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Therapeutic Targeting of Skeletal Muscle Nix in Early-Onset Insulin Resistance

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Background:

Fetal exposure to diabetes during pregnancy increases the risk for early-onset insulin resistance in the offspring; however, the key molecular regulators responsible for fetal metabolic programming have not been characterized in muscle tissue. Previously, we demonstrated that the expression of a mitochondrial death gene Nix, was elevated in the skeletal muscle of rats exposed to gestational diabetes.

Objective:

We hypothesize that a novel phosphorylation residue, activated by clenbuterol treatment can prevent Nix-induced mitochondrial dysfunction in muscle cells.

Methods:

C2C12 skeletal muscle myotubes were exposed to 200 µM palmitate, or vehicle control. To assess mitochondrial membrane potential, cells were stained with TMRM, and imaged through epifluorescence. Plasmid-based PKA biosensor was used to identify clenbuterol activation. Cellular localization of Nix was determined by cell fractionation and protein expression by western blot. Phospho-peptide mapping was performed by mass spectrometry and custom phopho-specific antibody was generated. One-way anova determined multiple comparisons between groups and student t-test compared mean differences.

Results:

Nix knock-down in cultured myotubes significantly improved mitochondrial membrane potential, diacylglycerol accumulation and insulin sensitivity ($p<0.05$). Through mass spectometry of Nix, we identified a novel phosphorylation residue within the transmembrane domain, that is modulated by PKA activating agents, such as adrenergic agonist clenbuterol and the phosphodiesterase-4 inhibitor cilomilast. Treatment of myotubes with these agents prevented Nix-induced mitochondrial dysfunction and restored insulin sensitivity ($p<0.05$). Furthermore, Nix knock-down or clenbuterol treatment rescued palmitate-induced inhibition of insulin signaling by reducing phosphorylation of Ser1101 on the insulin receptor substrate-1 (IRS-1). Using organelle-targeted calcium biosensors, we demonstrated that Nix triggers sarco/endoplasmic reticulum (SR) calcium (Ca^{2+}) release ($p<0.05$), and calcium-dependent inhibition of IRS-1. Moreover, calcium buffering by mitochondrial led to inner membrane depolarization.

Conclusion:

Our data supports the hypothesis that Nix regulates mitochondrial metabolism and insulin signaling in myotubes and suggest a possible therapeutic strategy to circumvent the mitochondrial dysfunction characteristic of insulin resistance.