Differential Effects of Continuous and Intermittent Salt-Inducible Kinase Inhibition on Cortical and Cancellous Bone Responses to Mechanical Loading

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Disclosures: MH (N). FPR (N). MCHv/d M (N). MNW has received research funding from Radius Health and is a co-inventor on pending patents regarding the use of SIK inhibitors for osteoporosis. MNW is a consultant for Relation Therapeutics, Sitryx Therapeutics, Aditum Bio & GLG.

Introduction: Osteoporosis, affecting one in three women, is the most common metabolic bone disorder, characterized by low bone mass and compromised tissue quality, leading to fractures[1]. Intermittent parathyroid hormone (PTH), one of the few FDA-approved anabolic treatments, enhances bone formation, especially at loaded sites[2]. PTH acts within cells by inhibiting Salt Inducible Kinases (SIKs)[3]. However, PTH's effectiveness is limited to two years[4] and requires self-administered daily injections, making alternative treatments essential. SIK inhibitor treatment is orally available, and genetic knockout or administration of SIK inhibitor stimulated bone formation similarly to PTH, increasing cancellous bone in young male mice[5]. Continuous PTH induces hyperparathyroidism, increasing cancellous bone mass and decreasing cortical bone mass, whereas intermittent PTH increases cancellous bone without reducing cortical bone and is synergistic when combined with loading[2,6]. However, the differential mechanism of continuous and intermittent SIK inhibition on bone has not been elucidated. Here, we extended our previous work on continuous SIK inhibition[7] by comparing its effects with intermittent inhibition in adult female mice and evaluating potential synergism with mechanical loading across multiple skeletal sites.

Method: Following IACUC approval, 16-week-old female C57BL/6J mice (n=10/grp) were administered the SIK inhibitor SK-124 via either intraperitoneal injection[7] (continuous, IP) or oral gavage (intermittent) at 40 mg/kg, or were given a vehicle solution (sterile water, oral gavage/IP, VEH) for six weeks (5d/wk). Concurrently, daily in vivo cyclic mechanical loading was applied to the left tibia (L, 5d/wk, 9N, 4Hz, 1200 cycles)[8], and the right limb served as the contralateral control (C). On day 41, the animals were euthanized. Blood was collected via cardiac puncture to analyze bone serum markers and residual SK-124 levels 24 hours after the last treatment dose. Tibiae were dissected and prepared for microCT imaging. MicroCT scans were analyzed at the cancellous core and cortical shell of the metaphysis, and the mid-diaphyseal cortex. Statistical significance was determined using a linear mixed model with loading and treatment as fixed effects, and mouse as a random effect, with significance at $p<0.05$. A Tukey HSD post-hoc test was conducted when the interaction term was significant.

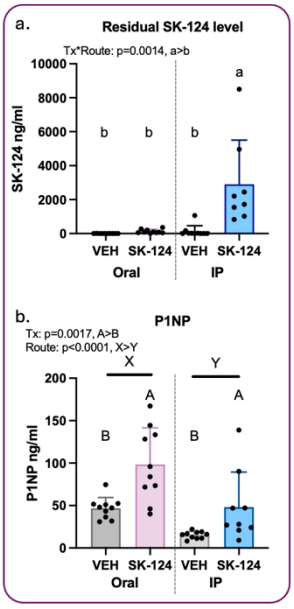
Results: Continuous SIK-inhibited animals had a significantly increased residual SK-124 level compared to VEH administration of both routes and SK-124 via oral gavage (Fig. 1a), confirming IP injection induced continuous exposure; in contrast, post-treatment oral gavage levels were similar to VEH. Both continuous and intermittent SIK inhibition increased a bone formation marker, P1NP (Fig. 1b). Intermittent SIK inhibition increased cancellous bone volume fraction (BV/TV) and trabecular thickness (Tb.Th). However, the response to loading in SIK inhibited animals was diminished; loading led to a greater cancellous increase in VEH compared to SK-124 group (Fig. 2a,b). Continuous SIK inhibition also increased cancellous BV/TV and did not affect the response to loading (Fig. 2a). Both metaphyseal shell and mid-diaphysis cortical bone thickness (Ct.Th) increased with intermittent SIK inhibition, although loading was not synergistic with SIK inhibition (Fig. 2c,d). In contrast, continuous SIK inhibition (achieved via IP drug administration) decreased Ct.Th and led to an amplified response to mechanical stimulus in which the SIK inhibition-induced bone loss was rescued by loading (Fig. 2d).

