

## P2Y2 Inhibition Modifies the Anabolic Response to Exercise in Adult Mice

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**INTRODUCTION:** Osteocytes serve as a sensory network that responds to dynamic loading of the skeleton and play a crucial role in regulating bone mass while maintaining bone quality [1]. Osteocytes' induction of bone formation in response to loading is extremely age dependent, such that clinical studies have shown exercise to be less and less effective as we age [2,3]. As a result, modifying osteocytes' mechanosensitivity may enable older adults to better capitalize on the anabolic nature of exercise to increase bone mass and reduce fracture risk. Based on our previous work, P2Y2 activation down-regulates the mechanosensitivity of bone cells by increasing actin-stress fiber formation, and thereby presents a novel mechanism that limits the anabolic response to loading [4]. Therefore, the central hypothesis states that inhibiting P2Y2 activity can enhance the anabolic response to loading. To test this hypothesis, the anabolic response to exercise was examined by treating adult mice, which typically display a minimal response, with the P2Y2 inhibitor AR-C118925XX.

**METHODS:** Male 9-month old C57/B16-J mice were divided into 4 weight-matched groups: Sedentary + Vehicle, Exercise + Vehicle, Sedentary + ARC, Exercise + ARC. Mice were treated with a vehicle control (0.8% saline) or AR-C118925XX (ARC, 10mg/kg) via intraperitoneal injection. Exercise groups were subjected to treadmill exercise (15m/min on a 5% incline) for 30 minutes each day for 5 weeks. Tibia samples were isolated for histomorphometry and micro-CT analysis. A second batch of mice were exercised for only 1-week and then euthanized to isolate mRNA samples from osteocyte-enriched cortical bone samples. Primary bone marrow stromal cells (BMSC) were isolated from 9-month old C57/B16 mice and cultured for one day before isolating the non-adherent Hematopoietic stem cells (HSC). The HSC's were cultured in differentiation media (Rankl + MSCF) supplemented with either DMSO or ARC (10μM). After 9-days, multi-nucleated osteoclasts that stain for tartrate resistant acid phosphatase (TRAP) were counted. Adherent BMSC's that remained were split and cultured in osteogenic media (100 μM l-ascorbic acid and 2 mM β-glycerophosphate) supplemented with either DMSO or ARC (10μM). The degree of mineral formed after 9-days was quantified by staining Alizarin red and reading the absorbance at 562 nm. Throughout the study statistical differences between groups were identified using a student t-test with a p-value < 0.05 considered statistically significant. Animal procedures were conducted under Institutional Animal Care Use Committee (IACUC) approval at the Henry Ford Hospital.

**RESULTS:** Based on histomorphometry we found periosteal mineralizing surface (PsMS/BS) increased with exercise in ARC treated mice compared to the sedentary controls as well as vehicle treated mice exposed to the same exercise regimen (Fig 1A). Although mineral apposition rate at the periosteum was unaffected by treatment, the resulting periosteal bone formation rate displayed a similar increase in response to ARC treatment during exercise. MicroCt analysis at the mid-diaphysis found a significant increase in Ct.Ar in response to exercise for both vehicle and ARC treated mice (Fig 1A). For vehicle treated mice cortical thickness (Ct.Th) increased by 6% ( $0.205 \pm 0.014$  vs.  $0.218 \pm 0.009$ ,  $p < 0.05$ ), while ARC mice displayed a 13% increase in Ct.Th following exercise ( $0.180 \pm 0.019$  vs.  $0.205 \pm 0.187$ ,  $p < 0.05$ ). The moment of inertia about the anterior-posterior axis (MOI) also significantly increased in response to exercise for both Vehicle and ARC treated mice; however, the MOI for sedentary and exercise mice treated with ARC was significantly greater than either sedentary or exercise vehicle treated mice (Fig 1A). At the cellular level, exercise failed to decrease Sost mRNA expression in vehicle treated mice similar to our previous work [5]. In contrast, ARC treated mice exercise displayed a significant decrease in Sost expression compared to sedentary controls as well as vehicle treated mice exposed to the same exercise regimen (Fig 1B). Independent of loading, ARC had no effect on osteoblast differentiation and mineralization (Fig 1C) or osteoclast differentiation (Fig 1D).

**DISCUSSION:** These findings suggest that antagonizing P2Y2 function in adults has the potential to increase the sensitivity to loading, namely treadmill exercise. Although the increase in bone formation measured via histomorphometry didn't lead to greater bone mass (Ct.Ar), the corresponding increase in MOI is likely to enhance bone strength. The increase in bone formation under loading was associated with an increase in osteocyte responsiveness, given that ARC appears to have no direct effect on osteoblast or osteoclast function independent of loading. Future studies will begin to examine how osteocytes' response to loading, specifically fluid flow is impacted by ARC treatment alongside the interaction between osteocytes with osteoblasts and osteoclasts.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study presents a novel use of ARC and its ability to enhance the potential to increase bone formation under loading. These findings are significant because increasing the responsiveness of bone to loading can reduce fracture risk in an aging population.

**REFERENCES:** [1] Bellido, *Calcif Tissue Int*, 2014, 94:25-34; [2] Gomez-Cabello et al., *Sports Medicine*, 2012;42(4):301-25; [3] Marques et al., *Age*, 2012;34(6):1493-515; [4] Gardinier et al., *Am J Phys Cell Phys*, 2014, 306(11):C1058-67; [5] Gardinier et al., *Bone Reports*, 2018;8:29-37.

