Intergenerational Effects of Maternal Obesity on Offspring Mitochondrial Reactive Oxygen Species Production and DNA Damage

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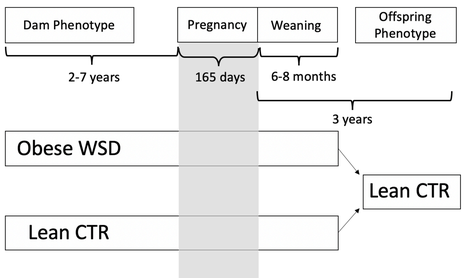
Abstract

Epidemiological studies have shown that offspring from pregnancies complicated by maternal obesity have a 4-fold greater risk for developing childhood obesity and symptoms of metabolic syndrome. The developmental origins of health and disease (DOHaD) hypothesis states that certain environmental exposures during critical windows of development may have consequences for an individuals long term health. DOHaD may explain a portion of the continual increase in obesity rates among children. In a non-human primate model, offspring of obese dams become sensitized to obesity-induced metabolic disruptions, including insulin resistance and mitochondrial disfunction. Increased reactive oxygen species (ROS) production contributes to mitochondrial defects observed in obesity. Oxidative stress, which is caused by overproduction of ROS, can lead to mitochondrial DNA (mtDNA) mutations, decreased copy number, reduced membrane permeability and subsequent suppression of mitochondrial respiratory chain activity. Therefore, I hypothesize that maternal obesity increases offspring mitochondrial ROS production leading to mtDNA damage without loss of mtDNA abundance. To study the effect of maternal obesity, we used a previously established Japanese macaque model of fetal programming. Dams were fed either a control (CON) diet or western style diet (WSD) prior to and during pregnancy and lactation. Offspring were then weaned at 8 months and fed a healthy CON diet. Skeletal muscle biopsies from offspring were collected at 3 years of age and relative mtDNA abundance was measured using quantitative PCR (qPCR) amplification of short regions of mtDNA. No differences were measured in the amount of mtDNA between offspring groups. Moving forward, I will test for elevations in ROS-induced mtDNA damage by qPCR amplification. Overall, these data indicate that exposure to maternal obesity and WSD during fetal development does not reduce mitochondrial abundance in skeletal muscle of adolescent offspring. Further tests are needed to determine whether observed reductions in mitochondrial homeostasis are linked to elevated ROS production.

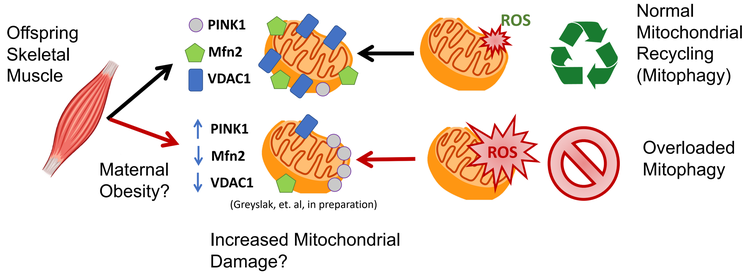
Introduction

Non-human Primate Model of Maternal Obesity

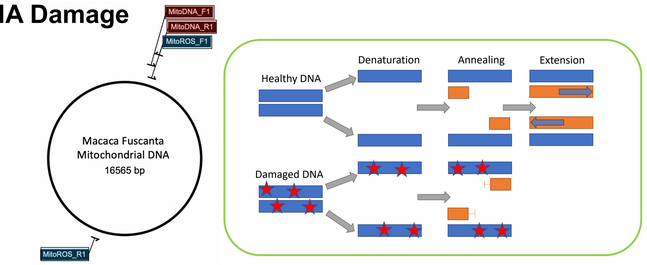


In order to understand the effect of maternal obesity on lean offspring, a previously established model of Japanese macaques was used. Dams were placed on either a control or western-style diet for 2-7 years prior to pregnancy. 6-8 months after birth, offspring from obese dams on a WSD were weaned to a CON diet, and offspring from lean dams on a CON diet were maintained on the CON diet. Skeletal muscle mitochondria was studied from offspring at 3 years of age.

Does Maternal Obesity Lead to Increased Mitochondrial Damage in Offspring?



Quantitative PCR Based Method to Measure Mitochondrial DNA Damage



Measuring mitochondrial DNA abundance and ROS production by quantitative PCR. To measure relative mitochondrial DNA abundance and ROS damage, I will use quantitative PCR with two different primer sets that anneal to different sites of the Japanese macaque mitochondrial genome. Primers placed closer together were designed to detect relative mitochondrial DNA abundance (MitoDNA), while primers placed further apart were designed to measure mitochondrial DNA damage via reactive oxygen species (MitoROS). I expect to observe reduced amounts of product from the distant primers in the mW/C offspring, indicating increased ROS damage.

