Research Reports from Undergraduate Students

Receiving Support from the Shackouls Honors College

Via the Honors Summer Undergraduate Fellowship Program

Summer 2016

Program Director:

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Name: Alexander, Audrey
Major: Psychology
Faculty Advisor: Kristina B. Hood, Ph.D.

Project Title: *Analyzing Connections of Personal Factors with Stressors and Coping Mechanisms*

The purpose of my research beginning in the summer of 2016 was to look at how college age peoples' personal factors like gender, race and socioeconomic status predict what specific stressors they have. These stressors could be things like school work, family problems, health issues, or trouble sleeping. The study also aims to explore how problem solving and emotion focused coping strategies are used to deal with stress.

Problem-solving coping strategies use tactics that work to resolve the stressful circumstance while emotion-focused coping strategies emphasize efforts to control the emotional consequences of the stressful circumstances (Taylor & Seeman, 1999). Personal factors' ability to predict specific coping strategies will also be explored. Other types of coping include adaptive and maladaptive. Adaptive coping is seen as positive actions taken to reduce or alter the stressful problem while maladaptive could increase the stress or further exacerbate the problem (Carver, Scheier, & Weintraub, 1989). Planning out how to best deal with the stressor is an example of adaptive coping while disengaging and essentially giving up on trying to reduce the stressor is a common maladaptive coping strategy (Carver, Scheier, & Weintraub, 1989). College-age students will be the focus of this study as research shows millennials have the highest levels of stress and are the most likely to report being lonely or isolated due to stress (American Psychological Association, 2014).

It is important to study the cause of stress for this age group and determine how they try to cope with the stress in order to have an increased knowledge on the subject. The better we can understand why millennials are the most stressed out generation and what might predict their stress and coping skills, the more we can improve University counselling centers to meet specific needs. The results of this study may shed light on new ways to educate college students on how to handle stress in a healthy manner or possibly cater coping mechanisms to college students with specific personal factors.

Participating in an Undergraduate Research Summer Fellowship allowed me to explore the topics of stress and coping on a deeper level and write a concept paper. During the fall semester I have begun the process of completing an IRB to run an online survey through SONA systems with the psychology research program. I have also used my concept paper to begin work on writing my honors thesis. I plan to get my survey online and start collecting data by the end of the fall semester in order to complete my thesis and defend it in the spring of 2017.

Name: Allred, Clayton
Major: Psychology
Faculty Advisor: Jared W. Keeley, Ph.D.

Project Title: *The Impact Social Withdrawal in Depression has on Social Functioning*

I was interested in the relationship of social withdrawal to depression, specifically whether social withdrawal predicted social functioning above and beyond the symptoms of depression and anhedonia, or loss of interest. In my review of the literature, I was unable to find a measure of social withdrawal, so I developed my own—the Social Withdrawal Scale (SWS). I compiled a survey which included measures of depression and social functioning, along with my measure of social withdrawal. Using funds I received from the Honors College, I conducted my study on Amazon MTurk and paid participants $0.75 to complete my survey. Using MTurk workers offers several advantages over using free participants, such as undergraduate psychology students. For example, college students are usually healthier overall and younger when compared to the general population. MTurk workers represent a much more diverse sample set. Five hundred one participants initially completed my survey, but 127 participants were denied credit due to inadequately completing the survey or missing too many validity questions. This deduction resulted in 374 participants whose results were analyzed.

Initial factor analysis showed that the SWS potentially measured two factors, but further analyses demonstrated that the SWS functioned best as a single factor measure. I conducted a hierarchical regression where I was able to see that the SWS added significantly to the prediction of social functioning measures when included in a model with a depression measure and a measure of loss of interest. Using Pearson's product-moment correlations, the SWS demonstrated good convergent and divergent validity with the other measures included in the survey. It also showed good convergent and divergent validity with the demographic variables. The results of my study indicate that social withdrawal is indeed a distinct construct that predicts social functioning above and beyond depression and anhedonia. Hopefully the SWS will be used in future studies as well as in clinical settings to better predict social functioning impaired by depression.

This experience of developing a study and carrying it through to completion would not have been possible without a summer research fellowship. I learned so much in regards to data collection and analysis, as well as how to articulate my findings in a concise and understandable way. My experience of completing my honors thesis will serve me well as I apply for graduate school because it is a demonstration of research potential. It also taught me much about statistics that will be useful in the future. I will attempt to publish my findings in an academic peer-reviewed journal. I will defend my honors thesis on November 11. I also plan on presenting at the Southeastern Psychological Association Annual Meeting, the National Conference on Undergraduate Research, and MSU's Undergraduate Research Symposium in the spring.

Name: Brotherton, Bailey

Faculty Advisor: Dr. Kristina Hood

Project Title: *Personality traits and sexual behaviors*

During the summer of 2016, I worked alongside Dr. Kristina Hood, formerly of Mississippi State's Psychology Department. My project's ultimate goal is to gain greater knowledge of personality traits associated antisocial personality disorder (APD) and how these traits interact with, or predict, risky sexual behaviors, including unsafe or unprotected sex and promiscuity. Ultimately, we hope that this information may be useful in better understanding the sexual actions of individuals with APD-like traits, and may be beneficial in figuring out how to protect such individuals from unsafe or risky sexual behaviors.

I spent the summer conducting an extensive literature review on previous studies, researching, finding, and inputting scales into Qualtrics that we will use to measure APD-like traits and risky sexual behaviors, and submitting our study to the Institutional Review Board (IRB). The purpose of the literature review was to better understand the gaps in the scientific literature that our study hoped to fill. For example, previous research has shown that APD-traits are predictive of risky sexual behaviors, but details on the particular sexual behaviors as well as what traits specifically may be driving these behaviors (e.g. impulsivity, compulsivity) has not yet come to light. After completion of the literature review, I pursued measures and scales that would be able to parse out the information I was looking for. This was a long and rigorous process, and I ultimately settled on a total of 18 scales. Finally, I knew my project could not move forward without the compliance of the IRB; therefore, the final month of my summer was spent applying to them and making edits as they saw fit.

As of early October, the IRB has approved my study, and we may move forward with the data collection process. Once completed, data will be cleaned, analyzed, and written up in the hopes of an eventual publication.

Thanks to the funding I received from the Honors Undergraduate Summer Research Fellowship, I was able to make multiple trips back and forth from home to Starkville in order to obtain materials. I was also able to find and make use of several scales that I would not have otherwise had access to. The results of my work this summer may one day be published in an academic journal, further enhancing the psychological community's knowledge of these pertinent and sensitive issues.

Name: Cato, Rachel
Faculty Advisor: Kristen Campbell, Ph.D.

Project Title: *Un-Social Media: Internet Addiction, Cyber-Pornography, and Social and Emotional Loneliness*

Introduction

Previous research has found that Internet pornography use is strongly correlated with social isolation, loneliness, relational regression, depression, anxiety, and impaired sexual and interpersonal relationships (Yoder, Virden, & Amin, 2005; Corely & Hook, 2012; Morgan, 2011; Philaretou, Mahfouz, & Allen, 2005). Depression is also a significant factor in the development of pathological Internet use (Young & Rogers, 1998), and Internet addicts typically experience shyness, depression, low self-esteem, and perceive difficulty in their interpersonal relationships (Yang & Tung, 2007). Therefore, the aim of the current study is to better understand the relationships between problematic Internet usage, consumption of cyber-pornography, feelings of social and emotional loneliness, and the presence of individual mood, obsession, and compulsion problems.

