Increase in Bone Volume Fraction Precedes Architectural Adaptation in Growing Bone

E. TANCK, J. HOMMINGA, G. H. VAN LENTHE, and R. HUISKES

Orthopaedic Research Laboratory, University of Nijmegen, Nijmegen, The Netherlands

In mature trabecular bone, both density and trabecular orientation are adapted to external mechanical loads. Few quantitative data are available on the development of architecture and mechanical adaptation in juvenile trabecular bone. We studied the hypothesis that a time lag occurs between the adaptation of trabecular density and the adaptation of trabecular architecture during development. To investigate this hypothesis we used ten female pigs at 6, 23, 56, 104, and 230 weeks of age. Three-dimensional morphological and mechanical parameters of trabecular bone samples from the vertebra and proximal tibia were studied using microcomputed tomography and micro-finite element analysis. Both bone volume fraction and stiffness increased rapidly in the initial growth phase (from 6 weeks on), whereas the morphological anisotropy started increasing only after 23 weeks of age. In addition, the anisotropy reached its highest value much later in the development than did bone volume fraction. Hence, the alignment of trabeculae was still progressing at the time of peak bone mass. Therefore, our hypothesis was supported by the time lag between the increase in trabecular density and the adaptation of the trabecular architecture. The rapid increase of bone volume fraction in the initial growth phase can be explained by the enormous weight increase of the pigs. The trabeculae aligned at later stages when the increase in weight, and thus the loading, was slowed considerably compared with the early growth stage. Hence, the trabecular architecture was more efficient in later years. We conclude that density is adapted to external load from the early phase of growth, whereas the trabecular architecture is adapted later in the development.

Key Words: Bone; Growth; Trabecular architecture; Microcomputed tomography μ-CT; Mechanical properties; Anisotropy.

Introduction

It is generally accepted that mechanical load plays an important role in development, maintenance, and adaptation of the skeleton. Early long bones are developed from mineralized cartilage and gradually transform into their eventual structure, whereas density and trabecular orientation are adapted to mechanical load.

Few quantitative data are available on the development of architecture and mechanical adaptation in juvenile trabecular bone. Korstjens et al.15 analyzed the two-dimensional trabecular patterns of the distal radius in children, aged 4–14 years, using radiographs and digital imaging. They found that the fine trabecular patterns of young children had coarsened in the older ones. In a medieval Nubian population, aged 0–60 years, vertebral morphology was investigated by Kneissel et al.14 who showed that the highest bone volume fraction was present during adolescence when the trabeculae were also more plate-like than in the youngest children. The recent development of microcomputed tomography (μ-CT) has made it possible to analyze the three-dimensional (3D) architecture of trabecular bone accurately.5 To date, this technique has been used to study adult cancellous bone and to investigate the effects of aging.2,3,8,21 In the adult stage, the trabeculae are aligned to the principal stress directions, as described by Wolff more than a century ago.25 In this way, trabecular bone is believed to be adapted to the typical external loads of daily living in both density and architecture. During growth, load increases gradually, which implies that density and architecture would change as well. As an increase in density due to increased loading would only have to involve bone formation, whereas the adaptation of architecture must involve both formation and resorption, we hypothesize that there is a time lag between these processes. In a computer simulation of bone-cell-based modeling and remodeling,9 it was found that adaptation of density (trabecular thickness) due to increased loading would occur much faster than trabecular adaptation due to changes in loading orientation. We investigated this hypothesis by checking whether this time lag occurs in growing bone. For this purpose, pig bone between 6 and 230 weeks of age was used. Three-dimensional trabecular bone structures in the vertebra and proximal tibia were studied using μ-CT and micro-finite element analysis (μ-FEA).

Materials and Methods

Materials

Bone from ten female pigs from a Dutch farm (Central Animal Laboratory, Nijmegen, The Netherlands) was used. The pigs were 6, 23, 56, 104, and 230 weeks of age (two per age group). Total body weight increased from 12 kg at 6 weeks of age to 212 kg at 104 weeks of age, after which it stabilized (Figure 1). The growth plates in the proximal tibiae of the 104-week-old pigs were nearly closed, whereas they were completely closed in the 230-week-old pigs. From these observations it can be estimated...
that 104 weeks in pigs is roughly equivalent to late adolescence (about 15 years) for humans, whereas 230 weeks is adulthood (about 30 years) for humans. Postmortem bone cylinders of 8.5 mm in diameter and about 50 mm in length were drilled out from the weight-bearing area of the proximal tibia (i.e., lateral or medial condyle) and the fifth lumbar vertebra (L-5) of each animal. These sites were chosen because both are loaded predominantly in axial compression, and were subject to studies of adult specimens in the literature. The axes of the bone cylinders were parallel to the longitudinal axes of the bones. Institutional approval was obtained for all experiments.

