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THERAPEUTIC TARGETING OF SKELETAL MUSCLE NIX IN EARLY-ONSET INSULIN RESISTANCE

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Background:
Lipotoxicity is a form of cellular stress caused by the accumulation of lipids resulting in mitochondrial dysfunction and insulin resistance in muscle. Previously, we demonstrated that Nix is a lipotoxicity-responsive gene that accumulates in response to diacylglycerols induced by high-fat and sucrose (HFS) feeding and exacerbated by exposure to gestational diabetes (GDM) during fetal development.

Objective:
Here we identify a novel phosphorylation residue, activated by clenbuterol treatment that 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 pharmacological PKA activation by clenbuterol and cilomilast. 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:
In a series of gain-of-function and loss-of-function experiments in rodent and human myotubes, we demonstrate that Nix accumulation triggers mitochondrial depolarization ($p<0.05$), fragmentation ($p<0.05$), calcium-dependent activation of DRP-1, and mitophagy ($p<0.05$). In addition, Nix-induced mitophagy leads to myotube insulin resistance through activation of mTOR-S6K inhibition of IRS-1. Finally, we demonstrate that Nix-induced mitophagy and insulin resistance can be reversed by direct phosphorylation of Nix by PKA, leading to the translocation of Nix from the mitochondria and sarcoplasmic reticulum to the cytosol.

Conclusion:
These findings provide insight into the role of Nix-induced mitophagy and muscle insulin resistance during an overfed state. Furthermore, our data supports the hypothesis that Nix

regulates mitochondrial metabolism and insulin signaling in myotubes and suggest a mechanism by which pharmacological activation of PKA may circumvent the mitochondrial dysfunction characteristic of insulin resistance in children.