This will be accomplished through an online survey. Upon completion of the data collection, the primary investigator for will analyze the data for any significant relationships between the characteristics of interest. Based on a thorough review of the available literature on adolescents, Internet addiction, and cyber-pornography, there are three expectations for the current study. First, younger participants will spend more hours in any given activity online than older participants will. Second, participants whose Internet use pattern is detrimental—identified via scores on the Internet Related Problems Scale Revised (Boies, Cooper, & Osborne, 2004)—will feel lonelier; experience more stress, anxiety, and depression; perceive themselves as having less offline social support; and exhibit more obsessive-compulsive symptoms. Finally, participants who perceive themselves as addicted to cyber-pornography—identified via scores on the Cyber-Pornography Use Inventory-9 (Grubbs, Volk, Exline, & Pargament, 2015)—will feel

lonelier; perceive themselves as having less social support in all domains; experience more stress, anxiety, and depression; and exhibit more sexual compulsivity.

Over the summer, background research and a literature review was conducted, and, during the fall semester, IRB revisions were completed and approval was obtained. Currently, I—the primary investigator—am working to enter the survey items into Qualtrics. Upon completion of this task, the questionnaire, consisting of 219 items, will be posted on Amazon Mechanical Turk, until 400 participants have responded. Upon completion of the data collection, the data will be analyzed for statistical significance. These results will be important because the problems associated with problematic internet and cyber-pornography use can include decreased relational fulfillment, lower levels of intimacy, increased loneliness and low self-esteem, and mental health problems such as anxiety or depression —all of which may lead to lower overall life satisfaction. Understanding the correlation between certain online activities, individual characteristics, and the instance of these problems will allow psychologists to better predict who specifically is at risk for developing these detriments. Furthermore, better understanding the social and sexual implications of self-perceived problematic pornography use, as well as other online activities, will have huge implications for the mental health treatment of this technological generation.

Name: Crawford, Haily
Faculty Advisor: Dr. Jarrod Moss

Project Title: *Poor Strategy Selection by Students: Failure of Metacomprehension or Lack of Effort?*

Accomplishments of Summer 2016:

One of the most significant accomplishments of the summer is that we finalized the research methods and design. The experiment has four conditions and uses three strategies. The summary strategy requires participants to read the text and then summarize it in their own words, as if they were explaining it to a friend. The recopy strategy requires participants to retype the text passage word for word, exactly as it is. Our original plan was to have the participants recopy the entire text. For the sake of time, we decided that participants will only recopy two sentences from each paragraph. They will choose the two sentences that they think are most important to the text. The rereading strategy requires participants to simply read the text a second time. Each condition has three trials. In the first condition, participants use the summary strategy first, then the recopy condition, and then are given a choice between the two strategies. In the second condition, participants use the recopy strategy first, then the summary strategy, and then are given a choice between the two strategies. In the third condition, participants use the summary strategy first, then the rereading strategy, and then are given a choice between the two strategies. In the fourth condition, participants use the rereading strategy first, then the summary strategy, and then are given a choice between the two strategies. The four different conditions ensure that the conditions are counterbalanced. The first participant gets the summary-recopy condition, the second participant gets the recopy-summary condition, so on and so forth. This ensures that we get equal participation in each condition. We were not able to completely counterbalance the strategies that we chose. One is high effectiveness and high effort (summary strategy), one is low effectiveness and low effort (rereading strategy), and one is low effectiveness and high effort (recopy strategy). We are not using a strategy that is high effectiveness and low effort because, to our knowledge, such a strategy does not exist.

A second significant accomplishment from this summer was the creation of a program file. The program file was created in E-Prime 2.0. The first step in this process was learning how to use the E-Prime program. I started by creating a conceptual program in Microsoft PowerPoint. I did this so that I knew exactly what I wanted participants to see during the experiment and what information I wanted to collect from participants before trying to create the run file. The conceptual program allowed me to copy and paste what the participant sees into E-Prime so that I could focus on the "behind the scenes" part of the program. After I created the conceptual program, I had several meetings with my advisors. We went through the conceptual program together and addressed anything that needed to be altered or added. Finally, I created my program in E-Prime.

We were not able to collect data over the summer because our subject pool is not available during the summer. We get our participants from the Research Participation Program in the Psychology Department. Participants sign up for the experiment through a website called "SONA." Because the vast majority of students take General Psychology in the fall and spring, SONA participation is not offered over the summer.

Accomplishments of Fall 2016:

During the fall, we obtained IRB approval to run this study. This approval is a significant accomplishment because without this approval, we would not be allowed to collect any data. After getting IRB approval, we were able to obtain approval to post experiment sessions on the SONA website. At the point, I ran my pilot study. The purpose of the pilot study was to determine which texts would be best to use in the actual experiment. We ran a one way ANOVA to determine if any of the texts were significantly different from each other. The only significant difference that was found was that the average score on text one (42.47%) was lower than the average score on text four (61.56%), (p = .003). No other texts differed from each other. We chose texts three, six, and seven. These texts were chosen because they had the most similar means and confidence intervals. This means that performance on these three texts was very similar across participants. Therefore, the difficulty of the texts has been controlled, which will allow for comparisons to be made. This data can be seen in the table below. The text topics are DNA, plant growth, and viruses, respectively. After the texts has been chosen, I narrowed down the questions that would be used in the experiment. To make the program run smoothly, all texts needed to have the same number of questions. I eliminated two questions from the DNA text. The two questions were ones that the majority of participants got wrong. There were no such questions that needed to be eliminated from the other two texts. I then added simple questions to each text to serve as an attention check. The attention check questions got all texts up to ten questions. For example, in the DNA text the attention check questions is "Do humans have DNA?" The purpose of these questions is to make sure that participants are reading each question and answering carefully rather than mindlessly hitting enter all the way through the experiment.

The next step was to check the program for any bugs. "Bugs" includes things like unclear instructions, awkward wording, typos, or unexpected crashes. The undergraduate research assistants in the lab performed this task for us. They went through the experiment as if they were a participant and took detailed notes on a debugging sheet that I created for them. They were instructed to try to cheat their way through the experiment, such as by pressing random buttons over and over, as well as to catch any other unexpected occurrences.

After the debugging process, I made final changes to the program. At that point, we created a run schedule to decide which lab I would be running experimental sessions in and at what time. Data collection began on October 19th and is expected to conclude by the end of the semester.

Descriptive Statistics for PSS Text Selection

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
text1	37	.4247	.19486	.03203	.3597	.4897	.00	.79
text2	37	.5000	.17342	.02851	.4422	.5578	.14	.82
text3	36	.5472	.26212	.04369	.4585	.6359	.00	.90
text4	37	.6156	.20388	.03352	.5476	.6836	.22	1.00
text5	26	.4860	.26716	.05239	.3781	.5939	.00	.82
text6	28	.5404	.17731	.03351	.4716	.6091	.20	.80
text7	28	.5571	.21676	.04096	.4731	.6412	.10	1.00
Total	229	.5243	.22009	.01454	.4956	.5529	.00	1.00

Name: Davis, Margaret
Faculty Advisor: George E. Howell, Ph.D

Project Title: *Effects of exposure to persistent organic pollutants on hepatocyte glucose metabolism*

Background:

In 2012, 29.1 million people in the U.S. or 9.3% of the population were reported to have either diagnosed or undiagnosed diabetes mellitus [1]. The hallmark of diabetes is hyperglycemia which results, in part, from abnormal glucose metabolism given the liver plays a major role in maintaining blood glucose levels [2]. A significant positive association between diabetes and elevated serum concentrations of persistent organic pollutants (POPs), such as oxychlordane, trans-nonachlor, and DDE has been observed [3]. Recent studies show the strongest correlations between POPs exposures and diabetes existed for organochlorine (OC) pesticides or their metabolites, especially DDE and trans-nonachlor [4].

