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TUNING MUSCLE ENERGY HOMEOSTASIS: THE ROLE OF NIX DURING CONTRACTILE ACTIVITY.

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Background:
Exercise is instrumental in reversing derangements to muscle metabolism, such as insulin-resistance during type 2 diabetes. Mitochondrial quality control is important for efficient use of metabolic fuels in muscle, but the mechanism remains unclear. In an *in vitro* model of skeletal muscle, I observed an increase in the protein Nix after contraction; this protein mediates the specific removal of dysfunctional mitochondria (mitophagy).

Objective:
I used an *in vitro* model of skeletal muscle to test the hypothesis: Nix protein is elevated in contracting skeletal muscle to increase the removal of dysfunctional mitochondria, aiding in the fine tuning of energy homeostasis in muscle.

Methods:
An *in vitro* model of skeletal muscle (C2C12 myotubes) was used to investigate the role of Nix-mediated mitophagy in muscle. Contraction was induced by applying an electrical field (12V, 1Hz, 2ms) for 1 hour. Levels of Nix and mitochondrial biogenesis proteins (PGC-1α, NRF2) were determined by immunoblotting whole-cell lysates. Next, fluorescent biosensors were used to detect levels of mitochondrial and nuclear calcium, and mitophagy. The contribution of Nix protein was assessed in knockdown experiments using lenti-viral delivered siRNAs in myotubes.

Results:
Following contraction, I found elevated levels of Nix (1.9-fold), and mitochondrial-biogenesis proteins PGC-1α (2.5-fold) and NRF2 (1.4-fold). Next, I examined mitochondrial clearance by assessing mitochondrial acidification, which increased (~1.4-fold) following contraction as would be expected once mitochondria enter the acidic lysosome. Next, I observed an increase in nuclear-calcium (~2-fold) and a small but significant rise in mitochondrial calcium, suggesting changes in gene expression and elevated metabolic output, respectively. Importantly, knockdown of Nix in myotubes prevented the contraction-induced rise in mitochondrial clearance and nuclear calcium.

Conclusion:
Together these data uncover the potential contribution of Nix to muscle metabolism and adaptation for the efficient use of metabolic fuels which is disrupted by the onset of insulin-resistance in diabetes.