Adaptive Response to Loading is Impaired in a Mouse Model of Osteogenesis Imperfecta

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INTRODUCTION: Osteogenesis imperfecta (OI) is a connective tissue disorder characterized by increased bone fragility and frequent fractures. A contributing element to elevated fracture rates lies in the consistently diminished cross-sectional dimensions of long bones, specifically evidenced by a low periosteal circumference in the diaphysis among individuals with OI [1]. The rate at which bone tissue is deposited on the periosteal surface plays a crucial role in determining periosteal perimeter and cross-sectional area, implying that a reduced apposition of bone tissue on this surface likely contributes to increased long bone fractures. Given the hypermineralization and increased brittleness [2] of OI bone tissue, it is plausible that these long bones exhibit a lesser-than-normal deformation in response to applied loads [3]. Thus, we hypothesize that reduced diaphyseal cross-sectional area in OI is the result of an altered adaptive response to mechanical loading. The aims of this study were as follows: 1) To determine strains engendered in tibias during locomotion and assess strain-load relationship, and 2) To examine the bone formation and resorption response in OI compared with wild type (WT) littermate control mice in response to 2 weeks of in vivo tibial loading.

METHODS: Ten-week old female *Brtl*$^{+/-}$ mice and WT littermates (n=7/genotype) underwent a surgical procedure where a strain gauge was attached to the antero-medial surface of the left tibia. By analyzing the strains engendered during walking and controlled in vivo cyclic tibial compressive loading (3N to 12N), the load-strain relationship for each genotype was quantified. Following this, adaptive response to two weeks (5 days/week) of in vivo tibial loading at 10N was assessed. This evaluation was conducted in both 10-week-old female *Brtl*$^{+/-}$ mice and WT littermates (n=10/genotype), with the left tibia subjected to loading and the right tibia serving as a nonloaded control (**Figure 1A**). Changes in bone mass and microstructure (Δ=day 15-day 0) were characterized via in vivo μCT. Further, μCT-based timelapse morphometry [4] was performed to assess endocortical (Ec.) and periosteal (Ps) mineralized and eroded volumes (MV; EV) and surface areas (MS; ES) normalized to bone volumes (BV) and surfaces (BS) over the 15-day interval (**Figure 1B**). Activity analysis of mice, dynamic fluorochrome-based histomorphometry, assessment of bone formation markers (P1NP & TRAP5b), and characterization using synchrotron tomography were also performed. Statistical analysis of acquired data was performed via ANOVA, Tukey post-hoc tests, and paired t-tests ($p<0.05$). All experiments were approved by an animal ethics committee.

RESULTS: Strain gauging revealed similar strains engendered in the tibial midshaft during locomotion for *Brtl*$^{+/-}$ (225με) and WT mice (300με). Furthermore, similar whole-bone stiffness was determined as the slopes of the load-strain regressions not significantly different between the *Brtl*$^{+/-}$ (-0.0070 ± 0.0012 N/με) and WT mice (-0.0061 ± 0.0011 N/με). Thus, a load magnitude of -10N (inducing 1500 με) was used for loading both genotypes. At baseline (day 0), as expected, *Brtl*$^{+/-}$ mice showed significantly reduced cortical area (CtAr), total area (TtAr) (**Figure 1C**), marrow area (MaAr), second moment of area (Imax and Imin) and cortical thickness (CtTh) (**Figure 1C**) compared to WT littermates. Following two weeks of loading, ΔCtAr, ΔCtTh, Ec. MV/BV, Ec. MS/BS were significantly increased in loaded compared to non-loaded limbs for both genotypes. However, strikingly, ΔTtAr (**Figure 1C**), Ps. MV/BV (**Figure 1C**), and Ps. MS/BS were significantly increased in the loaded vs non-loaded limb of WT mice, but not in the *Brtl*$^{+/-}$ mice, suggesting an altered formation response on the periosteal surface. In terms of resorption, both Ec and Ps EV/BV and ES/BS were reduced in the loaded compared to control limb of both genotypes. Analyses of additional outcomes are ongoing.

