Osteogenic Voluntary Wheel Running in Mice

Ted S. Gross, Brandon J. Ausk, Phillipe Huber, Steven D. Bain, Edith M. Gardiner, Sundar Srinivasan
University of Washington, Seattle, WA
tgross@uw.edu

Disclosures: Ted S. Gross (N), Brandon J. Ausk (N), Phillipe Huber (N), Steven D. Bain (N), Edith M. Gardiner (N), Sundar Srinivasan (N)

INTRODUCTION: Voluntary wheel running (VWR) provides a non-invasive means to explore how exercise influences rodent physiological systems. While modest trabecular and cortical augmentation is observed when young mice are provided in-cage wheels, VWR by adult mice is not perceived as an osteogenic stimulus (1,2). Interestingly, this adult phenotype accurately models the muted skeletal response of adult humans to exercise and may explain, in part, why interventions derived from highly anabolic skeletal loading models have not been successfully translated into exercise strategies that enhance bone morphology in adult humans. However, if osteogenic VWR protocols were identified, subsequent insights from the model may reduce this barrier to human translation. In this study, we explored one such strategy by supplementing VWR with low dose cyclosporine (CsA). Based on previous *in vivo* and *in vitro* studies (3,4), we hypothesized that VWR+CsA would enhance periosteal cortical bone formation but neither VWR or CsA alone would alter normal periosteal bone formation.

METHODS: Female C57BL/6 mice (n=26, 16 wk, group housed) were randomly assigned to one of four groups: naïve cage mice (Naïve; n=6), CsA (3.0 mg/kg, s.c.; n=6), VWR (2 hr exposure between 8am and 12:00pm for 3 wk, 5 d/wk; n=7), or VWR+CsA (3.0 mg/kg, s.c., 30 min prior to the same activity protocol as the VWR group; n=7). CsA mice received injections at the same time as VWR+CsA mice. On the Thursday and Friday preceding the experiment start, VWR and VWR+CsA mice underwent acclimation to locked low-profile wheels (Med Associates Inc) for 2 hr. During acclimation (and subsequent wheel exposures), each mouse was isolated a cage with its own wheel. The cages were place in a sound and light attenuated activity monitoring cabinet (4 isolation cages at a time). Beginning the following Monday and on each subsequent experimental day, all mice (group housed) were moved to the activity room at 8am. VWR and VWR+CsA mice underwent wheel exposure, while Naïve and CsA mice remained in their group housed cages (excepting for CsA injection). Following VWR, mice were returned to their group cages. During VWR, wheel counts (binned in 1 min increments) were recorded. All mice received calcein labeling (10 mg/kg) on d10 and d19 and were sacrificed on d22. For this study, left and right tibiae were removed, sectioned at the mid-shaft, ground to 200 μm, imaged, and standard cortical dynamic histomorphometry was performed to quantify periosteal mineralizing surface (p.MS), mineral apposition rate (p.MAR), and bone formation rate (p.BFR). Left and right tibia data were averaged for each mouse. As the data did not demonstrate homogeneous variance across groups, Mann-Whitney non-parametric t-tests (p=0.05) were used to compare wheel count data, while Kruskal-Wallis non-parametric one-way ANOVA followed by post-hoc Dunn's test were implemented to assess the dynamic histomorphometry data (p=0.05). This study was approved and conducted in compliance with the University of Washington IACUC.

RESULTS: Average daily wheel turns for VWR and VWR+CsA mice were not significantly different (p=0.18) although VWR+CsA mice ran less (31% lower daily average). VWR+CsA mice demonstrated five-fold greater variability in average daily wheel turns than VWR mice (CV: 0.55 vs 0.11, respectively). VWR alone did not alter p.MS, p.MAR, or p.BFR vs Naïve mice. CsA mice demonstrated elevated p.MS vs Naïve mice (p=0.02) and VWR mice (p<0.01). VWR+CsA mice demonstrated significantly elevated p.MS, p.MAR, and p.BFR compared to Naïve and VWR mice (all p<0.001). p.MS in VWR+CsA was not significantly elevated vs CsA mice (p=0.55). However, VWR+CsA demonstrated profoundly elevated p.MAR (281%) and p.BFR (362%; Fig 1) vs CsA mice. Total wheel counts were poorly correlated with all osteoblast function parameters in the VWR group (r^2<0.05) and with p.MS and p.BFR in the VWR+CsA group (r^2<0.001). A slightly stronger, but non-significant, correlation was observed for VWR+CsA p.MAR (r^2=0.16), but arose via a negative correlation (i.e., the mice that ran the most, demonstrated the smallest p.MAR).

DISCUSSION: Superimposing low dose CsA (approx. 1/3 of immunosuppressant levels) upon VWR transformed a brief 2 hr exposure into a highly osteogenic intervention. This outcome was foreshadowed by previous externally controlled *in vivo* loading model data and *in vitro* studies of CsA enhancement of osteoblast function. However, to our knowledge, these data are the first to demonstrate that a brief exposure to VWR is capable of enhancing cortical periosteal osteoblast function in adult mice. While our protocol allowed 2 hr of daily VWR, previous studies have exposed mice to VWR for either 12 or 24 hr/d and it is possible that the constrained overall activity in our study contributed to elevated osteoblast function. In this context, however, the lack of responsiveness in the VWR group is consistent with previous VWR studies. One additional limitation of our study is that the most effective CsA dosing range or its interaction with varied duration VWR has not yet been explored. The variability observed in the p.BFR response of the VWR+CsA group (in which all mice demonstrated > p.BFR than the maximum observed in the CsA group) suggests that if it was possible to generate parameters that more accurately model the highly heterogenous skeletal loading activity during VWR exposure and across exposures (e.g. variability of distance traveled across days) it may be possible to associate specific activity behaviors with greater osteogenic responses (as all mice received the same CsA dose). Although the lack of correlation between osteoblastic response and distance run is consistent with the literature (5), much larger group sizes are required to confirm this observation given the substantially elevated p.MAR and p.BFR observed in the VWR+CsA group. Despite these limitations, these data support our general hypothesis and suggest that low dose CsA supplementation holds potential to overcome a primary barrier to using VWR as a translational model for exercise induced bone augmentation.

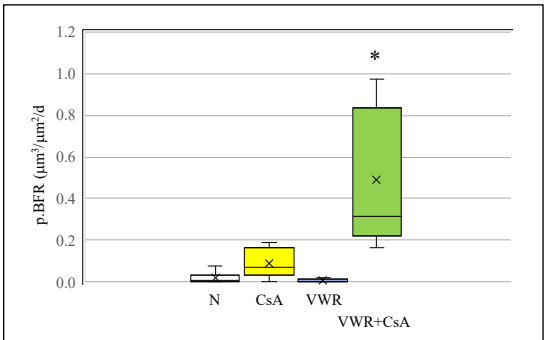

Fig 1. *VWR was osteogenic only when combined with low dose CsA.* Box and whisker plot of p.BFR for Naïve (N), CsA, VWR, and VWR+CsA groups (X=mean, horizontal line=median). No differences were identified between Naive, CsA and VWR groups. VWR+CsA demonstrated significantly increased p.BFR vs all other groups (p<0.01; *).

SIGNIFICANCE/CLINICAL RELEVANCE: The *in vivo* pre-clinical exercise model that is most relevant to humans (VWR) does not induce bone formation in adult mice. Here, we report on a novel VWR protocol that overcomes this barrier and, we believe, will enable unique exploration of what characteristics of voluntary activity are associated with enhancing bone formation.

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