras have reduced the necessary dose for good image quality and the first generation of *in vivo* scanners have now reached the laboratories.

Even though the necessary radiation has been reduced to

The authors have no conflict of interest.

Corresponding author: H. Weinans, Erasmus Medical Center, Department Orthopaedics, Rotterdam, The Netherlands
E-mail: h.weinans@erasmusmc.nl

Accepted 31 July 2005

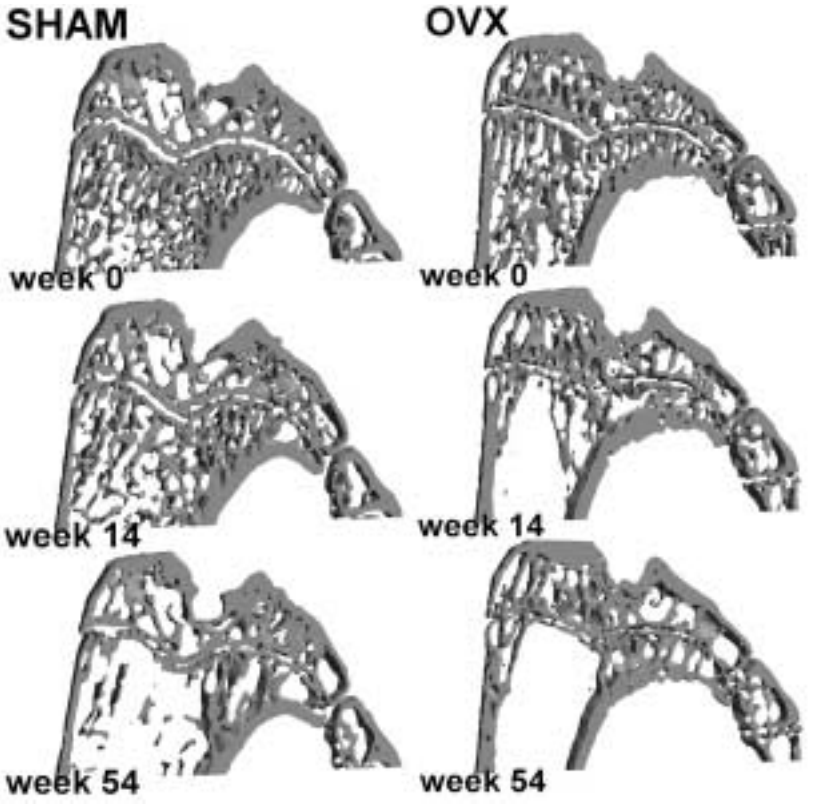

Figure 1. 3-D slices out of the proximal tibia of a sham operated (normally aging) rat and an ovariectomized rat, imaged at week 0, prior to operation, and at week 14 and week 54 post-operation. Notice how the preserved trabeculae align across the growth plate.

a great extent, the dose needed to obtain usable images at high resolutions of 20 microns or less are still 10 to 100-fold higher than in clinical CT scanners. Long-term stochastic effects of radiation are not an issue as they are in human CT scanners, but radiation between 100 and 1,000 mGy can be harmful. Even though it is not lethal to the animal, it can have serious consequences for the experiment since it might affect bone metabolism and therefore interact with and confound the sought experimental findings. The only option is to scan at lower resolutions, or work with poor image qualities.

A very important and unfortunately often ignored topic in micro-CT scanning is the segmentation of the raw reconstructed images in which the grey value of each voxel represents an attenuation co-efficient in Hounsfield units, into binary images that only represent bone and non-bone. In other words, for each voxel in the data set a decision needs to be made whether a voxel is bone or not. This issue becomes even more important when the quality or the resolution of the images is limited as with *in vivo* micro-CT. Simple segmentation methods using a single global threshold value are far from optimal in this case. We have therefore developed an algorithm using local thresholds that tries to segment the non-optimal *in vivo* images in an optimal way[7]. This method maximizes the potential of *in vivo* micro-CT by giving good structural representation without the need to use longer scanning times that would increase absorption of harmful X-ray radiation by the living tissue.

Perhaps the most exciting possibility of *in-vivo* microCT is that changes in bone architecture can be followed over time as it occurs in individual animals. Due to the high resolution, it is in theory possible to follow changes at the level of single trabecula. For this, scans made at different time points need to be positioned on top of each other. This is, however, not trivial. The leg of an animal cannot be positioned in exactly the same position during each scan. Furthermore, bones change over time, trabeculae are resorbed, new trabeculae are formed, and longitudinal growth increases the size of the bones. We have tackled this issue by using three-dimensional registration algorithms. Although these algorithms can give the optimal mathematical match, this is often not the same as the optimal biological match, in particular when the animal is still growing. In an optimal biological match, a bone package that is present at one time point needs to fall on top of the same bone package at another time point. In order to obtain the biological match on the tibia of one-year-old rats, we applied the registration separately on epiphyseal and metaphyseal parts of the tibia. To perform the registration we used certain details like arterial openings in the cortex and trabeculae that were present in all sequential scans. Comparing the follow-up scans with histology validated the

result of this match. New bone formation as suggested by the follow-up scans corresponded perfectly with calcein labels in the histological sections[8]. This shows that *in vivo* micro-CT can visualize bone apposition similar to histology. Besides apposition, *in vivo* micro-CT also can visualize bone resorption, which is impossible when using histology or conventional micro-CT.

