Sex Specific Effects of Osteocytic Cx43-Deficiency on Unloading-Induced Bone Loss

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Disclosures: None

INTRODUCTION: Previous research has shown that gap junction communication and mechanical stimulation, or lack thereof, influence bone development and remodeling, however, the mechanisms by which this occurs remain unclear. Our lab and others have previously shown deficiency of connexin 43 (Cx43), bone's predominant gap junction protein, in early osteoblasts/osteocytes protects against unloading-induced bone loss in mice.[1,2] It is unclear, however, if this protection is due to Cx43 deficiency in osteoblasts or osteocytes (Osteocalcin Cre), the latter being the most abundant and primary mechanosensory bone cell. To address this, we used a Cx43-deficient mouse targeting later osteoblasts/osteocytes (DMP1 Cre) to test the hypothesis that osteocyte-specific Cx43 deficiency protects against hindlimb unloading by tail suspension (HLS)-induced bone loss.

METHODS: All animal procedures were approved by the VCU IACUC. At 6 months of age, male and female osteocyte-specific Cx43 deficient mice ($Cx43^{\Delta OCY}$) and their wildtype littermates (WT) on C57Bl/6J backgrounds underwent HLS or normal ambulation (control) for 21 days (n=8-11 per group). MicroCT scans were taken on days 1 (baseline) and 22 (endpoint) and images of the left femur were analyzed. 2-way ANOVAs with Bonferroni's post-hoc tests were used to assess baseline scans for significant effects of sex and genotype. 3-way ANOVAs with Sidak's post-hoc tests were used to assess the change the change in groups over time for significant effects of sex, genotype, and HLS ($p<0.05$).

RESULTS: Statistical analyses on baseline scans showed consistent sex and genotype effects ($p<0.05$) on most metrics of both cortical and trabecular bone (**Figure 1).** Overall, Cx43 deficiency increased trabecular bone in the epiphyseal and metaphyseal regions, shown by BV/TV (**Figure 1)** in male mice, but not females. These results were further supported by increased Tb.N and decreased Tb.Sp in $Cx43^{\Delta OCY}$ male mice but not female mice compared to WT (data not shown). Cx43 deficiency also resulted in decreased cortical bone in both males and females, shown by CtAr/Tar and Cs.Th **(Figure 1).** After 21 days of HLS, significant effects of HLS were found in all epiphyseal and metaphyseal metrics measured as well as in Ct.Ar/T.Ar and cortical Cs.Th (**Figure 2**). Significant differences in change from baseline of epiphyseal BV/TV were seen between control and HLS mice in female WTs and male WT and $Cx43^{\Delta OCY}$, but not in female $Cx43^{\Delta OCY}$ mice (**Figure 2A**). Cx43 deficiency did not affect HLS-induced decreases in metaphyseal or cortical bone in either male or female mice (**Figure 2B** and **2C)**.

DISCUSSION: The basal differences between WT and $Cx43^{\Delta OCY}$ mice show osteocytic Cx43 plays a site-specific role in postnatal bone volume, with sexually dimorphic differences due to genotype in the metaphysis. We found it interesting that Cx43 attenuated the rate of disuse-induced epiphyseal bone loss in female mice but not male mice. Our previous work with an osteoblast and osteocyte specific knockout model showed attenuation in trabecular bone loss in the proximal tibial metaphysis in male mice. This difference in results suggests that the role of Cx43 in regulating bone metabolism could differ by both sex and cell type, with Cx43 deficiency in earlier osteoblasts being protective against disuse-induced bone loss. These differences also suggest that the role of osteocytic Cx43 in unloading-induced bone loss differs by sex. Future studies plan to further examine the mechanism of how Cx43 deficiency attenuates bone loss by using a double knockout mouse model to determine if Cx43 attenuates disuse-induced bone loss via a mechanism involving β-catenin signaling.

SIGNIFICANCE/CLINICAL RELEVANCE: These results help establish that osteocyte-specific Cx43 deletion causes increased trabecular bone and decreased cortical bone as a basal phenotype. The data also demonstrate that, at least in female mice, Cx43 deficiency in osteocytic cells only is sufficient to protect against disuse-induced bone loss.

1. Grimston et al. J Bone Miner Res. 2011. 2. Lloyd et al. J Bone Miner Res 2012.

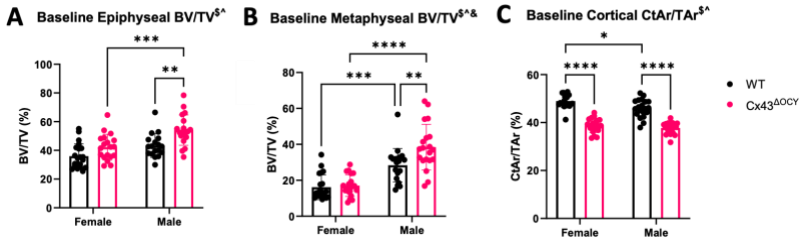

Figure 1. Baseline microCT data for WT and $Cx43^{\Delta OCY}$ female and male mice analyzed by 2-way ANOVA. $ Significant main effect of sex. ^ Significant main effect of genotype. & Significant interaction of sex and genotype. * p<0.05; ** p<0.005; **** p<0.00005 by Bonferroni post-hoc.

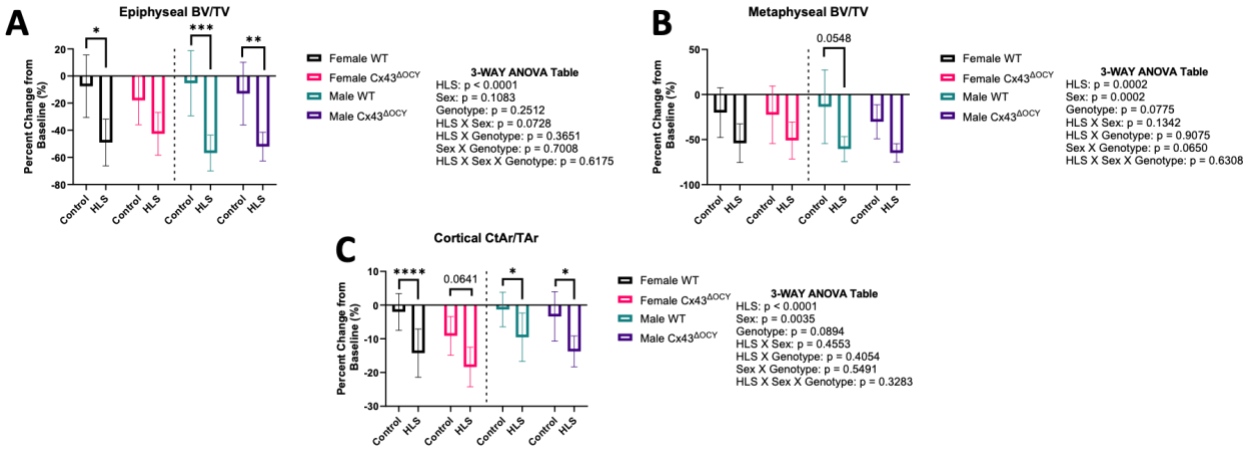

Figure 1. Percent change from baseline microCT data after 21 days of HLS. Females and males 3-way ANOVA. * p<0.05; ** p<0.005; *** p<0.0005; **** p<0.0001 by Sidak post-hoc.