**Microcomputed Tomography Scanning**

All bone cylinders were scanned in a μ-CT apparatus (μCT 20, Scanco Medical AG, Zurich, Switzerland) with an isotropic spatial resolution of 28 μm. From each a 4 × 4 × 4 mm³ volume of interest (VOI) was selected. In the vertebra, the VOI was taken from the center of the vertebral body. In the proximal tibia, one VOI was selected from the epiphysis and one from the metaphysis. In the epiphysis, it was taken approximately midway between the articular cartilage and the growth plate. In the metaphysis it was taken at a distance of about 5 mm from the growth plate. In the pigs with the closed growth plates, the cement line was used to determine the position. The VOIs were represented in 22 × 22 × 22 μm³ voxels and segmented using an individually optimized density threshold value. Segmentation was performed visually by comparing slices before and after segmentation for a range of threshold values. The threshold that resulted in the best fit between the two was used. The sensitivity of this method was tested.

**Morphometric and Mechanical Analysis**

The morphological anisotropy was determined from the mean intercept length\(^2\) ( MIL\(_{max} \)/MIL\(_{min} \)) directly from the segmented scans. The bone volume fraction (BV/TV) was determined from these scans as well. The voxel meshes were subsequently converted to μ-FEA models with element sizes of 22 × 22 × 22 μm³ for bone cubes of the 6- and 23-week-old pigs, and 44 × 44 × 44 μm³ for bone cubes of the older pigs. The element coarsening of the latter group was done to reduce computer time, without changing the trabecular structure significantly. For one sample we tested the difference in maximal stiffness for the two element sizes and determined that the change was only 0.1%, and hence insignificant. Data reduction in the 6- and 23-weeks-old pigs was performed by reducing the VOI to 2.6 × 2.6 × 2.6 mm³, leaving at least five trabeculae in every direction. The apparent mechanical properties were determined with the μ-FEA models as were the orientations of the three principal mechanical axes. These μ-FEA models have been shown to give accurate estimates of the apparent elastic moduli of cancellous bone. A tissue modulus of 5 GPa was assumed for all specimens.

Data analysis was performed between age categories per site (i.e., for the vertebra, tibial epiphysis, and tibial metaphysis) separately, using one-way analysis of variance (ANOVA) with age as the grouping variable. If the result was significant, it was followed by a pairwise multiple comparison procedure using the Tukey test. In addition, an average value of the three sites was determined for each animal. This resulted in mean and standard deviation (SD) data for the pigs in each age category on which similar statistical data analysis was performed; that is, one-way ANOVA with age as grouping variable, followed by the Tukey test if required. Differences were considered statistically significant at \(p < 0.05\).

**Results**

The 3D reconstructions showed clear differences in trabecular structure with age (Figure 2). For the youngest bones, the structure was refined, whereas in older bones it developed into a much coarser trabecular structure.

The bone volume fraction (BV/TV) increased rapidly in the initial growth phase, followed by a slight decrease and then a stabilization (Figure 3). The increase was significant between 6 and 56 weeks (Figure 3). BV/TV and total body weight did not exactly follow the same trend; whereas total body weight reached a maximum at 104 weeks of age, and was stable from there on, BV/TV reached its maximum at 56 weeks, decreased (although
not significantly), and stabilized from 104 weeks on (Figures 1 and 3).

On average, the early morphological anisotropy was relatively low (Figure 4); it did not increase from 6 to 23 weeks. After 23 weeks of age, anisotropy increased significantly until 104 weeks of age, after which it remained about the same.

On average, the maximal ($E_{\text{max}}$) elastic moduli increased with age until 104 weeks, after which they stabilized (Figure 5). For the separate locations, the trends for $E_{\text{max}}$ vs. age were similar, but increase with age was significant only for vertebrae.