We have performed an *in vivo* micro-CT study that demonstrates the power and potential of this new method[9]. During one year we followed the changes in trabecular architecture due to aging and ovariectomy in the tibia of mature rats. The registered scans revealed local results of the remodeling process. Some trabeculae were slowly resorbed while their neighbors increased in thickness. Other trabecular structures were merged into one big strut. An interesting phenomenon that could be seen in both groups was the alignment of trabeculae across the growth plate (Figure 1). Co-ordinated remodeling resulted in the removal of metaphyseal trabeculae that did not have an opposite neighbor on the epiphyseal side of the growth plate, while trabeculae that did have such a neighbor were preserved, straightened and increased in thickness.

The local changes in bone architecture that were revealed by the registered *in vivo* scanning strongly suggest local regulation of bone remodeling and recall theories of regulation of cell activity through positional information[10]. The nature of the changes, especially the alignment of the trabeculae, suggest that the mechanical environment might deliver this positional information. This issue has been a constant item for many bone researchers. Some studies on bone adaptation have utilized computer simulations of the bone remodeling process[11] showing the possibility of trabecular alignment as a response to loading. A drawback of these studies is the unfeasibility to validate the outcome of the simulation with the situation in the 'real world'. The introduction of the *in vivo* micro-CT can take away this drawback. When the scan made at a certain time point is used as input for the computer simulation, the follow-up scans show the result of the 'real world' remodeling that can be compared to the outcome of the remodeling simulation. We have performed some preliminary simulations, in which the high-resolution scan of the tibia of a rat served as input of a model that uses local mechanical conditions to simulate the remodeling process. The output of the simulation showed a strong resemblance with the follow-up scan of the same bone. In both the real world as in the simulation, more centrally located trabeculae were resorbed, while remaining trabeculae aligned across the growth plate, increased in thickness and got straightened.

Findings in bone cell biology, biochemistry and genetics result in more and more detailed information about regulation of bone remodeling. These results stress how complicated the interaction and regulation of bone cells is. All the pieces of this jigsaw puzzle can only be put together when the image of the puzzle is known. *In vivo* micro-CT, especially when combined with image registration and computer simulation, is a very powerful tool that helps to understand what the image of the jigsaw puzzle of bone remodeling should look like. Together with other techniques in bone research *in vivo* micro-CT might bring us closer to unraveling the bone remodeling process and thus give us a better understanding of the bone diseases that affect the elderly, which are principally a failure of the bone remodeling process.

References

1. Ciarelli TE, Fyhrie DP, Schaffler MB, Goldstein SA. Variations in three-dimensional cancellous bone architecture of the proximal femur in female hip fractures and in controls. J Bone Miner Res 2000; 15:32-40.
2. Homminga J, McCreadie BR, Ciarelli TE, Weinans H, Goldstein SA, Huiskes R. Cancellous bone mechanical properties from normals and patients with hip fractures differ on the structure level, not on the bone hard tissue level. Bone 2002; 30:759-764.
3. Day JS. Bone Quality: The Mechanical Effects of Microarchitecture and Matrix Properties, PhD Thesis Erasmus University Rotterdam, Dept. Orthopaedics, Erasmus University Medical Center; 2005.
4. Homminga J, Van-Rietbergen B, Lochmuller EM, Weinans H, Eckstein F, Huiskes R. The osteoporotic vertebral structure is well adapted to the loads of daily life, but not to infrequent "error" loads. Bone 2004; 34:510-516.
5. Kuhn JL, Goldstein SA, Feldkamp LA, Goulet RW, Jesion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. J Orthop Res 1990; 8:833-842.
6. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987; 2:595-610.
7. Waarsing JH, Day JS, Weinans H. An improved segmentation method for *in vivo* micro-CT imaging. J Bone Miner Res 2004; 19:1640-1650.
8. Waarsing JH, Day JS, van der Linden JC, Ederveen AG, Spanjers C, De Clerck N, Sasov A, Verhaar JA, Weinans H. Detecting and tracking local changes in the tibiae of individual rats: a novel method to analyse longitudinal *in vivo* micro-CT data. Bone 2004; 34:163-169.
9. Waarsing JH, Day JS, Verhaar JAN, Ederveen AGH, Weinans H. Bone loss dynamics result in trabecular alignment in aging and ovariectomized rats. J Orthop Res (in press).
10. Wolpert L. Positional information revisited. Development 1989; 107(Suppl.):3-12.
11. Huiskes R, Ruimerman R, van Lenthe GH, Janssen JD. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. Nature 2000; 405:704-706.