Results

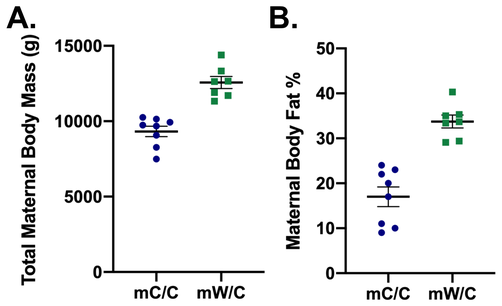


Figure 1: Dams placed on WSD became obese. (A) Dams fed WSD had a higher body mass (g) than those fed a control diet ($p = 0.00003$). (B) Dams fed WSD had a higher body fat (%) than those fed a control diet ($p = 0.00003$). Data was analyzed by unpaired t-test.

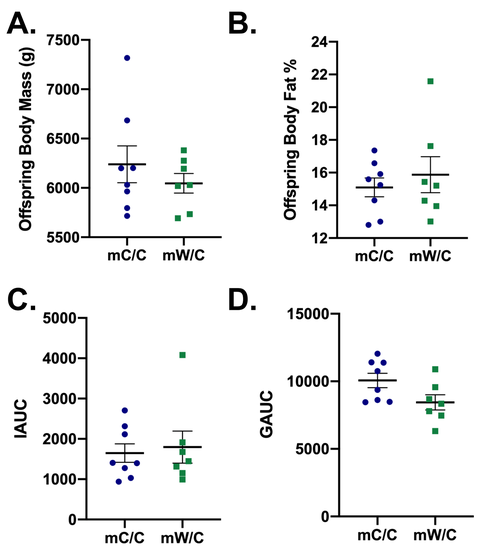


Figure 2: Offspring of obese dams do not show increased adiposity or altered glucose handling at three years of age. (A) Offspring body mass (g) between mC/C and mW/C are not statistically different ($p = 0.4$). (B) Offspring body fat (%) between mC/C and mW/C are not statistically different ($p = 0.5$). (C) Offspring insulin area under the curve (IAUC) between mC/C and mW/C, calculated with 0 as the baseline, are not statistically different ($p = 0.7$). (D) Offspring glucose area under the curve (GAUC), calculated as 0 as the baseline, are not statistically different ($p = 0.055$) between mC/C and mW/C. Data was analyzed by unpaired t-test.

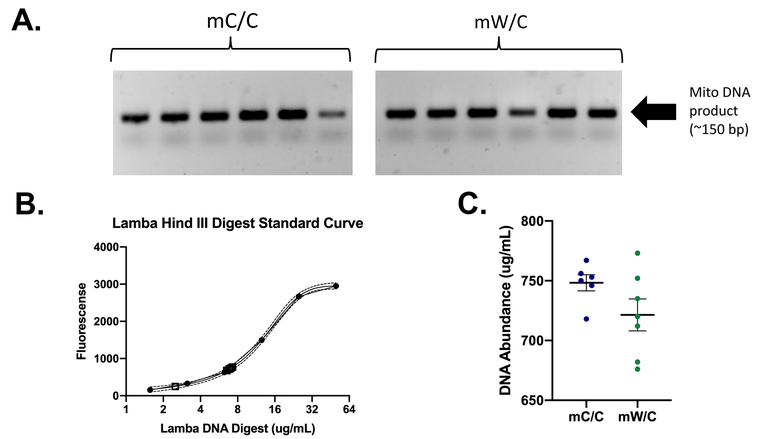
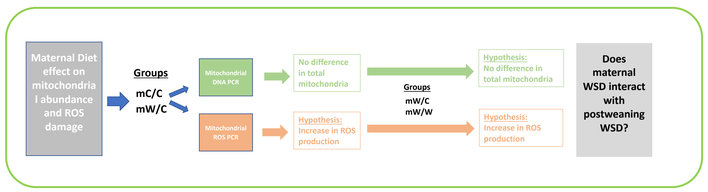


Figure 3: Offspring from obese dams do not have a significant reduction in mitochondrial DNA abundance compared to offspring from lean dams. (A) Agarose gel of PCR product from the reaction using DNA from each offspring shows a single product. (B) Standard curve of PicoGreen stained Lambda Hind III DNA used to quantify PCR product from mitochondrial DNA PCR. (C) PicoGreen fluorescence was used to measure the amount of PCR product. Maternal diet did not affect the amount of mitochondrial DNA PCR product, indicating that there is no difference in mitochondrial content. Fluorescence, as a measure of DNA abundance, displays no significant difference between the offspring from the Ob/WSD dams and Ln/CON dams ($p = 0.1$). Data was analyzed by unpaired T test.

Summary

- Previous studies have shown that maternal obesity results in increased mitophagy related signaling events in offspring skeletal muscle, suggesting increased mitochondrial damage.
- Maternal obesity does not alter skeletal muscle mitochondrial DNA abundance, suggesting that mitochondrial abundance is not affected by maternal diet, and previously observed defects in cellular respiration are not caused by a global decrease in mitochondrial abundance.
- If hypothesis is true, we expect that maternal obesity increases offspring mitochondrial ROS production, which ultimately leads to mitochondrial DNA damage.

Future Directions



Funding

This research was funded by NIH 1R24DK090964-01 to Carrie E. McCurdy, as well as by the Undergraduate Research Opportunity Program Mini-Grant.