POPs are highly lipophilic, have long half-lives, and are environmentally widespread compounds. Epidemiological studies have explored this proposed association between POPs and type 2 diabetes (T2D), many of which explore an exposure to TCDD through contact with herbicide Agent Orange. Recent studies also suggest that a major class of POPs is composed of certain OC pesticides, which are positively associated with insulin resistance, diabetes, and metabolic syndrome [5]. As mentioned, previous studies show elevated serum concentrations of POPs, not obesity, promotes diabetes is positively associated with diabetes in human populations, and even low levels of POPs can lead to other metabolic disturbances [5]. However, *empirical studies delineating the mechanisms through which POPs exposure can promote metabolic dysfunction associated with T2D are lacking and are the focus of the present study*.

Lab Techniques:

Each week, primary hepatocytes were isolated from male Sprague Dawley rats. The student assisted in culturing these cells in twelve-well plates in one two main media: glucose uptake media or glucose production media. In each twelve-well plate, there were two wells including each of the following treatments:

1. Control
2. Vehicle (DMSO 0.025%)
3. Trans-nonachlor 2 μM
4. Trans-nonachlor 20 μM
5. DDE 2 μM
6. DDE 20 μM

Typically, there were two plates of cells for both glucose production and glucose uptake were cultured and measured each week. In addition to culturing cells in glucose production and glucose uptake medias, the student also assisted in running western blots to probe for antibodies, including Pepck, JNK, Actin, and p-JNK. The student helped make the blot by assisting with gel electrophoresis, membrane transfer, blocking, and antibody probing. This process was done for Rats 23, 24, 25, and 26. Each animal was probed for 6 different antibodies, including the ones previously listed.

Finally, the student worked to isolate total RNA in order to synthesize cDNA. The process was done using the same primary hepatocytes isolated for the glucose production and glucose uptake studies. The RNA was isolated from adherent cells and was done for Rats 27, 28, 31, and 32. cDNA was also synthesized from the isolated RNA from each of these animals. *These data are currently being analyzed and are therefore not included in the present report which is limited to the physiological assays governed by these molecular targets.* Throughout these studies, the student amassed a great deal of pipetting skill while culturing these cells, experience in experimental design, and data collection.

Results:

<u>DDE and trans-nonachlor are not cytotoxic at the concentrations tested</u>. Rat primary hepatocytes were exposed to DDE or trans-nonachlor (0-80 µM) for 24 hours then cellular viability was determined. There was little variation in cytotoxicity with increasing concentration of OC compounds (Figure 1). Therefore, the chosen concentrations of DDE and trans-nonachlor are not cytotoxic and potential observed effects will not be due to cytotoxicity.

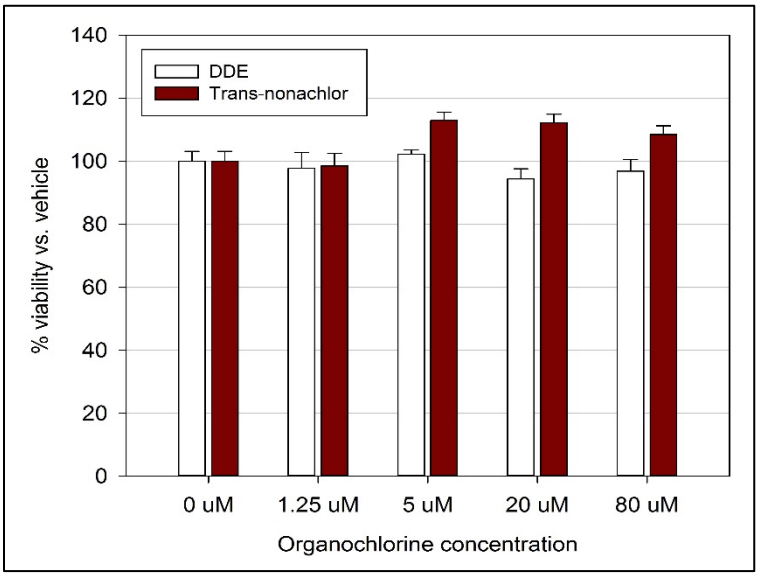

Figure 1: Exposure to DDE or trans-nonachlor was not cytotoxic. Data represent the mean ± SEM of n=5-6 animals per group.

<u>Exposure to DDE or trans-nonachlor decrease hepatocyte glucose production.</u> Glucose production assays were done with vehicle (0.025% DMSO), 2 µM or 20 µM of DDE or trans-nonachlor (Trans). Primary hepatocytes were incubated in normal growth media followed by glucose production media plus either vehicle or an organochlorine compound (DDE or Trans) for a total of 24 hours. In order to determine glucose production, the media was taken off of the cells and analyzed using Amplex Red Glucose Reagent (Life Technologies). The glucose production was compared between vehicle (0.025% DMSO), 2 µM and 20 µM of both DDE and trans-nonachlor

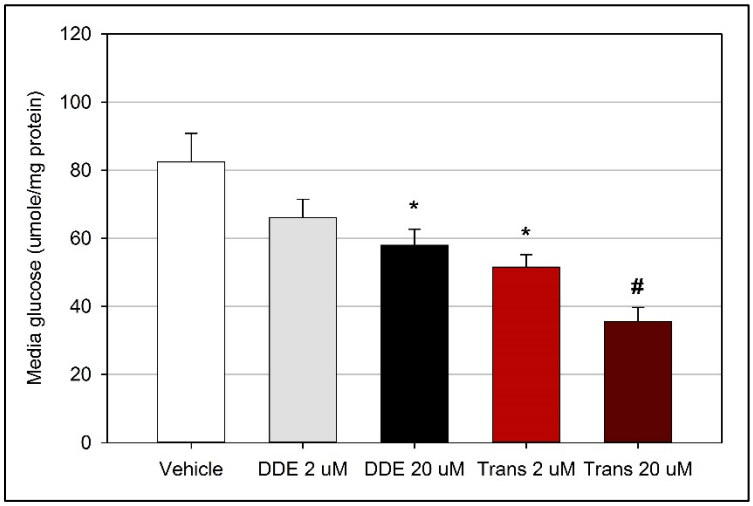

Figure 2: Exposure to both DDE and trans-nonachlor decrease hepatocyte glucose production in rat hepatocytes. Data represent the mean ± SEM of n=7 animals per group. *P<0.05 vs. vehicle; #P<0.01 vs. vehicle.

(Figure 2). Exposure to the highest concentration of DDE (20 µM) significantly decreased hepatocyte glucose production compared to vehicle. Whereas both concentrations of trans-nonachlor (2 and 20 µM) significantly decreased hepatocyte glucose production in a concentration-dependent manner.

Exposure to DDE or trans-nonachlor did not alter hepatocyte glucose uptake. Glucose uptake assays were done with vehicle (0.025% DMSO) and 2 µM and 20 µM of both DDE and trans-nonachlor (Trans). Primary hepatocytes were treated with organochlorine compounds for 16 hours in a low glucose (5 mM) media. Then treated with glucose uptake media which included 6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG), plus either vehicle or organochlorine compound for 30 minutes. In order to determine glucose uptake, cells were lysed to assess intracellular 6-NBDG (ex. 485/em. 528) and measured using a fluorescent plate reader (Figure 3). The glucose uptake was compared between vehicle (0.025% DMSO), 2 µM, and 20 µM of both DDE and trans-nonachlor. Exposure to DDE or trans-nonachlor did not significantly affect hepatocyte glucose uptake when compared to vehicle treated cells.

