A low dose of WNT-agonist molecules stimulates bone formation in aged mice additively with mechanical loading in strain-dependent manner

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INTRODUCTION: Osteoporosis is the most common metabolic bone disorder in humans. It is characterized by loss of bone mass and increased risk of fracture. WNT signaling is a key pathway regulating bone mass and can be activated pharmacologically or in response to mechanical loading to increase bone mass. However, old mice have diminished bone formation response to loading, due in part to reduced upregulation of WNT ligand expression. At last year's meeting, we showed a novel tetravalent antibody-based molecule ("FL5"), that acts as a WNT agonist by binding to FZD and LRP5 co-receptors, can act synergistically when combined with mechanical loading in young-adult (5-mo) mice. In particular, FL5 treatment at sub-therapeutic doses of 1 and 5 mg/kg stimulated periosteal bone formation synergistically with tibial loading that generated 2200με peak compressive strain. Here, we examined whether mechanical loading in combination with low-dose FL5 would synergistically stimulate bone formation and rescue reduced loading response in aged (22-mo) mice.

METHODS: Animal studies were approved by IACUC. The effect of low-dose FL5 combined with mechanical loading was evaluated in 22-mo B6 female and male mice, which model 65-year-old humans. Mice were dosed via IP injection 3 times/wk for 3 wks with either IgG control, 1 mg/kg FL5 or 5 mg/kg FL5. Starting on day 1 of treatment, the right tibia of each mouse was loaded to a peak compressive force of either 7 or 9 N for female mice, 9 or 11 N for male mice, 60 cycles/day, 5 days/wk, for 2 wks. These forces generate 2200 and 3000 με peak compressive strains, respectively, shown previously to induce modest periosteal lamellar bone formation in 22-mo female B6 mice. Calcein and alizarin were injected at the end of weeks 1 and 2 to label bone formation for dynamic histomorphometry. Before day 1, the right (loaded) and left (non-loaded) tibias were microCT scanned *in vivo* (pre-scan; Scanco, 10.5 mm voxel size). At the end of the 3-wk study, each tibia was re-scanned (post-scan). Scans were analyzed for cortical bone morphology, and temporal changes were determined by computing post- minus pre- values (Δ). After the post-scans, both tibias were dissected and embedded undecalcified in plastic. Transverse diaphyseal sections (100 μm) were cut and analyzed to determine mineral apposition rate (MAR), mineralizing surface (MS/BS), and bone formation rate (BFR/BS) on periosteal (Ps) and endocortical (Ec) surfaces separately (Bioquant). Two-way ANOVA was used to evaluate the effects of loading (loaded vs. non-loaded) and WNT agonist treatment (IgG, 1 mg/kg FL5, 5 mg/kg FL5) and their interaction; the two loading magnitude groups and sexes were analyzed separately. A significant ($p<0.05$) drug-loading interaction was the criterion to infer a synergistic effect.

RESULTS: Determined from dynamic histomorphometry and microCT data, mice loaded to generate 2200με compressive strain displayed a significant drug (Rx) effect. However, a loading effect was not observed, nor was there a synergistic effect of combined treatment. Mice treated with 5mg/kg FL5 had 0.7 $\mu m^3/\mu m^2$/day higher Ps.BFR ($p = 0.01$, Fig. 1A) and 0.03 mm higher increase in cortical thickness (ΔCt.Th, $p <0.0001$, Fig. 1B) compared to IgG control mice. Males and females loaded to 2200με compressive strain showed similar response and data were pooled together. Determined by microCT data, both female and male mice loaded to 3000με compressive strain showed significant drug (Rx) and loading effects. The effects were additive; no synergistic effect of combined treatment on microCT outcomes was observed. Among female mice, loaded limbs treated with 5 mg/kg FL5 had 0.04 mm increase in Ct.Th compared to IgG controls ($p<0.0001$, Fig. 2A). Similarly, male mice treated with 5 mg/kg FL5 had 0.035 mm increase in Ct.Th in loaded limbs compared to corresponding IgG controls ($p<0.0001$, Fig. 2B). Similar results were seen for cortical area and medullary area. Dynamic histomorphometry analysis for this group is ongoing.

