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Examining the Oxidative Stress Effects in Rats Exposed to 2-Aminoanthracene In Utero and High Fat Diet Three Months After

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Examining the Oxidative Stress Effects in Rats Exposed to 2-Aminoanthracene In Utero and High Fat Diet Three Months After

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in the *Department of Chemistry and Biochemistry.*

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ABSTRACT

Polycyclic aromatic hydrocarbons are organic compounds that are created synthetically or found in partially burned oil, gasoline, and coal. 2-aminoanthracene (2AA) is a common example of a PAH and can affect lipid and carbohydrate metabolism and resulting in inflammatory problems and oxidative stress. Oxidative stress is an imbalance between production and storage of reactive oxygen species. Diabetes is a group of endocrine diseases involving high blood sugar levels. The objective of the research project is to investigate oxidative stress in relation to diabetes in rats exposed to 2AA *in utero* and a high fat diet later in life. Pregnant dams were separated into treatment groups based on their dosages of 2AA; 0 mg/kg (control), 50 mg/kg (low), 100 mg/kg (high). During the gestation -postpartum period, the dams were fed a 2AA contaminated diet. Three months post wean the rats were assigned to regular rat food then a moderately high fat diet for six weeks. Adipose tissue from the rat's abdomens were removed and protein levels, glucose concentration, lipase activity and total antioxidant capacity were measured. Protein levels in the treatment groups compared to the control were elevated. Glucose levels showed a significant reduction in the treatment groups. Lipase activity displayed increased activity between the treatment groups but not significant. Antioxidant capacity in the adipose tissue was slightly reduced in the treatment groups compared to controls. A gene expression analysis was also performed on the mRNA and the genes RGS16, DBP, and ABCB1B demonstrated an upregulation. In conclusion, it appears 2AA ingestion during gestation had a mild effect on oxidative stress in rat progeny.

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Introduction

Embryonic development is one of the most delicate biological processes that humans experience, due to all of the differentiation that occurs. Environmental exposure to chemicals, such as polycyclic aromatic hydrocarbons, can stunt this process and lead to the development of life altering diseases. Polycyclic aromatic hydrocarbons are organic compounds that are produced synthetically or found naturally in the environment as a result of partially burned oil, gasoline, and coal (CDC, 2022). There are three forms of PAH's: pyrogenic, petrogenic, and biogenic (Patel, A.B., et al., 2020). Pyrogenic PAH's are formed by the unintentional "incomplete combustion of organic materials at very high temperatures" or in conditions with little to no oxygen available (Patel, A.B., et al., 2020). Petrogenic PAH's are found in petroleum and all of its byproducts (Patel, A.B., et. Al., 2020). Biogenic PAH's "are synthesized by biological [species] like microorganisms, phytoplankton, algae, and plants during slow biological conversion of organic materials" (Patel, A.B., et al., 2020). When large amounts of polycyclic aromatic hydrocarbons are inhaled, ingested, or absorbed through the skin, severe blood, kidney, skin, and liver problems may arise (CDC, 2022). This compound tends to target fat tissue and can result in cancer.

This compound, 2-Aminoanthracene (2-AA), is the targeted example of a PAH in this experiment. This molecule is found in manufacturing dyes, chemicals, inks, and resins. 2-AA is a carcinogen and a mutagen therefore it can affect DNA directly and increase the rate of genetic mutations (National Center for Biotechnology Information 2023). This compound dissolves easily in adipose tissue and affects lipid and

carbohydrate metabolism as well as causes inflammatory problems and oxidative stress in the body (CDC, 2022).

The introduction of environmental chemicals to the womb during pregnancy may result in the development of malformations. About “6% of births” per year around the world involve birth defects (WHO 2006). During the embryonic stage, the fetus’ organs are in a most delicate state because they are in the beginning stages of differentiation (WHO 2006). As a result of this, organs are susceptible to a variety of issues (WHO 2006). The type and severity of the malformity is dependent upon the dosage of the chemical and the life stage’s susceptibility. Over the recent years, research has demonstrated that the intrauterine environment can cause an onset of adult diseases like “hypertension and type 2 diabetes” (WHO 2006). Problems may arise during the exposure period or later in life (WHO 2006).

Diabetes is a chronic disease that affects the way the body turns food into energy. As of June 29, 2022, 11.3% of the United States population has been diagnosed with diabetes; about 1 in 10 have diabetes (Center for Disease Control and Prevention 2022). Adipose tissue is a factor in the formation of diabetes. There is fat tissue that is found directly under the skin and fat that lines organs called visceral fat. Visceral fat tissue is linked to insulin resistance and when this tissue is damaged in any way it will become dysfunction and may lead to diabetes (Centers for Disease Control and Prevention 2022). This disease can result in a decrease in the overall quality of life, affecting the mental, physical, and emotional states (Centers for Disease Control and Prevention 2022).

Oxidative stress is the result of a continuous imbalance between the “production of free radicals” and the body’s “ability to counteract or detoxify the reactive

intermediates" (Maurya, 2017). This oxidation-oxidation imbalance can lead to the development and progression of diabetes (Zhang, Pengju et al. 2020). Exposure to environmental stressors, such as 2-AA, can induce prolonged oxidative stress and the production of reactive oxygen species and subsequently oxidative damage. (Shihori et al. 2022). Reactive oxygen species (ROS) are produced in the mitochondria and can cause direct or indirect toxic effects, such as apoptosis and severe oxidation on proteins, lipids, and DNA (Pawan and Chandra, 2017). Examples of ROS include O_2^-, OH, and H_2O_2 (Pawan and Chandra, 2017). Oxidative stress can result in long term issues like insulin resistance and chronic low-grade inflammation (Maurya, 2017). Due to recent advancements, oxidative stress can be measured by "assaying the product of… oxidative damage in clinical samples", by measuring "antioxidant potential of an organ, tissue, or body fluids to withstand further oxidation" or by using nano diagnostic methods (Pawan and Chandra, 2017). The detection of the enzyme SOD also indicates an increase in the oxidative defensive system. Both oxidative stress and ROS levels are inspected during this study due to the fact that past research has shown a correlation between them and the pathogenesis and progression of type 2 diabetes (Zhang, Pengju et al 2020).

