

Parathyroid hormone alters the transcriptomic response of bone to mechanical loading in a site-specific manner

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Introduction: Parathyroid hormone (PTH) is an FDA-approved anabolic therapeutic used to treat osteoporosis. PTH exhibits both anabolic and catabolic effects on bone tissue, driven by salt inducible kinase control of HDAC4/5 and CRT2 transcription factors¹. A combination of PTH and mechanical loading elicits a greater anabolic response than the additive benefits of either intervention independently^{2,3}. Further, PTH treatment resulted in earlier and greater load-induced increases in bone mass in regions of compression compared to regions of tension⁴. However, the molecular mechanisms that drive the synergistic effects of PTH and mechanical loading are unknown. In this study, we explored these mechanisms through transcriptomic analysis, hypothesizing PTH alters the transcriptomic response to mechanical loading as compared to saline vehicle (VEH).

Methods: Following IACUC approval, 16-week-old female C57BL/6J mice were subcutaneously injected with PTH (40 ug/kg, n=5) or saline vehicle (n=4) for 1 week concurrent with in vivo cyclic tibial compression. Left limbs were loaded (1200 cycles, 4Hz, 9N peak load) and right limbs served as contralateral controls⁵. Animals underwent five consecutive days of concurrent treatment and loading, followed by two days of rest, and euthanasia on day 8. Following previously established protocols, tibiae were dissected, and bone marrow was removed⁶. Cancellous and cortical bone from the proximal metaphysis and cortical bone from the mid-diaphysis were isolated and stabilized in Trizol prior to RNA isolation⁷. 3' RNA sequencing was performed (Illumina NextSeq500). STAR was used to align transcripts to the mm10 genome and provide gene counts. Samples contained >1 million reads and had an average of 71.48% uniquely mapped reads. Normalized gene counts were calculated across all tissue segments (DESeq2⁸). Tissue segments from loaded and control limbs were compared for differential gene expression (EdgeR⁹, FDR<0.05) and enrichment of biological processes (GSEA, FDR<0.25).

Results: PTH altered the number of differentially-expressed (DE) genes with loading at all tissue sites compared to VEH-treated animals. In VEH-treated mice, 8 genes were DE in the mid-diaphysis and 1 gene in cancellous bone. No genes were DE in either of these sites with PTH treatment. The metaphyseal shell had the greatest number of DE genes in both VEH- (53 genes) and PTH-treated (69 genes) groups, with 3 DE genes (*Sp100*, *Pdcd11*, and *Coll14a1*) overlapping between the two groups (Fig. 1). In the VEH-treated animals, 2 genes that encode histones (*H2afy2* and *Hist3h2ba*) were DE with loading at the metaphyseal shell. Although no genes related to histone encoding were DE with loading at this site in the PTH-treated animals, genes related to modifications of DNA and post-transcriptional RNA were DE. Specifically, these genes are related to deacetylase activity (*Ndst1*), RNA methyltransferase activity (*Trmt10c*), histone demethylase (*Kdm4c*), and methylated histone binding (*Zcwpw1*, Fig. 2). In the metaphyseal shell, 17 biological pathways were enriched with loading and PTH treatment. These processes included positive enrichment of regulation of cAMP-mediated signaling and negative enrichment of multiple pathways associated with the muscular and nervous systems (Fig. 3). No biological processes were enriched in the metaphyseal shell of VEH-treated animals.

Discussion: The transcriptional response of cortical bone to combined mechanical loading and PTH treatment differed between the proximal metaphysis and mid-diaphysis. This effect may be due, in part, to the different loading modes at these locations. When the tibia is loaded, the proximal metaphysis experiences predominantly compression while the mid-diaphysis undergoes bending, resulting in distinct compartments of both tensile and compressive strains. The differences in DE genes involved in histone encoding and DNA and post-transcriptional RNA modification between PTH and VEH treated metaphyseal cortical bone suggest that novel epigenetic mechanisms may drive the anabolic synergism of PTH and mechanical loading. Transcription factors HDAC4/5 and CRT2 have previously been shown to control osteocyte response to PTH¹, suggesting the role of epigenetic control of gene expression under PTH treatment. Our work indicates that epigenetic mechanisms may similarly drive synergism in the anabolic response of PTH and mechanical loading, allowing us to better understand the increased combined benefit of the two treatments compared to the additive effects.

Significance: Understanding the molecular mechanisms driving the synergism of PTH and mechanical loading may help direct therapeutic intervention and physical therapy regimens in treating osteoporosis, maximizing the benefits of combined treatment.

References: ¹Wein et al. 2016. ²Sugiyama et al. 2008. ³Hagino et al. 2001. ⁴Rooney & McNeill et al. 2022. ⁵Fritton et al. 2005. ⁶Kelly et al. 2016. ⁷Chlebek et al. 2022. ⁸Love et al. 2014. ⁹Robinson et al. 2010.

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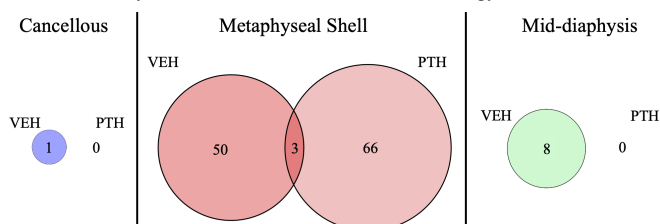


Figure 1: The number of DE genes at all tissue sites. The greatest number of DE genes were expressed in the metaphyseal cortical bone.

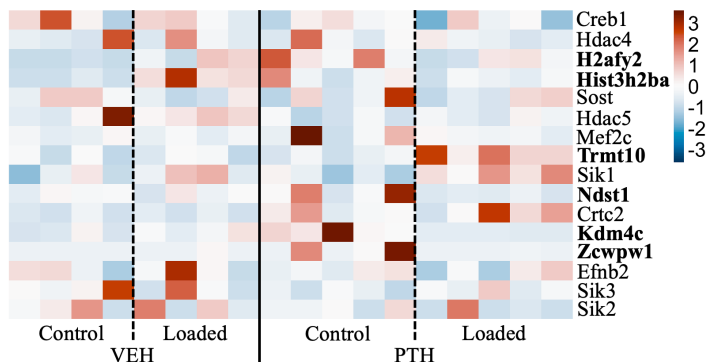


Figure 2: Normalized counts of genes of interest from the metaphyseal shell. Bolding indicates DE gene with load in VEH or PTH group.

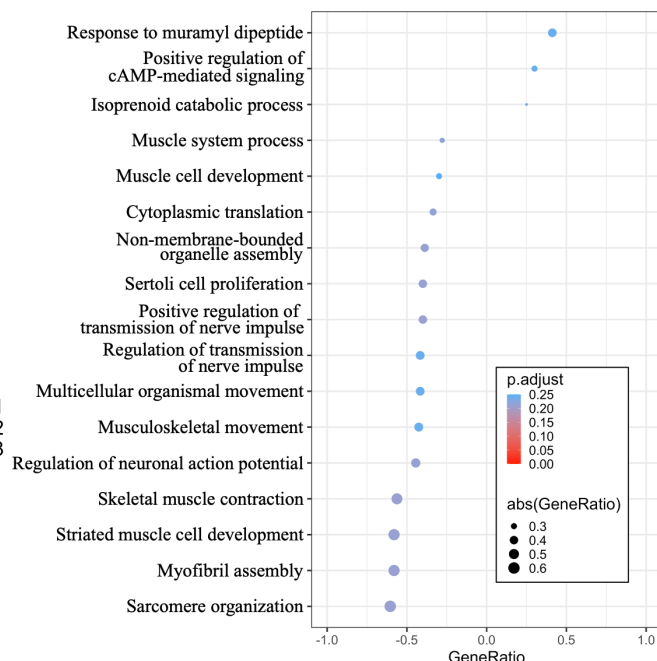


Figure 3: GSEA biological pathway enrichment in the metaphyseal shell of PTH-treated animals, with corresponding gene ratios and adjusted p-values.