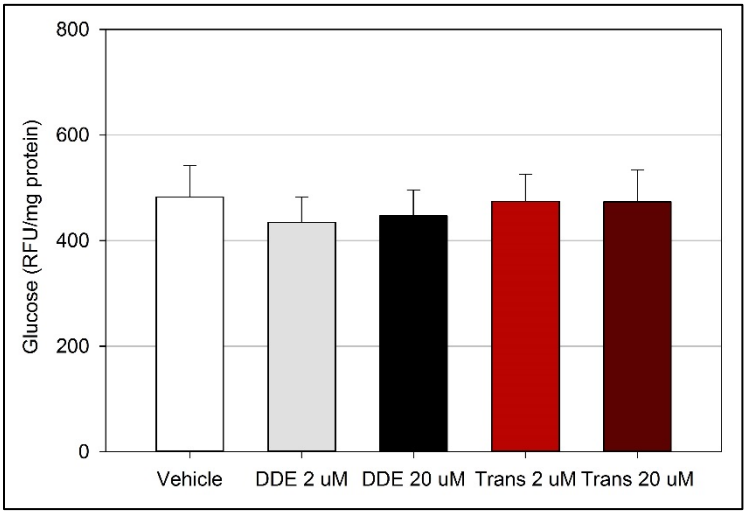

Figure 3: Exposure to DDE or trans-nonachlor did not significantly alter hepatocyte glucose uptake. Data represent the mean ± SEM of n=7 animals per group.

Hepatocyte glycogen content is unaltered following exposure to DDE or trans-nonachlor. Glucose is taken up into the hepatocyte and either goes through glycolysis and the TCA cycle or is stored as glycogen. Glycogenolysis is the breakdown of glycogen into usable glucose. If hepatocyte glycogen content is altered by organochlorine content, a decrease in glycogenolysis is possible, which could potentially decrease glucose production. Therefore, hepatocyte glycogen content in the presence of trans-nonachlor was measured to determine whether the organochlorine has an effect on glycogen storage rather than affecting glucose production in another way (Figure 4).

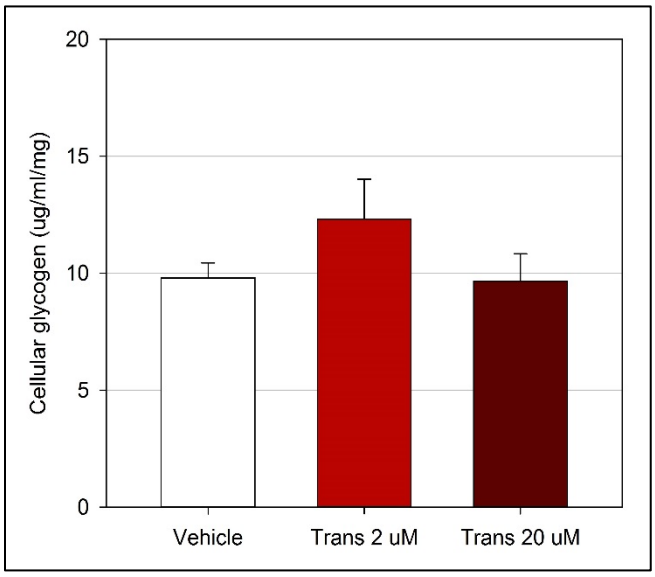

Figure 4: Exposure to DDE or trans-nonachlor did not significantly alter hepatocyte glycogen content. Data represent the mean ± SEM of n=3 animals per group.

Exposure to trans-nonachlor (2 or 20 μM) does alter cellular glycogen content, and implicates alteration of glucose production through another means such as gluconeogenesis.

Summary:

Hepatic glucose metabolism plays a pivotal role in maintaining blood glucose levels. This buffering capacity of the liver is affected by both hepatic glucose uptake and production. In past studies, an emphasis has been placed on risk factors such as lifestyle, age, weight, and genetic predisposition. However, these risk factors do not sufficiently explain continuous increase in the prevalence of T2D. Therefore, environmental exposures have been explored recently as a potential risk factor. In this study, the effects of exposure to the organochlorines DDE and trans-nonachlor on hepatocyte glucose metabolism were explored. Based on the presented data, direct exposure to both DDE and trans-nonachlor decrease glucose production but did not significantly affect glucose uptake in primary hepatocytes. Interestingly, these data indicate exposure to these compounds may promote hypoglycemia and not hyperglycemia as observed in T2D. Therefore, more in-depth studies are warranted to delineate the actions of these organochlorine compounds on hepatic and systemic glucose metabolism.

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Name: Ferguson, Lucas

Major: Biochemistry and Molecular Biology

Faculty Advisor: Dr. Henry Xiu-Feng Wan

Project Title: *Interferon-Induce GTP-Binding Protein Mx1 Diversity in Avian Species of China*

Introduction:

Influenza A virus (IAV) is a segmented, negative sense single-stranded RNA virus which belongs to the *Orthomyxoviridae* family, and is found worldwide infecting a wide range of species included avian, human, swine, equine, canine, and sea mammals. IAV can cause seasonal outbreaks globally and are closely tracked by international organizations to evaluate the evolution of and prepare vaccines against these outbreaks. Due to IAV's evolving antigenicity resulting from mutations (antigenic drift) and reassortments (antigenic shift), continuous and comprehensive surveillance and study are imperative to combating IAV.

In China, avian influenza virus (AIV) outbreaks are of particular concerns due to the emergence of highly pathogenic H5N1 and low pathogenic H7N9 and H9N2 AIV strains in domestic poultries and the circulation of these viruses, as well as other subtypes, in the live-bird markets. The H5N1, H7N9, and H9N2 viruses can cause significant respiratory morbidity and mortality in humans, posing a threat to public health. Although waterfowl species serve as a natural reservoir of AIVs, there is a sizable diversity of domestic poultry species in China. Limited knowledge exists regarding which species could act as the primary host or hosts maintaining influenza virus circulation in the live bird markets, making further study difficult.

Previous study has demonstrated that Mx1 gene in birds codes an interferon-induced GTP-binding protein that can lead to influenza-specific antiviral state. The goal of this project is to evaluate the single nucleotide polymorphisms (SNP) of the Mx1 genes among various avian species found in live bird markets in China. We hypothesize that some variants of this gene in certain species may not adequately initiate an antiviral response, resulting in bird species which may shed virus for longer periods of time during infection giving the virus the opportunity to spread for a longer period of time.

Methodology:

Sample Collection: Blood was collected from poultry in China (Table 1). Peripheral mononuclear blood cells (PMBC) were isolated from blood samples using Ficoll-Paque (GE Healthcare Life Sciences, Marlborough, MA). Cells were counted and diluted in Roswell Park Memorial Institute (RPMI) medium (Gibco® Life Technologies, Grand Island, NY) with 10% fetal bovine serum (Gibco® Life Technologies) and 1% PenStrep (Gibco® Life Technologies) . 1mL of $2x10^6$ cells/mL suspended in supplemented RPMI media were aliquoted into two separate wells of a 24-well plate. One of these two wells was challenged with 1μg/mL lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO) prior to incubation for 24 hours (Table 1).

RNA Purification and Sequencing Preparation: mRNA from these cells was exctracted using TRIzol® LS Reagent (Thermo Fisher Scientific, Waltham, MA) using the manufacturer's protocol. Endogenous DNA was denatured with RQ1 RNase-free DNase (Promega Corp, Madison, WI) using the manufacturers protocol. Complimentary DNA (cDNA) of the mRNA were synthesized using Superscript III Reverse Transcriptase (Thermo Fisher Scientific) supplemented with RNAse OUT (Thermo Fisher Scientific). Primers were designed based on the limited sequences available on the NCBI GenBank Database (Table 2)

Results and Discussion:

Mx1 transcripts from this study have not been sequenced at this time. Once completed, it will be possible to design a realtime PCR probe which can be used to quantify the amount of Mx1 mRNA transcription.

