INTERGENERATIONAL EFFECTS OF MATERNAL OBESITY ON OFFSPRING MITOCHONDRIAL REACTIVE OXYGEN SPECIES PRODUCTION AND DNA DAMAGE

by

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

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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 individual's long term health. DOHaD may explain a portion of the continual increase in obesity rates among children. In a nonhuman 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. Similarly, no differences were measured in the amount of mtDNA damage between offspring groups. Overall, these data indicate that exposure to maternal obesity and WSD during fetal development does not reduce mitochondrial abundance or alter mitochondrial homeostasis that is linked to ROS production in skeletal muscle of adolescent offspring.

Acknowledgements

I would like to thank Byron Hetrick and Carrie McCurdy for helping me succeed in the laboratory setting. I am incredibly thankful for Byron Hetrick, as he has guided me through my entire thesis project and taught me many laboratory techniques that I will use in my future education. Byron also aided my editing process through writing this thesis report and has been an extraordinary mentor throughout my past three in this lab. I would also like to give a huge thank you to Carrie McCurdy, who has always been an incredible mentor. Carrie has dedicated numerous hours of her time to teaching me about research and has guided me through applying for grants and scholarships. I am extremely thankful for the privilege of having such amazing and brilliant mentors who were willing to guide me through this demanding but also valuable journey. I would like to thank Professor Brian McWhorter, Dr. Carrie McCurdy, and Dr. Byron Hetrick for serving on my thesis committee. Thank you to the Clark Honors College for granting me with a Thesis Research Grant and providing me with the education necessary to complete my thesis. Thank you to the Undergraduate Research Opportunity Program (UROP) for providing me with a Mini-Grant and allowing me to present this research at the Undergraduate Research Symposium in Spring 2020. Lastly, I would like to give a huge thank you to my mom and dad for being my number one supporters throughout my time in college.

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Introduction

Physiology is the study of how living organisms function, and can range from as small as cells and molecules, to as large as an entire animal. Physiological explanations are extremely important, being that they benefit everyone, and allow individuals to understand how we create movements, fluctuate our weight patterns, produce offspring, etc. Developments and breakthroughs in physiology have allowed doctors to make diagnoses and recommend treatments such as proper nutrition, exercise, and medications. Physiology studies how the human body adapts to stressors over time, such as physical activity and disease. This study determines how the chemistry and physics of structures within the human body work simultaneously. Physiological laboratory experiments allow scientists to better understand how functions are carried out, as well as to determine what chemical compounds make up the body (OpenStax, 2013). Analyzing body systems as this microscopic level allows for the understanding of disease through the study of physiology.

Currently in the U.S. adult population, 73.6 % of adults are included under the categories overweight or obese ("FastStats - Overweight Prevalence," n.d.). This means that measures must be taken to counteract this immense epidemic. An analysis was completed based on the National Health and Nutrition Examination Surveys from 2003 to 2012, which displayed obesity prevalence being increased in adults ages 20-39 years of age, as well as those above age 60 (Toth and Palmer, 2018). This paper points out the great need for a decreased obesity prevalence in the population of the U.S. Obesity is a significant issue due to its associations with disease. A study by Giridhara Babu et al. in 2018 showed that obesity has a significant and plausible association with hypertension

and type 2 diabetes mellitus (Babu et al., 2018). The study of physiology in relation to the functions and causes of obesity is necessary to diminish the high prevalence rates of this epidemic (Babu et al., 2018). Specifically, it is vital to understand how metabolic diseases are passed down from mother to offspring, and if our society is creating a more sensitive population via fetal programming. Fetal programming describes developmental adaptations caused by an individual's intrauterine environment that persist throughout life, and can result in predisposition to disease later in life. Understanding the role of fetal programming allows for the discovery of intervention strategies (Kwon & Kim, 2017). The developmental origins of health and disease (DOHaD), hypothesized by David Barker, is a current approach to understand the connection between prenatal and perinatal exposures to various parental diets and weight patterns, and how this affects development of diseases later in life. The DOHaD hypothesis was originally theorized when studying undernutrition during gestation, which was understood to be a vital early origin of adult cardiac and metabolic-related disorders caused by fetal programming (Wadhwa, Buss, Entringer, & Swanson, 2009). We study this process by understanding how organelles function and how they are influenced at the cellular level by exposure to maternal obesity during development.

