Poster Number 28

Abstract 0240_0346_000089

Tuning muscle energy homeostasis: The role of Nix during contractile activity.

Jared Field, University of Manitoba, Max Rady College of Medicine; **Matthew Martens**, University of Manitoba, Max Rady College of Medicine; **Simone da Silva Rosa**, University of Manitoba, Max Rady College of Medicine; **Joseph Gordon**, University of Manitoba, Rady Faculty of Health Sciences

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 a short period of 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.

Results:

Following 1 hour of contraction, I found elevated protein levels of Nix (1.9-fold), and mitochondrial biogenesis proteins PGC-1α (2.5-fold) and NRF2 (1.4-fold). Next, I examined mitochondrial distribution and found greater clustering of mitochondrial after contraction, suggesting clustering of mitochondria prior to, or following, incorporation in lysosomes. Importantly, these cells also possessed a complex and intact mitochondrial network. Furthermore, mitochondrial acidification increased (~1.4-fold), as would be expected in the acidic lysosome. Finally, I observed a substantial increase in nuclear-calcium load (~2-fold) and a small but significant rise in mitochondrial calcium, suggesting changes in gene expression and elevated metabolic output, respectively.

Conclusion:

Together these data uncover the potential contribution of Nix protein to muscle metabolism and the efficient use of metabolic fuels that is disrupted by the onset of insulin-resistance in diabetes.