The antiviral activity of Mx1 during IAV infections works by inhibiting the interaction between IAV polymerase basic 2 (PB2) and nucleoprotein (NP) proteins to prevent their assembly with polymerase acid (PA) and polymerase basic 1 (PB1) proteins to form the ribonucleoprotein complex (RNP)[1]. By inhibiting assembly of the RNP complex, Mx1 reduced viral progeny by blocking the assembly of the viral replication machinery[1]. Moreover, it has been shown that the murine Mx1 protein varied in sensitivity to various influenza strain's NP proteins, suggesting Mx1's interaction with NP is strain specific and important to initiation of the influenza-specific antiviral response[1].

Previous studies have shown mutations to chicken Mx1 gene can affect host antiviral response. Using mouse 3T3 cells, *in vitro* study noted that S631N (G2,032A) mutation of the Mx1 gene resulted in an increase in antiviral activity[2,3]. Two further *in vitro* study using chicken embryonic fibroblasts found neither the S631 nor the N631 isoform led to a significant interferon-mediated resistance to IAV infection, nor did the mutations have an effect on IAV replication[4,5]. Regardless, Chinese chicken breeds more often have the S631N mutation compared to commercial chicken breeds, suggesting the mutation has some purpose among Chinese chicken breeds[6]. More recent investigations have shown that homozygous S631 chicken embryo fibroblast Mx1 mRNA expression levels were higher compared to the heterozygous S631 chicken embryo fibroblast Mx1 mRNA expression levels[7]. However, the mRNA expression level was significantly higher for S631/N631 heterozygous chicken embryo fibroblast cells[7].

Future Work:

Samples which were challenged with LPS recovered from this study will be sequenced using Sanger sequencing. Multiple sequence alignment will be performed on recovered sequences, and SNPs will be recorded. In order to observe the evolutionary relationship between the recovered sequence, phylogenic analysis will be performed using PAUP*[8]. Using the recovered sequences, a realtime PCR primer and probe set will be designed. With this, quantitative PCR can be performed in order to compare Mx1 transcription levels across multiple samples.

Based on the findings, an *in vitro* study of various SNPs will be conducted using cells transfected with cloned plasmids containing the cDNA transcript of the recovered Mx1 mRNA sequences. Transfected cells will be challenged with IAV in order to evaluate the antiviral activity of the Mx1 transcripts on viral replication. This study could shed light on the likely poultry host maintaining avian influenza circulation in live bird markets, and thus help develop an effective strategy for avian influenza prevention and control.

Table 1: Summary of domestic poultry species sampled in China.

Common Name	Latin Name	Replicates
Jianghan White Goose	*Anser Anseritormes Anatidae*	3
Mallard Duck	*Anas platyrhynchos*	2
Hyline Variety Brown	*Gallus gallitormes phasianidae*	1
Xueshan indigenous chicken black	*	3
Xueshan indigenous chicken	*	2
xueshan partridge chicken	*	3
Black Chicken	*	2
Barred Chicken	*	1
White Leghorn Chicken	*	3

Notes: *Latin names do not exist for these breeds, however, they are recognized as *Gallus gallus domesticus*

Table 2: Primers designed for sequencing.

Primer Name	Sample	Primer Sequence	Direction	Start Site	Tm	Expected Sequence Coverage	Purpose
Chk_F_1	Chicken	ATGGGCCTAAGTTCGAAAAT	FWD	1	53.2	NA	Amplification
Chk_F_566	Chicken	TGGGACATTGACGTGAAGCA	FWD	566	59.89	600 to 1500	Sequence
Chk_R_585	Chicken	TGCTTCACGTCAATGTCCCA	REV	585	59.89	540 to 0	Sequence
Chk_R_771	Chicken	TGCTCAGGCCAGAATTGGTT	REV	771	59.89	700 to 0	Sequence
Chk_F_1034	Chicken	ATGTCCGAAACTCTCTGCGG	FWD	1034	60.11	1100 to 2000	Sequence
Chk_F_1375	Chicken	CTCTCTTGCTGGATTGCGGA	FWD	1375	60.11	1400 to 2300	Sequence
Chk_R_1405	Chicken	TGTCCTTCACCTCCGCAATC	REV	1405	60.04	1350 to 450	Sequence
Chk_F_1561	Chicken	GTGGGATCCACCTCTTGAGC	FWD	1561	60.11	1600 to 2500	Sequence
Chk_F_2373	Chicken	AGCTGAGCTGAAGTTGGACC	FWD	2373	59.96	2400 to 2545	Sequence
Chk_R_2490	Chicken	CAAGAGTGGTCGGTGTCGAT	REV	2490	59.76	2400 to 1500	Sequence
Chk_R_2545	Chicken	CGCCGACTTACATCAATTAA	REV	2545	53.2	NA	Amplification
Duk_F_1	Duck	ATGATGCACAGTAGACAAAG	FWD	1	53.2	NA	Amplification
Duk_R_335	Duck	CCGAGTTAATCGGCTCAGCA	REV	335	60.18	300 to 0	Sequence
Duk_F_440	Duck	GTCGCCGAAGTCATGAAGGA	FWD	440	60.11	500 to 1400	Sequence
Duk_F_1159	Duck	CTATTGTGGGTGTGCCTCGT	FWD	1159	60.04	1200 to 2200	Sequence
Duk_R_1179	Duck	GACGAGGCACACCCACAATA	REV	1179	60.04	1000 to 100	Sequence
Duk_F_1999	Duck	CGAGGATGGAGCTTTTCCCA	FWD	1999	59.75	2100 to 2503	Sequence
Duk_R_2116	Duck	ATCCGGCCTTGCATTGATCT	REV	2116	59.82	2000 to 1100	Sequence
Duk_R_2119	Duck	AAGATCCGGCCTTGCATTGA	REV	2119	60.03	2000 to 1100	Sequence
Duk_R_2503	Duck	TTGCTGCCAGACAGGAAC	REV	2503	56	NA	Amplification
Goo_F_1	Goose	CTACAGACAGCTAAAGGTCTTG	FWD	1	58.4	NA	Amplification
Goo_R_909	Goose	AAGTACAGACGAGGCACACC	REV	909	59.68	850 to 0	Sequence
Goo_F_881	Goose	TCTATTGTGGGTGTGCCTCG	FWD	881	59.75	900 to 1800	Sequence
Goo_F_1421	Goose	CTCACAGCCACTCTGGCAAT	FWD	1421	60.32	1500 to 2112	Sequence
Goo_R_1539	Goose	GTTGTGGCTGGTACAAAGCG	REV	1539	60.04	1500 to 400	Sequence
Goo_R_1702	Goose	TCTTCCTAGGGGCAACGGTA	REV	1702	59.96	1650 to 700	Sequence
Goo_R_1904	Goose	TGCACGAAGGAGAACAGGAC	REV	1904	59.97	1850 to 1000	Sequence
Goo_R_2112	Goose	ATGTACCACAGAAGTCCCAAG	REV	2112	57.9	NA	Amplification

Notes: Primers were designed based on NCBI GenBank sequences for geese, ducks, and chickens.

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Name: Gruich, Cameron

Faculty Advisor: Dr. Veera Gnaneswar Gude

Project Title: *A Kinetic and Thermodynamic Study of Microwave/Ultrasound-assisted Transesterification of Rapeseed Oil for Biodiesel Production*

Objective: Determine common thermodynamic and kinetic data for biodiesel production via microwave and ultrasound based irradiation. Specifically, calculate reaction rate constants, Gibbs Free Energy change, entropy change, enthalpy change, activation energy, and the pre-exponential factor.

