Scleraxis Targeted Collagen V Deletion Affects Bone Morphology with Altered Skeletal Loading

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Introduction

Classic Ehlers-Danlos syndrome (EDS) is characterized by abnormalities in connective tissue due to mutations in collagen V, with the most common mutation being haploinsufficiency in COL5A1. This syndrome is characterized by hypermobility, instability, hyperextensible skin, and abnormal wound healing [1,2]. Scleraxis-driven collagen V null (Col5a1Δten/Δten) mice have been developed to determine the role of collagen V in tendon [3,4]. In addition to differences in tendon, Col5a1Δten/Δten mice demonstrate decreased body size and exhibit reduced cortical bone polar moment of inertia (pMOI) [5,6], even though the role of collagen V in bone is believed to be limited [7]. The cause of this change in pMOI in these mice is unlikely, given the specificity of scleraxis, and as such, the cause of alterations in cortical bone morphology is unknown. Therefore, the objective of this study was to determine whether the observed bone morphological changes could be due to reduced skeletal loading. We hypothesized that any changes in bone morphology in the Col5a1Δten/Δten would be due to decreased skeletal loading.

Methods

Humeri from day 60 Col5a1+/+ (WT, n=8) and Col5a1Δten/Δten (NULL, n=13) mice, and ribs from day 120 Col5a1+/+ (WT, n=10) and Col5a1Δten/Δten (NULL, n=8) mice (IACUC approved) were prepared for high-resolution micro-computed tomography (μCT). Rib bone was chosen as a skeletal structure that is relatively load-independent and therefore its structure would not be affected by decreased mechanical loading. High-Resolution micro-CT. All samples were scanned using a μCT 35 (Scanco Medical AG). The epiphyseal and metaphyseal regions of the humeri, defined as proximal and distal to the growth plate, respectively, as well as the ribs at the cortical midshaft, were scanned at an isotropic resolution of 6μm. Standard cortical and trabecular morphometry parameters (trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp)), were measured for all humeri, while ribs only underwent cortical evaluation. Samples were kept hydrated with phosphate buffered saline (PBS) during scanning.

Data Analysis

The maximum pMOI for the cortical regions was calculated using custom code (MATLAB) and standard cortical and trabecular evaluations provided by Scanco were used to obtain all other parameters. Statistics Unpaired t-tests ($p<0.05$) were used to compare between WT and NULL mice for both humeri and ribs.

Results

Body weight was significantly decreased in the NULL mice when compared to the WT mice for both the day 60 and day 120 mice (Fig.1A-B).

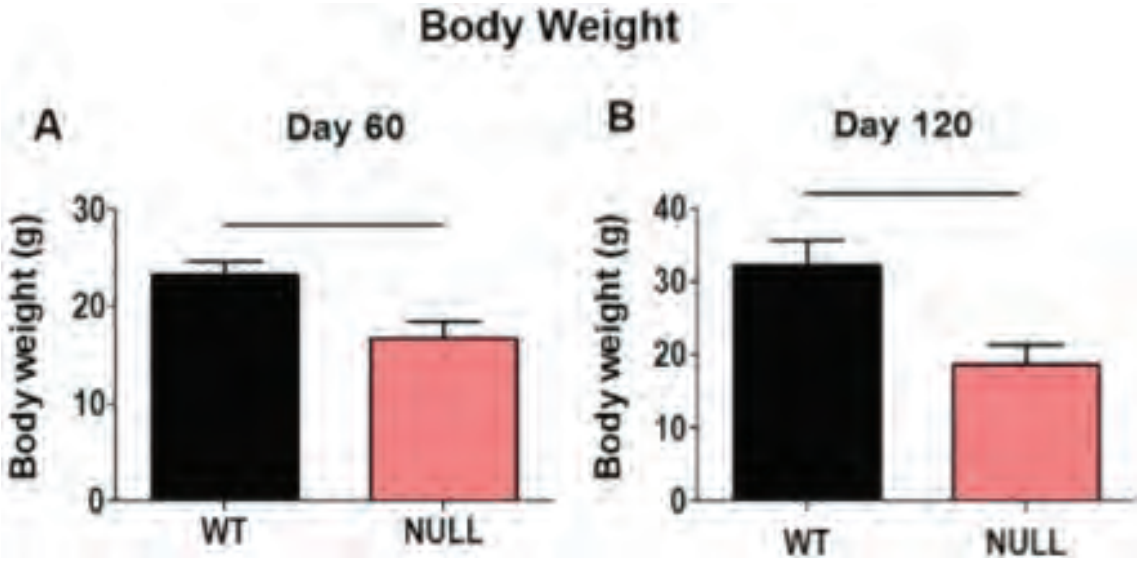

Figure 1. Body weight in WT was significantly greater than NULL at **(A)** day 60 and **(B)** day 120

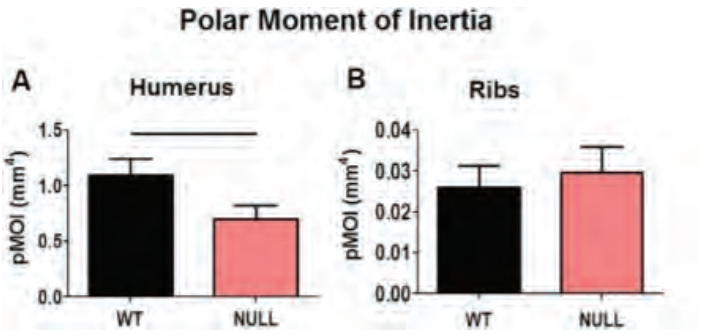

Figure 2. (A) pMOI was greater in the WT than the NULL for the humeri but **(B)** no changes were shown in the ribs.

