

## **The Regulation of The Autophagy-Lysosome System in Response to Hindlimb Denervation**

**Triolo, M** & Hood, D.A.

*Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, Canada.*

Skeletal muscle displays a high degree of plasticity. In response to contractile stimuli like exercise, the metabolic capacity of the muscle is enhanced. The opposite is true during chronic muscle disuse, in which fibre atrophy occurs due to decreases in protein synthesis and elevations in the degradation of proteins and intracellular organelles. One system which regulates intracellular degradation is the autophagy-lysosome pathway. This includes the targeting of damaged intracellular components by autophagosomes, followed by delivery of these to the lysosomes for degradation. The regulation of these processes is not well characterized in skeletal muscle disuse, and even less is understood about the regulation of the lysosomes as an end stage for degradation. Thus, the objective of this work is to better understand the autophagy-lysosome system in context of muscle disuse. Accordingly, we employed a hindlimb denervation protocol in which we unilaterally sectioned the peroneal nerve of one hindlimb, using the contralateral limb as a control in Sprague-Dawley rats. Protein measurements of autophagy and lysosome markers were made at 1, 3 and 7 days post-denervation. We observed significant 25-30% reductions in tibialis anterior (TA) and extensor digitorum longus (EDL) muscle mass by 7 days post-denervation. Elevations in the autophagy proteins (Beclin1 and ATG7) were measured at 7 days post-denervation. To investigate the changes in autophagy flux, we treated a subset of animals with colchicine (4mg/kg/day) for 2 days to inhibit autophagic breakdown in response to denervation. Autophagy flux was enhanced by 55% as early as 3 days post-denervation, but it then declined by 30% following 7 days of denervation. To assess how denervation may enhance autophagy flux, we looked toward the lysosomes and observed significant elevations in lysosomal protein. To uncover why this may be the case, we measured TFEB protein level, a transcription factor that regulates the expression of genes responsible for the lysosomal biogenesis. The nuclear localization of TFEB was enhanced by 70% after 1 day, while TFEB protein was elevated by 50% at 7 days serving to promote the expression of lysosome-associate genes. Cumulatively, these data suggest that the intrinsic activity of the autophagosomal breakdown pathway is sufficient in the early time course but may be upregulated later to meet the demands of prolonged denervation.