Discussion: Continuous SIK inhibition increased cancellous bone mass but reduced cortical bone, which aligns with the bone phenotype observed in hyperparathyroidism. Mechanical loading was essential for maintaining cortical bone mass at both the cortical metaphyseal shell and mid-diaphysis. In contrast, intermittent SIK inhibition was anabolic at both cancellous and cortical sites but did not act synergistically with mechanical loading. Instead, intermittently SIK-inhibited animals had a blunted response to loading in cancellous bone. These results may reflect that cancellous bone volume was already near its maximum with SIK inhibition and mechanical loading, leaving little capacity for further increases. Overall, the efficacy of intermittent SIK inhibition as an oral therapy was evident through its anabolic effects in female mice and the importance of administration route and dosing is emphasized. Future studies should evaluate the potential of intermittent SIK inhibition in clinical settings.

Significance: Understanding differential effect of continuous and intermittent SIK inhibition and mechanical loading at different skeletal sites will provide insights for clinical therapeutics for osteoporosis.

References: [1]Imel et al., 2014. [2]Sugiyama et al., 2008. [3]Wein et al., 2016. [4]Aslan et al., 2012. [5]Sato et al., 2022. [6]Uzawa et al., 1995. [7]Huang et al., 2024 ORS [8]Fritton et al., 2005.

Acknowledgments: Funding provided by Robert and Helen Appel Fellowship, and NIH R01-AR081943. We thank the Cornell CARE Staff.



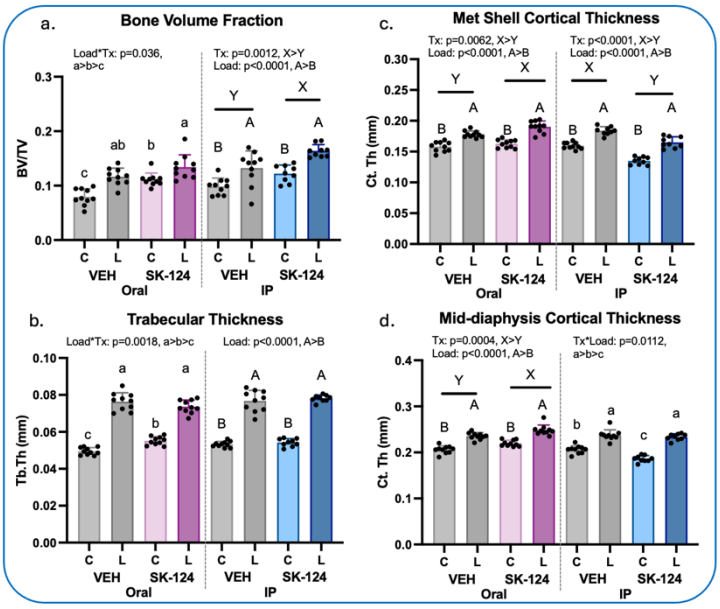


Figure 1 (left) a) Animals administered SK-124 via IP injection had higher residual levels 24 hours after the final dose; **b)** Both continuous and intermittent SIK inhibition increased bone formation marker, P1NP

Figure 2 (right) a) & b) Animals administered SIK inhibitor SK-124 via oral gavage (intermittent) or IP (continuous) had an increased cancellous bone mass. Response to loading in the oral gavage group was blunted; **c) & d)** Animals administered intermittent SIK inhibitor via Oral Gavage had an increased cortical bone mass; SIK inhibitor administered via IP led to a decreased cortical bone mass while loading recused the loss (C: Control; L: Loaded)