Research Progress: The bulk of the experimental setup was designed over the summer to undergo this study. Pure canola oil was used as a feedstock. A high methanol-to-oil molar ratio was used to shift the equilibrium reaction towards the products side. 200 watts and 10 mL biodiesel samples were used because it was found that 20 watts/mL is an effective power density for high yields. Sodium hydroxide was used as a catalyst in a ratio of 0.5% of the oil's weight per sample. The reaction vessel shape stayed constant. All of these conditions were chosen by judging results from previous research articles. However, the methods for recording biodiesel yield over multiple samples proved to be a creative challenge.

Biodiesel samples were made over 30 second intervals and up to 2 minutes for microwave, ultrasound, and microwave/ultrasound irradiation. Reactions were done under 35 degrees Celsius, but the planned 45 degrees and 55 degrees Celsius tests were postponed. It is very important to retain the product to get an accurate yield reading, especially since the sample volume is small. The first yield method was a direct yield by extracting the distinct biodiesel phase in the sample and weighing it. This weight was compared to the ideal stoichiometric amount of biodiesel. However, it was found that this ratio was much higher than 100% yield, and further miscibility investigations found that the biodiesel phase contained oil and small amounts of methanol. Likewise, glycerol is a product of the reaction that forms a distinct phase below the biodiesel phase, and it was found that this phase has large amounts of methanol, soap, and trace amounts of biodiesel. These observations led to an interesting learning exercise. Because distillation is not very energy efficient and gas chromatography was not readily available for so many samples, a glycerol purification and back-calculation of the yield was proposed to get accurate yields.

The approach of this glycerol method and the problem of refining it for accurate scientific results is the current state of this research project moving forward. The current process is made up of a centrifugation stage and an evaporation stage. A small amount of castor oil is added to the samples before centrifugation to separate product phases and also catch nonpolar impurities. After the first centrifugation, castor oil is removed and solid soap byproduct is filtered out with a mechanical filter. This process is repeated again, and then the samples are put

in the oven for 45 hours. Glycerol is then weighed from the evaporated samples and compared to the stoichiometric glycerol amount to produce a yield.

While this method has many promising qualities about it such as glycerol's high boiling point and consequent high retention, there are numerous challenges. Namely, the optimum centrifugation and evaporation times need to be determined. A liquid better to handle than castor oil could be used. Additionally, liquid sticking to the mechanical filter is also a problem. The mathematics to determine the kinetic and thermodynamic properties are already planned out, but much of the broad physical procedure for calculating yield has to be refined to have the yields be scientifically rigorous.

Name: Hargrove, Shaquela
Major: Psychology
Faculty Advisor: Kristina B. Hood, Ph. D

Project Title: *Acceptance of Interracial/Interethnic & LGB Couples*

Marginalized couples are couples that are deemed different from a socialized "norm al" standard (Lehmiller & Agnew, 2006). Marginalized couples may include lesbian, gay, or bisexual (LGB) relationships, interracial/interethnic couples, and age gap couples. Currently, the general U S's acceptance is around 87% for interracial relationships (Newport, 2013) and 55% for same-sex couples (Changing Attitudes on Gay Marriage, 2015). The current study focused on interracial/interethnic and LGB couples due to the increase in these types of relationships over the past few decades--6.3% of all marriages were interracial in 2013 (Wang 2015) and less than 1% in 2012 (Schwarz, 2014).

Much previous literature has explored acceptance of both interracial/interethnic couples. First, previous research suggests that most people who accept interracial/interethnic relationships are male (Yancey, 2002; Perry, 2013c), non-White (Perry, 2013c), more politically liberal (Eastwick, 2009; Yancey, 2002; Perry, 2013c; Johnson, 2005), and highly educated (Johnson, 2005). Religiosity is also a significant factor in acceptance of interracial/interethnic couples, such that the less religious a person is, the more likely they are to approve of interracial/interethnic dating (Sasser, 2015). When it comes to racial composition of couples, those that were White/Asian were approved of far greater than those that were White/Black couples (Field, 2013).

As for LGB couples, past literature demonstrates that women (Perry, 2013b; Horn, 2007), non-White (Perry, 2013d), non-Republicans (Perry, 2013b; Horn, 2007), and highly educated individuals (Perry, 2013d) were more accepting of LGB couples. Religiosity, as with acceptance of interracial/interethnic couples, affected acceptance of LGB couples significantly, such that the more religious a person is, the more likely they are to have negative attitudes towards homosexuality (Jäckle & Wenzelburger, 2015). Acceptance is at an all-time high for LGB couples (currently around 55%; Changing Attitudes on Gay Marriage, 2016). However, prejudice still exists, particularly toward bisexual individuals since their sexuality is sometimes viewed as illegitimate or a "phase" (Boyer & Galupo, 2015). One study found that bisexual individuals were viewed more negatively than Lesbians and Gay males (Eliason, 1997). Highly religious and more politically conservative individuals are more likely to oppose both LGB marriage and interracial marriage (Haider-Markel, 2005).

There has been little research on how the acceptance of one type of marginalized relationship can predict the acceptance of another. The theory is that the acceptance of one could predict the acceptance of the other due to the fact that people who oppose interracial couples have similar characteristics of those who oppose LGB couples. This is important because perceived social disapproval and opposition to marginalized relationships have been associated with less overall relationship commitment, less satisfaction, and stress (Lehmiller, 2006). The current study seeks to confirm which personal factors are correlated with the acceptance of both interracial couples and LGB couples. Additionally, a primary goal was to determine whether the acceptance of one type of couple can predict the acceptance of the other. Further, the study aimed to find whether composition of couples affected acceptance of them, such that will race, sex, and sexual orientation play a role in the acceptance of one couple over the rest. For example,

will a black female/white male relationship be more accepted if they are both heterosexual versus a black female/white male couple where at least one is bisexual.

During the Fall 2015 semester, I designed the study in conjunction with Dr. Hood, submitted my IRB for approval, and began collecting data the following semester (Spring 2016). The study recruited almost 300 undergraduate students enrolled in psychology courses via SONA-System, a department-run website where students can sign up for studies and keep track of their earned credits. In exchange for participating in the study, subjects received 1 credit hour that could be used towards one of their classes. After collecting data and receiving participant feedback, it was determined that 1 credit hour was not enough, as people were taking 1.5 hours on average to complete the survey. During Summer 2016, I updated my IRB application to change the credit hour from 1 to 1.5 as well as other items that needed to be addressed in my updated IRB. I plan to collect more data before the current semester ends (Fall 2016). If I am unable to, I will collect more data at the beginning of the Spring 2017 semester.

With the data that was collected and cleaned I did begin to discover that attitudes towards one type of marginalized relationship does significantly predict acceptance of the other, even with several factors controlled for. I will be presenting these preliminary findings at the 2016 Society of Southeastern Social Psychologists (SSSP) conference in November and the Spring 2017 Undergraduate Research Symposium. I am also currently working on configuring my Honors Thesis paper so that I can defend in Spring 2017.

I feel certain that this information is vital because of the negative effects of social discrimination and disapproval of interracial/interethnic and LGB couples. There are still a great number of individuals who oppose or discriminate against these couples, so finding out what factors are associated with the acceptance of these marginalized couples could lead to educational programs tailored to a specific group to educated them on the harms prejudice can cause.

Name: Herz, David

Major: Biological Engineering

Faculty Advisor: Dr. Mark Welch

Project Title*:* *Investigations into the hybridization of Grand Cayman Blue Iguana (Cyclura lewisi) founders.*

The Grand Cayman Blue Iguana, *Cyclura lewisi*, is an IUCN-listed endangered species endemic to the island of Grand Cayman[i]. Prior to the early 2000's, *Cyclura lewisi* was a critically endangered species, with only about 25 wild individuals in existence.[i] One of the largest threats to iguanas and other fauna on Grand Cayman is the impact of humans, causing great stress on many of the island's local populations. Predation by feral dogs and cats, along with road traffic, have become major threats to *Cyclura lewisi* in the past few decades.[ii] Dealing with these problems has taxed conservationists in the Caymans attempting to prevent extinction of several species on the islands. However, thanks to the recent conservation efforts of the Blue Iguana Recovery Program (BIRP), the population has rebounded to almost 500 mature individuals.[i] Directed by F. J. Burton, BIRP has been the leader in Iguana conservation efforts in the Caymans, and is entirely responsible for the Blue Iguana captive breeding program. This captive breeding program deposits young adult iguanas into the three protected areas of Grand Cayman, consisting of the QE II Botanic Park, Salina Reserve and Colliers Wilderness Reserve.[iii] Now that *Cyclura lewisi* has been downlisted from its former critically endangered status, restoring a viable wild population of these iguanas will depend on closer attention to factors such as genetic diversity.

Hybridization poses a major threat to the genetic distinctness of rare species, in some cases leading to hybrid swarms and local extinctions. For example, the Pecos Pupfish (Cyprinodon pecosensis) was, prior to the early 2000's, a critically endangered species due to its hybridization with the Sheepshead Minnow.[iV] Without conservation efforts, it is likely that hybrids between these two species would have caused the Pecos Pupfish to become extinct, due to the hybrids' increased rate of survivorship compared to the Pupfish.[v] Due to a lack of source information from the early years of the breeding program, there is reason to suspect that a portion of BIRP's founders were in fact hybrids with the Cuban Iguana (*Cyclura nubila nubila*) or Sister Isles Rock Iguana (*Cyclura nubila caymanensis*). The purpose of this research is to assess the severity of hybridization within the local *Cyclura lewisi* breeding population, so that steps may be taken to minimize any loss of genetic distinctness within the species.

Methods

To achieve the objectives of this project, we will genotype animals at 25 microsatellite loci, and analyze haplotypes from mtDNA sequencing to assess hybridity. DNA will extracted from Blood samples taken from 70 putative *C. lewisi* in the Blue Iguana Recovery Program using a Maxwell® Blood DNA Purification System. PCR amplification of microsatellites will be performed in a 10 µl volume containing 1 µl extracted DNA using standard protocols[Vi]. PCR products will be sent to Arizona State University for fragment analysis and mtDNA sequencing. To score the microsatellite genotypes, the software Peak Scanner™ v1.0 will be used. Mitochondrial sequence variation also obtained from sequencing analysis will be analyzed to

determine the unique haplotypes within the *Cyclura lewisi* samples, so that they might be compared to that of other species within genus *Cyclura* to determine possible hybridization.

Should the mitochondrial sequence data indicate that some individuals within the *Cyclura lewisi* population share or have a very similar haplotype to that of *nubila* or *caymanensis*, there may be good reason to believe that hybrid individuals existed within the founder population of BIRP's captive breeding program. Should there be any question as to whether an individual is a hybrid, microsatellite data can be used as a deciding factor. Because there is a fairly representative sampling performed in this project, the severity of hybridization within *Cyclura lewisi* may be assessed, and the resulting information sent to BIRP for consideration.

Summer Research Completed

Thus far, all conditions have been optimized for each microsatellite locus, and all microsatellite loci have been screened and sent to Arizona State University for fragment analysis. After receiving results back from Arizona State, the majority of samples have been genotyped and archived for future use. A portion of mitochondrial sequence data has been received from Arizona State, and haplotype analysis has begun. As mtDNA sequencing progresses, we will start getting a better idea of how hybridized these founder individuals are.

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Name: Hunt, John
Major: Biomedical Engineering
Faculty Advisor: Dr. Nicholas Fitzkee

Project Title: *Chemical Shift Assignment of the Regulatory Domain of Calcineurin Using NMR Spectroscopy*

Abstract

Calcineurin (CaN) is a serine/threonine phosphatase that is universally expressed in eukaryotic cells. In humans, CaN participates in cardiovascular and central nervous system development, and is known for its role in T-cell activation. Because of its signaling activity, regulatory problems that occur with CaN have been associated with several human diseases, including Alzheimer's disease and cardiac hypertrophy. CaN is activated by increased levels of intracellular calcium ions (Ca^{2+}). These ions bind to a regulator protein, Calmodulin (CaM), which activates CaN by binding to CaN's calmodulin binding region (CaMBR). The binding site is located within the regulatory domain (RD) of CaN. The RD is intrinsically disordered until Ca^{2+}-bound CaN binds, whereupon the RD folds and releases the previously bound autoinhibitory domain (AID). Our research is centered on understanding the molecular interactions that facilitate binding of CaN to the RD. To accomplish this, we used 2D and 3D NMR techniques to examine the structural determinants of binding and completed the protein's backbone and sidechain assignments in the solution phase. Our protein production methods included expressing protein from transformed *E. coli* and purifying the cell lysate on a Ni-NTA column followed by a CaM-Sepharose column. Purity was affirmed using SDS-PAGE and LC-MS.

Research Methods and Instrumentation

Our proteins are produced by transforming competent *E. coli* cells and growing them in several liters of minimal (M9) media. This process yields approximately 4.284 mg of pure protein per liter of media. The protein is purified using Fast Protein Liquid Chromatography (FPLC), a type of affinity chromatography. The FPLC works by passing the impure solution containing the desired protein through a column containing a matrix (Calmodulin-Sepharose) that will selectively bind that protein. The rest of the impurities are washed though, and a chelating agent is passed through the column. This agent (we use EGTA) will strip the Ca^{+2} ions from the calmodulin, causing the RD to transform back to its unbound state and fall from the column into solution. The elution can then be concentrated if necessary and its identity verified using mass spectrometry (LCMS).

This purification procedure is crucial for establishing well-controlled measurements of binding. Any impurities present in the sample will complicate accurate measurements of protein concentrations, and they could also potentially introduce side reactions that interfere with measurement of binding. This is particularly true of small fragments: For example, an impurity that is similar in structure to the regulatory domain may compete with the actual regulator domain, altering the observed equilibrium. After production of our proteins is complete, the solution's purity can be assessed using polyacrylamide gel electrophoresis.

Extensions and Project Continuation

The manuscript of the current extent of this project, to be published in a scientific journal, is currently being written. CaN's backbone and sidechain protein NMR peak assignment is complete for the protein in the solution phase. Future directions could involve altering the solution's composition to mimic more closely the intracellular environment where human calcineurin functions. Many proteins in the human body that are easier to work with than CaN have been studied in the solution phase, yet their behavior inside the actual cell remains ambiguous. This is currently a frontline area of biophysical research. There are also different instruments available with which to explore the CaN-CaM binding equilibrium. Isothermal Titration Calorimetry (ITC), for instance, is a method often used to study the binding of small molecules (such as medicines or smaller proteins) to macromolecules (such as large proteins). Utilizing this instrument could generate very precise measurements of binding affinity and enthalpy changes that occur as calcium-loaded calmodulin binds calcineurin.

Name: Jackson, Anna
Faculty Advisor: Dr. Mark Welch

Project Title: *An Analysis of the effect of Anthropogenic Stresses on Mating Behaviors Displayed by Sister Isles Rock Iguanas*

Background

Within the scientific community, it has long been understood that mating between closely related individuals poses a genetic risk in the concentration of rare and possibly deleterious alleles (Wright 1977, Ralls and Ballou 1986, Shields 1987, Cronkrak and Roff 1999). A reduction in reproductive fitness due to inbreeding and increased homozygosity can lead to stark declines in population numbers of what may be an already suffering species. This evolutionary pressure is particularly severe in small populations (<1,000 individuals) (Lynch *et al*. 1995). Significant loss of genes within a population's respective gene pool tends to result in marked reduction of genetic variability and thus inbreeding depression may ensue. For some small populations, inbreeding depression may be so inevitable that the selective pressure may play only a minute role in fitness (Shields 1993). In accordance with this finding, newly emerging studies regarding the inbreeding avoidance behaviors displayed by animals within small, isolated island populations have begun to arise. Inbreeding avoidance may represent a unique reproductive strategy that influences animal mating behavior.

