Exercise Improves Structural Properties in Load Bearing Bones of Female but not in Male SPARC$^{-/-}$ mice

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Introduction: Secreted protein acidic and rich in cysteine (SPARC) is the most abundant glycoprotein in bone and is considered a "matricellular protein" as it mediates interactions between cells and the extracellular matrix (ECM). In bone, SPARC is expressed by osteoblasts and is thought to play a critical role in bone remodeling and homeostasis, although the exact mechanism is not clear. Sex as a variable effecting health, illness and treatment efficacy has not been well studied is not well understood. The purpose of this study was to quantify differences in the structural and biomechanical properties between the calvarial and femoral bone from male (M) and female (F) wild-type (WT) and SPARC null (SPARC$^{(-/-)}$) mice. We also examined whether voluntary wheel running can rescue bone health in these bones and if there were significant differences in bone quality between the two sexes.

Methods: 7-9 month old male and female WT (n = 40) and transgenic SPARC$^{(-/-)}$ (n=43) mice were randomly assigned to exercise or sedentary group (n>= 12). Exercise groups were given a running wheel while in the sedentary groups the wheel was fixed. After 6 months, the mice were sacrificed and the calvaria and femur harvested. Bone structural parameters were quantified *ex-vivo* using micro computed tomography (µCT). Biomechanical properties were evaluated using push out testing (calvaria) and three-point flexural testing (femurs). Morphological features of the bone were examined via histological staining.

Results: Calvaria: µCT found that M-SPARC$^{(-/-)}$ had significantly greater bone mineral density (BMD) compared to M-WT (Fig. 2A). WT mice of both sexes had significantly greater bone volume (BV) and bone volume ratio (BS/BV) compared to SPARC$^{(-/-)}$ mice (Fig. 2B-C). Conversely, F-SPARC$^{(-/-)}$-SED had significantly greater connective density compared with F-WT groups and F-SPARC$^{(-/-)}$-RUN (Fig. 1D). M-WT calvaria had significantly greater connective density compared to M-SPARC$^{(-/-)}$. WT had significantly greater trabecular thickness compared to SPARC$^{(-/-)}$ groups for both sexes (Fig. 1E). Push out test showed a significant reduction in load to failure and toughness in the SPARC$^{(-/-)}$ compared to WT for both sexes (Fig. 3A-B). Stiffness was also significantly reduced in the SPARC$^{(-/-)}$-SED for both sexes (Fig. 3C).

Femur: F-SPARC$^{(-/-)}$-SED mice had significantly lower BMD than all other female groups (Fig. 2A). BV was significantly greater for WT compared to SED males with M-SPARC$^{(-/-)}$-RUN femurs having greatest reduction (Fig. 2C). The only significant difference in BV among females was between SPARC$^{(-/-)}$-SED and WT-RUN. BS/BV was significantly greater in M-SPARC$^{(-/-)}$-SED compared to M-WT-RUN, while in females BS/BV was significantly greater in F-SPARC$^{(-/-)}$-SED compared to other groups. Three point flexural testing demonstrated significantly lower ultimate load to failure in the F-SPARC$^{(-/-)}$-SED compared to the F-SPARC$^{(-/-)}$-RUN and F-WT-SED (Fig. 3D). Running significantly increased the toughness of F-SPARC$^{(-/-)}$ femurs but not M- SPARC$^{(-/-)}$ femurs (Fig. 3E). M-SPARC$^{(-/-)}$-SED femurs were significantly stiffer than M-WT-SED (Fig. 3F). Running further increased the stiffness of M-SPARC$^{(-/-)}$ femurs.

Discussion: This study emphasizes significant differences in the structural, biomechanical, and morphological properties of bone in regards to the gender and exercise in SPARC$^{(-/-}$ and WT mice. In males, exercise had little to no effect on rescuing structural and biomechanical properties, with only stiffness being affected. Similar reductions in structural and biomechanical strength were observed in both male and female SPARC$^{(-/-)}$ calvaria, besides BMD which was elevated in M-SPARC$^{(-/-)}$ mice. Most measures were not significantly affected by exercise, however, exercise did increase the compromised stiffness observed in male and female SPARC$^{(-/-)}$ calvaria. This suggests that the effect of SPARC$^{(-/-)}$ was greater than exercise on calvaria bone structure and function. In femurs, the effect of SPARC$^{(-/-)}$ was detected in the structural and biomechanical parameters of both sexes. It was, however, more noticeable in females with bone quality rescued with exercise. Further research of the mechanisms that cause these differences is needed.