The angle between the stiffest direction and the axial loading direction (angle $\alpha$) decreased with age (Figure 6). The standard deviation of angle $\alpha$ showed a decrease with age (Figure 6); at 6 weeks of age, the angle varied between $0^\circ$ and $90^\circ$, whereas it remained $< 23^\circ$ from the age of 104 weeks on. After 104 weeks, angle $\alpha$ of the vertebra was constantly below $7^\circ$, whereas the values for the tibia varied more (Figure 6). This may be explained by the fact that the vertebra is primarily loaded in axial compression, whereas the loads on the tibia are more variable.

**Discussion**

This study has shown that density is adapted to external load from the early phase of growth, whereas the trabecular architecture is adapted later in development. During initial growth, bone becomes denser by trabecular deposition, as was also found from histomorphometry in rats. The rapid increase of bone volume fraction (BV/TV) in the initial growth phase might be explained by the explosive weight increase during early development. It is likely that this results in increased mechanical loads to the bones as, in growing pigs, body weight increases relatively faster than bone mass and bone cross-sectional area. The trabeculae align at a later stage, when the increase in weight, hence in the loads, is slower compared with the early growth stage. This suggests that, in the early growth stage, when the trabecular architecture is not yet optimally adapted to external loads, mechanical adaptation is achieved through increased bone mass, and that the trabecular architecture is more efficient in later years.

The hypothesis supporting a more efficient trabecular structure in later years is confirmed by the development of morphological anisotropy, which, on average, only started after 23 weeks of age. At 56 weeks of age bone volume fraction peaks, whereas trabecular alignment still progresses until 104 weeks of age. Furthermore, the reduced variation of angle $\alpha$, between the principal material axis and the load, with age, also illustrates the increasing structural organization in later years.

Since the submission of this article, Nafei et al. published a study of morphological and mechanical properties in the growing bones of sheep. In general, the results of the present study are in agreement with their observations. Among other findings, they observed that the elastic modulus, bone volume fraction, and architectural anisotropy correlated positively with increasing age. In addition, the values of all parameters differed significantly between younger and older sheep.
Maximal stiffness ($E_{\text{max}}$) is determined by three parameters: tissue modulus; bone volume fraction (BV/TV); and architecture. In this study, the tissue modulus was assumed to be constant, whereas, in reality, the modulus increases during growth due to a higher mineral content per tissue volume. Hence, the relationship between real stiffness and age is stronger, and would probably lead to more significant results. The development of stiffness ($E_{\text{max}}$) can be explained by the combined development of volume fraction and anisotropy. The increase of $E_{\text{max}}$ from 6 up to 56 weeks of age was caused mainly by the increased volume fraction, whereas the increase from 56 up to 104 weeks of age was caused by the increased anisotropy. As stiffness and strength are highly correlated, trabecular bone also becomes stronger during growth.

There are some limitations in the present study. First, there may have been slight differences in drill direction for obtaining the bone cylinder and the axial loading direction. Second, the axial loading direction may differ from the principal stress direction. These two possible inaccuracies affect only the value of the angle $\alpha$. The maximal effect on angle $\alpha$ is estimated to be $23^\circ$, based on the result that angle $\alpha$ is not zero but maximal $23^\circ$ after the equilibrium stage of 104 weeks of age (Figure 6). However, this does not affect the conclusion that the variation of angle $\alpha$ decreases with age. Another limitation is the small number of pigs per age category. One of the consequences of small numbers per age category is the difficulty in obtaining significant results. However, the results showed some noteworthy significance data for all parameters. Furthermore, the analysis of trends of different parameters improves the insight in trabecular bone adaptation.

In summary, this study supported our hypothesis. The results showed the presence of a time lag between increase in trabecular density and adaptation of trabecular architecture. Bone volume fraction increased rapidly in the initial growth phase, whereas anisotropy started to increase at a later stage. In addition, anisotropy reached its highest value much later in development than did bone volume fraction. This is in agreement with computer simulations of bone-cell-based modeling and remodeling, in which adaptation of density due to increased loading occurred much faster than trabecular adaptation due to changes in loading direction. In conclusion, we found that density is adapted from the early phase of growth, whereas trabecular architecture is adapted later in development, during which mechanical adaptation produces a more efficient architecture.

Acknowledgments: This project was sponsored by the Dutch Organization of Research, Medical Science Section (NWO/GbMW, Grant No. 902-36-065/903-41-193). The authors thank Dr. T. Hara for his assistance in obtaining the bone specimens.

References