YAP and TAZ Mediate Mechanical Load-Induced Bone Formation

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Introduction

Osteoporosis, a disease characterized by low bone mass and structural deterioration, affects over 200 million people globally and increases the risk of fractures[1]. Mechanical signals have been shown to regulate bone structure and mass[2,3]. Therefore understanding how bones sense and respond to mechanical forces is critical for identifying new treatment strategies.

Bone adapts to mechanical cues through mechanotransduction[4] : Yes-associated protein (YAP) and Transcriptional co-activator with PDZ-binding motif (TAZ) regulate gene expression through interactions with other proteins[5,6]. These proteins mediate bone mechanotransduction by transducing mechanical signals into gene expression[6,7]. Although YAP and TAZ lack DNA-binding domains, they translocate to the nucleus upon loading and activate transcription by partnering with TEAD factors, driving anabolic bone formation[8]. However, the molecular mechanisms underlying this response remain poorly understood.

To investigate the regulatory role of YAP/TAZ in load-induced bone formation, we used pharmacological approaches to inhibit YAP/TAZ using two orthogonal inhibitory drugs, verteporfin (VP) and MGH-CP1 (CP1). Here, we hypothesize that inhibiting YAP/TAZ signaling will impair their regulatory role in mediating load-induced bone formation. Understanding this pathway may offer new insights into skeletal diseases such as osteoporosis and inform the development of targeted therapies to improve bone strength.

Materials and Methods

To assess the role of YAP/TAZ in mediating the load-induced bone formation in mice with normal developmental histories, we employed pharmacological methods to acutely inhibit YAP/TAZ activity. Fourteen-week-old C57BL/6 mice were intraperitoneally (i.p.) injected, every day for two weeks, with either VP or CP1, which prevents TEAD from interacting with YAP/TAZ.

Cyclic compressive mechanical loading (9N peak load, 4 Hz frequency, 1200 cycles/day) was applied to one tibia for five days/week over a two-week period, while the contralateral tibia remained unloaded to serve as a control. Dynamic fluorochrome labeling was used to quantify bone formation in cross sections of cortical bone at the site of maximal strain (37% away from the proximal end), as well as in longitudinal sections of trabecular bone in the proximal tibia metaphysis. Calcein (0.6%) and alizarin complexone (1%) were administered via intraperitoneal injection on days 5 and 11, respectively.

Regions containing single- and double-labeled surfaces in tibial periosteal surface and proximal metaphyseal trabeculae were imaged using the Axio Observer Z1 (Zeiss) with a 10x objective. Labeled surfaces were then imported into OsteoMeasure and manually traced to quantify the extent of the mineralized surface as a fraction of bone surface (MS/BS) and the mineral apposition rate or the distance between the two labels, normalized to the time between two labels (MAR). Bone formation rate (BFR/BS) was calculated as (MS/BS × MAR) according to standard dynamic histomorphometry protocols[9].

This approach enabled precise, site-specific quantification of bone formation in both cortical and trabecular bone.

Results and Discussion

Cortical Bone: Following mechanical loading, our dynamic histomorphometry analysis of cortical bone revealed a significant increase in MS/BS in DMSO-control tibiae in response to mechanical loading **(Figure 1A-D)**. This response, however, was abrogated in both VP and CP1 treated groups. Similarly, MAR was significantly elevated in loaded DMSO-controls, however, this effect was absent in VP and CP1 groups **(Figure 1E)**. BFR/BS showed a significant response to mechanical loading in DMSO-controls, whereas VP and CP1 samples showed no statistically significant change. Additionally, a significant drug effect was observed in DMSO-loaded samples compared to CP1-loaded in the cortical bone **(Figure 1F)**. These findings suggest that YAP/TAZ inhibition abrogates the response to mechanical loading in tibial cortical bone.

