

# Muscle-derived FGF21 may negatively regulate bone homeostasis in DMD via promoting bone marrow adiposity

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**INTRODUCTION:** Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy seen in children which affects both muscle and bone. Poor bone health is a significant problem for DMD patients which concomitantly contributes to progressively reduced mobility and low quality of life. To date, the pathogenesis of bone abnormalities in DMD is still poorly understood and effective therapies to treat poor bone health in DMD are still lacking. We have previously identified a novel myokine, fibroblast growth factor 21 (FGF21), typically not expressed in skeletal muscle under physiological conditions, is dramatically upregulated in skeletal muscles in DMD mouse models. Systemic neutralization of circulating FGF21 significantly improved bone quality in dystrophic mice. However, how dystrophic muscle-derived FGF21 contributes to the disease progression in DMD, specifically to the poor bone health, has yet to be fully understood.

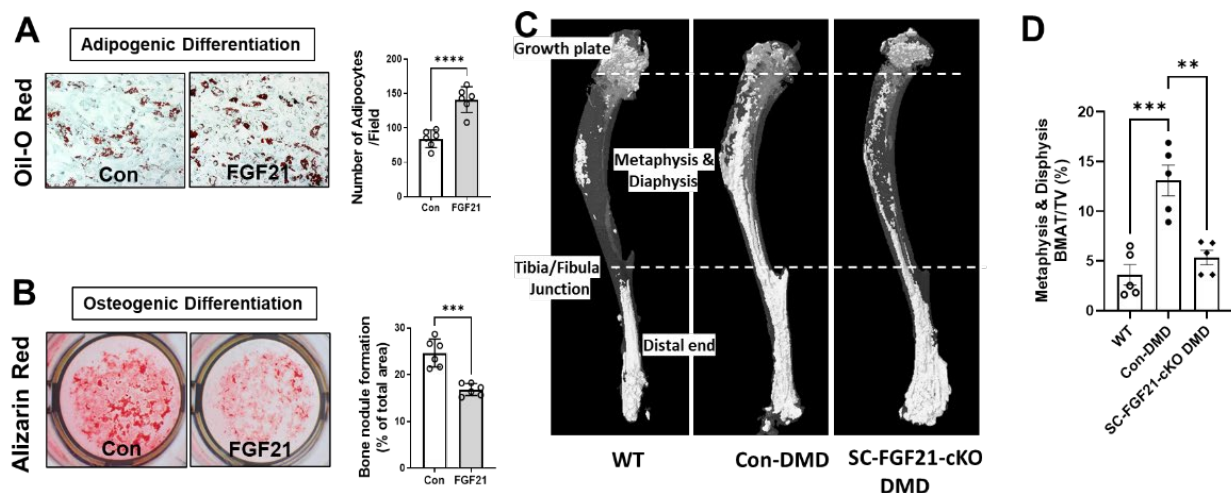
**METHODS:** Mdx mice were used in this study. Both a murine bone marrow mesenchymal stem cells (BMSCs) cell line (ST2 cells) and primary BMSCs isolated from mdx mice were used to test the effects of FGF21 on osteogenic and adipogenic differentiations. Muscle conditional FGF21 KO dystrophic mice were generated by cross-breeding mdx, Pax-7 Cre, and FGF21 loxP mice. The muscle specific FGF21 KO were induced by Tamoxifen gavage. Bone marrow adiposity were accessed via micro-CT analysis on decalcified tibia stained with osmium tetroxide.

**RESULTS:** We found that rFGF21 treatment significantly increased BMSCs adipogenesis and concomitantly inhibited osteogenesis (Fig. 1 A & B); Knockdown of beta-klotho (KLB, an obligate co-receptor of FGF21) using siRNAs blocked the effects of FGF21 on promoting adipogenic differentiation of BMSCs. Our data demonstrate that FGF21 directly regulates BMSCs differentiation favoring adipogenesis. We further tested *in vivo* whether elevated FGF21 increases bone marrow adiposity in DMD. We used osmium tetroxide staining with microCT to visualize and quantify bone marrow adipose tissue (BMAT) in tibia of dystrophic mice. Significantly increased bone marrow adiposity was observed in dystrophic mice when compared with WT mice. Excitingly, significantly reduced bone marrow adiposity along with improved bone mass and bone microstructure were observed in muscle conditional FGF21 KO dystrophic mice when compared with their dystrophic loxP controls (Fig. 1 C & D).

**DISCUSSION:** Our observations suggest an important role of muscle-derived FGF21 in regulating bone marrow adiposity and affecting bone homeostasis in DMD.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study reveals a new pathological pathway that contributes to DMD bone pathology and further identify muscle-derived FGF21 as a negative regulator for the bone health in DMD.

**IMAGES:**



**Figure 1:** Bone Marrow Mesenchymal Stromal Cells (BMSCs) were isolated from long bones of dystrophic mice. BMSCs were cultured in adipogenic or osteogenic differentiation media with or without rFGF-21 (10ng/ml). The effects of FGF21 on adipogenesis and osteogenesis of BMSCs were evaluated. **A.** Representative images of Oil-O red stained BMSC adipogenic cultures and quantification of Oil-O red positive adipocytes per field. **B.** Representative images of Alizarin red S stained BMSC osteogenic cultures and quantification of percentage of Alizarin red positive bone nodule area. Decalcified tibiae were stained with osmium tetroxide and the content of BMAT was visualized by  $\mu$ CT. **C.** Representative images of BMAT of tibia. **D.** BMAT quantification in the in metaphysis & diaphysis of the tibia. Student *t*-tests were used. Data were expressed as mean  $\pm$  SEM. One-way ANOVA is used to compare values among groups. \*\**P* < 0.01, \*\*\**P* < 0.001, and \*\*\*\**P* < 0.0001.