



IS INTACT ENDOTHELIAL CELL AUTOPHAGY NECESSARY TO OBSERVE TRAINING-INDUCED VASCULAR ADAPTATIONS?

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Macroautophagy is operational during basal conditions to maintain intracellular organelle and protein quality control, but is upregulated during cellular stress (e.g, dynamic exercise) to adapt to changing nutritional and energy demands. We tested the hypothesis that intact endothelial cell (EC) autophagy is required to observe training-induced vascular adaptations. Rationale for this hypothesis was provided by an earlier report that obese mice with germline, whole body mutation of a protein requisite for autophagy i.e., Bcl2-AAA mice were refractory to training-induced improvements concerning glucose homeostasis (*He et al., Nature, 2012*). First, we demonstrated that : (i) workload achieved during a maximal treadmill test; (ii) soleus muscle citrate synthase activity; (iii) mRNA and protein expression of vascular autophagy; and (iv) intraluminal flow-mediated vasodilatory responses (FMD) of femoral arteries examined *ex vivo*, were greater (all $p < 0.05$) in ~ 4-month old male C57Bl/6 mice that completed 10-weeks of treadmill-training vs. age-matched sedentary animals ($n=10$ mice per group). These findings indicate that our efficacious training protocol improves vascular autophagy and arterial function in adult mice. Next, ~ 4-month old male mice on a C75Bl/6 background with inducible Cre/LoxP- based impairment of autophagy-related gene 3 (Atg3) specifically in ECs (*iecAtg3KO* mice) and their Cre negative littermates (WT) were treated with tamoxifen. Two weeks later, one cohort of *iecAtg3KO* mice initiated a treadmill training program (ETR; 10-60 min per day x 0-20% grade x 6 days per week x 10 weeks) or maintained familiarity with the treadmill by running 10 min per day x 5% grade x 1 day per week (SED). Two cohorts of WT littermates were treated identically. After 10-weeks efficacy of the training protocol was established as described earlier and verification of our mutant was assessed. With regard to the latter, primary ECs obtained from the carotid artery and aorta indicated Atg3 mRNA and Atg3 protein, respectively, was minimal ($p < 0.05$) in *iecAtg3KO* vs. WT mice, whereas vascular smooth muscle cell Atg3 was similar between groups. As expected, intraluminal FMD responses were greater ($p < 0.05$) in WT-ETR vs. WT-SED mice, while vascular smooth muscle responses to sodium nitroprusside were not different between groups. Further, as anticipated, intraluminal FMD was blunted ($p < 0.05$) in *iecAtg3KO*-SED vs. WT-SED mice, indicating the importance of intact EC autophagy to FMD. Contrary to our hypothesis, however, training-induced vascular adaptations indeed were observed ($p < 0.05$) in *iecAtg3KO*-ETR vs. *iecAtg3KO*-SED mice, while vascular smooth muscle responses were not different between groups. These findings indicate that intact EC Atg3 is not necessary for training-induced vascular adaptations to occur.