# Effects of bone anabolic treatments on cortical bone porosities in a mouse model of osteoporosis

**Elisabeth Millard[1], Bryant Roberts[1,2], Enrico Dall'Ara[1,2]**

[1]Insigneo Institute for *in silico* Medicine, [2]Department of Oncology & Metabolism, University of Sheffield, UK

INSIGNEO
Institute for *in silico* Medicine

## Background

In an ovariectomized (OVX) mouse model of osteoporosis (OP), combining the bone anabolics parathyroid hormone (PTH(1-34)) and mechanical loading had increased benefit to cortical bone properties than in independent treatments alone [1]. However, due to the limited voxel size of *in vivo* microCT used in that work, the networks of intracortical porosities such as the osteocyte lacunae and vascular canals could not been accurately resolved. Thus, it remains unclear what the individual and combined effects of these bone anabolics are on these small features of the bone.

The aim of this project was to quantify the combined and individual effects of PTH(1-34) and mechanical loading on tibia intracortical porosities in the OVX mouse model of osteoporosis.

## Methods

30 female C57BL/6 mice were included. 26 mice were ovariectomized at 14-weeks old and were randomized into five groups (n=4-6/group): untreated mice **[OVX]**; or treated with **[PTH]** PTH(1-34); **[ML]** mechanical loading; **[ML+PTH]** concurrent treatment; and **[ML+PTH$_{alt}$]** alternating treatment of PTH(1-34) and mechanical loading;. There were 4 untreated, intact, wild type controls **[WT]**. Mice were treated and the tibia µCT-scanned (10.4µm voxel size) per the below schedule (Fig. 1). After 24 weeks of age, the mice were sacrificed and the tibia synchrotron radiation µCT (SR-µCT) scanned (1.62µm voxel size). SR-µCT images were registered to the µCT scans to identify the cortical midshaft which was cropped for further analysis (Fig. 2). Then, the SR-µCT images were processed (including despeckling to reduce noise and ROI-shrink rap to generate a region of interest (ROI) to generate the cortical region of interest and isolate intracortical pores (Fig. 3) For example, a despeckling operation was implemented to reduce noise and ROI-shrink rap to generate a region of interest (ROI). Intracortical pore properties were quantified using software CTAn (Skyscan-Bruker, Kontich, Belgium), including: tissue volume (TV), vascular canal volume (CV), osteocyte lacuna volume (Lc.V), number of lacuna (Lc.N). Differences in pore properties among the treatment groups were evaluated using one-way ANOVA in GraphPad (GraphPad Prism 8.0, San Diego, USA) was used to for statistical analysis. Statistical significance was set to p<0.05.

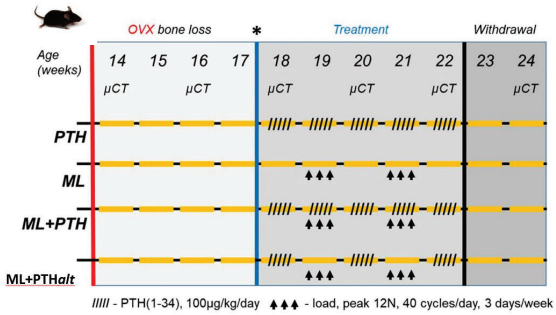



**Figure 1.** Anabolic treatment schedule for the ovariectomized mice

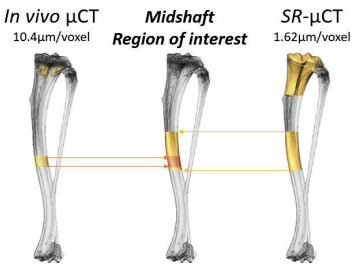



**Figure 2**. SR-µCT images were registered to the µCT scans to identify cortical midshaft (in red).

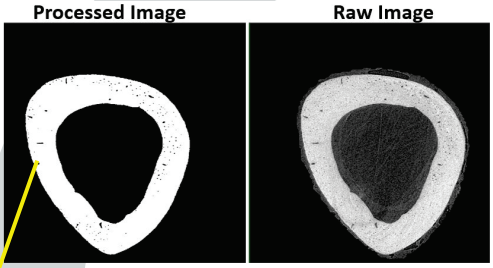
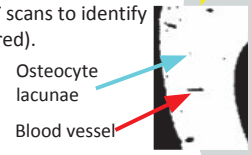



**Figure 3 (above).** Raw and processed SR-µCT images. ROIs were created for cortical bone, blood vessels and osteocyte lacunae. Intracortical porosities were then measured.
**Left.** Magnified subregion of the processed image showing blood vessels and osteocyte lacunae.




