

Early Protection of Compound Amino Acids Against Stress Fracture via Hippo-YAP/TAZ Axis

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Abstract

Objective: Stress fracture is a prevalent form of overuse skeletal injury, arising when repetitive mechanical loading exceeds the intrinsic self-repair capacity of bone tissue. Nutritional interventions based on amino acids have shown beneficial effects on skeletal health; however, a specifically formulated compound amino acids (CAAs) preparation for the prophylactic purpose against stress fractures remains to be established. This study aimed to identify the optimal proportion of CAAs blend (glutamine, branched-chain amino acids, and alanine) and to clarify its protective effects and mechanistic basis in stress fracture pathogenesis. **Method:** The 5-month-old male Sprague-Dawley rats were given intragastric pretreatment with CAAs of different concentration and proportioning for two weeks, followed by two-week axial fatigue loading to construct ulnae stress fracture models. The protein expression levels of osteogenic biomarkers in ulnae were examined by western blotting assays to ascertain the optimal strategies of CAAs administration. Then, the bone metabolism, remodeling activity and biomechanical properties of ulnae with fatigue loading after pretreated with the optimized CAAs were assessed via SPECT/CT imaging, histomorphological analysis, osteocytic functional detection and three-point bending test. Transcriptome sequencing was performed to predict differential genes and key signaling pathways. In vivo rescue assays using Hippo-YAP/TAZ pathway inhibitors were conducted, and the phenotypes of stress fracture injuries pretreated with CAAs were further evaluated after the pathway was inhibited. **Result:** i) The optimal proportion of CAAs for the early protection against stress fracture was glutamine: alanine: branched-chain amino acids at a ratio of 2:2:1. ii) The radiotracer ^{99m}Tc-MDP uptake and osseous micro-damage (including linear microcracks and diffuse microdamage) in ulnae were decreased in CAAs-treated rats followed by fatigue loading. iii) The activity of osteocytes and their regulatory effects on bone formation and resorption were also activated in CAAs-treated rats after fatigue loading. iv) The inhibition of ultimate load and stiffness of ulnae after fatigue loading were significantly reversed

by CAAs pretreatment. v) Mechanically, CAAs can up-regulate RUNX2 expression and stimulate osteogenesis by activating the Hippo-YAP/TAZ pathway collaborating with Homeobox TF family. Inhibiting Hippo-YAP/TAZ or Homeobox obviously reversed CAAs-induced bone protection and osteogenic biomarker changes. Conclusion: Fatigue-induced microdamage accumulation, coupling with bone biomechanical property deterioration can be reversed by early intervention with the prophylactic supplementation of optimized CAAs. The restrain of stress fracture occurrence and progression are regulated by Hippo-YAP/TAZ-mediated osteogenic signaling pathways. In summary, this study offers new theoretical basis and nutritional intervention directions for high-risk populations like athletes and military staff.

Keywords

Stress Fracture, Compound Amino Acids, Bone Remodeling, Hippo-YAP/TAZ Signaling, Osteogenesis, Mechanical Loading, RUNX2