Introduction: Very little quantitative data is available about the development of architecture and mechanical competence in juvenile trabecular bone. Early bone is developed from mineralized cartilage and gradually transforms into its eventual structure, with density and trabecular directionality apparently adapted to its typical mechanical load [1]. During this development, the external loads on the bone increase considerably. Early in growth most bone is added to non-resorbed surfaces (modeling), while later on more is deposited on resorbed surfaces (remodeling) [2]. We suspect that these processes are stimulated by mechanical signals. If this is true, then it must be reflected in the 3-D morphological and mechanical properties of growing and mature bone. We tested this in post-mortem pig specimens.

Methods: We used female pigs at 6, 23, and 230 weeks of age (two per age group; average weights about 12, 60 and 180 kg). Institutional approval was obtained for the experiments. At 6 and 23 weeks the bones still have growth plates. At 230 weeks of age, the growth plates are closed but remain visible as dense contours on X-rays. Bone cylinders of 8.5 mm in diameter were drilled out of the proximal medial tibiae (epiphysis and metaphysis) and vertebrae (L5) of the animals.

The bone cylinders were scanned in a micro-CT (µCT 20, Scanco Medical AG, Zürich, Switzerland) with a spatial resolution of 28 µm [3]. A 4x4x4mm³ volume of interest was segmented and represented in 22x22x22 µm³ voxels. An individual, optimized threshold for every sample was used. The voxel meshes were used to determine the morphological anisotropy from the mean intercept length (MILmax/MILmin) [4]. The voxel meshes were converted to µFEA models, which were used to determine the mechanical properties [4]. The orientations of the 3 principal mechanical axes (fabric axes) were determined, as were the three elastic moduli, shear moduli and Poisson’s ratios. A tissue modulus of 5GPa was assumed for all specimens. One-way ANOVA was used to statistically analyze the data of the different age groups. Since in vivo all samples sites are loaded predominantly in axial compression, the samples of the vertebral body and the proximal tibia were combined. Hence, for each pig, mean values for three samples were calculated.

Results: The 3-D reconstructions showed clear differences in trabecular structure with age (Fig 1). Bone volume fraction increased significantly with age (Fig 2A). The morphological anisotropy was similar in bone specimens from the 6 and 23-week-old pigs, whereas the specimens from the adult pigs showed significantly higher anisotropy (Fig 2B). The average and the maximal elastic moduli increased significantly with age (Fig 3A). The other elastic constants did not show clear patterns in time. The angle between the main mechanical axis (maximal modulus) and the axial anatomic direction decreased with age, as did its standard deviation (Fig 2C).

![Image](image1.png)
![Image](image2.png)

Figure 1: 3D reconstruction of pig vertebral cancellous bone specimens at different ages. The specimens of the iliac epiphysis and metaphysis showed a similar pattern.

![Image](image3.png)

Figure 2: Bone volume fraction (A) and morphological anisotropy (B) for the bone specimens at different ages. *p<0.05 versus other age groups. Error bars indicate standard deviations.

![Image](image4.png)

Figure 3: Maximal elastic modulus $E_{\text{max}}$ (A), and angle of $E_{\text{max}}$ – associated axis relative to the primary load direction (B) for the bone specimens at different ages. *p<0.05 versus other age groups. Error bars indicate standard deviations.

Discussion: Our results show that during initial growth bone becomes denser by trabecular deposition, as was also found from histo-morphometry [2]. Relative to the weight of the animals, more bone is gained between 6 and 23 weeks than between 23 weeks and maturity. The increase in volume fraction effects in stiffer (hence also stronger) bone, particularly in the principal loading direction. However, this increase in stiffness is more directly related to body weight than volume fraction is. This points to a more efficient architecture in later years. This is confirmed by the development of morphological anisotropy, which only commences after 23 weeks. Hence, in the initial phase of growth the bone is relatively unorganized. This is nicely illustrated also by the angle between the principal stiffness and principal loading directions, which reduces towards maturity, pointing at a gradual alignment of the structure with the loads. The reduction of the standard deviation in these values strengthens this idea of gradual structural organization.

We propose that the trabecular structure, which is initially unorganized, is formed by the influence of mechanical stimuli to osteoblasts. In the early growth stage, when loads increase rapidly, bone is deposited rapidly as well, relative to which osteoclastic resorption is insignificant (modeling). In a later stage, when loads increase less rapidly, the formation rate also reduces, and approaches the resorption rate. Only then can the architecture be remodeled to an efficient, mechanically adapted structure. Until eventually, in adulthood, resorption and formation rates are balanced (remodeling).


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