Microbiome-Mediated Vertebral Trabecular Bone Thinning is Reversed via Exercise Intervention

Sarah M. Staller[1], Cameron X. Villarreal[1], Deva D. Chan[1]

[1]Purdue University, West Lafayette, IN.

smstalle@purdue.edu

DISCLOSURES: SM Staller (N), CX Villarreal (N), DD Chan (N).

INTRODUCTION: Spinal degenerative diseases, such as spinal stenosis and degenerative disc disease, are prevalent in society, particularly in the aging population.[1] These diseases are not only a significant burden on the healthcare system and economy but can also lead to low back pain for those afflicted, affecting the patient's quality of life.[1,2] These disorders impact spinal biomechanics and morphology of the discs, vertebrae, and surrounding tissue.[3] Changes in these parameters can influence spinal flexibility, loading patterns, and shock absorbance.[3] While spinal degeneration is a common problem, the specific mechanisms behind its pathogenesis and progression remain poorly understood. Research indicates a relationship between the gut microbiome and musculoskeletal biomechanics.[4,5] The gut microbiome is the summation of all genetic material present in the gastrointestinal tract. This environment is affected by lifestyle factors such as exercise, diet, and medications and has potential impacts on many physiological systems. Recent work has shown antibiotic-induced disruption of the gut microbiome reduced whole bone strength in mice.[6] Other studies have found that exercise increased microbiome microbial diversity and metabolic capacity, and prebiotic dosing increased production of beneficial microbiome metabolites.[7,8] Given that microbiome manipulation has been shown to alter whole bone mechanics in murine long bones, we tested whether vertebral trabecular bone exhibits similar systemic or region-specific responses to microbiome disruption and whether these effects can be restored with intervention.[6,9]

METHODS: Under IACUC approval, we treated mice (n=5+, per sex, per group) with 1 g/L ampicillin + 0.5 g/L neomycin from 5 to 16 weeks of age to disrupt the gut microbiome. At 16 weeks, mice were assigned to continue antibiotics (ABX), cessation of antibiotics alone (Cess), cessation with moderate treadmill exercise at 13 m/min for 5 days/week (Cess+E), cessation with prebiotic supplementation of 10 g/L inulin in drinking water (Cess+P), or cessation and both exercise and prebiotic (Cess+E+P). Control mice (n=6 M/F) received normal drinking water. At 24 weeks, mice were euthanized and lumbar spines collected. L5 vertebrae were isolated and scanned via micro-computed tomography (Bruker SkyScan 1272; 60 kV, 167 µA, 0.5 mm Al filter). Trabecular bone was segmented and analyzed with Dragonfly (Comet Technologies Canada Inc.)[10] to determine microarchitectural trabecular properties, including bone volume fraction (BV/TV) and trabecular number (Tb.N), spacing (Tb.Sp), and thickness (Tb.Th). To evaluate whether antibiotic treatment altered lumbar trabecular parameters, control and ABX were compared using Mann-Whitney U Test. A Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction was used to determine how cessation and intervention groups compared to continued antibiotics. Data are reported as mean ± standard deviation, and statistical significance was set at $\alpha = 0.05$.

RESULTS: In male mice, Tb.Th was significantly lower in ABX (0.039 ± 0.001 mm; n=8) compared to controls (0.041± 0.002 mm; n=7) ($p = 0.024$). No significant differences were detected between ABX and control groups for BV/TV, Tb.N, or Tb.Sp. Kruskal-Wallis testing revealed significant differences in Tb.Th among ABX and intervention groups ($p = 0.020$); post hoc analysis indicated Tb.Th was significantly higher in Cess+E (0.043 ± 0.001 mm; n=8) compared to ABX ($p = 0.008$). In female mice, no significant differences were detected between ABX and control groups for BV/TV, Tb.N, Tb.Sp, or Tb.Th.

