Fetal Programming and the Effects of Maternal Diet on Offspring Cellular Quality Control

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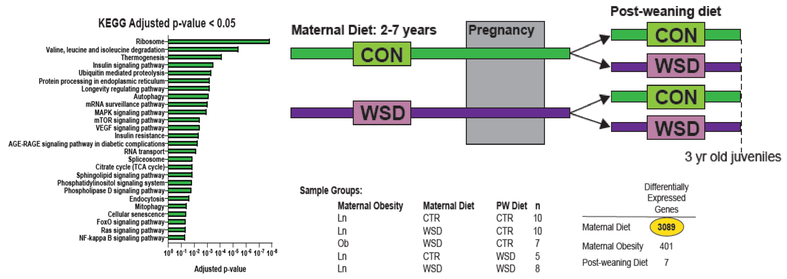
Background

- Maternal obesity has been shown to negatively impact offspring metabolic health, leading to a greater and earlier risk for the development of metabolic diseases such as type 2 diabetes.
- This presents a serious concern as obesity prevalence has been steadily rising over the past four decades.
- The mechanisms behind these intergenerational effects are still unknown.



Maternal diet exposure results in alterations in offspring skeletal muscle gene expression. Recently the McCurdy lab conducted an experiment in a non-human primate model which analyzed the effects of maternal obesity and maternal diet on the gene expression of offspring skeletal muscle. Over 3000 genes were found to be dysregulated based on maternal diet exposure. Many of the genes dysregulated in response to maternal western style diet are associated with the cellular quality control pathways of autophagy and mitophagy.

- Autophagy is the process of recycling intracellular components for nutrients during times of starvation or to mediate clearance of damaged intracellular components to maintain quality control. This process occurs through the engulfment of cellular components in the autophagosome and subsequent fusion with lysosomes results in the breakdown of components into their representative building blocks.
  - Mitophagy refers to the specific autophagic clearance of mitochondria.
- To test if these alterations in gene expression have a functional effect, we developed two flow cytometry-based assays to measure the relative cellular abundance of damaged mitochondria and lysosomes in offspring myoblasts in response to the mitochondrial uncoupler CCCP and lysosome biogenesis inducer rapamycin.
- Based on the maternal diet induced changes in gene expression associated with these pathways, we hypothesized that mitophagy and lysosome biogenesis would be functionally impaired.
  - If this hypothesis is correct, then we predict that offspring exposed to a maternal western style diet should have an increased accumulation of damaged mitochondria and fewer lysosomes in response to cellular stress.

Acknowledgments

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NIH National Institute of Diabetes and Digestive and Kidney Diseases

Results

Figure 1: Assessing the sensitivity of the assays to measure differences in markers of cellular quality control.

