

## **Raman Spectroscopy Demonstrates PTH Prevention Of Radiation-Induced Damage On Bone Mineral Properties And Blunting Of Bone Matrix Damage In An Irradiated Mouse Model**

Gong B, Oest M, Mann K, Damron T, Morris M.

SUNY Upstate Medical University and University of Michigan Medical Center

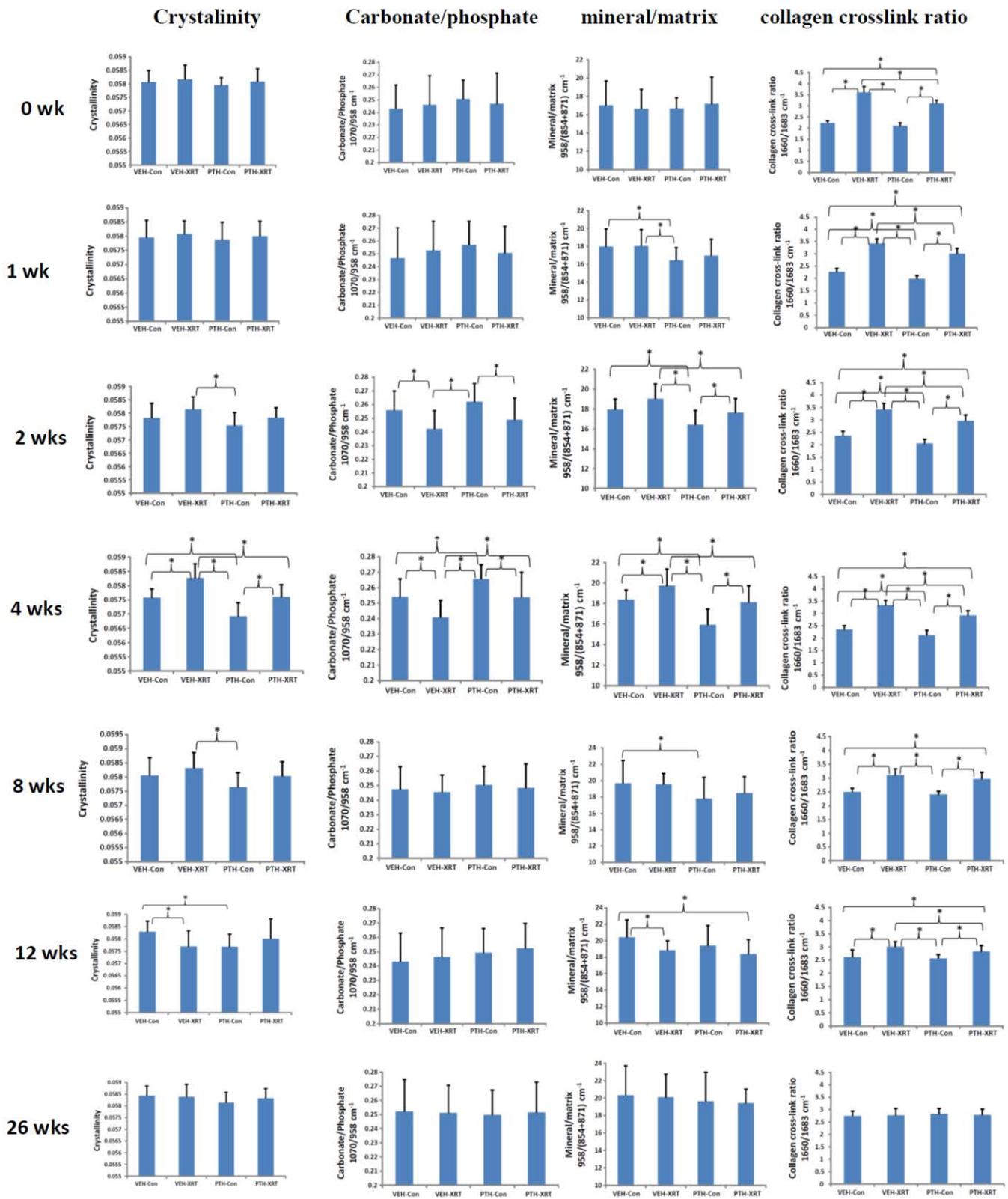
**Background:** Post-radiation fractures after radiotherapy are prevalent in specific anatomic locations and may lead to devastating complications including amputation in extremity sites such as the femur after treatment for soft-tissue sarcoma. Lack of progress in developing strategies for prevention or treatment is limited by poor understanding of underlying pathophysiology and presents a particular challenge to clinicians currently. Recombinant parathyroid hormone (PTH (1-34)) is an approved anabolic hormonal therapy with demonstrated benefits to improve bone quality for osteoporosis. Intermittent administration of PTH enhances the bone regeneration process, maintains more functional and heterogeneous collagen orientation and increases bone mineral density. In this study we examine the effects of radiotherapy and PTH administration on bone chemical composition, which is an important predictor of bone functional quality. We use Raman spectroscopy, which provides measures of bone mineral composition, bone matrix composition, and collagen cross-linking and can be used on fresh as well as on fixed and embedded bone tissue. We employ a mouse hind limb irradiation model to study effects of PTH intervention on radiation-induced bone damage as reported by changes in bone chemical composition measured by Raman spectroscopy. We hypothesize that PTH mitigates radiation-induced bone damage that causes measurable changes in chemical composition, especially collagen cross-linking, that have been previously linked to adverse changes in bone biomechanical parameters.

