INTRODUCTION:
Bisphosphonates (BPs) are commonly prescribed drugs for the treatment of osteoporosis, a condition associated with increased fracture risk. BPs inhibit bone resorption and decrease bone turnover and bone loss. Long-term use of BPs may be associated with changes in the mechanical integrity of the bones of some patients. Odvina et al [1] were the first to report on a group of nine patients who suffered spontaneous non-vertebral fractures with delayed or absent fracture healing while on long-term (3 to 8 years) alendronate, a commonly prescribed BP. The patients received alendronate either alone or in combination with estrogen or prednisone, resulting in a condition of severely suppressed bone turnover (SSBT). Several cases and case series have since been published.

The quality of bone tissue in patients with low-energy fractures is not completely understood. Mineralization variability at the micrometer scale level may be related to the mechanical behavior of bone tissue, for example in the ability of the material to prevent crack propagation [2].

In this study, we report mineralization densities of trabecular bone regions from iliac crest biopsy specimens measured using quantitative backscattered electron microscopy (qBSE). We hypothesize that the mineralization densities at the microscopic level are different in SSBT compared to young and old normals and untreated osteoporotic patients.

METHODS:
Mineralization measurements were made using qBSE microscopy on 55 human iliac crest biopsy specimens. All biopsies were from female patients or normal subjects with the exception of one male SSBT patient. Biopsies were from 12 SSBT patients (aged 49-77y), 11 age-matched osteoporotic patients with vertebral fracture (aged 53-76y), 12 age-matched normal subjects (aged 49-74y), and 20 young normal subjects (aged 20-40y). SSBT biopsies were from patients reported in two publications [1,3]. The three comparison groups (osteoporotic fracture patients, and the young and age-matched normal subjects) were not treated with BPs. Biopsies were prepared at Henry Ford Hospital (Detroit, MI) and Southwestern Medical Center (Dallas, TX), where they were embedded in poly methyl-methacrylate (PMMA) and analyzed for histomorphometry. The blocks were cut to expose bone, and the cut surfaces were polished to a mirror finish with successively finer grades of carborundum paper and polishing powders (0.05 μm particle final polish). To make the surfaces electrically conductive, the blocks were sputter coated with carbon.

Quantitative backscattered electron microscopy (qBSE): Grayscale levels in backscattered images of bone have been shown to correlate with atomic number and mineral content [4]. The bone specimen blocks were scanned at 300× magnification (Figure 1) using a 30-kV excitation voltage and 15 mm working distance (Philips XL 30 SEM). Area of each region of interest for mechanical property measurements. Collagen orientation, collagen cross-linking, and other factors, which are not detected by qBSE, may contribute to the variability in mechanical properties.

RESULTS:
Mean mineral content was calculated as the area-weighted average atomic number of the five backscattered images collected for each biopsy. The main effect of group was significant for mean mineral content (Figure 2, ANOVA, p=0.018). Tukey post-hoc pair-wise analysis did not yield significant differences between pairs, however two pairs—SSBT/age-matched normals, and SSBT/osteoporotics—showed marginally insignificant differences (p=0.059 and 0.062, respectively).

Post-hoc analysis was performed using Student’s t-test, these groups differed significantly (p<0.05). Standard deviation of mineral content was not different among groups (p=0.08), but high mineral content was associated with greater variation in mineral content (Figure 3, R2=0.20, p=0.001). Mean mineral content was not correlated with bone formation rate measured previously [1, unpublished data]. Average micro-mechanical elastic modulus and hardness measured previously by nanoindentation were not correlated with mean mineral content (p=0.76 and 0.36, respectively). One SSBT biopsy outlier (more than 2.4 standard deviations greater than the group mean) was excluded from the statistical analyses.

REFERENCES:

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Figure 1: Backscattered electron image of a single trabecula. Regions of high mineral content appear lighter (higher grayscale level).

Figure 2: Overall, mean atomic number was different among groups (ANOVA, p=0.018). Asterisks (*) denote groups that were significantly different from SSBT (post-hoc Student’s t test).

Figure 3: Mean atomic number correlated significantly with standard deviation of atomic number (R2=0.20, p=0.001).