DISCUSSION: Antibiotic-treated male mice exhibited reduced trabecular thickness compared to controls, while exercise intervention following cessation restored trabecular thickness above antibiotic-treated mice levels. This suggests that mechanical loading can effectively counteract deleterious vertebral trabecular bone changes resulting from gut microbiome disruption in males. Interestingly, this trend was not observed with either prebiotic intervention group. Inulin has been shown to aid in mineral metabolism, including calcium absorption, and increase whole-bone mineral density and content in rats.[11] However, inulin administered after antibiotics was not significantly different than spontaneous recovery at mitigating microbiome dysbiosis in mice.[12] This indicates that post-antibiotics inulin may have limited efficacy as an intervention method to restore microbiome-mediated trabecular bone changes. Inulin may have mitigated exercise benefits in the combined intervention by exerting opposing effects on gut motility. No vertebral trabecular parameter differences were observed between control and antibiotic-treated female mice. This is consistent with prior studies, which identified a sex-dependent trabecular bone response in murine femora and tibiae following antibiotic-induced microbiome disruption.[13,14] Comparable reductions in male mice trabecular microarchitectural parameters at multiple skeletal sites suggests that gut microbiome disruption exerts systemic effects on trabecular bone.[13,14]

SIGNIFICANCE/CLINICAL RELEVANCE: The results of this study aid in characterization of the gut-spine axis by demonstrating that mechanical loading can restore microbiome-mediated trabecular bone thinning in males and that gut microbiome manipulation may have systemic effects on trabecular bone. These findings indicate a need to consider sex-dependent trabecular bone responses in future investigations involving gut microbiome manipulation and may help guide the development of sex-specific clinical treatment plans to promote skeletal health.

REFERENCES: 1. Kalichman, et al. *Spine Jl*, 2010. 2. Luoma, et al. *Spine*, 2000. 3. Patentaeu, et al. *Sci Rep*, 2021. 4. Wang, et al. *Gut Microbes,* 2023. 5. Li, et al. *Gut Microbes,* 2024. 6. Luna, *J Bone Miner Res,* 2021. 7. Denou, *Am J Physiol Endocrinol Metab*, 2016. 8. Nakajima, et al. *Nutr Metab (Lond)*, 2022. 9. Guss, et al. *J Bone Miner Res,* 2017. 10. Gendron, et al. *Microsc Microanal,* 2021. 11. Roberfroid, et al. *J Nutr,* 2002. 12. An, et al. *Food Funct,* 2024. 13. Pusceddu, et al. *J Orthop Res*, 2020. 14. Hathaway-Schrader, et al. *Am J Pathol,* 2019.

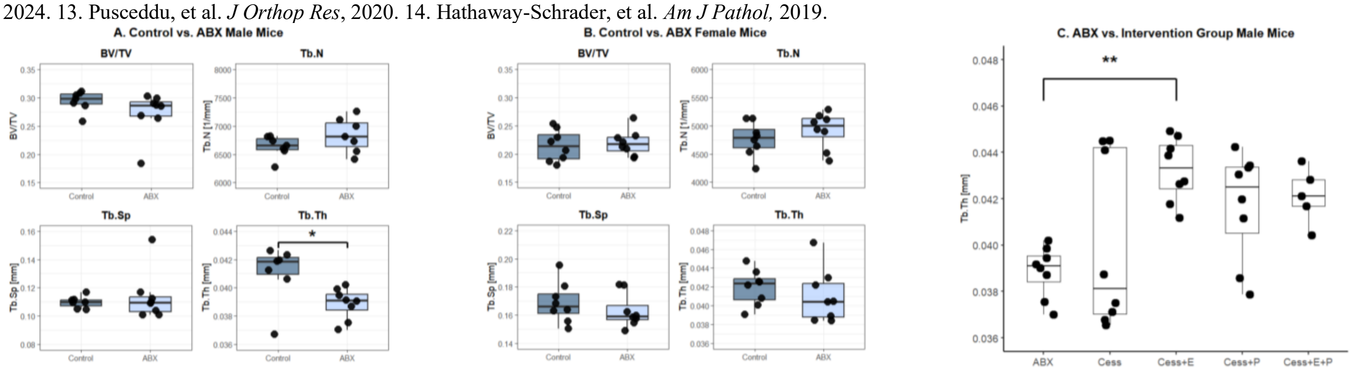

Figure 1. A: BV/TV, Tb.N, Tb.Sp, and Tb.Th results for control vs. ABX male mice. Tb.Th was significantly higher in control vs. ABX mice. **B:** BV/TV, Tb.N, Tb.Sp, and Tb.Th results for control vs. ABX female mice. **C:** Tb.Th results for ABX vs. intervention group male mice. Tb.Th was significantly higher in Cess+E vs. ABX mice. Groups with $p \leq 0.05$ are denoted by * and $p \leq 0.01$ by **.