ESRF	**Experiment title:** Evaluation of bone architecture on mice 3D High Resolution Computed Microtomography for studying the genetic determinism of immobilization-induced bone loss	**Experiment number:** LS-1393
Beamline:	**Date of experiment:** from: 10/11/1999 to: 15/11/1999	**Date of report:** 28/02/2000
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Report:

The purpose of this experiment was to study the genetic influence on a model of osteoporosis. For this, two strains of mice with different skeletal characteristics (bone mineral density high for the same body weight and similar bone size) were used. They were submitted to the model of bone loss by hind limb unloading produced by tail-suspension developed at the LBBTO. On each strain, three groups of distal metaphyses of femurs were constituted: basal control (BC), sacrificed at the beginning of the experiment, tail-suspended group (S) and attached non-suspended control (C) both sacrificed after a 14-d. period. On the different groups, the distal metaphyses of one femur was evaluated by histomorphometry at the LBBTO, and we imaged the distal metaphyses of the second femur using 3D SR μCT on ID19 at ESRF.

The images were acquired using a 6.65μm spatial resolution to get a good description of the trabeculae (approximately 20-30 μm for mice). The samples were placed in little polypropylene tubes with alcohol within a conical contention system that ensured they would not move during data acquisition. The energy was set to 18 keV. The experiment allowed to image 56 samples from the two strains C3H/HeJ (called here C3H) and C57BL/6J (called B6) distributed in the following groups:

- Strain C3H: 10 basal control (BC), 10 attached non-suspended control (C) and 10 tail-suspended (S), 21 days.
- Strain B6: 10 basal control (BC), 8 attached non-suspended control (C) and 8 tail-suspended (S), 14 days.

For practical reasons, the reconstruction was limited to a 512x512x600 region, corresponding to a cylinder of radius 3.4 mm and height 4 mm including the entire femur above the growth cartilage. Figure 1 shows a slice through a reconstructed volume in the same plane than the images used in histomorphometry. The white line indicates the trabecular region to be analyzed. Figures 2a) and b) and 3 show 3D views of cubic sub-volumes extracted from the trabecular region of interest (ROI) for the two strains of mice. It may be observed that strain B6 has thinner trabeculae than strain C3H. Parameters quantifying 3D bone architecture have to be computed to characterize bone loss in the two strains.

We selected a cubic trabecular sub-volume of 150x150x150 pixels (about 1 mm^3) within the ROI for each volume. We measured several morphological and topological parameters related to the architectural characteristics of bone samples. The results of the Bone Volume to Total Volume ratio (BV/TV), the mean trabecular thickness (Tb.Th) and the Euler Connectivity (CEuler), which is a 3D parameter related to the connectivity of trabecular bone are presented in Figure 3a) and b).

From these preliminary results, we can already announce the following conclusions:

- Strain C3H has thicker trabeculae and higher bone density (BV/TV) than strain B6.
- Both strains lose connectivity after suspension.
- Strain B6 has a substantial BV/TV diminution with suspension, while strain C3H does bot significantly change.

Future works on these data will consist of defining automatically larger non cubic ROIs of about 4 mm^3 in the trabecular region to get more representative parameters. In addition, new direct 3D parameters will be calculated, and the results obtained from 3D SR µCT will be correlated to histomorphometry.

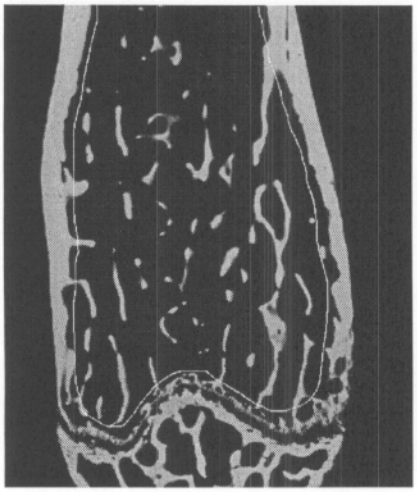
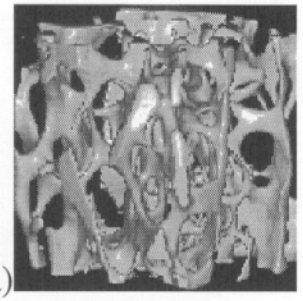
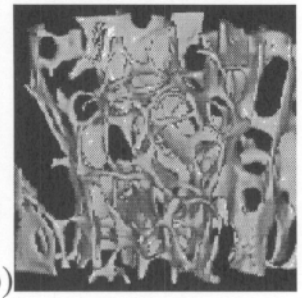


Figure 1:
slice reconstructed through a femur methaphyse volume

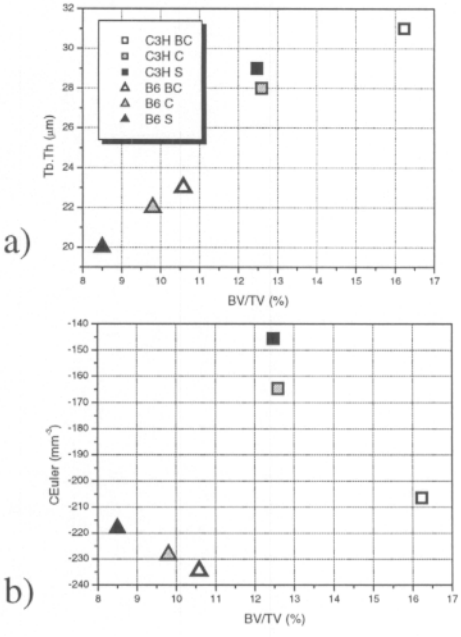


a) Strain C3H



b) Strain C3H

Figure 2: 3D displays



a)

b)

Figure 3 : Parameters (BV/TV, Tb.Th and Ceuler) computed on the three groups