INTRODUCTION: Bone remodeling renews and adapts trabecular bone in a coupled process of osteoclastic bone resorption and osteoblastic bone formation. Osteoporosis is associated with an imbalance of the remodeling process whereby excessive osteoclast resorption occurs without adequate new bone formation. Conventional hormone treatment (HT) drugs prevent this imbalance by inhibiting osteoclast activity and reducing bone resorption [1].

The efficacy of drug treatments has been tested using rat models. Using ovariectomy to induce osteoporosis, it has been found, for example, that treatment with Tibolone [2,3] and estrogen/Risedronate [4] increases the trabecular bone strength by maintaining bone mass and trabecular architecture. A concern with some HT drugs is that they inhibit the remodelling process resulting in an increase in the amount of old bone, so that, although bone mass is maintained, bone strength at the tissue level may be impaired.

To investigate whether or not this is the case, we have developed a method to evaluate the effect of drug treatment on the mechanical strength of single bone trabeculae. A micro-tensile testing apparatus was developed that minimizes errors due to misalignment and stress concentrations at the grips. We use the method to test the hypothesis that the strength of single trabeculae will differ for normal, ovariectomized, and drug treated rat bones over the course of ageing.

METHODS: Three groups of ten-month-old female Whistar rats were treated as follows: (i) a ‘sham operated’ control group, (ii) a group that were ovariectomized (OVX), and (iii) an OVX group that was treated with a suboptimal dose of Tibolone. Bones were harvested after 0, 4, 14 and 34 weeks of treatment. Sections of cancellous bone were cut from the right proximal tibiae and were cleaned of marrow using a water jet. Using a scalpel blade and forceps individual trabeculae (~3 per tibia) were excised under a stereomicroscope and stored in normal saline before testing.

An MTS Tytron 250 testing machine, capable of applying loads to a resolution of 0.001 N, was used to perform micro-tensile testing of the trabeculae, which are typically 100 µm in diameter. This system was modified by customizing a microscope assembly and a grip system. The procedure for gripping trabeculae used the shafts of hypodermic needles (inner diameter = 0.3 mm) as grip rods. A close tolerance fit between an alignment sleeve and the grip rods ensured co-axiality of the grip rods. The specimen was aligned using a cyanoacrylate adhesive. The specimen was aligned under 30X magnification, see Fig. 1.

RESULTS: There was no significant difference found between the yield strength of control specimens at 0, 4, 14 and 34 weeks (p=0.27, ANOVA). Ovariectomy caused a significant increase in the yield strength of bone tissue relative to the control over the course of 34 weeks (p=0.015, ANOVA). Compared to the control, the yield strength of OVX bone tissue was significantly higher at 34 weeks [50.4MPa ± 24.7MPa (n=9) vs. 19.2MPa ± 13.9MPa (n=6); p = 0.0014]. The yield strength of OVX was significantly higher than the drug-treated group at both 14 weeks [51.1MPa ± 25.0MPa (n=8) vs. 19.2MPa ± 19.6MPa (n=7); p = 0.008] and 34 weeks [50.8MPa ± 19.9MPa (n=9) vs. 27.9MPa ± 13.4MPa (n=10); p = 0.005]. However, there was no significant difference between the yield strength of the control relative to the drug treated tissue over the course of 34 weeks treatment; therefore the drug-treatment maintained bone strength near normal levels (Fig. 2). Looking at the strength of the trabeculum as a whole, it is found that the drug-treated is stronger than the control after 34 weeks treatment, though not as strong as trabeculae from the ovariectomized bone (Fig. 3).

DISCUSSION: This is the first study to report the effect of drug treatment on single trabeculae. Our results indicate that ovariectomy in rats increases the strength of the mineralized tissue constituting trabecular bone relative to normal, and that drug treatment inhibits this increase and normalizes it to control levels; we found this inhibition to be significant at 14 and 34 weeks. However, a limitation of this study is that the choice of test specimens was biased; thicker trabeculae were chosen in order to facilitate gripping and alignment of micro-specimens.

Since previous studies showed that drug treatment improves the structural mechanical behaviour of osteoporotic bone [2,3,4], the implication from this study is that the increase found in such studies is due only to increase in bone mass, not to an increase in the strength of the mineralised matrix constituting the trabecular tissue itself.

It is interesting that ovariectomized bone tissue is stronger than both normal and drug-treated. This agrees with the results of a study that found that trabecular bone samples from subjects with osteoporotic fractures had a higher stiffness relative to their volume fraction than subjects without disease or fracture [5]. It is intriguing to speculate that the increase in the yield strength from ovariectomy is due to some mechanism whereby bone attempts to compensate for the decrease in bone mass by altering the mechanical properties of the tissue, perhaps through increasing the degree of mineralization of the tissue. This may suggest that the tissue has become more brittle, which may be less functional in responding to loads; this is an aspect that can be examined in future studies.

In conclusion, by measuring the tensile strength of individual trabeculae, we have found that the bone tissue of trabeculae is significantly stronger in osteoporotic bone compared to normal, and that Tibolone treatment normalizes the strength to normal levels.

REFERENCES

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ACKNOWLEDGEMENTS: Funded by EU project “MIAB”