Mitochondrial function is altered by obesity, due to the fact that increased reactive oxygen species (ROS) are seen in macaques and mouse models with a high-fat diet (HFD) (Furukawa et al., 2004). Present in nearly all human eukaryotic cells are double membrane-bound organelles, commonly known as mitochondria. They are found in the fluid surrounding the nucleus of a cell, which is called the cytoplasm. The main function of mitochondria is to generate energy for the body, in the form of

adenosine triphosphate (ATP). They also carry out other vital functions including storing calcium for cell-signaling activities, producing heat, and arbitrating cell growth as well as cell death. The abundance of mitochondria present varies throughout the human body. Muscle cells are known to have a large abundance of mitochondria ("mitochondrion | Definition, Function, Structure, & Facts | Britannica," n.d.). While a cell does have deoxyribonucleic acid (DNA) stored in the nucleus, mitochondria also contain DNA, which is known as mitochondrial DNA. Human mitochondrial DNA covers about 16,500 DNA base pairs, which is only a small fraction of the total DNA in a single cell. In total, mitochondrial DNA is composed of 37 genes, with 13 of the genes allocating information on creating enzymes that function in oxidative phosphorylation, which is a process that uses oxygen as well as simple sugars to create ATP. The other 14 genes of mitochondrial DNA hold instructions for producing transfer RNA and ribosomal RNA, which carry information on how to create the primary building blocks of proteins, commonly known as amino acids ("Mitochondrial DNA: MedlinePlus Genetics," n.d.).

Epidemiological studies have shown that children from obese pregnancies have an increased risk for developing obesity and metabolic syndrome (Glastras, Chen, Pollock, & Saad, 2018). Disruption of skeletal muscle mitochondrial function is associated with obesity related metabolic diseases, including type 2 diabetes (Chanséaume & Morio, 2009). A study completed in 2016 exploring the effects of maternal obesity on oxidative capacity in fetal skeletal muscle of Japanese macaques found that the obese macaque mothers placed on a western-style diet had fetuses with decreased mitochondrial abundance, as well as reduced oxidative capacity and

mitochondrial efficiency in muscle. The conclusion of the study suggested that maternal obesity, alone or in conjunction with western-style diet, results in reduced oxidative metabolism in fetal muscle (McCurdy, 2016). In summary, altered mitochondrial function is closely tied to development of metabolic disease.

Further studies have also shown that obesity, caused by the consumption of a high-fat diet can lead to oxidative damage and mitochondrial dysfunction. A study researching the brains of diet-induced obese rats and diet-resistant rats found that the diet-induced obesity resulted in mitochondrial dysfunction and oxidative stress (Ma et. al, 2014). Furthermore, many studies have suggested a connection between obesity prevalence in mother to offspring. The maternal environment has the ability to influence the development of the fetus in utero, as well as impact the offspring's chances of acquiring obesity and type 2 diabetes. For instance, a study completed in Chicago researching diabetes in pregnancy found that offspring born from multiethnic women with type 1 diabetes, had excessive growth (Dabelea & Crume, 2011). The studies explained above adhere to the concept known as the developmental origins of health and disease, which declares that stimuli exposed at a critical period of developmental plasticity in early life leads to alterations towards normal growth and development, as caused by one genotype inducing various phenotypes based upon the surrounding environmental conditions exposed to mothers (Williams et. al, 2014). This developmental programming suggests permanent effects towards the function and metabolism of vital organs, ultimately resulting in increased risk of type 2 diabetes. Previous studies have demonstrated that both maternal and paternal dietary

manipulation lead to increased risk of the development of metabolic diseases in offspring (Ozanne, 2015).

Oxidative stress, which is caused by the overproduction of reactive oxygen species (ROS), can lead to mitochondrial DNA mutations, impairment to the mitochondrial respiratory chain, change membrane permeability, and impact mitochondrial antioxidant defense systems. All in all, oxidative stress and mitochondrial damage result in impaired signaling, causing poor insulin secretion function as well as reduced immune responses. These factors in turn lead to the development of insulin resistance and diabetes (Newsholme, Cruzat, Keane, Carlessi, & de Bittencourt Jr, 2016). The overproduction of free radicals causes disturbances to the balance between ROS and antioxidants, which leads to many cellular disturbances. Examples include DNA damage, breakdown of lipids (fats) and proteins, and neurodegenerative diseases. Specifically, mitochondrial DNA is thought to be extremely susceptible to damage via oxidative stress from ROS production. The overproduction of ROS ultimately leads to metabolic syndrome, which is characterized by conditions including obesity, hypertension and insulin resistance (Roberts & Sindhu, 2009).

Using a previously established non-human primate model of maternal obesity, I propose to investigate the effect of maternal obesity and in utero western style diet (WSD) exposure on 3-year-old juvenile offspring. Female Japanese macaques were placed on either a control (CON) diet or WSD for 2-7 years prior to pregnancy. WSD describes the continuous availability of high-fat, high-sugar foods provided to the primates in this study (Odermatt, 2011). Offspring were then weaned onto CON diet until age 3, creating two sample groups: maternal CON, postweaning CON (mC/C) and

maternal WSD, postweaning CON (mW/C). Non-human primates are used as a model for this experiment due to the fact that they have similar developmental changes in their placenta, islets, and brain to humans, as well as are the only known animal models to be accessible to all of the metabolic diseases acquired in humans (Friedman, 2018).

Previously, our lab has shown that a maternal WSD, with increased saturated fats and sugars, alters offspring skeletal muscle functions. Increased markers of mitochondrial damage have been found to be compounded with decreased release of (reactive oxygen species) ROS with maternal obesity, suggesting altered ROS handling may increase mitochondrial damage (McCurdy, 2016). I expect that exposure to maternal WSD and obesity will increase the mitochondrial DNA damage in offspring at 3 years of age. My research is significant because it will determine whether maternal obesity predisposes offspring to metabolic disease through increased mitochondrial damage.

Methods

Experimental design

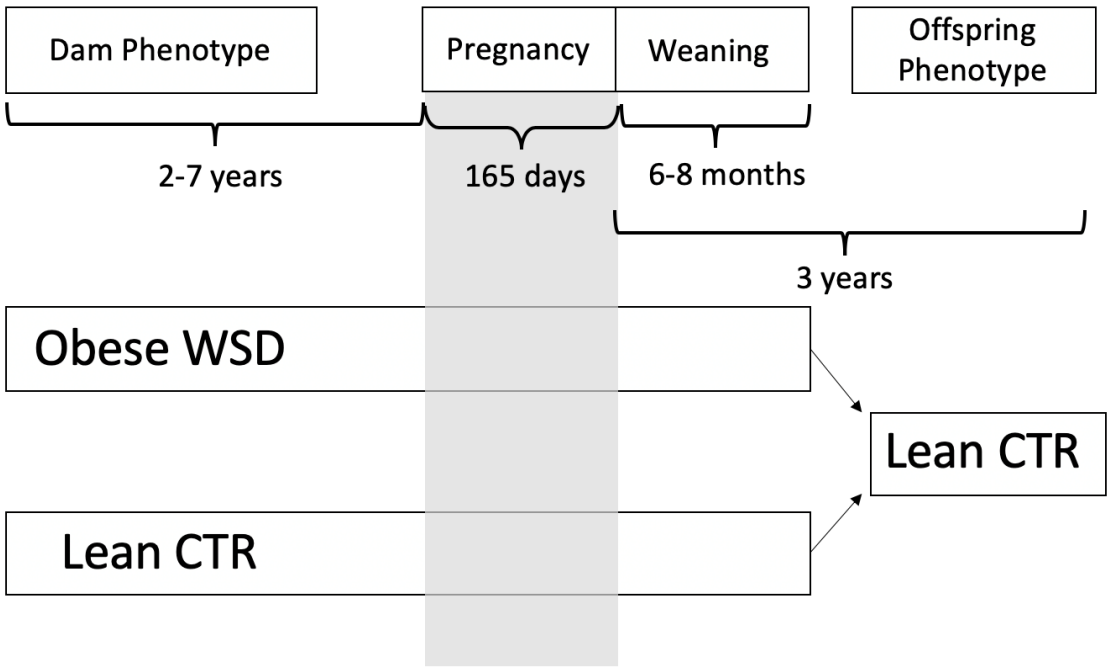


Figure 1: Previously established model of Japanese macaques

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 were studied from offspring at 3 years of age.

In order to understand the effect of maternal obesity on lean offspring, a previously established model of Japanese macaques was used (Figure 1). Adult Japanese macaque dams were socially housed in indoor/outdoor pens, while fed a CTR diet (15% calories from fat) or WSD (37% calories from fat) for 2 to 7 years prior to pregnancy. Measurement of body weight, fasting plasma for assay of glucose, and

insulin concentrations were measured before an in vitro glucose tolerance test (GTT) in the nonpregnant state as well as during the third trimester of pregnancy. The area under the curve (AUC) for glucose and insulin were calculated from baseline insulin and glucose values. Dual-energy X-ray absorptiometry (DEXA) was used to measure the percentage of body fat to determine if dams were to be classified as lean or obese. The baseline population used to define maternal obesity consisted of 33 age-matched adult females consuming the CTR diet. Percentage body fat was 16.5% +/- 7.5% (mean +/- SD). For dams consuming the WSD, a lean phenotype was defined as body fat less than 25% and obese as greater than 30% (~2 SD above baseline mean) (McCurdy et al., 2016). Females were allowed to breed seasonally, and gestational age was determined by ultrasound (AE et al., 2011). Pregnancies were then terminated by cesarean section at gestational day 130.

The obese dams were on the WSD for about 9 years, and maintained this diet throughout pregnancy (65 +/- 24 days). Offspring were than weaned to a control diet for a period of 6-8 months (mW/C). Lean dams on the control diet for the 9 years prior to pregnancy remained on this control diet through pregnancy. Offspring then maintained this control diet (mC/C). 8 mC/C dams and 7 mW/C dams were used for experimentation purposes in this study. Fetuses were delivered by cesarean section at gestational day 130. They were then necropsied. Fetal skeletal muscle from the soleus was dissected of fascia and sections were flash frozen in liquid nitrogen-cooled isopentane. Frozen tissue from these samples were then stored at -80°C. The 8 mC/C offspring (4 males and 4 females) and 7 mW/C offspring (2 males and 5 females) were then studied.

DNA Extraction

A Quick-DNA Miniprep Kit was utilized to complete DNA extraction. Prior to extraction, tissue was pulverized on dry ice to break tissue into a powder. About 25 mg of pulverized tissue was added into a bead tube (composed of 2.8mm beads) with 500 µL of genomic DNA buffer. A bead ruptor was then applied for 2 rounds at 30 seconds each. This was then transferred into a microcentrifuge tube and centrifuged at 10,000xg for 5 minutes. The supernatant was transferred to a new column and 200 µL of DNA pre-wash was added. 500 µL g-DNA wash buffer was then added to the column after being centrifuged for 1 minute. The column was then transferred to a new collection tube and 50 µL of water was added. An incubation period of 2 to 5 minutes was set at room temperature, and then centrifuged at 10,000 xg for 30 seconds to elute the DNA. 25 mg of this eluted DNA was able to be used immediately for molecular based application post extraction or may be stored at -20 degrees Celsius for future experimentation.

Optimization

Mito primers were tested using PCR by creating a master mix of DreamTaq Buffer (5 µL) , dNTP mix (1 µL), forward (2.5 µL) and reverse (2.5 µL) primers, template DNA (7.14 µL), DreamTaq DNA polymerase (0.25 µL), and water (31.61 µL) and allocating this into 15 50uL PCR tubes, with one for every offspring sample. A gradient was run with a protocol that included initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 60-67°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

A 2% agarose gel was then poured by mixing 50mL TAE, 1g agarose, and 2.5uL SYBR safe gel stain. 10 uL of each sample was added to 2uL 6x loading dye, and compared to the lambda DNA Hind III digest mixture, which was composed of 2uL lambda DNA HindIII digest, 2uL 6x loading dye, and 8uL of water. This gel was run at 100V for 30 minutes.

To quantify the Quant-iT Picogreen Mito DNA PCR quantification, the Mito DNA PCR product, as described previously, was run with sample and controls. The 2 samples were made up of a full PCR with 1x genomic DNA and a full PCR with 0.5x genomic DNA. A standard curve was run alongside these controls and samples. 10uL of each PCR product in the controls and samples with diluted with 90uL TE buffer. 100uL of all standards and PCR samples were mixed with 100uL of diluted Quant-iT Picogreen (1 to 5 dilution factor). The fluorescence of each sample was then measured.

qPCR Amplification

The abundance of mitochondrial DNA as a reporter for mitochondrial abundance and the relative amount of mitochondrial DNA damage in skeletal muscle tissue was measured by quantitative PCR (polymerase chain reaction). DNA damaged by ROS is a poor template for replication by DNA polymerase. PCR amplification of mitochondrial DNA was used as a reporter for DNA damage by ROS. A comparison was drawn between the PCR amplification efficiency of large regions of mitochondrial DNA (~10 kilobases) from skeletal muscle of 3-year-old offspring exposed to either maternal CON, or WSD. Relative mitochondrial DNA abundance was measured by the PCR amplification of short regions of mitochondrial DNA (~150 bases), which are too

short to accumulate appreciable DNA damage. The florescence intensity of each PCR product was measured and compared to a standard curve, as described previously, to determine relative amount of mitochondrial DNA.

Because no previous studies have used relative PCR amplification efficiency of mitochondrial DNA as a reporter for DNA damage in Japanese macaques, optimizations of primers and PCR conditions were performed prior to beginning data collection, as described above. Firstly, the primer sequences were designed to align to sites in the mitochondrial DNA that were relatively close (~150bp apart) for total mitochondrial DNA measurements, or relatively far (~10kbp apart) for mitochondrial ROS DNA damage. Next, it was necessary to optimize PCR amplification conditions including the annealing temperature, cycle number, and perform a quantitative test to be sure a proportional yield of DNA was obtained. Total DNA extracted from previously collected skeletal muscle samples was then amplified using the optimized PCR conditions. The PCR product was then quantified by measuring the fluorescence of double stranded DNA specific binding dye and compared to a standard curve of HindIII digested Lambda phage DNA.

Results

In our model of maternal obesity, dams placed on a western style diet had increased body weight and body fat, in comparison to dams placed on a control diet (Figure 2A). Offspring body composition and glucose handling were then quantified. Offspring body mass and body fat percent were not different between maternal groups (Figure 2B). Whole body insulin response was assessed in offspring by glucose tolerance tests (also known as a GTT). There was no difference in insulin production during the GTT as measured by the insulin area under the curve (IAUC) (Figure 2C). The glucose area under the curve (GAUC) from the GTT reports on the rate of glucose clearance from the blood. The GAUC trended to be decreased with in the maternal WSD group, relative to the maternal control group. Taken together, there was no difference in body composition and glucose handling among offspring from both groups.

To measure relative mitochondrial DNA abundance and ROS damage, I optimized a previously established quantitative PCR method to measure relative mitochondrial DNA abundance and ROS damage (Furda, Santos, Meyer, & Van Houten, 2014) for use with Japanese macaque mitochondrial DNA with two different primer sets that anneal to different sites of the Japanese macaque mitochondrial genome. The first set (MitoDNA), placed ~150bp apart will report on total mitochondrial DNA abundance (Figure 3A). Amplification from a second primer set (MitoROS) ~10kbp apart will be more sensitive to ROS induced DNA damage, resulting in less PCR product with increased ROS damage (Figure 3B). I expected to

observe reduced amounts of product from the distant primers in the mW/C offspring, indicating increased ROS damage.

Prior to completing PCR to determine mitochondrial DNA abundance and DNA damage, the PCR protocol was optimized in order to obtain quantitative results. The product yield of this experiment depended on proper optimization of annealing temperature and cycle number. Total genomic DNA was used as the substrate for PCR. Low Annealing temperature may permit non-specific priming, while a temperature too high may reduce priming efficiency. This temperature was optimized by completing a temperature gradient PCR, beginning at 5 degrees Celsius below the lowest melting point of the forward and reverse primers. The gradient tested included the temperature range between 60-67 degrees Celsius. Using the gradient, the optimal annealing temperature was 63 degrees Celsius, being that this temperature displayed the greatest product band intensity on an agarose gel (Figure 4A and 4D). Following this, the cycle number was optimized in order to obtain a proportional product yield. One sample included half the amount of DNA that was in the second sample (Figure 4C). When the amount of template DNA per reaction was graphed against the adjusted volume on the agarose gel, a proportional yield was observed. These results indicate that 20 cycles was most beneficial in order to accurately amplify the target MitoDNA and produce an unbiased amplification (Figure 4B). After this optimization process was completed for each primer set, PCR was then used to determine mitochondrial DNA abundance, as well as ROS production.

To determine if differences in total mitochondrial abundance can explain previously observed differences in cellular respiration and mitophagy, we used the PCR

based method optimized above to measure relative amounts of total mitochondrial DNA as a reporter for total mitochondrial abundance. An agarose gel of PCR product demonstrates specific PCR priming (Figure 5A). The PCR product was stained with PicoGreen and a standard curve of PicoGreen fluorescence was used to quantify the amount of PCR product (Figure 5B). There was not a statistically significant difference in the amount of mitochondrial DNA PCR product between the two maternal diet groups, suggesting offspring from obese dams have the same mitochondrial abundance as offspring from lean dams (Figure 5C).

Utilizing results from figure 5, mitochondrial DNA damage via ROS was measured in a similar fashion to the procedure utilized for mitochondrial DNA abundance. ROS-induced mtDNA damage was thus measured via PCR amplification. This protocol was optimized to obtain accurate results. The gradient tested included the temperature range between 65 and 72 degrees Celsius. Using the gradient, the optimized annealing temperature was 68 degrees Celsius, being that this temperature displayed the greatest band intensity on an agarose gel (Figure 6A). Cycle number was then optimized to ensure product proportionality, as previously described in figure 4C (Figure 6B). The PCR product was then measured with a Qubit fluorimeter (Figure 6C). There was not a statistically significant difference in the amount of mitochondrial DNA PCR product between the two maternal diet groups, suggesting offspring from obese dams have the same mitochondrial DNA damage as offspring from lean dams (Figure 6D).

Discussion

My original hypothesis, maternal obesity increases offspring mitochondrial ROS production leading to mtDNA damage without loss of mtDNA abundance, was only partially correct. I was correct in that maternal obesity does not alter skeletal muscle mitochondrial DNA abundance. This suggests 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.

According to my hypothesis, we expected that maternal obesity increased offspring mitochondrial ROS production, which would ultimately lead to mitochondrial DNA damage. This hypothesis was based on previous study findings, which have shown that maternal obesity results in increased mitophagy related signaling events in offspring skeletal muscle, suggesting increased mitochondrial damage and elevations in ROS-induced mtDNA damage. Study results displayed no significant difference in MitoDNA damage between offspring from the maternal WSD group and the maternal control group.

More specifically, research completed in the Molecular Metabolism and Endocrinology lab found that lean offspring from WSD mothers had less ROS damage (MDA), as well as decreased VDAC1 abundance (mitophagy target and involved in ROS release). This decrease in VDAC1 means that there is less ROS released, in turn meaning less cellular ROS damage. This aligns with my original hypothesis of increased mitochondrial DNA damage in offspring from obese mothers. Experimental findings did not align with my original hypothesis, as results displayed no difference

between the two groups. This finding suggests that increased ROS damage is not the leading cause of elevated mitophagy signaling events caused by VDAC1.

Future experimentation should observe VDAC1 levels in the same subject groups as were used in this study. Previous experimental findings described above were observed in the gastric muscle, as opposed to the soleus muscle utilized in this study, as well as we observed in lean dams on a WSD, while this study observed obese dams on a WSD. These factors may serve as a significant role in explaining differences in study findings.

Figures

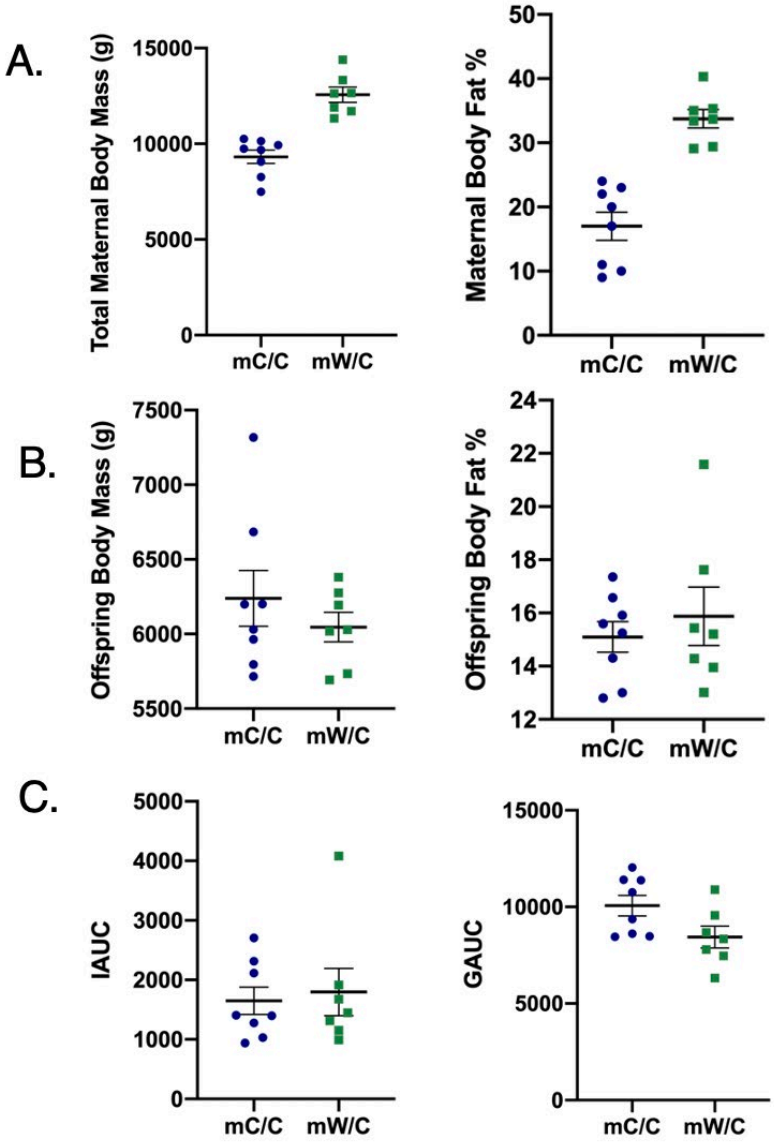


Figure 2: Physiological measures of maternal and offspring bodyweight, body fat, and glucose handling.

(A) Dams fed WSD had a higher body mass (g) than those fed a control diet (p = 0.00003). Dams fed WSD had a higher body fat (%) than those fed a control diet (p =

0.00003). (B) Offspring body mass (g) between mC/C and mW/C are not statistically different (p = 0.4). 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). 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.

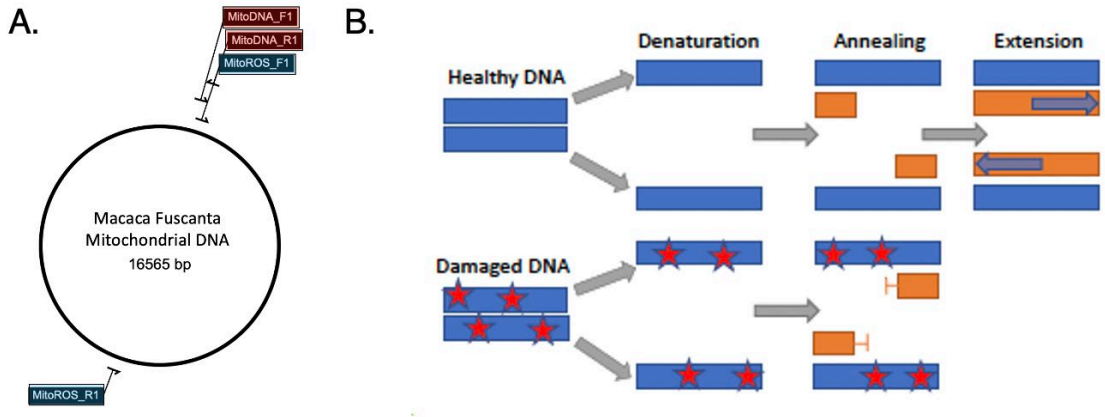
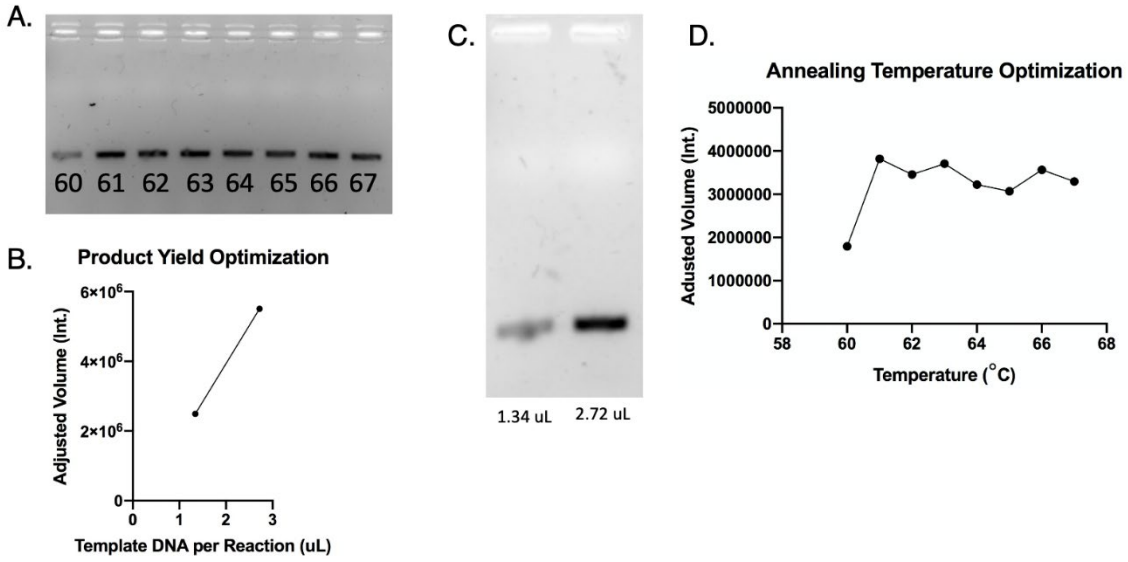


Figure 3: Measuring mitochondrial DNA abundance and DNA damage via ROS production by quantitative PCR.

(A) MitoROS and MitoDNA forward and reverse primer design. (B) Schematic displaying hypothesis of PCR product following application of MitoROS primers. It is hypothesized that increased mitochondrial DNA damage is present in offspring from obese mothers on a WSD.



(A) Agarose gel of annealing temperature (60-67 degrees Celsius) (B) Product Yield Optimization graph displaying 2x product yield (C) Cycle number and product yield (1x and 2x) optimization on agarose gel (D) Annealing temperature optimization graph, band volume vs. annealing temperature.

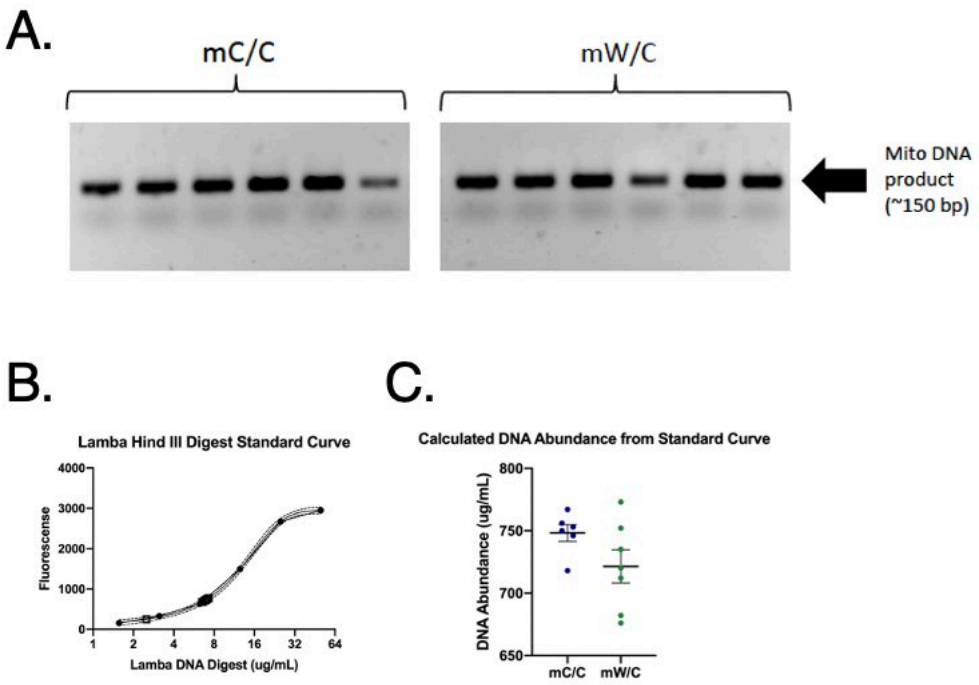


Figure 5: Offspring from obese dams do not have a significant reduction in mitochondrial DNA abundance compared to offspring from lean dams.

(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 digested DNA used to quantify mitochondrial DNA PCR product. (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.

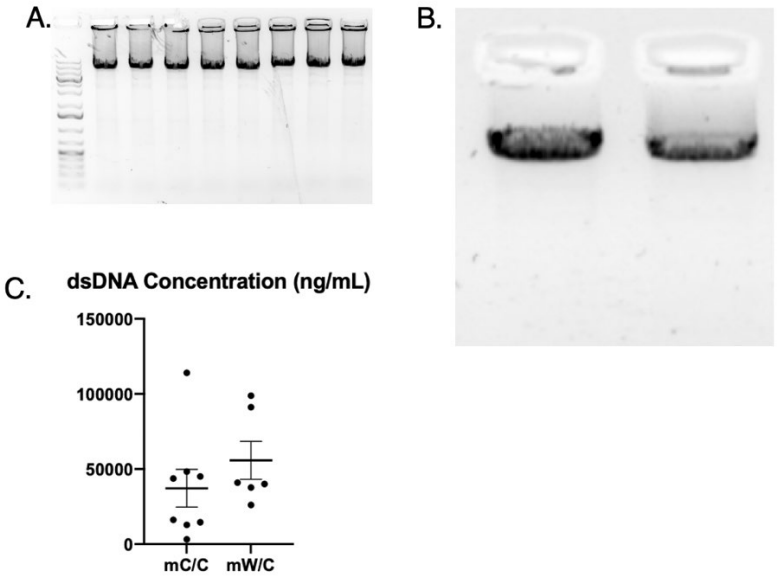


Figure 6: Offspring from obese dams do not have a significant increase in mitochondrial DNA damage compared to offspring from lean dams.

(A) Agarose gel of annealing temperature (65-72 degrees Celsius) (B) Cycle number and product yield (1x and 2x) optimization on agarose gel (C) Qubit 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 damage by ROS. Fluorescence, as a measure of DNA abundance, displays no significant difference between the offspring from the Ob/WSD dams and Ln/CON dams ($p = 0.3$). Data was analyzed by unpaired t-test.

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