**DECIPHERING THE ROLE OF MITOPHAGY IN THE HEART DURING FASTING**

**Q. Liang**

New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY, USA

Alternate-day fasting or starvation is beneficial to the heart, but the underlying mechanism remains speculative. Starvation activates general autophagy, which may contribute to the cardioprotective effect of fasting. Increased mitochondrial degradation or mitophagy is cardioprotective under certain conditions. However, it is unclear whether mitophagy is involved in fasting-induced cardioprotection. In this study, we investigated the functional significance of mitophagy in the heart during fasting. We created a novel mitophagy reporter transgenic mouse line that expresses mito-Rosella, a mitochondria-targeted dual-emission biosensor comprising a pH-stable RFP linked to a pH-sensitive GFP. These mice were subjected to fasting, and treated with vehicle or lysosomal inhibitors pepstatin A (pepA)/ E64d. Mitochondria that are being degraded in the lysosome are seen as red puncta on the overlaid confocal images. Mitophagy flux is measured as the difference in the numbers of red puncta in the presence and absence of pepA/E64d. A 24-hour fasting increased mitophagy flux in the heart by 44.2% as compared with normal feeding, which was accompanied by increased LC3-II protein levels in both cardiac tissue lysates and the mitochondrial fractions, suggesting that 24-hour fasting enhanced both autophagy and mitophagy. Surprisingly, the 48-hour starvation decreased mitophagy flux by 50.1% and 28% as compared to 24-hour fasting and normal feeding, respectively. Also, LC3-II protein levels were increased in cardiac tissue lysates but reduced in the mitochondrial fractions, suggesting that the 48-hour fasting enhanced autophagy but inhibited mitophagy, in contrast to the effects of 24-hour fasting. These results demonstrated time-dependent differential effects of fasting on cardiac autophagy and mitophagy, suggesting that mitophagy and autophagy must be regulated by different signaling pathways. Overexpression of E3 ligase Parkin, a positive regulator of mitophagy, restored mitophagy but impaired cardiac function after 48-hour fasting, suggesting that reduced mitophagy is an adaptive response essential for maintaining cardiac function during starvation.