## Results

Representative models of cortical bone and intracortical pores from each group are shown in Fig 4.

**Cortical bone parameters (Fig. 5A):**

- Sig. ↑ TV in ML+PTH and ML+PTH$_{alt}$ than OVX

**Blood Vessel parameters (Fig. 5B):**

- Sig. ↑ CV in ML+PTH than OVX

**Osteocyte Lacunae Parameters (Fig. 5C):**

- Sig. ↑ in Lc.V and Lc.N in ML+PTH and ML+PTH$_{alt}$ than OVX
- **Sig.** ↑ in percent lacunae volume in ML+PTH$_{alt}$ than OVX/WT

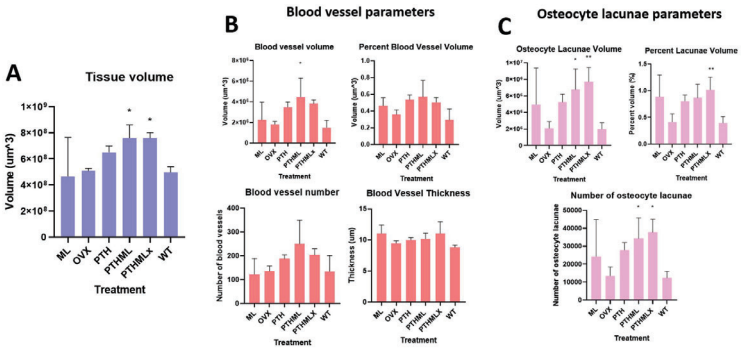



**Figure 5.** Graphs displaying mean and standard deviation (error bars) for midshaft intracortical pore properties in the six study groups. (A) tissue volumes (B) blood vessel parameters, (C) osteocyte lacunae parameters. *p<0.05 compared with OVX. **p<0.001 compared with OVX.

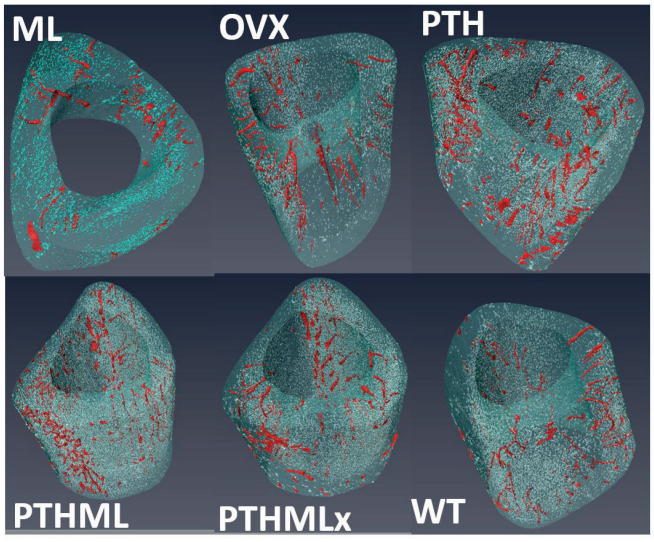



**Figure 4. (above)** Cortical bone midshaft (blue) from the six study groups. Blood vessels are shown in red, osteocyte lacunae in white

## Conclusion

The results suggest that combined and alternating treatments of mechanical loading and PTH have the greatest effect on bone intracortical porosities in an oestrogen-deficient mouse model of OP. This data extends upon previous research where micro-/nano- structures were unable to be accurately resolved due to limited resolution. In the future, it will be interesting to understand how intracortical porosities differ across the length of the bone and how these impact on mechanical properties of bone tissue.

## Acknowledgements

This research was partially funded by EPSRC grants EP/K03877X/1 and EP/S032940/1 and NC3Rs grant NC/R001073/1. The authors acknowledge the Diamond Light Source beamtime on the I13-2 beamline (proposal MG21628-1). EM was supported by a stipend for undergraduate research from the Insigneo Institute.

**References**

[1] Roberts, BC *et al.*, 2020. *Sci Rep.* 10(1):8889

The University Of Sheffield.

Sheffield Teaching Hospitals NHS
NHS Foundation Trust