A Leucine-enriched Diet Enhances Overload-induced Growth and Suppresses Markers of Protein Degradation in Aged Rat Skeletal Muscle

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Introduction: The hypertrophic response to overload in fast-twitch skeletal muscle is impaired in aged humans and rats, and upregulation of protein degradation pathways are hypothesized to be a contributing factor. Muscle growth occurs when protein synthesis is greater than protein degradation. Dietary supplementation of the essential amino acid leucine has been shown to reduce protein degradation in both young and aged skeletal muscle. Specifically, leucine acts in part by attenuating 5'-AMP-activated protein kinase (AMPK) activation as well as the translocation of the forkhead box transcription factor 3A (FoxO3, known to promote transcription of mRNAs encoding degradation pathway proteins) to the nucleus. Akt (a promoter of muscle growth) prevents translocation of FoxO3 into the nucleus by phosphorylating FoxO3 phosphorylation at $Ser^{318/321}$. However, AMPK, inhibits Akt's phosphorylation of FoxO3, allowing it to enter the nucleus and increase transcription of protein degradation pathway genes encoding ubiquitin ligase proteins such as muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx, or Atrogin-1). During the aging process, AMPK Thr^{172} phosphorylation (and thus its activation) is increased, purportedly inhibiting gains in muscle mass and strength. Although dietary leucine supplementation has been shown to enhance strength gians in response to resistance training in young humans, the potential for leucine supplementation to enhance overload-induced muscle hypertrophy in aged humans or animal models has not been examined. Thus, the aim of this study was to determine whether dietary leucine supplementation can attenuate markers of protein degradation and rescue hypertrophy during overload in the fast-twitch skeletal muscles of aged rats to levels comparable to their younger counterparts. It was hypothesized that dietary leucine supplementation during 7 days of fast-twitch plantaris muscle overload would enhance plantaris muscle hypertrophy in aged rats to levels observed in young adult rats not receiving leucine. It was also hypothesized that dietary leucine supplementation during the overload period would alter markers of protein degradation (enhance FoxO3 phosphorylation and reduce the levels of AMPK phosphorylation, Atrogin-1 protein content, and MuRF1 protein content) in the overloaded fast-twitch plantaris muscles of the aged rats to levels observed in young adult rats not receiving leucine.

Methods: Young adult (8 mo.) and old (33 mo.) male Fisher 344 x Brown Norway F1 Hybrid (FBN) rats underwent a 1-week unilateral overload of the fast-twitch plantaris muscles via tenotomy of the synergistic gastrocnemius muscle. Within each age group, animals were matched for body weight and separated into either a dietary leucine supplementation group (normal rat chow supplemented by an additional 5% leucine content in place of 5% of the carbohydrate content; n = 7/age group) or placebo group (normal rat chow; n = 6/age group). The leucine groups started the leucine-enriched diet 2 days prior to, and throughout, the overload intervention. All animals had ad libitum access to water and chow during the entire experiment; no differences in daily calorie consumption were observed between the placebo vs. leucine

groups within each age group. At the end of the overload period, sham-operated and overloaded plantaris muscles were harvested and analyzed via western blotting for the phosphorylations of AMPK and FoxO3 as well as total levels of Atrogin-1 and MuRF1. A 2x2x2 ANOVA with repeated measures was used for analyses of the effects of age, dietary intervention, and overload (the repeated measure) on muscle hypertrophy. A 2x2 ANOVA was used to measure the percent changes in hypertrophy and western blot analyses. Post-hoc comparisons were accomplished via a Fisher's Least Significant Difference test, with statistical significance being set at $p \leq 0.05$.

Results: Dietary leucine enrichment significantly ($p \leq 0.05$) enhanced overload-induced fast-twitch plantaris muscle hypertrophy in old, but not in young adult, animals. A similar effect was also observed in the slow-twitch soleus muscles, but western blotting analyses are only presented for the fast-twitch plantaris muscles. Sham and overloaded plantaris muscle AMPK phosphorylation was significantly higher in aged animals receiving normal chow compared to young adult animals; however, leucine supplementation in old animals reduced this AMPK phosphorylation to levels similar to young adult animals. Compared to placebo, leucine also non-significantly ($p = 0.07$) enhanced FoxO3 phosphorylation in the overloaded muscles of both young adult and old animals (thus theoretically reducing FoxO3 translocation to the nucleus). Accordingly, leucine also non-significantly ($p = 0.07$) reversed the overload-induced increase (from a 22.8% increase to a 17.0% decrease) in Atrogin-1 content in aged muscles and non-significantly ($p = 0.14$) enhanced the overload-induced decrease in MuRF1 content in the muscles of both age groups.

Discussion: These novel findings indicate that a leucine-enriched diet may potentially enhance overload-induced growth of aged fast-twitch muscle, in part by suppressing pathways known to stimulate protein degradation. This is in accord with previous findings of leucine's suppressive effect on protein degradation in both young adult and aged skeletal muscle under resting conditions. The fact that leucine supplementation enhanced overload-induced hypertrophy only in the old (and not the young) animals may reflect the high growth stimulus of the chronic overload model. That is, the balance of protein synthesis/degradation rates under such a large chronic growth stimulus may not be the limiting factor in young animals, in which muscle growth is not impaired (i.e., synthesis/degradation rates may reach futile levels, and another factor such as sarcomere assembly may be limiting). However, the impaired balance of protein synthesis/degradation rates may be the limiting factor to growth in aged muscle, and leucine may correct this imbalance to restore muscle growth to levels observed in young animals.