Amino Acid-induced S6K1 Activity In Human Skeletal Muscle Is Mediated By Increased mTor/Rheb Interaction

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Cell culture studies have shown that amino acids activate mTORC1 signaling by increasing the interaction between mTOR and its essential activator Rheb. However, the existence of this mechanism in human skeletal muscle remains to be determined.

**PURPOSE:** To determine if increased mTORC1 signaling in response to amino acids in human skeletal muscle is due to an increased interaction between mTOR and Rheb.

**METHODS:** Eight well-trained men performed resistance exercise on two separate occasions. In connection with the exercise, subjects were supplemented with flavored water (Pla) and essential amino acids (EAA) in a double-blind, randomized cross-over design. Muscle biopsies were taken in the vastus lateralis muscle before, immediately after and 90 and 180 min post exercise. Activity of the mTORC1 pathway was assessed by a radiolabeled in-vitro kinase assay for its immediate downstream target S6K1. Protein-protein interactions were determined by western blot following co-immunoprecipitation of mTOR with Rheb. Co-immunoprecipitation was performed on pooled muscle samples from three of the eight subjects.

**RESULTS:** Activity of S6K1 remained unchanged immediately after exercise in both trials. However, at 90 min post exercise, S6K1 activity increased by approximately 2- and 8-fold (p<0.05) from baseline the Pla and EAA trials, respectively. At the 180 min time point, S6K1 activity remained elevated in both trials being approx. 3-fold higher in the Pla trial and 5-fold higher (p<0.05) in the EAA trial. The fold-change in mTOR and Rheb interaction largely resembled the activity pattern of S6K1 in both trials; in the Pla trial the fold-change was 0.9, 1.3 and 1.4 while in the EAA trial the fold-change was 1.6, 2.9 and 1.9 immediately after, 90 min after and 180 min after exercise, respectively.
CONCLUSIONS: The large increase in S6K1 activity following EAA intake appears to be mediated by an increased interaction between mTOR and its proximal activator Rheb. This is the first time this mechanism has been demonstrated in human skeletal muscle.