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Original Article

Hylonome

Bone tissue material properties are altered during osteoporosis

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Introduction. Measurement of the effects of osteoporosis on the mechanical behavior of bone has been performed using whole bone testing, or testing of volumes of ovariectomized cancellous bone[1-3] and these studies revealed a decrease in macro-level bone strength. However, it is not clear whether these changes were due only to the reduction in bone mass or whether a reduction in tissue strength also contributed. A number of studies report that the mineral content is unchanged or slightly lower in the osteoporotic bone tissue[4] or that there is an increase in the mineral content and a lack of collagen[5-7]. Such microstructural changes suggest that a corresponding change in the mechanical behavior of the tissue should ensue during osteoporosis. However, to date, the tissue level properties and tissue level mineral content of osteoporotic trabecular bone have never been measured and so such ideas remain conjecture.

Methods. In this study we test the hypothesis that a change in bone properties and bone mineral content occurs at the tissue-level during osteoporosis.

Two groups of 44-week-old female Wistar rats were either sham operated or ovariectomized (OVX) and followed for up to 54 weeks. After sacrifice individual rod-like trabeculae (n ~ 3 per tibia) were excised from the metaphysis of the right proximal tibia under a stereomicroscope and stored in saline before testing. A novel method was developed to carry out micro-tensile testing on individual trabeculae, which minimized errors due to misalignment and stress concentrations at the grips. Finite element analyses of the trabeculae were developed at micro-resolutions to calculate the tissue levels strains and the stiffness of the tissue was determined from these mathematical models.

After 54 weeks of treatment mineral content was determined from the tibiae 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/cm^3) was included in each scan and the specimens were immersed in water (0 g/cm^3) to provide reference values for mineral content 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) were converted to mineral content g/cm^3 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/cm^3) of mouse cortical bone to values calculated from μ-CT data (Hounsfield Units) and phantom data[8]; in these experiments it was reported that this μ-CT system could detect differences in mineral content that were comparable or 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, see Figure 1. 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. We found significant increases ($p<0.05$) in the yield strength and stiffness of trabecular tissue compared to normal ageing at 14, 34 and 54 weeks post-ovariectomy. The results are summarized in Table 1. The tissue stiffness of the ovariectomized group was increased by 40–90% of the values of the control sham-operated tissue.

Significant increases were found in the bone mineral content (g/cm^3) of the OVX bone tissue as compared to control after 54 weeks treatment (0.93 g/cm^3± 0. 005 g/cm^3 vs. 0.84 g/cm^3 ± 0. 01 g/cm^3; $p = 0.05$).

Discussion. In this study it was shown that following ovariectomy, while overall bone mass and bone mineral density are reduced, the material properties at the tissue-level increase by 40-90% and the mineral content at the tissue-level increases by 11%. These results are confirmed by a study that

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	Treatment		p
Test Group	Control	OVX	value
4 Weeks			
Yield Strength (MPa)	33.9 ± 23.3	31.9 ± 21.0	0.42
Elastic Modulus (GPa)	2.31 ± 1.24	3.11 ± 2.13	0.15
14 Weeks			
Yield Strength (MPa)	50.7 ± 44.3	51.1 ± 25.0	0.49
Elastic Modulus (GPa)	2.67 ± 2.05	5.11 ± 3.89	0.05
34 Weeks			
Yield Strength (MPa)	23.2 ± 16.9	50.8 ± 19.9	0.01
Elastic Modulus (GPa)	2.61 ± 0.79	3.66 ± 1.95	0.05
54 Weeks			
Yield Strength (MPa)	34.1 ± 15.9	81.7 ± 43.4	0.03
Elastic Modulus (GPa)	2.81 ± 2.09	4.23 ± 2.86	0.18

Table 1. Effect of ovariectomy on the yield strength and elastic modulus of rat trabecular tissue.

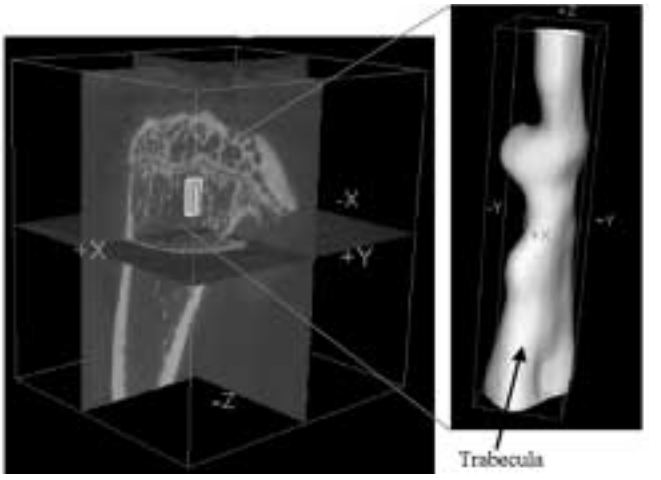

Figure 1. **(a)** Micro-CT image of bone obtained at 13 μm resolution **(b)** Individual trabecula chosen for mineral content analysis.

showed, using Back Scattered Scanning Electron Microscopy, that estrogen suppression increases the proportion of bone in the iliac crest with higher mineral content and that tibolone increases the proportion of bone with lower mineralization[9].

The precise mechanisms by which an increase in tissue-level properties can occur following ovariectomy are unclear. It may be that estrogen deficiency itself leads directly to changes in tissue properties, perhaps through increasing the degree of mineralization of the tissue. Alternatively, the observed increase may be a compensatory mechanism triggered by the loss of neighboring trabeculae, whereby the mechanical properties of the remaining trabeculae are altered by a shift in mineral content to counteract loss of structural strength. By comparing the local mineralization of single trabeculae from normal and ovariectomized bone using micro-CT images calibrated for bone mineral density assessment, we confirm that a change in bone tissue mineralization occurs at the local level. This data provides 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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