**Methods:** Female BALB/F mice aged 12 weeks underwent 4-day fractionated X-ray irradiation on left tibiae at daily 5 Gy doses (4×5Gy). From the first day of radiation the mice received daily subcutaneous injections of either PTH (1-34) or a vehicle (VEH) for eight weeks (5 days/week). The non-irradiated right tibiae (0 Gy) were used as controls. The mice were euthanized at 0, 1, 2, 4, 8, 12, and 26 weeks post-irradiation (n=6-7 per group at each time point). The 0 week post-radiation time point refers to the day following the last radiation. Raman spectroscopy was performed over the proximal cortical bone surface. Four commonly used bone Raman metrics were analyzed and compared in this study, including the mineral-to-matrix ratio, the carbonate-to-phosphate ratio, crystallinity and the collagen cross-link ratio (Fig.1).

**Results:** In comparison to the vehicle treated control group (VEH-Con), the collagen crosslink ratios significantly increased from 0 week to 12 weeks for the vehicle treated irradiation group (VEH-XRT). The PTH treated irradiation group (PTH-XRT) demonstrated a significant difference in the ratio when compared with both VEH-XRT and VEH-Con groups at all post-radiation time points except 26 weeks. Significantly lower crosslink ratios were found between PTH-XRT versus VEH-XRT, indicating PTH partially blunts radiation-induced damage of collagen crosslinks. Bone mineral crystallinity and carbonate to phosphate ratio show no significant difference between PTH-XRT versus VEH-Con groups at all time points, indicating PTH preserves bone mineral properties. By comparing PTH treated control group (PTH-Con) to VEH-Con, our results also show that PTH changes bone material properties at some time points, including decreased crystallinity at 4 and 12 weeks, decreased mineral to matrix ratio at 1, 2, 4 and 8 weeks, increased carbonate to phosphate ratio at 4 weeks and decreased collagen crosslink ratio at 1, 2 and 4 weeks. At 26 weeks post-irradiation, all metrics of four experimental groups had returned to normal.

**Conclusions:** Raman spectroscopy demonstrates that PTH prevents radiation-induced damage on bone mineral properties and blunts damage to bone matrix in an irradiated mouse model. Intermittent administration of PTH significantly affects both bone mineral and matrix. These findings may potentially impact clinical evaluation or

treatment of patients with post-radiation fractures.



**Fig.1.** Comparison of Raman bone metrics, including mineral crystallinity, carbonate to phosphate ratio, mineral/matrix ratio and collagen crosslink ratio, at different post-radiation time points. Asterisk represents  $p < 0.05$ .