Significance / Clinical Relevance: Research into gender differences can help understand, inform, and personalize treatment options for patients in order to treat patients more accurately.

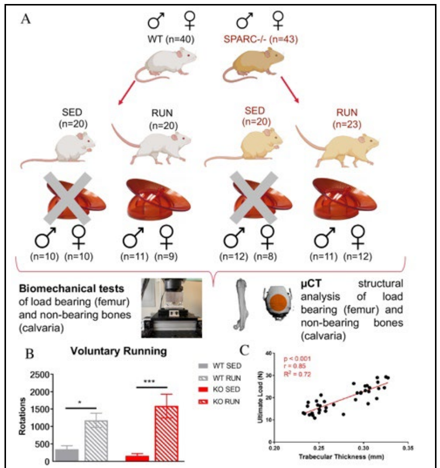

Fig. 1. Experimental design and methods authentication. **(A)** *Experimental design and groups.* **(B)** *Quantification of voluntary running in response to running wheel availability; *p<0.05, ***p<0.001.* **(C)** *Correlation between the ultimate load and trabecular thickness.*

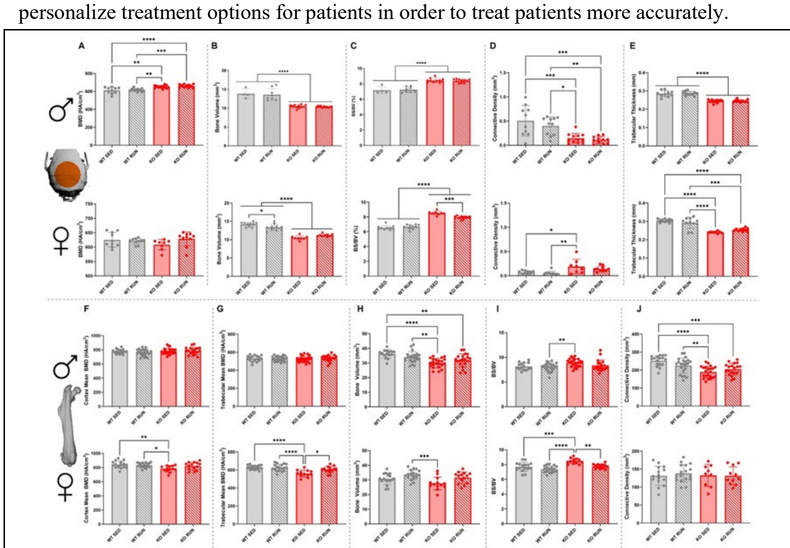

***Fig 2*: Structural properties demonstrated significant effects of SPARC deficiency in both calvaria (A-E) and femurs (F-G) regardless of sex and strain.** *(A) BMD, (B) Bone Volume, (C) BS/BV, (D) Connective Density, (E) Trabecular Thickness, (F) Cortex Mean BMD, (G) Trabecular Mean BMD, (H) Bone Volume, (I) BS/BV, and (J) Connective Density. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

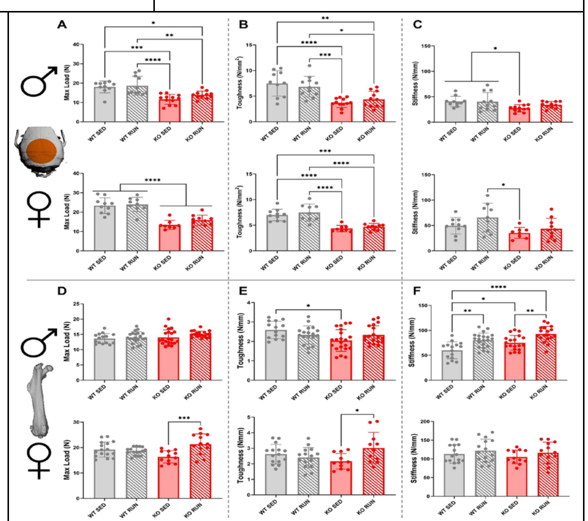

***Fig 3*: Biomechanical testing of calvaria (A-C) and femur (D-F) harvested from mice showed the significant effects of SPARC deficiency on bone quality. Exercise rescues bone stiffness in both sexes.** *(A, D) Max load, (B, E) Toughness, and (C, F) Stiffness.*