Cortical regions. pMOI in the WT mice was significantly greater than in the NULL mice for the humeri (Fig.2A); however no changes were detected in pMOI in the ribs between groups (Fig.2B). There were no changes found for the other cortical parameters (data not shown). Trabecular regions. Tb.N was greater in the WT mice than the NULL mice in the epiphyseal region, (Fig.3A), and greater in WT mice than NULL mice in the metaphyseal region (Fig.3B). Tb.Th was greater in WT mice than the NULL mice in the epiphyseal region (Fig.3C) and in the metaphyseal region (Fig.3D). Tb.Sp in WT was decreased compared to NULL in both the epiphyseal region (Fig.3E) and metaphyseal region (Fig.3F).

Discussion

This study investigated whether changes in bone morphology in Col5a1Δten/Δten were caused by reduced loading due to body weight. The WT mice had greater body weight than the NULL mice and therefore exhibited greater mechanical loading on load-bearing skeletal structures resulting in more numerous, thicker trabeculae in WT mice when compared to NULL mice. The only differences in cortical bone were observed in pMOI in the humeri, but not in the ribs. This indicates that altered skeletal loading due to a decrease in body weight is a strong contributing factor to the differences observed between the WT and NULL mice. In addition to reduced body weight, these mice exhibit altered mobility and diminished activity [7], which may also contribute to reduced mechanical loading. These results are also in agreement with previous studies demonstrating that increased volume of mineralized tissue is induced by increased loading, and that mechanical unloading leads to trabecular bone loss [8,9]. Previous research in EDS patients has shown a reduction in bone mineral density (BMD) and lower trabecular bone scores (TBS) [10]. Although we found no differences in BMD, we did observe reduction in trabecular morphological parameters. In conclusion, results indicate that bone loss consistent with EDS may be due to mechanical unloading of load-bearing skeletal

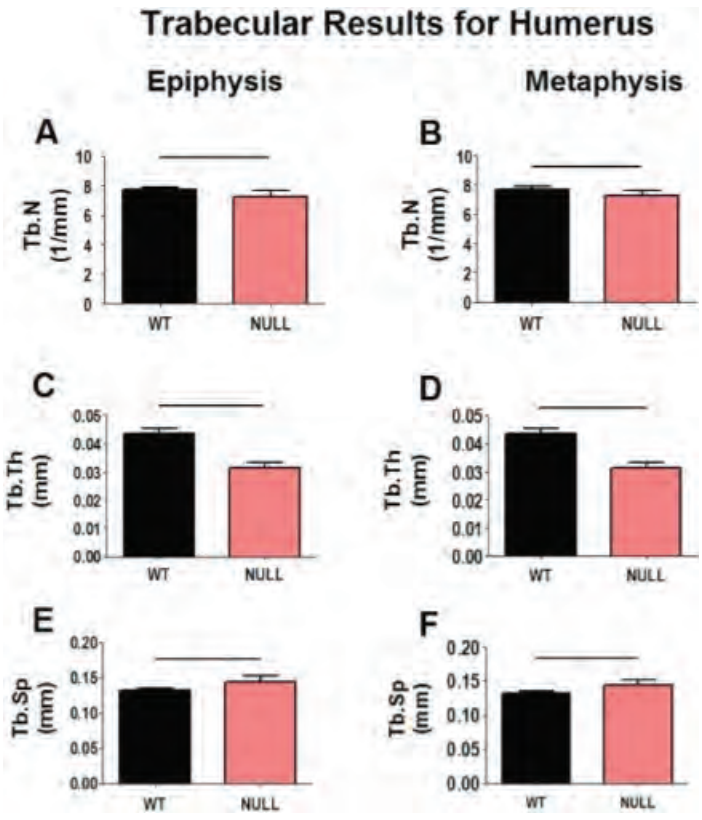

Figure 3. Tb.N was significantly greater in WT than NULL in both the **(A)** epiphysis and **(B)** metaphysis. Tb.Th was significantly greater in WT than NULL in both the **(C)** epiphysis and **(D)** metaphysis. Tb.Sp. was decreased in WT compared to NULL in both the **(E)** epiphysis and **(F)** metaphysis.

structures. However, other influences such as level of physical activity or aging might also contribute to this bone loss and future studies will investigate these potential influences further.

Significance

This study highlights the importance of monitoring bone integrity in patients suffering from classic EDS especially with reduction in physical activity and body weight during aging.

References

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