Intermittent PTH after Prolonged Bisphosphonate Treatment Improves Bone Structure by Inducing Substantial New Bone Formation with Decoupled, Inhibited Bone Resorption in Ovariectomized Rats

**Introduction**

Bisphosphonates (BP) are an osteoporosis treatment that acts to prevent bone loss by inhibiting bone resorption. While this adequately slows or stops further bone loss, it does not promote new bone formation. In addition, recent evidence has suggested that long-term bisphosphonate use may increase the risk of atypical femoral fractures. Intermittent parathyroid hormone (PTH) is the only FDA approved anabolic agent, which promotes bone formation. Using PTH in conjunction with bisphosphonates would provide the maximum benefit to severely osteoporotic individuals, both inhibiting resorption and promoting bone formation. Early studies showed conflicting results regarding the efficacy of combined treatment of bisphosphonate and PTH therapy, leading to the hypothesis that PTH’s anabolic effect may be dependent on prior resorption to initiate bone formation. However, recent studies have shown a positive effect of combined or tandem BP and PTH therapies, attesting to PTH’s anabolic effect in the absence of resorption. Therefore, we hypothesized that PTH is able to act through a resorption-independent pathway to promote modeling-based bone formation. A better understanding of this pathway would be advantageous for both understanding combined PTH and bisphosphonate therapy, as well as designing new treatments which could more directly target modeling-based formation.

In this study, the efficacy of PTH following 12-weeks of alendronate (ALN, a BP) treatment was tested in ovariectomized (OVX) rats. While current methods of investigating bone remodeling over time are limited to cross-sectional animal studies or indirect biomarkers, the current study employed a novel *in vivo* imaging technique to assess bone formation and resorption rate simultaneously and longitudinally. This allowed for an evaluation of the coupling between bone resorption and formation. We hypothesized that prolonged ALN prior to PTH treatment does not blunt PTH’s anabolic potential to activate new bone formation. Thus, PTH can be considered as a treatment for patients with a history of long-term BP treatment.

**Methods**

**Animals:** 30 female SD rats (n = 6/group) received surgery at 4-mo of age: 24 received a bilateral OVX, and 6 received a sham OVX surgery. The study began when all rats were 6-mo-old, after a 2-mo development of osteoporosis in the OVX rats. The treatment plan consisted of 2 phases (phase 1: weeks 0-12, and phase 2: weeks 12-16). The OVX rats were assigned to 4 groups: (1) a Veh group treated with saline for both phases; (2) an ALN group treated with ALN (28µg/kg 2x/wk) for both phases, (3) a Veh+PTH group treated with saline for phase 1, then switched to PTH (40 µg/kg 5x/wk) for phase 2, (4) an ALN+PTH group treated with ALN for phase 1, then PTH for phase 2 (Fig 1).

**In Vivo µCT Scans:** The right proximal tibia of all rats were scanned (Scanco VivaCT40, 10.5 µm) at week 0 (2 mo post surgery), then weekly from weeks 11-16 corresponding with phase 2 (Fig 1). Trabecular microstructure of subsequent scan images was precisely aligned to baseline using an iterative registration method to identify the same volume of interest (VOI). Bone microstructure was analyzed for the same VOI for weeks 0, 12, 14, and 16.

**In Vivo Dynamic Histomorphometry:** A bone sub-volume (1.575×1.575×1.05mm³) of each week in phase 2 was subtracted from the registered sub-volume of the previous week (weeks 11-15) to identify the newly formed bone voxels (green) and resorbed voxels (red) during each week (Fig 1 Right). New bone voxels were used to calculate the bone formation rate (BFR/BS), and lost voxels to calculate bone resorption rate (BRR/BS) weekly over phase 2.

**Femur 3-Point Bending:** The right femoral midshaft was scanned *ex vivo* for evaluation.
of cortical thickness and polar moment of inertia. Then the femur was subjected to a 3-point bending test for evaluation of stiffness and elastic modulus.

**Spine:** Lumbar vertebra, L2 was scanned ex vivo (Scanco µCT35, 3.5 µm) for microstructural and tissue mineral density (TMD) analysis. TMD was calculated for bone tissue at trabecular surface layers (sTMD), and central bone tissue (cTMD).

**Finite Element (FE) Analysis:** The trabecular bone subvolume of weeks 12 and 16 were converted to voxel-based FE models to estimate axial stiffness under compression.

**Statistical Analysis:** Longitudinal measures were compared over time and between groups using repeated measures ANCOVA adjusted for baseline, and cross-sectional measures were compared using ANOVA.