DISCUSSION: This study indicates that mechanical loading in old (osteoporotic) mice can additively increase cortical bone indices when combined with a WNT agonist, although only when the mechanical stimulation is relatively high (3000με peak strain). Previous results from our lab indicated that 15mg/kg FL5 dose itself induces a potent anabolic effect. In this combined treatment, an anabolic effect was seen using a relatively low dose of FL5 (1/3 of previous dose), suggesting that loading may potentiate the anabolic action of the WNT agonist. However, no synergistic effect was observed based on microCT . The dynamic histomorphometry analysis is in progress to determine if there is a synergistic effect on periosteal bone formation in old mice, as we previously observed in younger mice. Future work will elucidate the biological mechanism of such a combined treatment strategy for mitigating age-related osteoporosis.

CLINICAL SIGNIFICANCE: Osteoporosis occurs 1 in 3 women and 1 in 5 men. Combining drug treatment with mechanical loading can potentially lower the amount of drug required to reach the same treatment efficacy which reduces the risk of adverse effects caused by higher drug doses.

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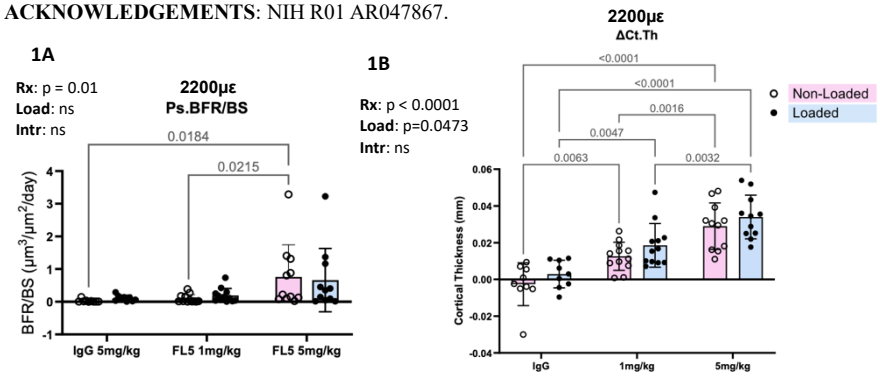

Figure 1. Mice loaded to 2200με compressive strain: Periosteal bone formation rate and cortical thickness showed dose dependent anabolic drug effect but no loading effect (female and male data pooled together) A) Only 5 mg/kg FL5 dose increased periosteal bone formation rate. No difference observed between control and lower dose nor was loading effect observed. B) Cortical thickness increased with the FL5 dose while loading has an overall impact. No pair-wise loading effect observed. (n = 9-13/ dose group)

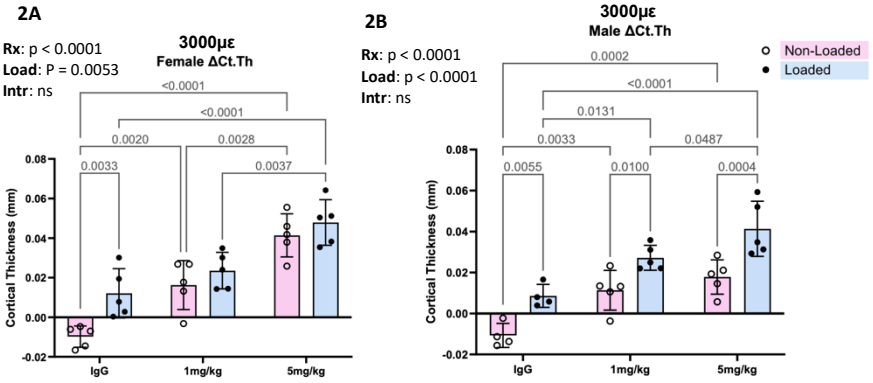

Figure 2. Mice loaded to 3000με compressive strain: A significant drug effect and dose dependent drug effect was observed cortical thickness. A) Only the control group among the female mice showed pair wise loading effect. FL5 treated groups had increased cortical thickness but no pairwise loading effect. B) Male mice showed additive effect of mechanical loading and FL5 treatment with dose dependent increase in cortical thickness. （n=5 per sex/dose group）