The overall objective of this research project is to investigate oxidative stress as it relates to diabetes in rats exposed to 2-aminoanthracene *in utero* and high fat diet later in life. Adipose tissue is the focus of this project due to its role in lipid and glucose homeostasis. 2-Aminoanthracene is known to cause an inhibition in carbohydrate as well as lipid metabolism, hence all of the tests on the adipose tissue. Noting abnormalities in the tissue under investigation can be a big indicator of diabetes.

Methods

1. *Description of study*

This study involved adipose tissues of male. Pregnant dams (n=9) were fed a diet which contained concentrations of 2-Aminoanthracene during the period of gestation. They were separated into treatment groups based upon their dosages of 2AA; 0 mg/kg (control), 50 mg/kg (low), 100 mg/kg (high). During the gestation/postpartum period, the dams were fed a 2AA contaminated diet. Three months post wean the rat pups were assigned to regular rat food then a moderately high fat diet for six weeks. Adipose tissue from the rat's abdomens were removed and tests were run to determine protein levels, glucose concentrations, lipase activity and antioxidant capacity. This part of the study focused on male rats exposed to 2AA *in utero* and later moderate high fat diet.

2. *Tissue Homogenization*

The tissues from the rats were extracted from the male rats' abdominal adipose tissue. There were tissues from the control, low, and high dosages of the 2AA diet. This tissue was stored in a freezer at -80 °C until it was needed. In order to study the tissue, it had to be homogenized. To create the solution, a 10X Phosphate Buffer Saline (PBS) was diluted to 1X by mixing 1.5 mL and 13.5 mL of deionized water in a test tube. This solution was frozen for 5 minutes. A cooler was filled with 1 liter of liquid nitrogen and the tissue samples were stored in it to ensure they stayed cold. Storage tubes were labeled appropriately and filled with 2 mL of 1X PBS solution. Approximately 1 gram of tissue was added to the tube. The mixture was blended using a TissueRuptor. Once fully blended, the tubes

were centrifuged for 15 minutes. Finally, the supernatant was removed and divided into 4 equal aliquots and stored in the freezer until further analysis.

3. Total RNA Isolation

Tissue weights varying between 20-30 mg were added a 2 mL Eppendorf tube containing 900uL of the lysis reagent. The solution was homogenized again using a TissueRuptor for 20-40 seconds. The homogenate was left at room temperature for 5 minutes in order to promote the dissociation of nucleoprotein complexes. Once completed, 100uL of gDNA eliminator solution was added to the homogenate and shaken vigorously for 15 seconds to reduce the genomic DNA contamination. Next, 180 µL of chloroform was added and the mixture was shaken again for 15 seconds. The tube was left at room temperature for 2-3 minutes. The tubes were centrifuged at 12,000 g for 15 minutes at 4°C, then incubated to room temperature (15°C-25°C). After all of this was completed, there were 3 layers in the sample tube, an aqueous phase (colorless with RNA), an interphase (white), and an organic phase (red). The complete 600 µL volume of aqueous phase was transferred into a new microcentrifuge tube. A 600 µL of 70% ethanol was then added and mixed using the pipetting method. A 700 µL of this solution was then transferred to a Qiagen's RNeasy mini spin column in a 2 mL collection tube and centrifuged for 15 seconds at 10,000 rpm. To wash the membrane, 700 µL of the Buffer RWT was added to the column and centrifuged again for 15 seconds at 10,000 rpm. It is crucial that the tube is removed carefully such that the column does not contact the flow through and is emptied completely. In the last stages of the isolation, 500 µL of the Buffer RPE was added to the

column and centrifuged (15s) and then another 500 µL were added and centrifuged from 2 minutes to fully wash and dry the column. This is all done in order to ensure no ethanol is carried over during the RNA elution. The RNeasy column is placed into a 2 mL collection tube and centrifuged for 1 minute, then to a 1.5 collection tube. In this new tube, 50 µL RNase free water is pipetted onto the spin column membrane and centrifuged for 1 minute. The RNA is aliquoted into 3 tubes and stored at -80°C.

4. Protein Assay BCA

Over the course of this experiment (9 standards, 6 unknowns) x (2 replicates) x (2 mL of WR per sample) were created, therefore a total of 60 mL volume of working reagent (WR) was needed. The working reagent was prepared by mixing 50 parts of BCA reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). To begin, 0.1 mL of each standard and unknown sample replicate was pipetted into the appropriate test tube. 2.0 mL of the WR is added to each tube and mixed well. The tubes are then covered and incubated at 37°C for 30 minutes. Once that was completed, the tubes were cooled to room temperature. Within 10 minutes, the absorbances of all of the samples were measured at 562nm. The average 562nm absorbance of the blank was subtracted from each of the standard and unknown replicates. A standard curve was created by plotting the average blank corrected 562nm measurement vs its concentration in µg/mL. This curve was used to determine the protein concentration of each unknown sample.

Table 1. Dilution scheme for standard test tube protocol and microplate procedure.

Standards

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA concentration (µg/mL)
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0=Blank

Table 2. Dilution Scheme for Enhanced Test Tube Protocol. Unknowns

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	700	100 of Stock	250
B	400	400 of vial A dilution	125
C	450	300 of vial B dilution	50
D	400	400 of vial C dilution	25
E	400	100 of vial D dilution	5
F	400	0	0=Blank

5. Reverse Transcription

Reverse transcription was performed using Bio-Rad reagents. To prepare the 180 µL of the reverse transcription master mix, 48 µL of the iScript RT Supermix and 132 µL of the nuclease-free water were mixed via pipetting up and down. A 15 µL of the master mix is added to 5 µL of RNA for each reverse transcription reaction. There were 2 replicates for each. The volumes were incubated in a thermal cycler by priming for 5 minutes at 25°C, reverse transcription for 20 minutes at 46°C, and RT inactivation for 1 minute at 95°C.