Trabecular Bone: In the trabecular bone of the metaphyseal proximal tibia, mechanical loading did not significantly alter the mineralizing surface (MS/BS) in any group **(Figure 2A-D)**. However, mineral apposition rate (MAR) increased in response to loading in DMSO-treated tibia but this effect was abrogated in VP or CP1 groups **(Figure 2E)**. As a result, bone formation rate (BFR/BS) was elevated in DMSO-loaded samples while there was no effect of loading in altering BFR/BS between unloaded and loaded VP and CP1 samples **(Figure 2F)**. These findings indicate that YAP/TAZ signaling is required for load-induced bone formation in trabecular bone.

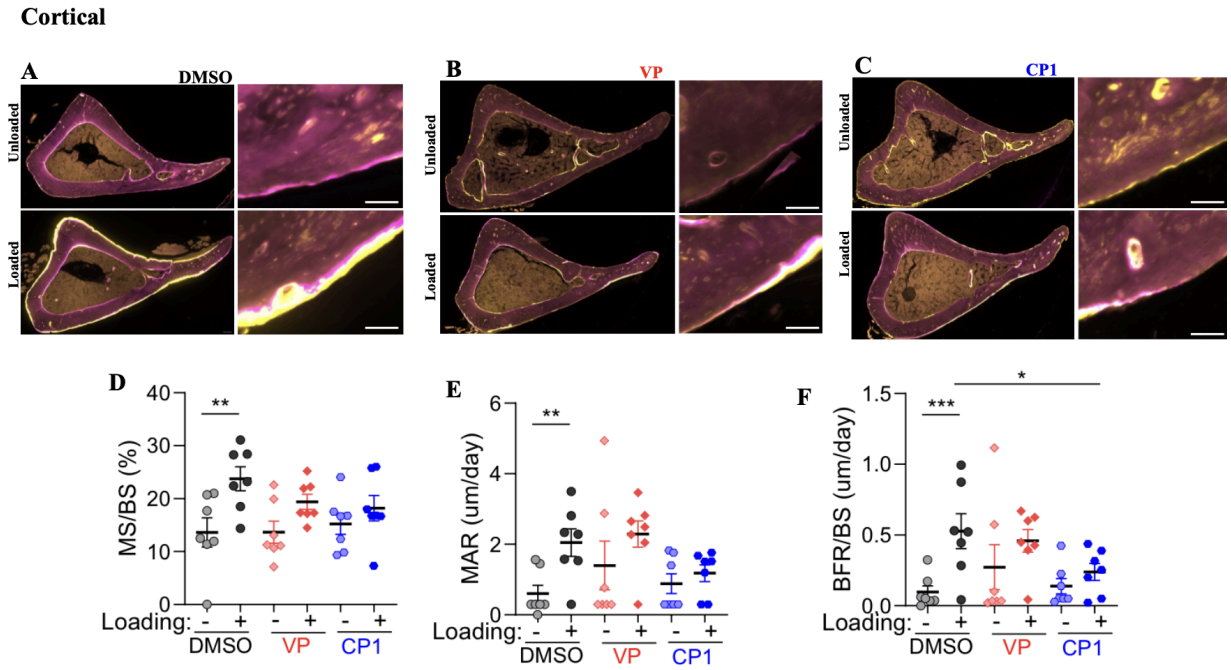

Figure 1. *Dynamic histomorphometry of cortical bone in response to mechanical loading and YAP/TAZ inhibition.*

Representative fluorochrome-labeled transverse sections of cortical bone (37% from the proximal tibia) from mice treated with DMSO (**1A**), VP (**1B**), and CP1 (**1C**), comparing unloaded and loaded limbs. Right panels show magnified views of the periosteal surface from the same sections. Alizarin complexone (pink) marks earlier mineral deposition, while calcein (yellow) marks later deposition. **(1D)** MS/BS significantly increased with loading in DMSO controls but not in VP or CP1 groups. (**1E)** MAR was elevated in loaded DMSO tibiae but remained unchanged in VP and CP1-treated limbs. **(1F)** BFR/BS increased with loading in DMSO controls, while no significant change was observed in VP or CP1 groups. Scale bar = 50µm.

