MICRO-CT BONE ASSESSMENT OF SEX DIMORPHISM IN A YOUNG MICE COHORT USING A NON-INVASIVE LOAD-INDUCED PTOA MODEL – PRELIMINARY RESULTS

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INTRODUCTION: One epidemiological feature of post-traumatic OA (PTOA) is the higher incidence in younger and more active adults, particularly those engaged in sports activities and military service. Anterior cruciate ligament (ACL) is the most commonly torn knee ligament, alone or associated with other structures. Therefore, animal models of PTOA that primarily induce non-surgical ACL lesions, such as load-induced models, are important research tools for simulating one of the most frequent PTOA mechanisms in humans. However, there is limited data on normal patterns of mice bone development and sex dimorphism, especially around skeletal maturity. To simultaneously investigate skeletal sexual dimorphism and bone alterations after acute trauma in the youth, our research utilized a single-load compression injury model of knee PTOA in a 12-week-old female and male mice cohort. We hypothesize that male and female mice at this age have differences in both macro and microstructure of the femur and tibia, as well as different responses to acute trauma, which can be assessed non-invasively by micro-CT.

METHODS: All procedures were conducted following an approved IACUC protocol. Female and male C57BL/6 mice (N=40 mice) were randomly assigned to control (N=10 female /10 male mice) and compression-induced injury (N=10 female /10 male mice) groups. At 12 weeks, mice were anesthetized, and the right limbs were placed in an ElectroForce 3100 instrument (TA Instruments) to undergo sham (0.6 N compressive pre-load only) or compression-induced injury (pre-load plus single ramp load with 1.7 mm displacement at speed 1mm/s]) according to assigned groups. Ketoprofen (5 mg/kg) was provided for post-procedure analgesia. The left knees were left unloaded and used as an internal control. Together, the right sham knees and left knees composed the uninjured group. ACL rupture was indicated based on auditory and mover displacement cues. Animals were anesthetized and sacrificed 3 days post-intervention. Body weight was measured before intervention (sham or compression-induced injury). Ex vivo bone assessments were performed using micro-CT (GE eXplore CT 120) (25 μm voxel size). The morphological and microstructure features quantitatively evaluated, including the abbreviations used herein, are shown in Figure 1. Cortical bone and trabecular bone microstructure were analyzed using a custom MATLAB code. The cortical features were assessed in the distal femoral and proximal tibial diaphysis. In addition, BMD was assessed in the femoral epiphysis. The trabecular microstructural features were assessed in the secondary spongiosa of the distal femoral and proximal tibial metaphysis. All variables were checked for normality using the Shapiro–Wilk test. Two-way analysis of variance (ANOVA) with post hoc test and paired t-test were used for normally distributed data. In contrast, Kruskal–Wallis and Wilcoxon rank tests were used for non-normally distributed data. Two-tailed $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS SECTION: ***Bone morphometry:*** There was a difference between the sexes in weight and tibial length in the uninjured (p<0.001) and injured groups (p= 0. 002 and p=0.006, respectively). In the uninjured group, male mice were heavier and showed longer tibias than female mice. There was an increase in the anterior tibial translation (in both compartments) in the injured group compared with the uninjured group in both sexes (p<0.001). A difference was observed in the morphometry of the femoral condyles between male and female mice in the uninjured (p< 0.001 for TCW, TCH-M, TCH-L) and injured groups (p=0.005, p=0.011, p=009, for TCW, TCH-M, TCH-L, respectively). Male mice had bigger femoral condyles compared to female mice (Fig. 2). However, no difference was observed in the ICNW between the sexes. There was a difference in TSM between male and female uninjured mice (p =0.044), which was not observed in the injured group. ***Bone microstructure:*** There was a difference in the femoral and tibial Cb.Th (p< 0.001) and femoral epiphyseal BMD (p= 0.005) in male and female mice in the uninjured groups, with male mice having greater Cb.Th and higher epiphyseal BMD (Fig. 3). In female mice, there was a difference in the BMD in the femoral diaphysis between the injured and uninjured groups (p=0.005). In the male mice, no significant differences in BMD and Cb.Th were observed between the uninjured and injured groups. TV, Tb.BV, and Tb.Th were higher in the femurs of male mice than in the femur of female mice in the uninjured group (p< 0.001, p< 0.003, p< 0.001, respectively). However, the proportion Tb.BV/TV was higher in the female mice compared to the male mice. Similar findings were observed in the tibia, except for Tb.BV, which was not significantly different among the sexes. The trabecular bone features differed between the sexes in the injured group, except Tb.BV in the femur and tibia.

DISCUSSION: Our study supports our hypothesis of bone sexual dimorphism in young mice. Overall, femur and tibia in male mice were larger, thicker, and with more trabecular bone than in female mice. Interestingly, Tb.BV/TV was higher in the female mice than male mice using micro-CT. In addition, our preliminary results suggest that within the short period of 3 days after injury, BMD changes were detected in the cortical bone, but only in female mice. However, additional sex-dependent differences may occur at later time points, which can be addressed in future studies. Despite some technical limitations inherent to micro-CT and the use of ex vivo samples, the technique allowed nondestructive assessment of the macro and microstructure of bone, with the possibility of selecting several different areas and planes for analysis as opposed to histology.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated the sex dimorphism in a young cohort of mice and different bone responses to acute trauma identified with micro-CT. These differences should be considered when planning and interpreting the results of murine models of PTOA.

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A

BONE MORPHOMETRY		
JOIN	FEMUR	TIBIA
Medial Tibial Translation	Intercondylar Notch Width (ICNW)	Tibial length
Lateral Tibial Translation	Total Condylar Width (TCW)	Medial Posterior Tibial Slope (TSM)
	Medial Total Condylar Height (TCH-M)	Lateral Posterior Tibial Slope (TSL)
	Lateral Total Condylar Height (TCH-L)	

B

BONE MICROSTRUCTURE	
CORTICAL BONE	TRABECULAR BONE
Bone Mineral Density (BMD)	Tissue Volume (TV)
Cortical Bone Thickness (Ct.Th)	Trabecular Bone Volume (Tb.BV)
	Trabecular Bone Volume/Tissue Volume (Tb.BV/TV%)
	Trabecular Thickness (Tb.Th μm)

Figure 1. Bone features quantitatively evaluated with micro-CT. A) Bone morphometry. B) Bone microstructure.

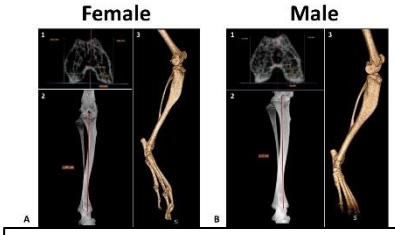

Figure 2. Micro-CT assessment of bone morphology in A) female and B) male mice. 1) MPR in axial oblique plane. 2) MIP in coronal plane. 3) 3D Volumetric rendering.

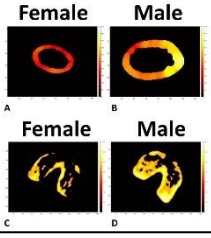

Figure 3. Cortical bone microstructure and comparison between the sexes. A-B) Cortical bone thickness. C-D) Bone mineral density.