DISCUSSION: Our findings, in line with our hypothesis, indicate an impaired bone formation response on the periosteal surface to mechanical loading in *Brtl*$^{+/-}$ mice. This reduced mechanoresponse likely contributes to the low bone mass phenotype in these mice. Further studies are ongoing to explore mechanisms underlying reduced mechano-adaptive response in mice.

SIGNIFICANCE AND CLINICAL RELEVANCE: Lack of periosteal apposition in the cortical diaphysis of long bones and a consequential reduction in bone cross-sectional area are important factors contributing to fracture risk in patients with OI. Our study addresses the mechanobiological mechanisms underpinning this phenomenon, leading to a greater understanding of low bone mass in OI and guiding eventual therapies targeting this phenotype.

REFERENCES: [1] Palomo, T., et al., *J Pediatr*, 2016. **169**: p. 232-7. [2] Indermaur, M., et al., *J Bone Miner Res*, 2021. **36**(7): p. 1364-1375. [3] Rauch, F., *J Musculoskelet Neuronal Interact*, 2006. **6**(2): p. 142-6. [4] Birkhold, A.I., et al., *Bone*, 2014. **66**: p. 15-25.

IMAGES AND TABLES:

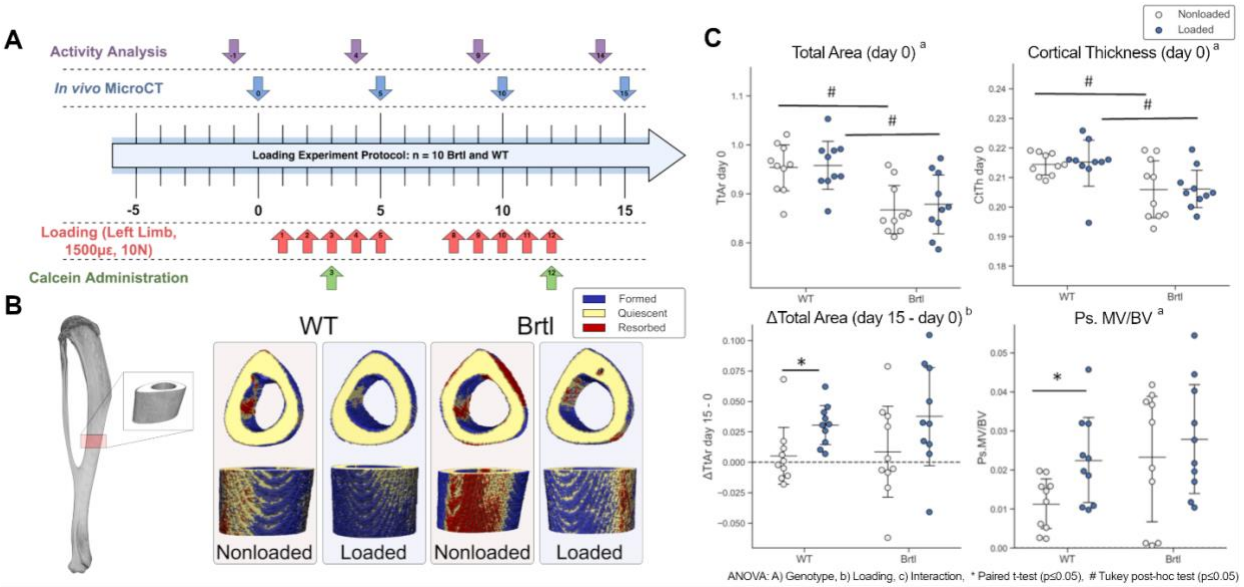

Figure 1: Two-week in-vivo loading tibial experiment in *Brtl*$^{+/-}$ mice and WT littermates. A) Experimental design and timeline. B) 3D images derived from μCT data and timelapse analysis showing formed, quiescent, and resorbed bone bone between baseline and the experimental endpoint at day 15. C) μCT analysis revealed significantly reduced total area and cortical thickness at baseline. After loading, change in total area increased for WT mice in loaded compared to nonloaded limbs, but this increase was not significant in Brtl mice. Ps. MV/BV was significantly increased by loading in WT, but not Brtl mice. Statistical analysis was carried out with ANOVA: a) Genotype, b) Loading, c) Interaction, as well as Tukey post-hoc tests (#) or paired t-tests (*), $p < 0.05$.