6. Quantitative Polymerase Chain Reaction (QPCR)

Quantitative PCR reaction was undertaken using Bio-Rad reagents. Thirty-six samples were created: 6 different genes and 6 RNA samples. To make the solution, 20 µL of supermix, 4 µL of forward primer, 4 µL of reverse primer, 4 µL of cDNA template, and 8 µL RNase free H2O, and 2 µL of the DNA template were combined. This process was done slowly and carefully to ensure everything went into the correct wells. Once everything was in place, the plate was cycled in the process seen in table 3.

Table 3. Cycling steps for QPCR

Cycling Step	Temperature (°C)	Time (s)	# Cycle
Enzyme activation	95	30	1
Denaturation	95	1s-5s	30-40
Annealing/Extension	55-60	1s-5s	
Melting Curve	65-95 (0.5 increments)	2-5 s/step	1

7. Antioxidant Assay

To begin, the reagents had to be prepared. Antioxidant assay was performed using Cayman Chemical, Michigan, 709001's antioxidant assay kit. To prepare the antioxidant assay buffer (10X), 3 mL of the assay buffer concentrate had to be diluted with 27 mL of HPLC-grade water. To reconstitute the chromogen, it was added to 6 mL of HPLC-grade water and vortexed well. The metmyoglobin was reconstituted by adding 600 uL of Assay buffer to the well and vortexed. Trolox was reconstituted by adding 1 mL of HPLC grade water and followed by vortexing. Lastly, 10 µL of the 8.82 M solution hydrogen peroxide was diluted with 990 µL of HPLC grade water, then diluted again by removing 20 µL and adding 3.98 mL of HPLC grade water.

The plate for the assay was set up to fill 32 wells (14 standards, 18 samples). Each of the standards were done in duplicates, and the samples were done in triplicates. The Trolox standard wells consisted of 10 µL Trolox standard, 10 µL Metmyoglobin, and 150 µL of Chromogen. The sample wells consisted of 10 µL of the sample, 10 µL of the Metmyoglobin, and 150 µL of Chromogen. The final volume in each well was 210 µL. The reaction was triggered by adding 40 µL of hydrogen peroxide working solution. The plate was covered and placed in a shaking incubator for 5 minutes. Once removed, the absorbance was measured at 750 nm using Tecan's plate reader model Safire.

Table 4. Trolox standard preparation

Tube	Reconstituted Trolox (µL)	Assay Buffer (µL)	Final Concentration (mM Trolox)
A	0	1000	0
B	30	970	0.045
C	60	940	0.09
D	90	910	0.135
E	120	880	0.18
F	150	850	0.225
G	220	780	0.33

8. Glucose Assay

To create the DNS reagent, at room temperature, 0.5g of 3,5-dinitrosalicylic acid was dissolved in 10 mL of 2M NaOH and 25 mL of deionized water in a volumetric flask. Once that was fully dissolved, 15 g of potassium sodium tartrate is added into the solution. Finally, the solution was diluted with dH2O. This solution is placed onto a stir plate with a stir bar and allowed to stir overnight.

To run the glucose assay, the glucose stock had to be made. This was done by adding 500 mg of sugar to 5 mL dH2O. A 2 mL of DNS reagent was added to each sample. Once mixed, the solution was placed into an actively boiling water bath for 5 minutes and a color change was observed. It was immediately allowed to cool in a cold-water bath for a few minutes and this stopped the reaction. A 5 mL of deionized water was put into each tube and mixed. The absorbances were measured at 540 nm.

9. Lipase Assay

To prepare the working reagent (WR) the color reagent was mixed into the assay buffer and mixed well according to QuantiChrom, Lipase Assay Kit (DLPS-100, Hayward CA). Addition of 0.8 mL BALB Reagent and the WR were completed. To prepare the wells, 150 µL of H2O and 150 µL calibrator were added into each. Also, 10 µL of each sample were pipetted into their respective wells. Lastly, 140 µL of the working reagent was added to the wells. The plate was read at 412nm at 10 minutes and at 20 minutes. This data was compared after completion.

Results and Discussion

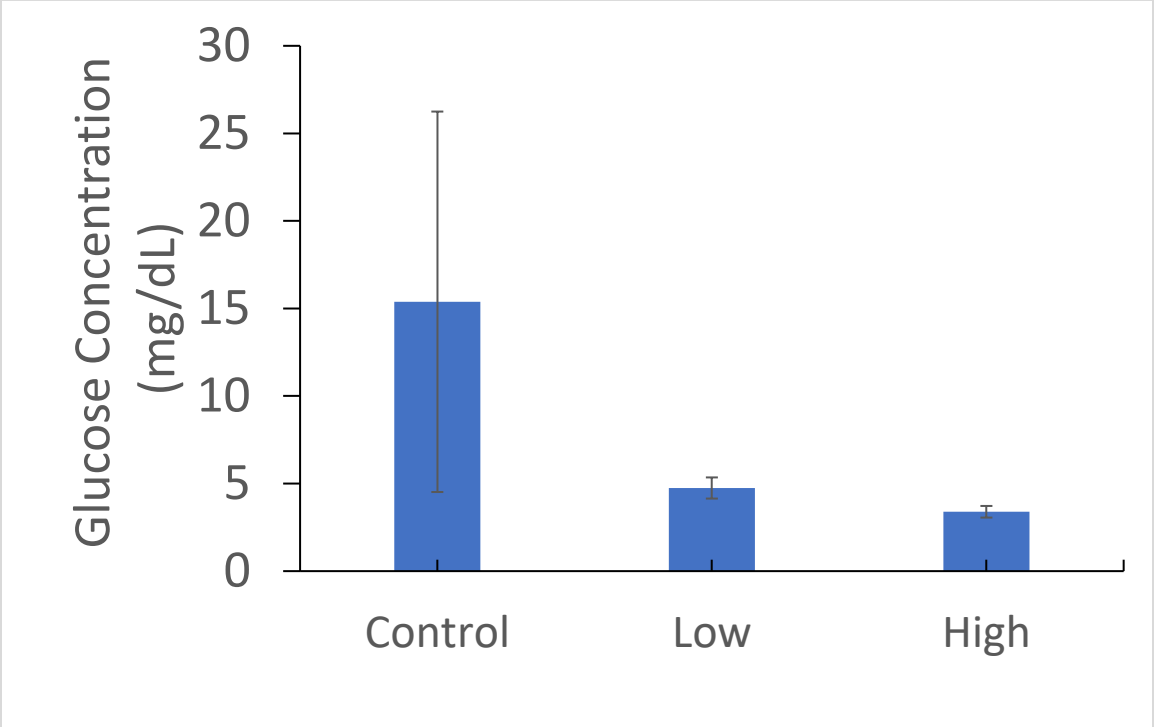

Figure 1. Average Glucose Concentration expressed in groups exposed to 0 mg/kg 2AA (Control), 50 mg/kg 2AA (Low), and 100 mg/kg 2AA (High)

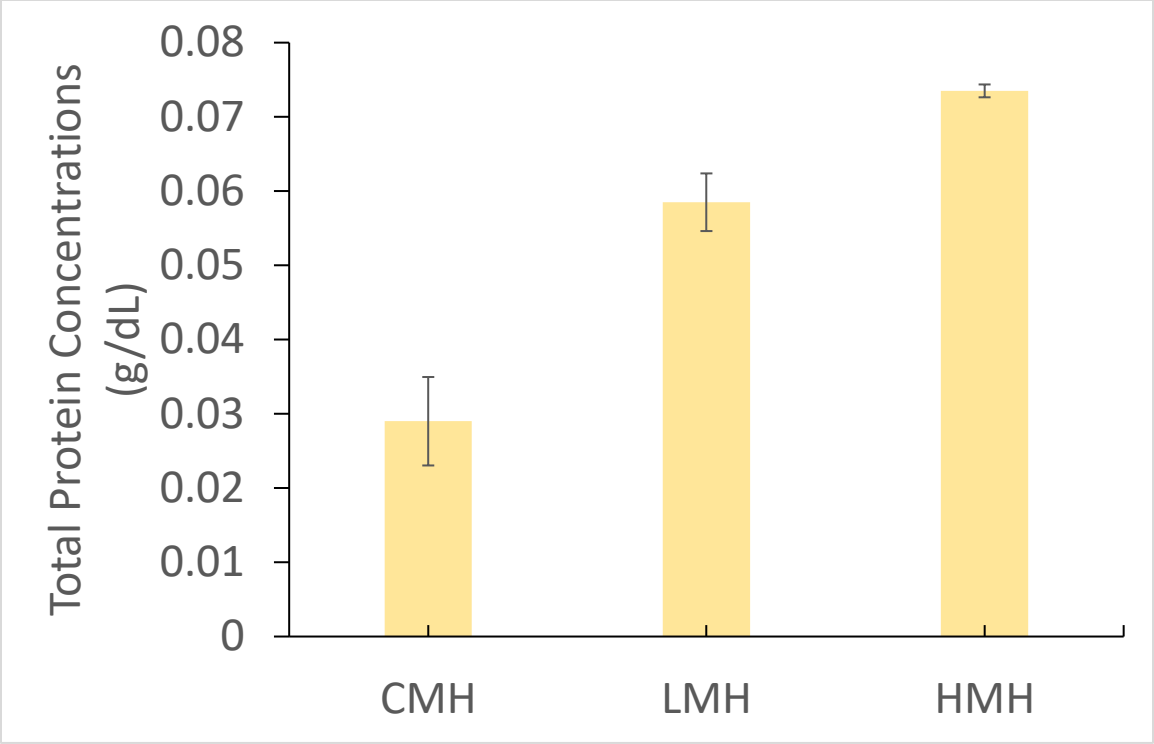

Figure 2. Average Protein Concentration expressed in groups exposed to 0 mg/kg 2AA (Control) 50 mg/kg 2AA (Low), and 100 mg/kg 2AA (High)

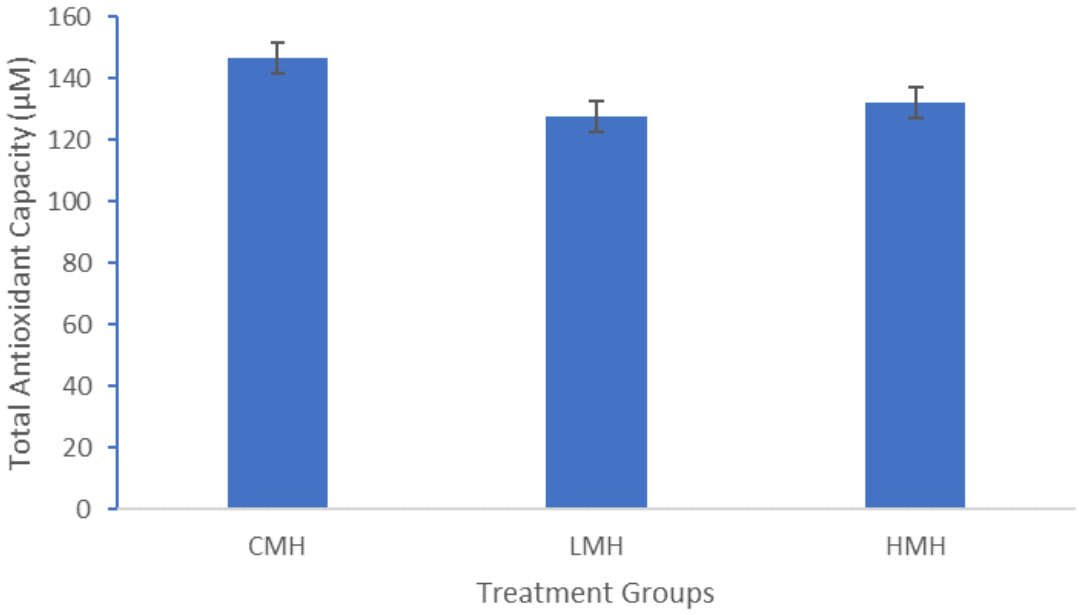

Figure 3. Total Antioxidant Capacity levels for treatment groups 0 mg/kg 2AA (Control), 50 mg/kg 2AA (Low), and 100 mg/kg 2AA (High)

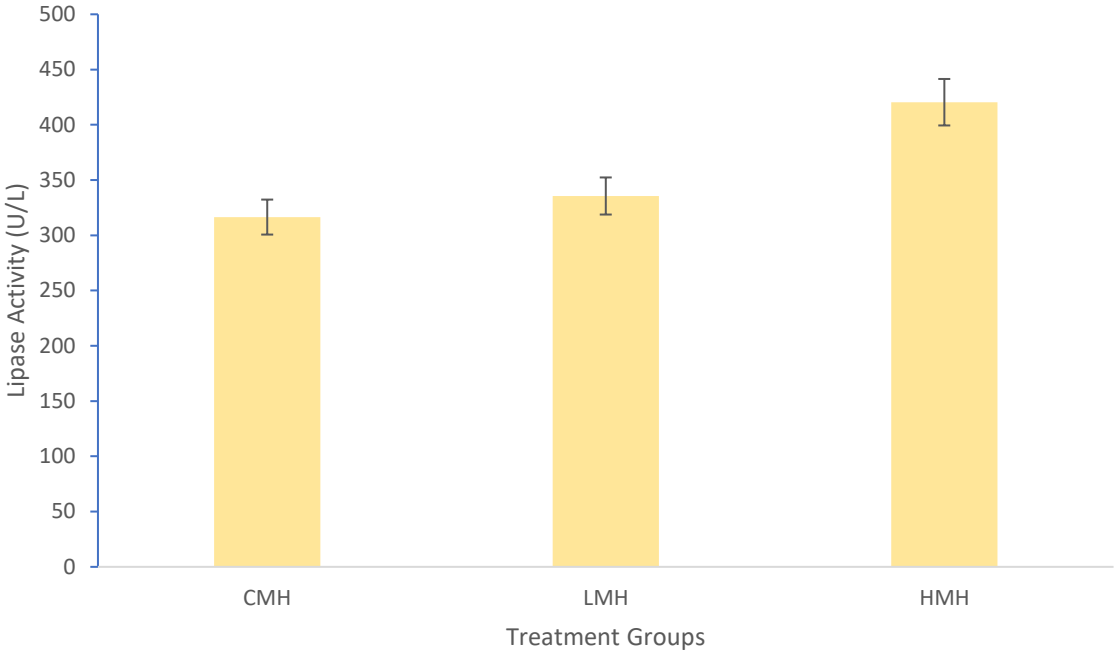

Figure 4. Average Lipase Activity levels for treatment groups 0 mg/kg 2AA (Control), 50 mg/kg 2AA (Low), and 100 mg/kg 2AA (High)

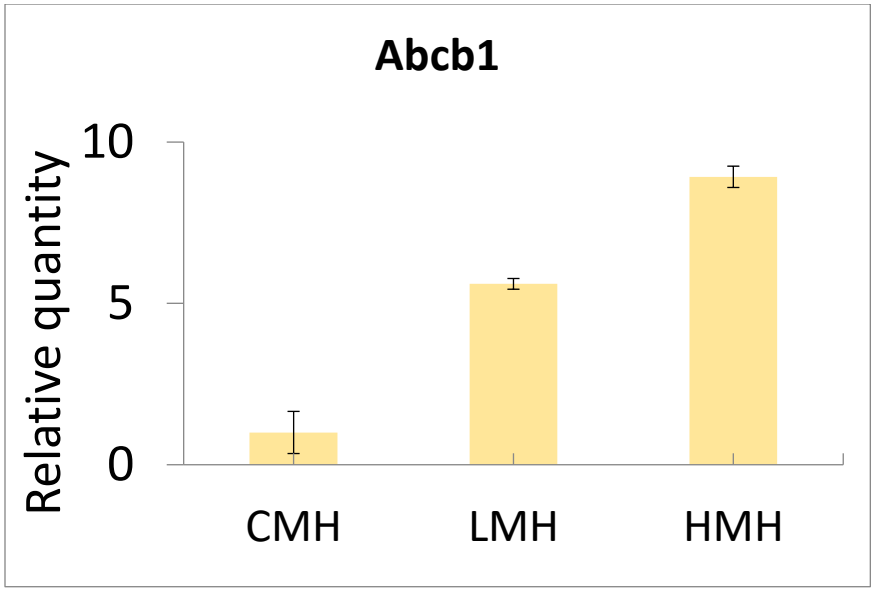

Figure 5. Abcb1 gene expression for in treatment groups control (1), low dosage (2), and high dosage (3).

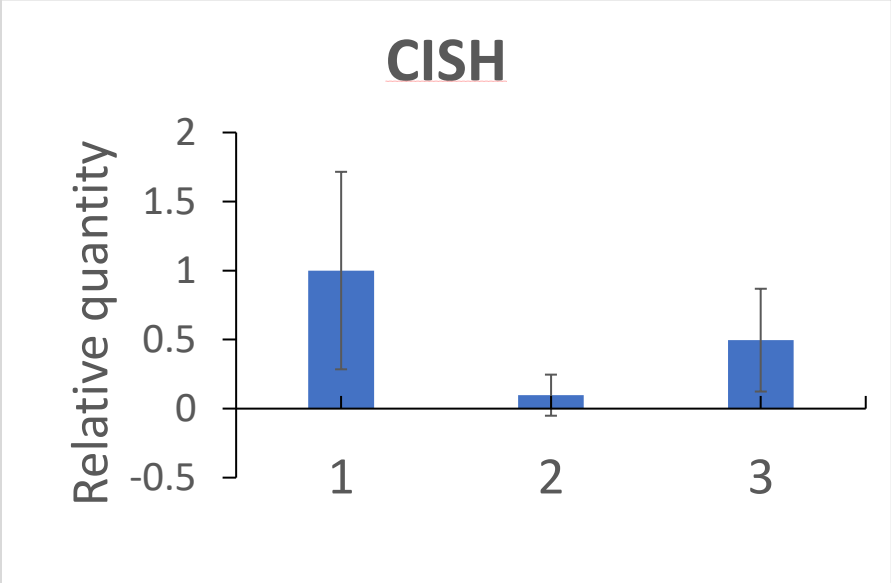

Figure 6. Cish gene expression for in treatment groups control (1), low dosage (2), and high dosage (3).

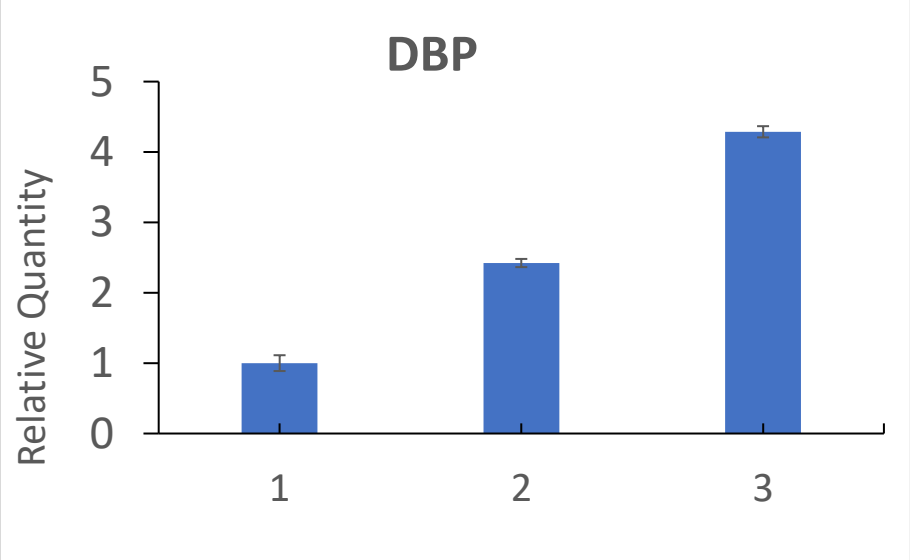

Figure 7. DBP gene expression for in treatment groups control (1), low dosage (2), and high dosage (3).

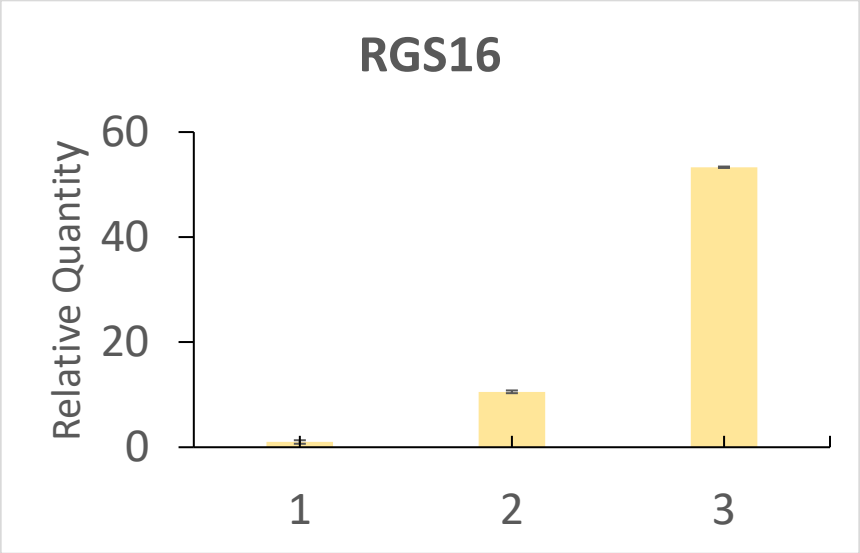

Figure 8. RGS16 gene expression for in treatment groups control (1), low dosage (2), and high dosage (3).

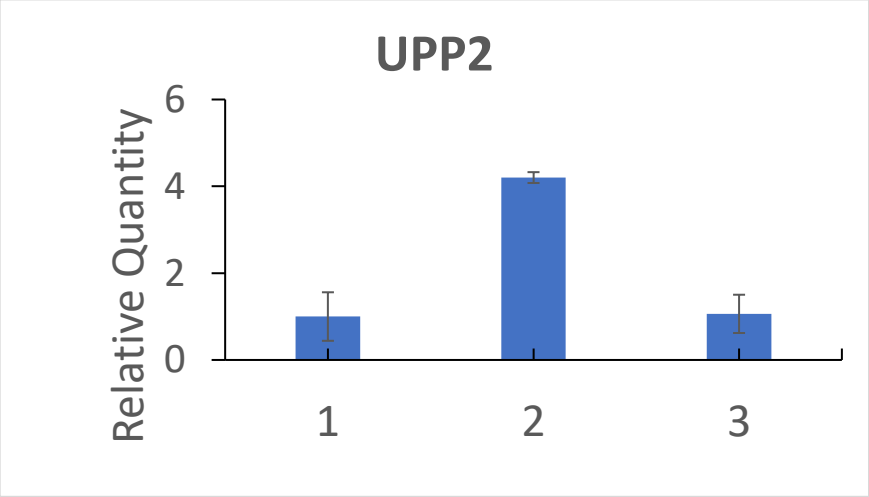

Figure 9. UPP2 gene expression for in treatment groups control (1), low dosage (2), and high dosage (3).

Glucose assay

Typically, diabetes is shown as elevated glucose levels due to insulin resistance. Oxidative stress can induce insulin resistance causing a spike in glucose levels. This seemed the opposite of what is demonstrated in our research. The glucose levels were monitored by use of a glucose assay. This assay showed a significant reduction in adipose tissues glucose levels amongst the treatment groups (control, low, and high) (Figure 1). The average glucose concentration of the control to the mean glucose of the low dose group decreased by approximately 66.7%. Our results were due to the fact that we investigated glucose levels within adipose tissue rather than blood. According to the National Library of Medicine, lipids are very sensitive to insulin and "insulin action on fat cells stimulates glucose uptake" (Richard 2020). It has also been shown that oxidative stress can "impair the body's response to insulin", resulting in insulin resistance (Dickson 2023). In this case, there is an increase in insulin resistance as a result of diabetes and oxidative stress, so there is no insulin action on fat cells stimulating glucose uptake. Therefore, as the concentration of 2-Aminoanthracene increases resulting in diabetes and oxidative stress, the amount of glucose in the fat tissue will ultimately decrease.

Protein assay

The results showed significantly elevated levels of protein in the treatment groups compared to the control (Figure 2). There was an increase by a factor of one-half from the control to the low dosage. Normally, diabetic patients do not show any differences in protein levels compared to patients without it. In instances of oxidative stress, diabetic

neuropathy can be induced because it modulates nerve cell function (Pang 2020). Diabetic neuropathy is defined as a "damage occurred in sensory neurons that causes neuropathic pain" (Pang 2020). This complication occurs in both type 1 diabetes and type 2 diabetes (Moawad 2022). Diabetic neuropathy is associated with a number of factors. Some of which are metabolic abnormalities like increased protein production (Moawad 2022). According to the research, protein levels should increase as the amount of 2-AA and oxidative stress increases. Thus, our results correlate with typical diabetes protein levels if the subject has diabetes neuropathy.

Antioxidant assay

The antioxidant capacity in the adipose tissue was slightly reduced within the treatment groups compared to controls (Figure 3). Antioxidants are molecules that remove free radicals and decrease oxidative damage by inhibiting oxidation. Antioxidants provide neutralizing effects that help to protect the body from oxidative stress (Eske 2019). When antioxidant levels are low, oxidative stress is more likely to occur because nothing is fighting off the free radicals. Once the free radicals truly begin to take over, antioxidant levels will continue to drop. This is directly shown in our results. The rats that were under investigation underwent oxidative stress which is the result of antioxidants not being effective in removing ROS. The difference between the groups, though not significant, showed that the treated samples had reduced levels of antioxidant capacity.

Lipase assay

Lipase is an enzyme which is responsible for the hydrolysis of lipids (fats) within the body. The lipase assay displayed increased activity between the treatment groups and the control, but not enough to be considered significant (Figure 4). As seen in type of diabetic 1 patients, lipid profiles are typically close to those of the general public (Feingold 2020). According to Feingold, there has been some research that shows that these patients may have slightly increased levels in lipid levels. A high percentage of diabetic patients are obese (retain fat) and insulin resistant (Feingold 2020). With this retention of lipids within the body, there will be an increase in lipase activity trying to break down these lipids. There was a not large difference between the treatment groups but there was a slight increase in lipase activity among treatment groups in comparison to the control rats.

Gene expression

Before beginning the gene expression assay, background research was done to see what genes are expressed specifically under conditions of oxidative stress in rodent RNA. The genes selected to study were ABCB1B, CISH, DPB, RGS16, and UPP2.

ABCB1 stands for “ATP-binding cassette sub-family B member 1” (ABCB1 Gene Spotlight 2021). This gene “encodes transporter and channel proteins” and pumps foreign substances out of the cells (ABCB1 Gene Spotlight 2021). This protein acts as a defense mechanism against harmful substances within the body, harmful substances like reactive oxygen species. The results from the assay indicate an increase in abcb1 gene

(Figure 5) expression as the 2-Aminoanthracene dosage increased. As the amount of toxins and oxidative stress increases in the adipose tissue the gene is overly expressed in order to try to lower oxidative stress levels.

CISH is a "cytokine-inducible SH2-containing protein" (Zhu, et al. 2020). Within the body, CISH acts a negative regulator of killer T cells (Zhu, et al. 2020). Killer cells are leukocytes that destroy infectious cells or particles within the body. As a negative regulator, CISH brings the killer T cell activity to an end. From the gene expression assay, it was shown that the CISH activity decreased as 2-AA dosage increased. This is predictable seeing as we introduced a toxin and oxidative stress to the tissues. CISH will allow the killer T cells to do their job and kill off foreign substances in the body. Once those levels decrease then CISH will activate and begin to slow the process. If there is a lot of foreign substances in the body, the T cell count will increase so ultimately CISH will increase in order to stop more T cells in the end. There was a bit of fluctuation between the graphs because the control had a moderate amount of CISH (Figure 6), the low dosage had low levels of CISH, and the high had increased levels of CISH. However, the overall CISH gene expression in adipose tissues was not consistent.

DBP is the D site of the albumin promotor binding protein (DBP Gene 2014). This gene "codes for a protein that binds to an upstream promotor in the insulin gene" (DBP Gene 2014). As it is a promoter of the insulin gene, it is associated with insulin resistance as well (DBP Gene 2014). The results from the test show that DBP was increased amongst the treatment groups (Figure 7). The body is unable to produce insulin in cases of type 1 diabetes. The assay results make sense because if the body is void of insulin the DBP will work on overdrive to continue to produce more but to no avail.

RGS16 was over-expressed in the adipose tissues of the rats exposed to 2-AA (Figure 8). RGS16 is a "novel regulator of beta-cell function that promotes insulin secretion" (Miaomiao, et al. 2022). Its function is to terminate the "downstream G-protein signaling pathway" which is responsible for signal transduction throughout the body (Miaomiao, et al. 2022). Issues involving the pathway can lead to multiple diseases such as diabetes (Miaomiao, et al. 2022). Due to the fact that the gene assists with signaling throughout the body it can affect "immune, inflammatory, and metabolic processes" (Miaomiao, et al. 2022). According to Functions of RGS16, this gene serves a critical role in T and B cell migration, similarly to CISH, this gene assists with fighting off foreign substances. As seen in an obese mouse model from Functions of RGS16, this gene played a role in reducing fat accumulation and insulin regulation and can provide treatment for diabetes.

UPP2 stands for uridine phosphorylate 2, a gene that "catalyzes the reversible cleavage of uridine" (UPP2 Gene 2022). According to the Uridine Metabolism article, previous studies have shown that uridine is a marker of insulin resistance among diabetes patients. The article notes that in the mice study, increased uridine levels led to glucose intolerance and insulin resistance (Zhang, et al. 2022). It was also noted that the biosynthesis of uridine occurs in the adipose tissues (UPP2 Gene 2022). Knowing all of this information, it can be said that it correlates with the results collected. Amongst the low dose treatment groups, UPP2 increased as 2-AA increased. However, in the high dosage treatment group, UPP2 was downregulated in comparison to the control (Figure 9). In the adipose tissue, uridine levels were already higher than in normal tissues. In this study, studying oxidative stress in relation to diabetes showed that uridine increases

insulin resistance therefore should increase with the amount of oxidative stress and this was demonstrated in our research.

Conclusion

The purpose of this study was to examine the oxidative stress effects on male rats that were exposed to 2-Aminoanthracene whilst *in utero*. It was hypothesized that addition of a polycyclic aromatic hydrocarbon to the diet would increase oxidative stress and induce diabetes in the rats. This hypothesis was supported by all of the results that were collected during this research study. It appears 2AA ingestion during gestation had a mild effect on oxidative stress in rat progeny. Future progress will involve observing the effects in female rats exposed to the 2AA *in utero* as well as observing the other tissues that were extracted. The similarities and differences will be compared and determined if there is any overarching correlation.

Works Cited

[1] Polycyclic Aromatic Hydrocarbons (PAHs) Factsheet. *National Biomonitoring Program, CDC.* https://www.cdc.gov/biomonitoring/PAHs_FactSheet.html.

[2] Patel, A. B.; Shaikh, S.; Jain, K. R.; Desai, C.; Madamwar, D. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Frontiers in Microbiology*, **2020**, *11*. https://doi.org/10.3389/fmicb.2020.562813.

[3] Maurya, Pawan Kumar. *Oxidative Stress*. Springer, **2017**

[4] Stagg, John C. Assessment of Oxidative Stress in the Livers of Rats Exposed to 2-Aminoanthracene for Twelve Weeks. *Honors College Theses*. 481, **2019**, https://digitalcommons.georgiasouthern.edu/honors-theses/481

[5] Zhang, Pengju et al. Oxidative stress and diabetes: antioxidative strategies. *Frontiers of medicine* vol. 14,5: 583-600. doi:10.1007/s11684-019-0729-1, **2020**

[6] National Center for Biotechnology Information. PubChem Compound Summary for CID 11937, 2- Aminoanthracene, **2023,** https://pubchem.ncbi.nlm.nih.gov/compound/2-Aminoanthracene.

[7] Principles for evaluating health risks in children associated with exposure to chemicals. *World Health Organization*. **2016.**

[8]Mays CA, Hunter DA, Yau W, Gato WE. Assessment of the link between in utero exposure to 2-aminoanthracene (2AA) and type-1 diabetes (T1D)". *J Diabetes Metab Disord*, **(January 28 2017).**

[9] Centers for Disease Control and Prevention. *National Diabetes Statistics Report website*, **(June 29 2022),** https://www.cdc.gov/diabetes/data/statistics-report/index.html.

[10] Shihori, Tanabe et al. Reactive Oxygen Species in the Adverse Outcome Pathway Framework: Toward Creation of Harmonized Consensus Key Events. *Frontiers in Toxicology* vol. 4, doi: 10.3389/ftox.2022.887135, **2022**

[11] Richard J. Allison, et al. Adipose tissue: Physiology to Metabolic Dysfunction. *National Library of Medicine*, **April 4 2020,** Adipose Tissue: Physiology to Metabolic Dysfunction - Endotext - NCBI Bookshelf (nih.gov)

[12] Dickson Alicia. How Oxidative Stress Affects Blood Sugar. *OptiHealth products*, **2023,** How Oxidative Stress Affects Blood Sugar – OptiHealth Products

[13] Lei Pang. Understanding Diabetic Neuropathy: Focus on Oxidative Stress. *Special Issue: Cellular and Molecular Mechanisms of Oxidative Stress in Nerve Injury, Hindawi*, **June 14 2020**. Understanding Diabetic Neuropathy: Focus on Oxidative Stress (hindawi.com)

[14] Moawad Heidi. Causes and Risk Factors of Diabetic Neuropathy. *Verywellhealth*, **August 20, 2022**, Diabetic Neuropathy: Causes and Risk Factors (verywellhealth.com).

[15] Eske Jamie. How Does Oxidative Stress Affect the Body? *MedicalNewsToday*, **April 3 2019,** What is oxidative stress? Effects on the body and how to reduce (medicalnewstoday.com)

[16] Feingold Kenneth. Dyslipidemia in Diabetes. *National Library of Medicine,* **August 10 2020,** Dyslipidemia in Diabetes - Endotext - NCBI Bookshelf (nih.gov)

[17] ABCB1 Gene Spotlight. *Genomind, The Science Behind Better*, **October 6 2021,** ABCB1 Gene Spotlight - Genomind.

[18] Zhu Huang, et al. Metabolic Reprogramming via Deletion of CISH in Human iPSC-Derived NK Cells Promotes In Vivo Persistence and Enhances Anti-tumor Activity. *National Library of Medicine,* **June 11 2020,** Metabolic Reprograming via Deletion of CISH in Human iPSC-Derived NK Cells Promotes In Vivo Persistence and Enhances Anti-tumor Activity - PubMed (nih.gov).

[19] DBP Gene- D-Box Binding PAR BZIP Transcription. *GeneCards, The Human Gene Database,* **July 2014,** DBP Gene - GeneCards | DBP Protein | DBP Antibody.

[20] Tian Miaomiao, et al. Functions of regulators of G protein signaling 16 in immunity, inflammation, and other diseases. *National Library of Medicine*, **September 2 2022,** Functions of regulators of G protein signaling 16 in immunity, inflammation, and other diseases - PMC (nih.gov).

[21] UPP2 Gene-Uridine Phosphorylase 2. *GeneCards, The Human Gene Database,* **April 2022,** UPP2 Gene - GeneCards | UPP2 Protein | UPP2 Antibody.

[22] Zhang Yumei, et al. Uridine Metabolism and Its Role in Glucose, Lipid, and Amino Acid Homeostasis, *Hindawi,* **April 15 2022,** Uridine Metabolism and Its Role in Glucose, Lipid, and Amino Acid Homeostasis (hindawi.com).