**Introduction** One potential cause of bone loss during osteoporosis is the incorrect sensing of bone strain by osteocytes, which may be influenced by the size and shape of osteocytes and lacunae. Previous studies addressing the size of lacunae in osteoporotic and normal individuals have been contradictory (1,2,3), and were limited to planar measurements on iliac crest biopsies. This study was designed to determine whether there are differences in the size or shape of osteocyte lacunae between osteoporotic and normal individuals, using 3-d confocal images of lacunae sampled from a site of high fracture risk.

**Methods** Trabecular bone specimens were obtained from 8 females 76 or 77 years old. Four subjects had tissue removed during arthroplasty following a non-traumatic hip fracture. The remaining specimens were obtained from individuals who died without fracture. The trabecular bone cube specimens were taken from the same relative location in the femoral head: near the superior surface of the neck, aligned with the principal trabeculae. This location was adjacent to the fracture site in most of the fracture patients. One additional cube was obtained from a human femoral head and used only for a calibration study. The specimens were fixed in 70% ethanol, stained with basic fuchsin (4), and embedded in methyl methacrylate.

Six sections from the calibration cube were ground and polished to thicknesses of 6 and 30 microns, mounted to slides using Permount, and imaged using a Bio-Rad MRC600 confocal microscope. Scans were obtained through the entire thickness at multiple bone regions, at 1 micron intervals. For each region, the number of images between two standardized brightness levels was calculated, which corresponded to a relative thickness of the bone region. The slope of the best-fit line between the measured and maximum calculated thicknesses, 0.8928, was determined as the correction factor for the depth. The R^2 value was >0.99, providing confidence that this method yielded a consistent correction factor.

Cover slips for the experimental samples were prepared by marking axes and random locations using a diamond scribe. The bone specimens were sectioned, polished, and mounted to the prepared cover slips using Permount. When dry, the specimen/cover slip constructs were loosely attached to slides with caulk. The specimens were then imaged as described above from the surface to a corrected depth of 22-27 microns, at .4464 micron intervals. The images appeared as shown in figure 1 for a small region.

The lacunae which were well-stained and completely imaged were analyzed, resulting in 29 from the osteoporotic group and 15 from the control group, with three to eleven lacunae from each individual. Otsu’s adaptive thresholding algorithm (5) allowed correction for brightness differences with depth. Image processing algorithms were used on each lacuna to close and smooth the contours. The principal axes of each lacuna were determined, and the maximum extent of the lacuna in each of these dimensions was calculated (actual dimensions). The dimensions of the ellipsoid that has the same volume as the maximum extent of the lacuna in each of these directions was calculated (ellipsoid dimensions).

Histograms of the ellipsoid dimensions, the actual dimensions, and the maximum dimension of the lacuna of interest were plotted. The results are shown in figures 2 and 3.

**Discussion** These results suggest that lacunae have the same maximum length in osteoporotic and normal individuals, but are more anisotropic in those with osteoporosis. Risk factors for osteoporosis may be related to the anisotropy of the lacunae and the potential variation in local mechanical conditions. These results are particularly intriguing when considered with the work of Ciarelli (6), which determined that the trabecular structure was also more anisotropic in individuals with osteoporosis.

**Previous studies** (1,2,3) have produced conflicting results regarding lacunae size differences between osteoporotic and normal individuals. This study deviates from their methods in two important ways. By measuring these parameters near the fracture site, we make no assumptions regarding the variation in lacuna measurements with anatomic location. In addition, measurements in three dimensions require no assumptions about the shape or orientation of the lacunae to satisfy the rules of stereology.

Continuing studies are focused on the potential structure/function implications of the contrast in lacunae shape and further exploration of these factors which may separate patients at high risk of fracture from normals.

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**References**

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