Metformin Promotes Femoral Fracture Repair through AMPK and Mitochondrial Pathways in Rats

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DISCLOSURES: All the authors: None.

INTRODUCTION

Bone fractures are among the most common traumatic injuries, affecting individuals of all ages and imposing a substantial socioeconomic burden. Current treatments carry risks and complications, and no pharmacologic agents are approved to accelerate fracture healing. Metformin (Met), a widely prescribed drug for type 2 diabetes, has been shown to promote tissue regeneration, including bone repair. However, conflicting evidence exists: some studies report no benefit or even impaired fracture healing with Met [1,2], whereas others demonstrate enhanced skeletal repair through angiogenesis, osteogenesis, and mineralization [3]. These discrepancies highlight the need to clarify Met's role in non-diabetic fracture healing. This study evaluated the therapeutic potential of Met to enhance femoral fracture repair in rats.

METHODS

Open femoral osteotomy stabilized with retrograde intramedullary nailing was performed in 60 female Sprague-Dawley rats (12 weeks old). Female rats were used to minimize sex-based biological variation. Animals were randomized to receive either oral Met (150 mg/kg/day) or PBS (control). All animals were administered NSAIDs and antibiotics for 7 days post-surgery. Fracture healing was assessed at 6 and 8 weeks (n = 6/group/timepoint) using: 1) **Histology** – Hematoxylin & Eosin (H&E), Safranin O/Fast Green (SFO&FG), and Picrosirius Red to examine callus composition and collagen distribution; 2) **Immunohistochemistry** – p-AMPK, NDUFB8, and mtTFA to evaluate mitochondrial activity; and 3) **Micro-CT** – bone volume (BV), tissue volume (TV), BV/TV ratio, trabecular number (TrN), thickness (TrTh), separation (TrSep), and connectivity density. Additionally, three-point bending tests were performed at 6 weeks in a separate cohort (n = 12), with intact femora serving as controls. All procedures were approved by the University of Pittsburgh IACUC. Statistical analysis performed using Mann-Whitney test.

RESULTS

At 8 weeks, both groups exhibited organized cortical bone with no apparent differences. At 6 weeks, however, control fractures contained abundant fibrovascular tissue and hyaline cartilage, dominated by collagen type III. In contrast, Met-treated fractures demonstrated partially ossified callus with strong collagen type I expression and reduced proteoglycan content (**Fig. 1**), consistent with accelerated endochondral ossification. Met treatment significantly increased expression of p-AMPK, NDUFB8, and mtTFA within the callus compared to controls (**Fig. 2**). Micro-CT analysis showed increased BV/TV and trabecular thickness, with reduced connectivity density, in the Met group relative to controls (**Fig. 3**).

DISCUSSION

Oral Met accelerated fracture healing in a rat open femur fracture model at 6 weeks post-surgery. Treated animals exhibited more advanced ossified callus, predominant collagen type I, and reduced proteoglycan content, indicative of accelerated endochondral ossification. These effects were accompanied by increased expression of AMPK and mitochondrial markers, supporting a mechanistic role in osteoblast differentiation and bone regeneration. Control fractures retained cartilage and collagen type III, reflecting delayed maturation. By 8 weeks, structural and histological differences had been resolved, suggesting Met primarily accelerates the timeline of healing rather than altering the final outcome. Micro-CT confirmed enhanced bone structure in Met-treated fractures, although biomechanical improvements were not statistically significant, indicating that early structural maturity may not immediately translate into mechanical strength.

SIGNIFICANCE

Metformin, an inexpensive and widely available drug, may represent a novel therapeutic option to accelerate fracture healing through metabolic modulation.

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ACKNOWLEDGEMENTS

This work was supported in part by the DOD/MTEC (W81XWH-22-9-0016) and the DOD (HT9425-23-1-0617).

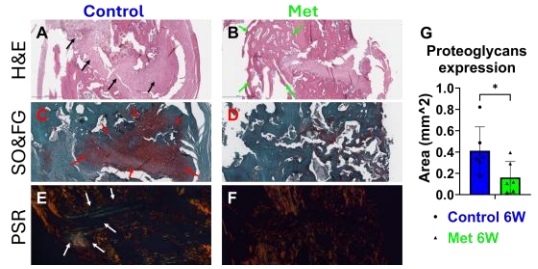

Fig. 1 Met accelerates femur fracture callus ossification at 6 weeks post-surgery. Control fracture site contains hyaline cartilage and proteoglycans, with predominant collagen type III expression (black, red, and white arrows in panels **A**, **C**, and **E**, respectively). Met-treated group shows ossification of hyaline cartilage (green arrows, **B**), formation of spongy bone (**D**), and predominant collagen type I deposition (**F**). Proteoglycan expression remains prevalent in the control group (**G**). n=6. *p < 0.05

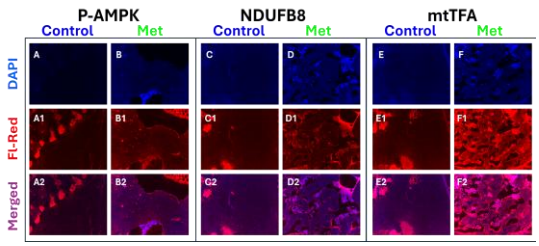

Fig. 2 Met enhances femur fracture healing through activation of p-AMPK, NDUFB8, and mtTFA. p-AMPK, NDUFB8, and mtTFA expression are reduced in the control group (**A–A1**, **C–C1**, and **E–E1**, respectively) compared to the Met-treated group (**B–B1**, **D–D1**, and **F–F1**, respectively) at the femur fracture healing site.

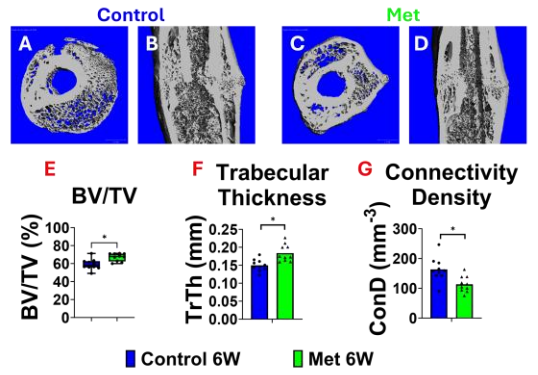

Fig. 3 Met accelerates femur fracture callus ossification. Met group (**C, D**) shows thick organized trabecular net in the stage of remodeling to cortical bone with prevalence of mineralized tissue, whereas control (**A, B**) shows disorganized, thin trabecular net with increased non-mineral tissue amount. BV/TV (**E**) and trabecular thickness (**F**) are increased, whereas connectivity density (**G**) is decreased in Met group compared to control. N =10, *p < 0.05