Introduction: Bone is a composite porous material with two functional levels of porosity: the vascular porosity and the lacunar-canalicular porosity that surrounds the osteocytes. It has been proposed that an alteration of the interconnectedness of the lacunar-canalicular network due to osteocyte death via apoptosis could affect interstitial fluid flow and alter the mechanical stimulus experienced by bone cells [1,2]. In recent work, we quantified bone porosities of normal and osteopenic rats in the tibia mid-diaphysis and found no significant differences between groups [3]. Because several studies have reported bone loss due to ovariectomy in the metaphyseal region of the rat tibia [4,5], the aim of this study was to further analyze the porosity and microstructure of normal and osteopenic bone using confocal and scanning electron microscopy.

Materials and Methods: Female Sprague Dawley rats were divided into two groups, with one group undergoing ovariectomy (OVX) at 5 months of age. Six weeks post-ovariectomy the OVX group (n=6) and the age-matched control (CTRL) group (n=6) were sacrificed, as approved by the IACUC. Tibiae were harvested and put in fixative for 24 hours. Metaphyseal sections were cut 2 mm below the growth plate and ground to a thickness of 50-80 μm. To label interstitial fluid space, sections were stained for 4 hours in a 1% FITC solution. Bone sections were visualized using a confocal microscope (Leica TCS SP2) with a 40x oil immersion lens and wavelength excitation of 488 nm. Images were taken at a resolution of 2048 x 2048 pixels, and a complete reconstruction of each tibial cross-section was obtained using Photoshop. Images were thresholded using Otsu’s method (ImageJ), and the total porosity (TP) was calculated as the percentage of bone area labeled with FITC. The vascular porosity (VP) was calculated by quantifying the total vascular pore area and dividing by the total bone area. The lacunar-canalicular porosity (LCP) was calculated as the difference between TP and VP. Comparisons between groups were statistically tested by student’s t-test (p<0.05).

To determine the distribution of mineral content, three sections from each group were visualized using an environmental scanning electron microscope in backscatter mode (FEI Quanta 600).

Results: The metaphyseal cross-sections showed uniform staining of the vascular and lacunar-canalicular systems, with the mineralized matrix impermeable to FITC. The high resolution images showed a compacted coarse-cancellous bone structure [6] consisting of older cancellous bone filled in with newer compact bone (Figure 1a). Because compacted coarse-cancellous bone represents an important aspect of bone growth [6], we separately quantified the ‘cancellous’ and ‘compact’ bone areas, and calculated the LCP of both regions.

Discussion: The differences in VP and LCP between the CTRL and OVX groups demonstrate a direct influence of osteopenia on bone macro- and micro-porosities. As revealed by the high-resolution images, metaphyseal sections from both groups present a compacted coarse-cancellous bone structure, consisting of two distinct patterns. The osteopenic group had more of the older ‘cancellous’ regions, although the LCP within this area had a similar porosity to the CTRL group. The filled-in ‘compact’ bone regions in the OVX group seemed to compensate for the greater presence of ‘cancellous’ regions by having a higher LCP than the ‘compact’ regions of the CTRL group. Interestingly, areas with a higher LCP had lower mineral density, while areas with lower LCP had higher mineral density, demonstrating that newer bone had a more extensive lacunar-canalicular network.

Acknowledgements: NIH/NIAMS (AR052866).