INTRODUCTION:

G Protein-coupled Estrogen Receptor 1 (GPER1) is a novel receptor that binds to estrogen with high affinity. The receptor is present on growth plate chondrocytes, osteocytes, osteoblasts, and osteoclasts and has been shown to play a functional role in promoted growth plate closure and bone remodeling [1]. The finding from a mouse study with the deficiency of GPER1 protein showed an increase of musculoskeletal growth and fat development. This suggests that GPER1 plays a significant role in bone development and remodeling [2, 4], but the role of GPER1 in bone repair and fracture healing is not clear. The purpose of this study was therefore to determine the role of GPER1 in bone healing utilizing an established mouse femur fracture model. We hypothesized that the deficiency of GPER1 enhanced bone repair by increasing bone mineral density (μCT), chondrogenesis, and biomechanical properties.

METHODS:

Surgical model: The retrograde intramedullary nailing and fracturing was done utilizing a previously established model [3]. Wild-Type (WT, GPER1+/+) mice and GPER1-knockout (KO, GPER1−/−) mice underwent retrograde intramedullary nailing of the right femur followed by creation of a closed femur fracture (Fx groups) and the left femur underwent a sham-operation (Control groups) to be used as a control.

Harvesting: The mice were sacrificed at 1 week (8 WT; 11 KO) and 6 weeks (7 WT; 8 KO) and both femurs were harvested and analyzed using histology, microCT and biomechanical testing (three-point bending stiffness). The femurs were placed in 1% PBS and stored in -20°C for micro-computed tomography.

Histology: The bones were embedded in paraffin, and 8 μm sagittal sections were taken through the mid-diaphysis. The sections were stained using Safranin O & Fast Green, and H&E.

RESULTS:

At 1 week after fracture, there was no difference in the histological appearance (Figure 1.) An increase staining of proteoglycan suggested an acceleration of chondrogenesis in the KO group (Figure 2). There was no change in bone mineral density (Figure 3) and three-point bending stiffness (Figure 4) between WT and KO deficient fracture groups.

At 6 weeks post fracture, there was a significant difference (p=0.04) between bone mineral density of the WT and KO groups. At 6 weeks, the three-point bending stiffness of the GPER1-deficient fractured femurs was also greater than those of the WT femurs (p = 0.02) and approached 74% of the control (unfractured) femur (Figure 5).

DISCUSSION:

Six weeks post-fracture GPER-1 deficient mice demonstrated increased bone mineral density and increased stiffness in three point bending stiffness relative to wildtype. These findings suggest that GPER1−/− mice have accelerated healing of a closed femur fracture at six weeks. Our findings suggest that GPER-1 plays a significant role in the regulation of fracture healing and the receptor may serve as a useful target for potential therapeutic modalities to enhance fracture healing.

SIGNIFICANCE:

Estrogen receptors are important for bone growth and osteoporosis. In this study, we determine the role of GPER1 in fracture healing.

ACKNOWLEDGEMENTS:

We thank Ms. Maia Frank for her technical assistance and financial support from NDDK T35 and UTSW Hofmann fund.

REFERENCES: