Introduction: Measurement of the effects of osteoporosis and anti-resorptive drug treatment using whole bone testing, or testing of volumes of ovariectomized cancellous bone, have revealed a decrease in macro-level bone strength during osteoporosis that is counteracted by drug-treatment [1-3]. However, these macro-level changes are primarily due to changes in bone mass rather than changes in bone tissue composition. We previously reported that tissue-level stiffness of ovariectomized rat bone is increased by 40-90% and that anti-resorptive drug treatment inhibits this increase and tissue-level properties remain the same as control levels. Such microstructural changes in mechanical behavior suggest that a corresponding change in the composition of the tissue should ensue [4]. Conflicting data exists regarding the osteoporotic bone tissue composition; some studies report that the mineral content is unchanged or slightly lower during osteoporosis [5,6] whereas others report an increase in the mineral content and a lack of collagen [7,8,9]. However, the mineral content of individual trabecula from ovariectomized bone is unknown and ideas regarding the causes of increased tissue-level stiffness are conjecture.

In this study we test the hypothesis that during ovariectomy-induced bone loss an increase in bone mineral content at the tissue-level occurs and that drug treatment inhibits this increase. We compare the local mineralization of single trabeculae from normal, ovariectomized and Tibolone-treated bone tissue using micro-CT images calibrated for bone mineral content assessment.

Methods: Three groups of 44 week old female Wistar rats were treated as follows: 1) a control group was sham operated and treated with vehicle (placebo-treated), 2) a group was ovariectomized (OVX) as a model of osteoporosis and treated with vehicle, and 3) an OVX group was treated orally with tibolone (2 mg/kg, d). As treatment in the current study tibolone was used. Tibolone is a tissue-selective compound with estrogenic effects on bone, vagina and brain. In a 2.5 mg dose it has been used for the prevention of bone loss [10]. After 54 weeks treatment mineral content was determined from the tissue of two animals from each group using μ-CT scanning. Bones were scanned using a GE eXplore Locus SP Pre-Clinical Specimen MicroCT (GE Medical Systems) operated at a 13-μm isotropic voxel resolution. Hydroxyapatite (1.13 g/cm³) was included in each scan to provide reference values for tissue density calculations. Beam hardening and flattening effects were minimized using hardware included in the system and scan optimization. Bone tissue was segmented from non-bone tissue using the thresholding algorithm provided by the μ-CT manufacturer. The output density data (Hounsfield Units) was converted to mineral content g/cm³ using the density data from the phantoms. Previous experiments have been performed to calibrate the system for bone mineral content assessment by comparing ash content (g/cm³) of mouse cortical bone to values calculated from μ-CT data (Hounsfield Units) and phantom data [11]; in these experiments it was reported that this μ-CT system could detect differences in mineral content that were comparable to superior to gold standard ash content values. Mineral content measures were determined from individual trabeculae (n=6 per group) that were selected for analysis and conformed to a volume of interest. Statistical analyses (Student’s t-test) were performed to analyze the effect of the treatments (ageing, OVX and tibolone treatment) on the bone mineral content.

Results: Significant increases were found in the bone mineral content (g/cm³) of the OVX bone tissue as compared to control (0.93 g/cm³ ± 0.005 g/cm³ vs. 0.84 g/cm³ ± 0.01 g/cm³; p = 0.05). The difference found between the mineral content (g/cm³) of the OVX and tibolone-treated bone tissue was significantly different (0.93 g/cm³ ± 0.005 g/cm³ vs. 0.86 g/cm³ ± 0.002 g/cm³; p = 0.03). There was no significant difference found between the density of the control and the tibolone-treated bone tissue (0.84 g/cm³ ± 0.01 g/cm³ vs. 0.86 g/cm³ ± 0.002 g/cm³; p = 0.33).

Conclusion: We have observed that an increase in mineral content of bone tissue confirms earlier findings of increased mechanical properties at the level of the bone trabeculae. These findings provide evidence that during osteoporosis an increase in mineral content occurs to strengthen bone at the tissue level. Whether this is a cause or an effect of loss of structural strength is yet to be determined.

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** Centre for Bioengineering, Trinity College Dublin, Ireland
*** Orthopedic Research Laboratory, Erasmus University Rotterdam, The Netherlands
**** Dept. of Pharmacology, N.V. Organon, Oss, The Netherlands

Figure 1: (a) Micro-CT image of bone obtained at 13 μm resolution (b) Individual trabecula chosen for mineral content analysis

Figure 2: Effect of Ovariectomy and anti-resorptive drug treatment on the Mineral content of rat trabecular tissue