Piezo2 Regulation in Chondrocytes Post-Exercise

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INTRODUCTION: Mechanical loading from an active lifestyle is essential for chondrocyte metabolism and cartilage homeostasis. Recent studies reveal the essential roles of Piezo1 mechanosensitive ion channels in osteoarthritis (OA) progression and mechanical injury-induced chondrocyte death. However, a significant knowledge gap exists concerning the roles of Piezo1 and Piezo2 mechanosensitive channels on cartilage maintenance post-exercise. Especially, the role of Piezo2 has been relatively understudied with respect to physiologic and hyper-physiologic mechanical cues than Piezo1 channel. TRPV4 channels have identified them as transducers of exercise-driven ECM remodeling, characterized by increased cartilage thickness and higher GAG/proteoglycan content. Our ultimate objective is to elucidate the functional expression of Piezo2 channels in response to exercise-driven physiologic loading. The goal of this report is to determine the gene regulations of Piezo1 and Piezo2 in exercised murine joints. We hypothesize that heterogeneous and exercise-driven gene regulations of Piezo2 channels in articular chondrocytes.

METHODS: Ethical approval was obtained by the UCAR committee (Protocol#: 2019-008). Eight-weeks old female C57BL6/J mice were subjected to Voluntary Wheel Running (VWR) system either with locked wheels (sedentary group) or unlocked wheels (exercise groups) for 1 or 2 weeks. Knee joints were harvested and fixed. Sagittal sections of medial side (thickness = 7μm) were labeled with anti-Piezo1 or anti-Piezo2 (ProteinTech, Inc.), and imaged by slide scanner (VS120) or Keyence. The fluorescent intensity was quantified by ImageJ and QuPath. All data was presented as Mean±SEM. Paired t-test or one-way ANOVA were used for comparison among sedentary and exercise groups.

RESULTS: First, we observed differential expressions of PIEZO1 and PIEZO2 channels in chondrocytes of femoral and tibial cartilage. Cartilage thickness and anti-Piezo1 intensity were statistically insignificant between femoral and tibial chondrocytes. However, the PIEZO2 expression level is significantly higher in tibial cartilage than femoral (Fig. 1). Second, PIEZO2 channels are augmented in exercised cartilage versus sedentary group. Interestingly, the number of PIEZO2-null chondrocytes were decreased post-exercise (~12% to 4%) and highly-PIEZO2-expressed cells were increased post-exercise (18% to 37% in femoral cartilage; 33% to 52% in tibial cartilage) (Fig.2).

DISCUSSION: Chondrocytes in knee articular cartilage experience a wide ranges of mechanical loading during daily activities, and chondrocytes may have differential cellular mechano-sensitivity and expression levels of mechanosensing ion channels. We observed heterogeneous Piezo2 expression in articular chondrocytes, from Piezo2-null to highly-expressed cells. We further observed the augmented Piezo2 channels in exercised chondrocytes in medial femoral and tibial cartilage, yet less significant in lateral sides. It has been known that the medial cartilage bears higher weight while standing and walking than lateral sides, our results suggest that physiologic joint loading may differentiate Piezo2 expression in chondrocytes. By showing the heterogeneity of Piezo1/2 expression level in femur and tibia, it is critical to limit the comparison in between certain regions. In addition to our previous finding that Piezo 1 was upregulated in osteoarthritis (OA) but not Piezo2, Piezo2 may play a chondro-protective role compensating Piezo1 upregulation. With figuring out the role of Piezo2 in cartilage health, potential agonists can be investigated to facilitate rehabilitation process and applied to clinical. Future work: We noticed the variation in sedentary group is larger than in exercise groups. For alleviating this limitation, we are planning to extend the exercise duration, at the meantime, separate the groups into low-activity and high-activity based on running distances. Since specific agonists or antagonists of PIEZO2 are unknown, cartilage-specific Piezo2-cKO mice will permit mechanistic elucidation of Piezo2-mediated chondrocyte mechanotransduction apart from Piezo1-mediated mechanotransduction mechanisms.

SIGNIFICANCE/CLINICAL RELEVANCE: This project emphasizes the character of Piezo2 channel in promoting cartilage health as well as the alternative role of Piezo1 other than in OA, which can bring attention to Piezo1 and 2 on promoting cartilage health in molecular level and make exercise-therapy more convincible to patients in rehabilitation.

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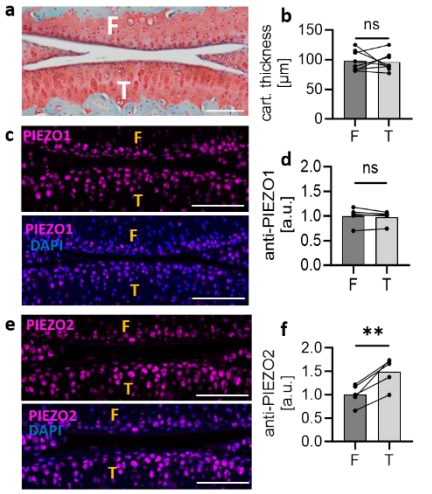

Fig 1. Heterogeneous expressions of Piezo1 and Piezo2 channels in femoral and tibial articular cartilage in mice. (a) A representative Saf-O-stained micrograph. (b) Cartilage thickness of femoral and tibal cartilage. (c-e) Representative IF images of anti-PIEZO1, anti-PIEZO2 (magenta) antibodies, DAPI (blue). Bar = 100μm. F=femur, T=tibia. (d-f) Fluorescence intensity of anti-Piezo1 and Piezo2. Higher expression of Piezo2 in tibial chondrocytes than femoral chondrocytes. N=5-7. Mean±SEM; paired student t-test were used for statistics; **p<0.002.

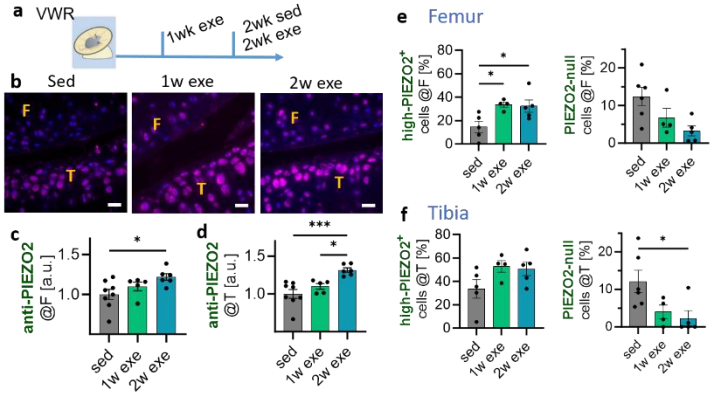

Fig 2. Upregulation of PIEZO2 post-exercise. (a) Timeline. (b-c) Representative anti-Piezo2 IF images of sedentary, 1wk exe and 2wk exe groups. Bar=20μm; increased anti-Piezo2 in femoral and tibial chondrocytes in exercised groups. (e-f) Increased high-Piezo2 expressing chondrocytes post-exercise, decreased Piezo2-null chondrocytes post-exercise. Quantified by ImageJ and QuPath. N=5-8/group. Mean±SEM; one-way ANOVA with Tukey's multiple comparisons. *p<0.05;***p<0.001.