INTRODUCTION:
Osteonecrosis of the femoral head is a disease where death of osteocytes with subsequent microarchitectural changes can lead to structural failure of the femoral head and secondary osteoarthritis of the hip joint. The purpose of this study was to investigate the changes in 3D microarchitecture and mineralization of trabecular and compact bone in the femoral head and acetabulum following osteonecrosis of the femoral head using high resolution QCT, digital radiography and standard histology.

METHODS:
Animals: Twelve unselected normal, male, 10-12month old New Zealand White Rabbits were purchased from a USDA-licensed dealer (Millbrook ImmunoServ, Inc., Amherst, MA). They were housed under standard conditions in accordance with guidelines from the Animal Care and Use Committee at Children’s Hospital Boston, MA.
Surgery: Under general anesthesia the left hip joint was approached laterally, and the femoral neck cauterized circumferentially. After ligation of the ligamentum teres, a 3 mm unicortical hole was drilled in the posterior aspect of the femoral neck, and the marrow cavity was cauterized briefly. The left hip in all of the animals was used for the production of osteonecrosis, the right side served as an internal control.
Tissue harvest: Six month post-surgery animals were sacrificed and the both proximal femurs as well as acetabuli were harvested, fixed in 10% NBF for seven days, and then transferred to 70% EtOH.
Radiography and micro-QCT: Digital radiographs of the bone specimen were taken on a Faxitron – MSX-20. Samples were then scanned on the eXplore M5 high-resolution QCT Scanner (GE Medical Systems). True Feldkamp reconstruction was used to reconstruct the images into 3D volumes. The reconstructed images were calibrated for air, water and hydroxyapatite (1024mg/cc). The resulting images have an isotropic resolution of 16 microns. To obtain accurate 3D data sets, micro-CT images were segmented using individual optimal thresholds corresponding to the point of inflexion between the marrow and the bone peak in the histogram. In subchondral compact bone in the femoral head and the acetabulum, the following parameters were determined: Thickness, Porosity – determined as void space in subchondral bone, volumetric Bone Mineral Density (vBMD: mg of mineral per cm³ tissue), Bone Mineralization (BM: mg mineral per cm³ bone) – determined as vBMD excluding void-voxels (vBMD*), normalized to Bone Volume Fraction (BVF); BVF=vBMD*/BVF. In the femoral head, measurements were taken in eight, and in the acetabulum in 16 standardized locations. In the trabecular region of the femoral head and the acetabulum, the following parameters were determined: Bone Volume Fraction (BVF), Trabecular thickness (Tb.Th.), Trabecular Separation (Tb.Sp.), Trabecular Number (Tb.N.), Bone Surface to Bone Volume Fraction ratio (BS/BV), Connectivity (Euler), vBMD, BM.
Histology: Specimen were decalcified in 15% formic acid containing 10% NBF, processed, embedded in paraffin, sections cut and stained with HE and Safranin-O. Statistical Analysis: Paired Student t-test (two-tailed) by Graph Pad Prism 4 software was used to evaluate significance between the groups. Statistical significance was set at the 95% confidence limit.

RESULTS
Digital-Radiography: Increased radiodensity could be observed in the osteonecrotic femoral head. Subchondral sclerosis could be observed in the osteoarthritic acetabulum.
Micro-QCT - Femoral Head: Volumetric BMD as well as the BVF were significantly increased in the trabecular region of the osteonecrotic femoral head. There were less and thicker trabeculae, with less separation and a lower bone surface to bone volume ratio. However the average BM was decreased in the osteonecrotic (ON) trabecular region. Thickness, porosity and mineralization were relatively constant throughout the entire normal subchondral bone in the femoral head. Thickness of the ON subchondral bone varied, but was on average thicker. The porosity of the ON-subchondral bone was significantly increased, and mineralization was (BM) decreased.
Micro-QCT - Acetabulum: There were regional differences in thickness, porosity, vBMD and mineralization (BM) of the subchondral bone in the normal acetabulum. The subchondral bone in the osteoarthritic (OA) acetabulum was thicker, had a higher porosity, lower vBMD and was less mineralized (BM), when compared to normal. In the OA acetabulum BVF and vBMD of the subchondral cancellous bone were significantly increased, but BM was less.

DISCUSSION:
Here we report for the first time the complex changes in 3D microarchitecture and mineralization in compact and trabecular bone of the entire femoral head and acetabulum, that take place during the repair of osteonecrosis of the femoral head and subsequent osteoarthritis of the acetabulum. Increased density observed on x-ray in the ON femoral head can be explained by an increase BVF and subsequent increase in vBMD in the cancellous region of the femoral head. However average BM is less, which could be explained by the fact that the newly formed bone is less mineralized than mature bone. Resorption of the dead subchondral bone in the femoral head increased its porosity and may weaken is structural properties. Subchondral sclerosis of the acetabulum as seen on x-ray, may be the result of an increase in undermineralized subchondral cancellous bone, which resulted in a net increase in subchondral cancellous vBMD. Subchondral bone in the osteoarthritic acetabulum is however thicker, more porous, less mineralized, and results in a net decrease in subchondral vBMD. We hope, our findings will further the understanding of osteonecrosis and osteoarthritis.