Conjugated linoleic acid promotes fracture healing in rats

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INTRODUCTION: Fracture healing in Tibetans is significantly better than Chinese Hans. The possible reasons of the superior fracture healing in Tibetans may attribute to factors from diet, altitude to physical conditions. The daily diet of Tibetans differs from that of Hans. The main portion of daily food intake of Tibetans, yak butter, beef and lamb are all high in the content of conjugated linoleic acid (CLA). It has been known that CLA functions include anti-cancer, anti-oxidation, anti-therosclerosis, lessening markers of catabolism, and decrease in body fat accumulation. Studies suggested that CLA is closely related to prostaglandins (PGs), peroxisome proliferator-activated receptor gamma (PPARγ) and osteoblast-like cells. PGs and PPARγ are multifunctional regulators of bone metabolism stimulating both bone resorption and formation. We hypothesized that CLA can significantly regulate fracture healing. In this study, we developed a rat fracture model to examine this hypothesis, and used structural, biomechanical and histological techniques to evaluate the outcomes of fracture healing.

METHODS: The experiment was performed on Sprague-Dawley rats (n=30, 200±15 g in weight) (approved by the institutional animal committee). The specimens were divided randomly into 2 groups equally: A. CLA (10 g CLA/kg diet) with the basal diet, and B. control with the basal diet only. The tibial fracture was created following a standard procedure [1]. The tibial tuberositas caudal to the patellar joint was palpated and a 0.8 mm K-wire advanced until the tarsal joint was delivered. The osteotomy line was performed manually using 2 mm osteotome from 15 mm caudal to the patellar joint resulting in fracture. Fracture healing process was first evaluated radiologically at week 2, 4 and 6. The degree of union was scored using a five point system [2], the evaluation score was 4.4±0.6. In contrast, the control group still had the vague fracture lines with matured callus and significant callus bridges. The evaluation score was 3.4±0.7 (p=0.002). The micro-CT images indicated that at week 4, both groups had no significant difference in CSA (3.7±0.7 in CLA and 3.0±0.5 in the control, p=0.07). At week 6, the fracture line in the CLA group vanished, and 40% specimens had complete callus absorption. The evaluation score was 4.4±0.6. In contrast, the control group still had the vague fracture lines with matured callus and significant callus bridges. The evaluation score was 3.4±0.5 (p=0.002). The micro-CT images indicated that at week 4, both groups had no significant difference in CSA (p=0.15), BMD (p=0.69) and BSI (p=0.006) (Figure 1). However, at week 6, the CLA group had 14.05±3.13 mm² in CSA, 0.98±0.04 mg/mm³ in BMD and 8.07±1.66 mg/mm³ in BSI, which were significantly greater than the control (9.38±1.87 mm² in CSA, p=0.02, 0.86±0.78 mg/mm³ in BMD, p=0.02, and 8.07±1.66 mg/mm³ in BSI, p=0.002). The 3-point bending test indicated that the load to failure of the CLA and the control groups was 78.12±10.03 N and 23.8±7.09 N, respectively. The difference was statistically significant (p=0.03). Histological results demonstrated the evidence of fracture healing in the CLA group at week 6 (Figure 2). Under 4X microscope, trabeculae were densely reconnected and medullary canal was redeveloped. The control group still showed disorganized and loose trabeculae. Under 10X microscope, The CLA group had matured callus, and woven bone spicules was observed predominantly with trabeculae significantly thicker than the control.

DISCUSSION: The present study demonstrated that dietary supplementation with CLA benefited to fracture healing in rats. Structural evaluation of X-ray indicated that fracture was healed in 6 weeks with the CLA intervention; and the evaluation scores were all between 4-5, significantly higher than those in the control. Micro-CT examination further showed fracture healing at the micro-structural level; and the BSI was significantly greater than the control at week 6, suggesting the improvement of overall strength being achieved via reestablishing trabecular connections. Biomechanical test of 3-point bending at week 6 also revealed the improvement of bending strength after CLA intervention. Histologically, the CLA group, in comparison with the control, showed the maturity of healing with solid network connection and thickening of trabeculae.

Fracture healing is a process that recapitulates certain aspects of skeletal development and growth involving a complex interplay of cells, growth factors, and extracellular matrix. Repair is typically characterized by four overlapping stages: the initial inflammatory response, the soft callus formation, the initial inflammatory response, and extracellular matrix. The evaluation score was 3.7±0.7 in CLA and 3.0±0.5 in the control, p=0.07). At week 6, the fracture line in the CLA group vanished, and 40% specimens had complete callus absorption. The evaluation score was 4.4±0.6. In contrast, the control group still had the vague fracture lines with matured callus and significant callus bridges. The evaluation score was 3.4±0.5 (p=0.002). The micro-CT images indicated that at week 4, both groups had no significant difference in CSA (p=0.15), BMD (p=0.69) and BSI (p=0.006) (Figure 1). However, at week 6, the CLA group had 14.05±3.13 mm² in CSA, 0.98±0.04 mg/mm³ in BMD and 8.07±1.66 mg/mm³ in BSI, which were significantly greater than the control (9.38±1.87 mm² in CSA, p=0.02, 0.86±0.78 mg/mm³ in BMD, p=0.02, and 8.07±1.66 mg/mm³ in BSI, p=0.002). The 3-point bending test indicated that the load to failure of the CLA and the control groups was 78.12±10.03 N and 23.8±7.09 N, respectively. The difference was statistically significant (p=0.03). Histological results demonstrated the evidence of fracture healing in the CLA group at week 6 (Figure 2). Under 4X microscope, trabeculae were densely reconnected and medullary canal was redeveloped. The control group still showed disorganized and loose trabeculae. Under 10X microscope, The CLA group had matured callus, and woven bone spicules was observed predominantly with trabeculae significantly thicker than the control.

SIGNIFICANCE: CLA improved the quality and mechanical strength of fracture healing in rats. The information may offer insight in development of new therapeutic strategies of fracture healing for general populations beyond Tibetans.

RESULTS SECTION: Radiological results indicated that fracture lines were clearly visible and fracture was not bridged in both CLA and control groups at week 2 with the evaluation scores of 1.3±0.9 and 1.0±0.5, respectively (p=0.23). At week 4, the fracture line became obscure in both groups. The CLA group appeared to have more callus formation than the control although they were not different statistically in evaluation scores (3.7±0.7 in CLA and 3.0±0.5 in the control, p=0.07). At week 6, the fracture line in the CLA group vanished, and 40% specimens had complete callus formation and fracture healing process was first evaluated radiologically at week 2, 4 and 6. The degree of union was scored using a five point system [2], the scores were evaluated by 3 surgeons independently and averaged. The healing process was also examined by micro-CT scanning at 4 and 6 weeks post-operation. The analysis determined the average cross-sectional area (CSA) of the callus, the bone mineral density (BMD) and the bone strength index (BSI). The mechanical properties of the tibia were measured using a three-point bending test and the load to failure was documented at week 6. Fracture healing was further examined at week 6 histologically. Mean and standard deviation in each group were calculated. The student t-test was performed with a significant difference among two groups (p<0.05).


IMAGES: Figure 1. Micro-CT examination at week 4 and 6. A) A typical micro-CT image revealing the fracture healing with CLA intervention at week 6 (the arrow pointed to the fracture site). B) The average cross-sectional area of callus. C) The bone mineral density of callus.

Figure 2. Histological evaluation at week 6. A) and B) were with the CLA intervention, and C) and D) were the control.