High-Resolution MicroCT Analysis of Cortical Bone Porosity and Vascularity in Methylphenidate-Treated Rats

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INTRODUCTION: Attention Deficit Hyperactivity Disorder (ADHD) is one of the most commonly diagnosed adolescent psychological disorders with a US incidence of ~9.5%. Methylphenidate (MP), a central nervous system stimulant, is one of the most commonly prescribed medications used in the treatment of ADHD due to its effectiveness of ~70% in reducing symptoms. Our laboratory has previously demonstrated that MP-treated male adolescent rats had smaller, less mineralized, and weaker appendicular bones, suggesting that adolescents being treated with MP for prolonged periods of time may be at higher risk for skeletal fractures. In adolescents being treated with MP, it is not uncommon to utilize intermittent breaks from treatment during weekends or school holidays in what is known as a 'drug holiday'. These breaks allow for the assessment of whether medication is still needed, as well as the management of adverse side effects. This study investigated the effects of MP treatment and Drug Holidays on cortical bone porosity and vascularity using high-resolution micro-CT.

METHODS:
Treatment & Dosing Paradigms: Four-week-old male Sprague-Dawley rats (n=36) were randomized into three groups: Water, MP (MP daily). Water groups received no MP, and treatment groups were given MP in a water-restricted, dual dosage paradigm where an initial dose of MP was given for the first hour, and a second dose was given for the next seven hours. Water was restricted for the remaining sixteen hours until the subsequent treatment. In order to test different drug dosing paradigms, Water, MP were further subdivided into Three Week On, One Week Off (3W, 1W), Five Days On, Two Days Off (5D, 2D), and continuous exposure. The 3W, 1W treatment groups cycled between a three-week period of receiving MP treatment and a one-week period of no treatment. The 5D, 2D treatment groups cycled between five days of receiving MP treatment and a two-day period of no treatment. The Continuous group received treatment for the entirety of the study period. Rats were euthanized after 13 weeks of this treatment regimen, and their hindlimbs were harvested for analysis.
MicroCT: Femora were scanned at the distal metaphysis (1-micron resolution) using a µCT system (SkyScan 1172, Bruker microCT, Belgium) for porosity and vascularity.
Porosity Analysis: A cylindrical (250 µm diameter x 2 mm height) intracortical volume was selected for analysis using ImageJv2. Briefly, images were stitched into a 3D image and thresholded to identify pores (2-3 µm). The images were analyzed for cortical pore number and volume.
Vascularity: The 3D images were analyzed for vascular structure in cortical bone, applying the Frangi Filter. The data were assessed for vessel number, volume, connectivity, and density.
Statistical Analysis: Data were normalized to the Water control groups within each of the three treatment paradigms. Kruskal-Wallis tests (SPSS) were used to determine statistical significance at an alpha of 0.05 and are presented as percentage change relative to the water group.

RESULTS:
Porosity: The pore volume was increased by 105% (p=0.0034) relative to the Water control. The pore numbers increased by 74% (p=0.0068). The Drug Holiday groups were able to resist the increase in pore volume and number (Figure 1).
Vascularity: Continuous MP exposure significantly decreased the vascular diameter by 31%(p=0.0046), decreased vascular diameter by 38%, increased vascular discontinuity by 633% (p=0.0027), and increased vessel density by 470% (p=0.0008). The Drug Holiday groups were not adversely impacted in terms of vascular diameter, volume, connection density, and discontinuity (Figure 2).

DISCUSSION: In this study, we utilized high-resolution MicroCT to examine the effects of MP and two MP 'drug holiday' dosing paradigms on the porosity and vascularity of the cortical bone. When choosing our treatment paradigms, we attempted to closely duplicate two dosing plans that are commonly clinically utilized – i.e., during school breaks (3W, 1W) and during the weekends (5D, 2D). We previously reported that increased MP-induced osteoclastogenesis and osteoclast activity led to increased bone loss in cortical bone. The data in this study emphasizes the detrimental effects of daily MP on the porosity and vascularity of cortical bone. The impairment in vascular structure shows MP effects on vessel continuity, with an increased number of small, disoriented vessels, implying MP-induced effects on vessel development, vasoconstriction, and angiogenesis. Future studies will evaluate the histological changes associated with these differences to understand the cellular mechanisms and tissue degeneration underlying our observed results on bone strength, healing, and vasoconstriction.

CLINICAL RELEVANCE: MP is one of the most prescribed ADHD medications that may lead to bone loss and an increased risk for fracture in long-term treatment plans and impaired fracture healing. The effects on vascularity may have systemic effects, including but not limited to muscular dystrophy, fatigue, and overall quality of life. Furthermore, the study indicates that changes in the drug paradigm may have significant clinical implications.

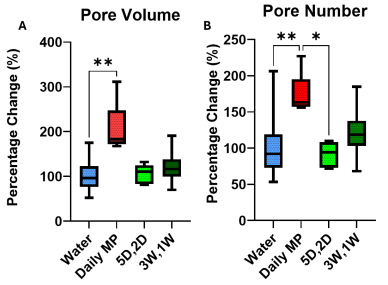

Figure 1: Porosity (A) Cortical bone pore volume (B) Cortical bone pore number. * (p<0.05) ** (p<0.001)

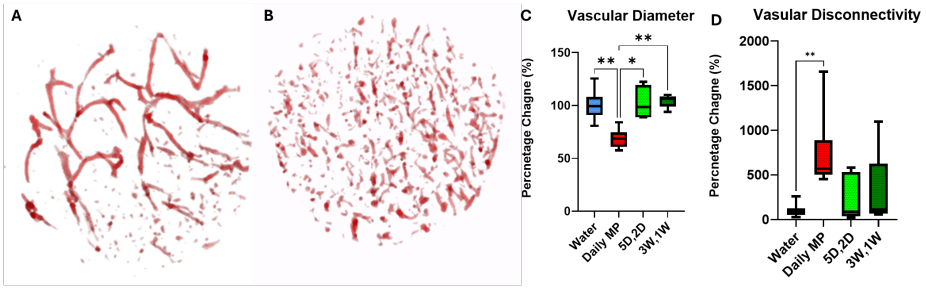

Figure 2: Vascularity (A) 3D Vascular structure in Water group, (B) 3D Vascular structure in continuous daily MP group (C) Vascular diameter illustrating Water, MP, and drug holiday groups. (D) Vessel structural impairment comparison between Water control, Daly MP, and Drug Holiday groups. groups failure * (p<0.05) ** (p<0.001)