*TACSM Abstract*

# Autophagy is Required for the Anabolic Response to Muscle Contraction

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ABSTRACT

Exercise is a key stimulus in regulating the behavior and metabolism of skeletal muscle, with exercise inducing muscular growth through activation of the anabolic mechanistic target of rapamycin kinase (mTOR). Separately, there is mounting evidence that exercise increases autophagy (one of the main routes by which intracellular proteins are degraded) and that the autophagic process may indeed be required for adaptations to exercise training. **PURPOSE:** To investigate the effects of autophagy inhibition on mTOR signaling and cellular anabolism after muscular contraction. **METHODS:** Cultured L6 myotubes were to exposed to electrical pulse stimulation using a stimulator set to deliver bipolar pulses of 30V at 100 Hz for 200 ms every fifth second for 60 minutes. Subsequently, cells received either vehicle control, or 100 µM NSC-185058, an antagonist of the key autophagy protein ATG4B and known inhibitor of autophagy. All groups were also exposed to 4% deuterium oxide, a stable isotopic tracer for measurements of protein synthesis. 24 hours post "exercise" bout, cells were lysed in ice-cold Norris buffer, and prepared for Western immunoblot of protein expression, or determination of protein fractional synthesis rate (FSR) of the myofibrillar fraction via mass-spectrometry analysis. Non-stimulated cells receiving vehicle control treatment served as controls, with a one-way analysis of variance and Tukey's post-hoc test used to test for any differences between groups. **RESULTS**: We found that phosphorylation of a key downstream target of mTOR, P70S6 kinase, was roughly seven times greater in cells subjected to EPS and vehicle control (710.3%) relative to control ($p<0.05$). NSC-185058 treatment almost completely abolished these changes, with no significant phosphorylation of P70S6K in cells receiving EPS and NSC-185058 (165.7%, $p>0.05$). While there was a trend for EPS treatment to increase expression of ATG4B, along with a reduction of ATG4B content as a result of NSC-185058 treatment, this finding did not rise to the level of statistical significance. There were no differences in FSR between cells exposed to EPS; however, NSC-185058 treatment significantly reduced FSR in EPS treated cells relative to controls (0.8712 %/hr vs 1.193 %/hr). **CONCLUSION**: These findings present two conclusions: high-intensity EPS as an *in vitro* model of exercise elevates mTOR signaling through P70S6K 24 hours post exercise, and mTOR activation as a result of muscular contraction is reliant upon autophagy in skeletal muscle. Further work will be required to elucidate the dynamics of this relationship, and the interplay between skeletal muscle autophagy and anabolism.