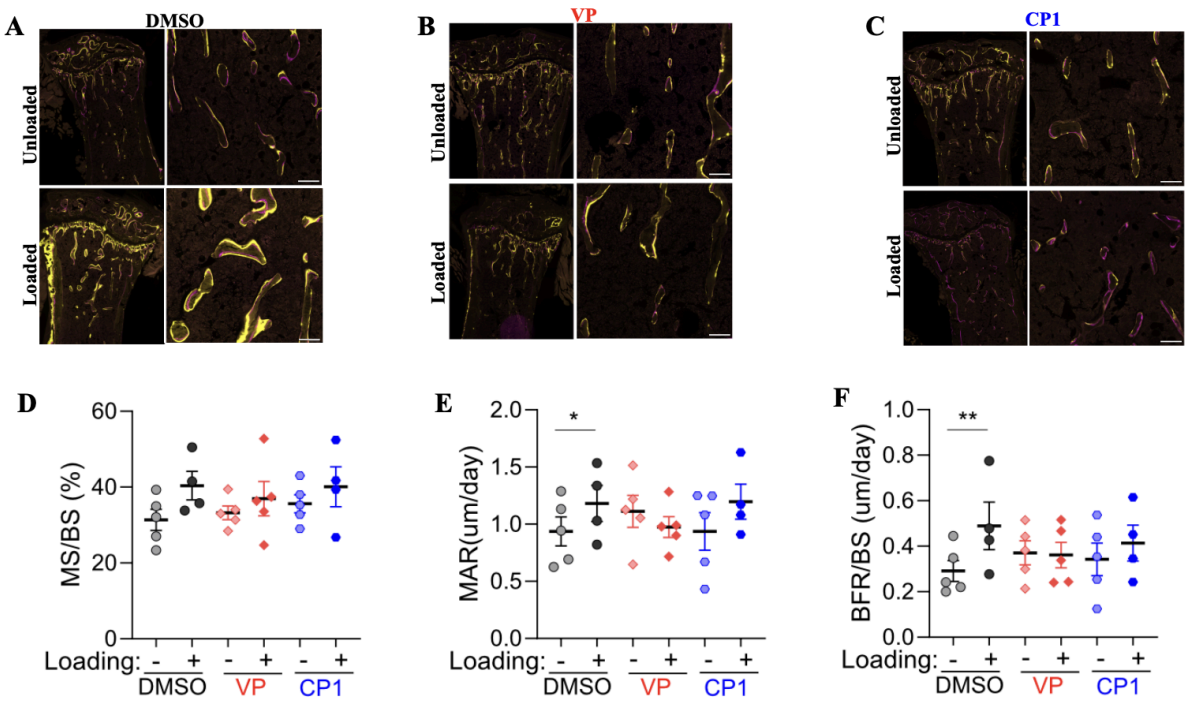

Figure 2. *Dynamic histomorphometry of trabecular bone in response to mechanical loading and YAP/TAZ inhibition.*

Representative fluorochrome-labeled longitudinal sections of trabecular bone in the proximal tibial metaphysis from mice treated with DMSO (**2A**), VP (**2B**), or CP1 (**2C**), comparing unloaded and loaded limbs. Right panels show magnified views of trabecular structures within the same section. Alizarin complexone (pink) marks earlier mineral deposition, while calcein (yellow) marks later deposition. **(2D)** MS/BS was unchanged by loading across all groups. **(2E)** MAR increased with loading in DMSO controls but not in VP or CP1 groups. **(2F)** BFR/BS was elevated in DMSO-loaded samples and decreased in VP and CP1 samples. Scale bar = 100µm.

Conclusion

Together, these findings demonstrate that YAP/TAZ signaling is essential for bone formation in response to mechanical loading, highlighting its potential as a therapeutic target for skeletal disorders such as osteoporosis, where mechanotransduction is impaired.

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