Targeting Apolipoprotein E to Improve Obesity-Impaired Fracture Healing

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Introduction: Obesity is a major risk factor for bone fracture, often leading to delayed union or nonunion; however, the underlying mechanism remains poorly understood. In this study, we observed that fracture healing was significantly impaired in mice with high-fat diet (HFD) compared with those on chow diet. Interestingly, ApoE knockout (ApoE-KO) mice fed on an HFD exhibited improved fracture repair compared to wild-type (WT) mice. We further investigated how ApoE promotes adipocyte differentiation within the fracture callus, thereby impairing bone repair.

Methods: *Mice* - All animal experiments were approved by IACUC and IRB. ApoE knockout (ApoE-KO) mice were purchased from the Jackson laboratory. *High-fat diet* - Four-week-old mice were fed either a high-fat diet (HFD; Bio-Serv F3282, 36% kcal fat, 20.5% protein, 35.7% carbohydrate) or a chow diet (Bio-Serv F4031, 7.2% kcal fat, 20.5% protein, 61.6% carbohydrate) until 4 months of age, at which point they underwent fracture surgery. The respective diets were continued throughout the healing period until sacrifice at 21 days post-fracture. *micro–CT Analysis of Fracture Callus* - Midshaft tibial fractures were induced in 4-month-old mice. After 21 days of healing, fracture calluses were harvested, fixed, and scanned using a Scanco vivaCT 80 at 8 µm resolution. *Histological Analysis of Fracture Callus* – Fracture calluses were decalcified, paraffin-embedded, sectioned, and subjected to histological staining. *Cell Culture* – BMSCs were isolated from the femurs and tibiae of unfractured mice based on plastic adherence. For adipogenic differentiation, cells were cultured in adipogenic induction medium. To assess the effect of exogenous ApoE (circulating ApoE), recombinant ApoE (50 ng/ml) was added to the medium. To examine endogenous ApoE (cell-derived ApoE), BMSCs from ApoE-KO mice were induced to differentiate. *Gene Expression Analysis* – Total RNA was extracted using TRIzol Reagent and reversed transcribed to cDNA. Realtime PCR was performed using the SYBR Green PCR Master Mix, with expression levels normalized to GAPDH. *Protein analysis* - Proteins were extracted using RIPA lysis buffer, separated by SDS-PAGE, and transferred onto a PVDF membrane. PVDF membranes were incubated with specific primary antibodies against the proteins of interest.

Results: Wild type C57BL/6 mice were fed either an HFD or chow diet starting at 4 weeks of age until sacrifice. Tibial fractures were induced at 4 months of age, and diets were maintained during the healing period. After 21 days of post-fracture, HFD-fed mice exhibited less bone volume and bone mineral density, along with increased adipocyte accumulation within the fracture callus compared with chow-fed mice (Fig. 1). ApoE plays a central role in the regulation of lipid metabolism, including lipid transport, uptake, and synthesis. To assess its function in obesity-impaired fracture repair, we examined fracture healing in HFD ApoE-KO mice. Notably, ApoE-KO mice on HFD showed significantly increased bone volume and bone mineral density and decreased adipocyte accumulation in the fracture callus compared with WT controls (Fig.2). BMSCs can differentiate into osteoblasts or adipocytes, and excessive adipogenesis under HFD conditions may impair osteogenesis. To further explore the cellular role of ApoE, BMSCs were isolated from WT and ApoE-KO mice and induced for adipogenic differentiation in vitro. ApoE-KO BMSCs exhibited markedly reduced adipogenic potential compared to WT BMSCs. Furthermore, with HFD feeding we found that circulating ApoE was elevated, prompting us to test whether this systemic, exogenous ApoE could directly influence adipogenesis. Indeed, treatment of WT BMSCs with recombinant ApoE (50ng/ml) significantly enhanced adipogenic differentiation.

Discussion:
Our findings identify ApoE as a key mediator of obesity-impaired fracture healing through its promotion of adipogenesis in both circulating and cell-intrinsic contexts. Accordingly, ApoE inhibition emerges as a potential future approach for the treatment of obesity-impaired fracture healing.

Significance/Clinical Relevance:
This study demonstrates that ApoE inhibition—either at the circulating or cellular level—can enhance fracture healing under obesity conditions. These findings support ApoE as a promising therapeutic target for improving bone repair in obese patients and warrant further exploration in clinical settings.

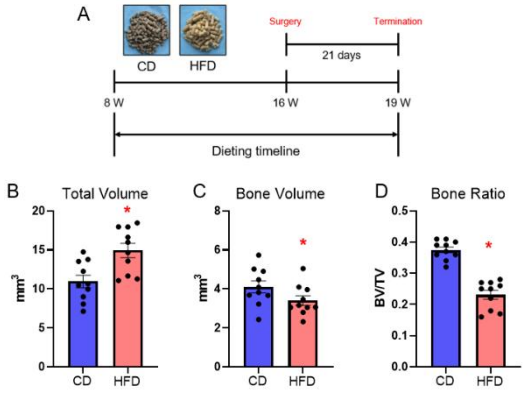

Figure 1 – Results of micro-CT evaluation showing decreased regenerate ability after mice with high-fat diet. A) Schematic diagram: Mice were fed either chow diet or high-fat diet starting at 4 weeks of age. At 4 months, mice underwent tibial fracture surgery and remained on their respective diets during the 21-day healing period. B-D) 21-day fracture calluses were assessed using µCT for total volume, bone volume, bone ratio within the callus. n=6. Data are expressed as mean ± 95% confidence interval. *Statistically significant, p<0.05.

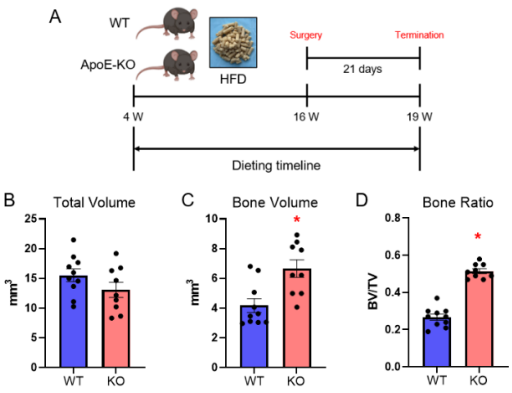

Figure 2 –Fracture healing improved in ApoE-KO mice with HFD. A) Schematic diagram: Mice were fed a high-fat diet (HFD) starting at 4 weeks of age. At 4 months, mice underwent tibial fracture surgery and sustained HFD during the 21-day healing period. B-D) 21-day fracture calluses were assessed using µCT for total volume, bone volume, bone ratio within the callus. n=6. Data are expressed as mean ± 95% confidence interval. *Statistically significant, p<0.05.