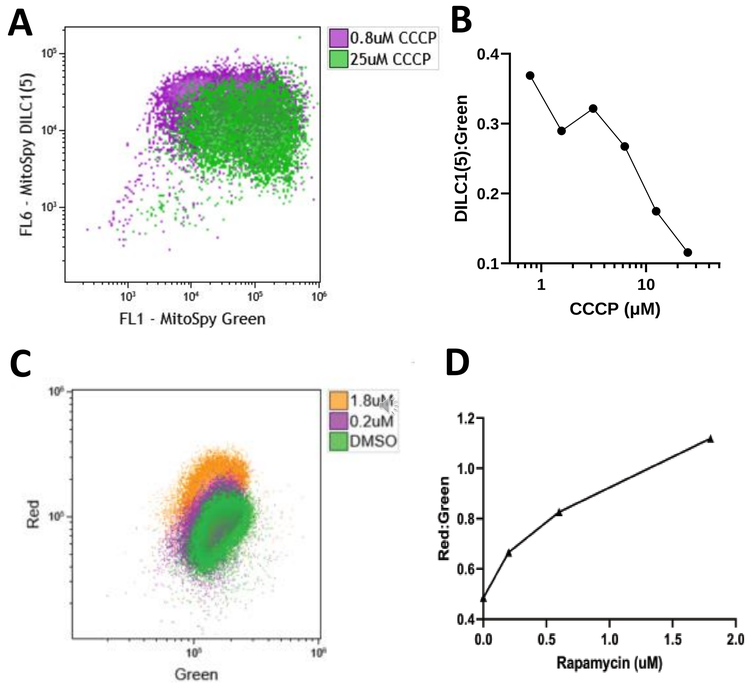

Figure 1: Detection of damaged mitochondria and lysosome biogenesis via flow cytometry in L6 rat myoblasts. Mitospy DILC1(5) stains for healthy polarized mitochondria. Mitospy Green stains for total mitochondria. Acridine orange fluoresces red in lysosomes and green in other cellular compartments. A) Bivariate plot of Mitospy DILC1(5) and Mitospy Green fluorescence intensity with varying degrees of CCCP treatment. B) Effects of CCCP concentration on the ratio of DILC1(5):Green mean fluorescence intensity. C) Bivariate plot of acridine orange red fluorescence and acridine orange green fluorescence with varying degrees of rapamycin treatment. D) Effects of rapamycin concentration on the ratio of acridine orange red:green mean fluorescence intensity.

Figure 2: Assessing the sensitivity of the assays to measure differences in markers of cellular quality control.

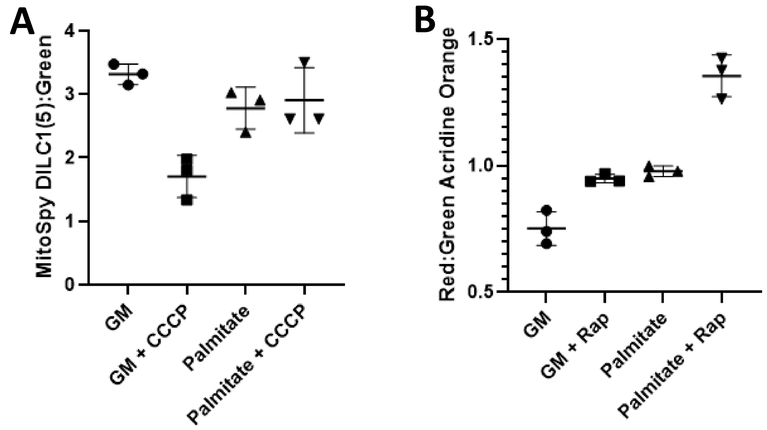

Figure 2: Cell stress induced by palmitate changes the response to induction of mitophagy and lysosome biogenesis in NHP primary myoblasts. A) Ratio of DILC1(5):Green mean fluorescence intensity with varying treatments. B) Ratio acridine orange red:green mean fluorescence intensity. Cells were treated overnight with either growth media (GM) or 0.4 mM palmitate media with or without 10 μM CCCP or 0.5 μM Rapamycin.

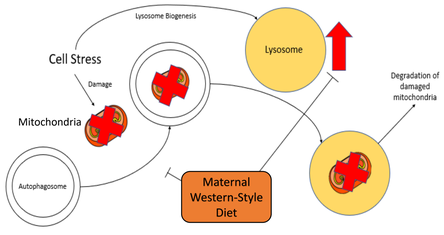

Figure 3: Proposed model of functional consequences of maternal diet exposure on cellular quality control mechanisms of autophagy and mitophagy. Cellular stress results in the accumulation of damaged organelles such as mitochondria. The process of autophagy normally clears these damaged organelles. Maternal diet exposure is hypothesized to reduce autophagic capacity and thus results in the accumulation of damaged organelles.

Future Directions

- At this point in time, the assays have only been conducted in a single non-human primate subject. Before conclusions can be drawn regarding if there are differences by maternal diet exposure, an adequate number of subjects exposed to maternal control and maternal western style diets need to be assayed.
- In addition, probing of protein levels of key targets involved in these signaling pathways would provide further insight into potential functional consequences of the altered gene expression seen in response to maternal diet exposure.

Summary

- Exposure to a maternal western-style diet has been associated with the dysregulation of over 3000 genes in offspring skeletal muscle of a non-human primate model.
- Many of these genes have been associated with the cellular quality control mechanisms of autophagy and mitophagy.
- It has not been studied whether these alterations in gene expression have functional consequences of the ability of these mechanisms to function.
- To probe for pathway function, two novel flow-cytometry assays were developed to measure the relative abundance of damaged mitochondria and lysosomes within cells.