### Results

**Tibia Microstructure:** 2 mo after surgery, OVX resulted in an average BV/TV of 0.13, in contrast to 0.50 in the SHAM group at week 0. BV/TV continued to drop in the Veh and Veh+PTH to 0.06 by week 12 (Fig 2). ALN treatment effectively stabilized BV/TV for ALN and ALN+PTH groups. Switching to PTH resulted in a dramatic increase in BV/TV by week 16 compared to week 12 (40% and 42%) driven by increased Tb.Th (33% and 25%) in both Veh+PTH and ALN+PTH groups (Fig 2).

**In Vivo Dynamic Histomorphometry:** During phase 2, the Veh+PTH group had a dramatic increase in BFR/BS by week 14, which began to stabilize by week 16. The ALN+PTH group had a similar increase in BFR/BS which remained elevated throughout phase 2 (Fig 3a). BRR/BS was low in all treatment groups after the onset of PTH therapy. This was further confirmed by TRAP serum ELISA (Fig 3b). Fig 3c showed that SHAM and ALN treated groups had highly coupled remodeling, with similar BFR/BS and BRR/BS. Resorption outpaced formation for the Veh group. Bone resorption and formation were decoupled in both Veh+PTH and ALN+PTH groups as shown by a substantially greater BFR/BS than BRR/BS.

**Femur:** No difference was found in cortical structure or mechanical parameters between groups.

**Spine Microstructure:** Compared to the tibia, OVX resulted in less reduced vertebral BV/TV (0.21 in Veh vs. 0.33 in SHAM). All 3 treatments groups resulted in BV/TV that was

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**Figure 1.** Left: Registered comparison of tibia bone segments at each timepoint with treatment indicated by the arrows between time points. Right: µCT-based in vivo bone dynamic histomorphometry, green indicates areas of new bone (formation), and red indicates lost bone (resorption) during the final week of treatment.
In healthy bone, resorption and formation are coupled and balanced to sustain bone mass. Their uncoupling due to OVX results in resorption outpacing formation, and subsequent trabecular bone loss which compromises the mechanical competence of the bone. ALN treatment effectively re-couples this balance, preventing additional bone loss. PTH, however, uncouples the balance in favor of formation, resulting in thickened bone and greater structural stiffness. Resorption activities after ALN, Veh, and ALN + PTH treatments were not different and remained significantly lower than those of Veh and Sham. While we may have expected a higher BRR/BS or TRAP in the Veh compared to Sham group, the resorption-formation balance of OVX had been re-adjusted to the slower formation rate 6 months after OVX. In summary, our investigation across multiple skeletal sites suggests that ALN followed by PTH is a viable treatment strategy to maintain and improve bone quality by stimulating substantial new bone formation.

**Spine TMD:** cTMD was higher in the SHAM than all treatment groups. cTMD of the Veh + PTH group was 7% and 4% lower compared to SHAM and Veh. sTMD was significantly reduced in the Veh + PTH (6%) and ALN + PTH (4%) compared to ALN.

**Tibia Stiffness:** The trabecular bone stiffness did not change during phase 2 in the SHAM, Veh or ALN groups. Stiffness improved in both Veh + PTH (97%) and ALN + PTH (114%) groups beyond that of all other groups.

**Discussion**

The results of this study clearly demonstrate the efficacy of PTH following BP therapy for stimulating new bone formation.
INTERMITTENT PTH AFTER PROLONGED BISPHOSPHONATE TREATMENT IMPROVES BONE STRUCTURE BY INDUCING SUBSTANTIAL NEW BONE FORMATION

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Significance
Long term ALN therapy following OVX can be further augmented by subsequent PTH treatment. This increases the BFR/BS to thicken the existing trabeculae while inhibiting bone resorption. The novel in vivo dynamic histomorphometry analysis provides direct evidence for PTH's anabolic effect in the absence of bone resorption, further confirming its capacity for modeling-based bone formation.

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References

Figure 3. (A) In vivo dynamic histomorphometry, BFR/BS week 12-16. Letters indicate differences between groups, a: ALN to ALN+PTH, b: ALN to Veh+PTH, c: ALN+PTH to Veh+PTH. (B) Serum TRAP concentration, bars indicate differences between groups. (C) In vivo dynamic histomorphometry at week 16. Left axis: BFR/BS (solid), right axis: BRR/BS (hashed). * indicates differences between formation and resorption, # indicates a trend difference (p < 0.1).