The Sister Islands Rock Iguanas (SIRI) *Cyclura nubila caymanensis* is a critically endangered iguana subspecies endemic to the Cayman Islands, specifically Cayman Brac and Little Cayman. It is estimated that fewer than 900 reproductively viable individuals currently persist between the two islands. Drops in population numbers are largely attributable to habitat degradation, commercial development, road traffic, and predation by feral mammals (Goetz, 2010; Goetz & Burton. 2012). Though featuring the most robust and widely dispersed population of SIRI, Little Cayman is subject to increasing human development (Gerber 2000, Goetz 2010, Goetz and Burton 2012). Further reductions in population size due to anthropogenic stressors elevates the risk of inbreeding and thus facilitates possible introduction of harmful alleles. As consequences of inbreeding tend to arise exclusively in small, isolated populations (Szulkin, Bierne, and David 2010), SIRI serves as an ideal candidate for further studies of these reproductive dynamics.

While natural selection represents an influential force in the purging of unfavorable alleles in nature, past research has suggested that animals within historically small and isolated populations have evolved mechanisms to promote outbreeding and preserve genetic diversity. The iguana's ability to store sperm, resulting in polyandry, increases the probability that offspring will receive at least a few favorable genes. This strategy may arise when females cannot distinguish between closely related iguanas (Petrie et al. 1992). We hypothesize that the pressures of inbreeding influence reproductive behaviors displayed by female SIRI, thus leading to multiple and non-random mating. We further hypothesize that living in close association with humans may disrupt the iguanas' normal behaviors, resulting in some isolated subpopulations displaying reduced rates of polyandry.

Methods/Progress

For the ongoing investigation of mating behavior and reproductive dynamics of SIRI, blood samples from adult and hatchling iguanas captured on Little Cayman during a field excursion summer of 2016 have been and continue to be utilized. The genetic data derived from these new samples will be added to a growing SIRI genotype database including samples collected from Little Cayman in 2015.

Funding provided through the Shackouls Honors College summer fellowship assisted in the travel expenses required for transportation to and from Little Cayman.

Marking the end of nesting and incubation periods, hatchling emergence began in early August 2016. In order to effectively corral and capture hatchlings after emergence, enclosures constructed with aluminum flashing and wooden stakes were erected around a total of 23 egg chambers. Special attention was given to known "pocket populations", areas of close human habitation where unusually high densities of iguanas have been found to exhibit altered social dynamics. Consistent monitoring of sites every two to three hours ensured early detection of hatchlings. Once an emergence hole and/or hatchlings were discovered, either within or outside the boundaries of an enclosure, excavation of nesting sites began with the extraction of remaining iguanas. Eggshells and unhatched eggs were removed from egg chambers as well for analysis of clutch success. Hatchlings within each clutch were captured, processed, and measured. Measurements recorded from hatchlings included: snout-to-vent length, vent-to-tail length, probe depth, and weight. Blood from the caudal vein was drawn from each hatchling for collection, and each animal received a subcutaneous passive integrated transponder (PIT) identification tag and color-coded beads for future mark recapture data. In addition to the nesting survey, adult and juvenile iguanas dispersed throughout the island were (re)captured, marked, and measured with documentation of GPS coordinates for determination of survivorship rates. A total of 118 adults, including 25 known dams, and 423 hatchlings representing 38 clutches were collected over the course of the entire field season.

Iguana blood samples were then brought back to the research laboratory at Mississippi State University for DNA extraction and genotyping. To compare multilocus heterozygosity (MLH) across adult and hatchling individuals, 16 microsatellite markers known to be polymorphic in the genus *Cyclura* were amplified for genotyping. Sampled clutches that match to known nesters will be analyzed for reconstruction of paternal genotypes using the software COLONY. Sibships will be assigned to pairs of hatchlings within known maternal clutches using a full-pedigree likelihood algorithm to estimate the most probable number of contributing sires per clutch. In addition, all sampled adults will be reviewed by COLONY for genotype compatibilities with progeny arrays, and in the absence of a high probability match, parental genotypes will be inferred by reconstruction. The information provided by these current investigations, compounded with recent data from past sampling excursions, will allow insight into the extent polyandry plays a role in female inbreeding avoidance behaviors while also comparing behaviors between animals within and outside close human contact zones.

Project Significance:

Conservation of biodiversity represents a pressing dilemma that falls under human responsibility. Rock iguanas play an active role in the maintenance of their ecosystem through plant cropping and seed dispersal. Removal of any member of an ecological niche may disrupt the flow of a natural system. By understanding the mate choice behaviors and reproductive dynamics of a potentially inbred population, we may pave the way for future conservation management plans aimed at preserving an array of animals with similar life histories. Our findings may also influence the way people consider small, isolated, and possibly inbred populations. With the information gained from this research, conservationists may be able to better design exclusive strategies aimed at restoring particular species.

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Name: Langford, Jaslyn
Faculty Advisor: James A. Stewart, Jr., Ph.D.

Project Title: *Role of RAP1A in Age/Rage-Mediated Signaling in Type 2 Diabetes Mellitus*

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease characterized by an increasing resistance to the biologic effects of insulin leading to hyperglycemia. Insulin resistance is thought to occur by either a desensitization of the insulin receptor to its ligand or by an uncoupling of the intracellular signaling cascade to the glucose transporter translocation mechanism. Prolonged exposure to hyperglycemia is now recognized as the primary causal factor in the majority of diabetic complications [1,2]

Nearly 40%-50% diabetic patients manifest cardiac abnormalities [3,4]. Chronic hyperglycemia is likely responsible for many of the pathophysiological complications in the cardiovascular system [5]. The exact mechanisms that link hyperglycemia to diabetic cardiomyopathy are unknown. However, several pathological alterations have been suggested, including 1) ventricular hypertrophy, 2) myocyte contractile dysfunction, 3) interstitial fibrosis and/or increased extracellular matrix (ECM) accumulation, 4) myocardial stiffening resulting from dynamic changes in structural and cellular components of the myocardium and 5) AGE accumulation and enhanced AGE/RAGE signaling. Our laboratory has recently found that a small monomeric G protein may play a role in exacerbating a number of diabetes mediated complications.

Rap1a, a member of the Ras superfamily of GTPases, is a small monomeric G protein that acts as a molecular switch coupling extracellular events to intracellular signaling. Rap1a is able to cycle between its GTP-bound form (active state) and GDP-bound form (inactive state). Little is known about the interaction of Rap1a in type 2 diabetes mellitus. Traditionally studied Rap1a pathways involve second messengers, such as cAMP, calcium and DAG, to activate Rap1a through the guanine exchange factor (Epac). This activation leads to downstream effector protein activation to elicit biological responses. In studies performed by Keiper, et.al, Epac stimulated ERK1/2 activation in a PKA-Epac-Rap1a manner [6]. Of note, it has also been shown that PKC-Epac-Rap1a, has been demonstrated by Miller et.al, in which Epac was capable of regulating cardiac myofibroblast activation and ECM synthesis when stimulated by TGF-β [7].

Recent studies have demonstrated Rap1a plays a role in dampening PI3K/AKT/mTOR signaling. This pathway is crucial to insulin receptor signaling and GLUT-4 translocation/recycling. In type 2 diabetes, the insulin receptor cascade uncouples from the PI3K/AKT/mTOR

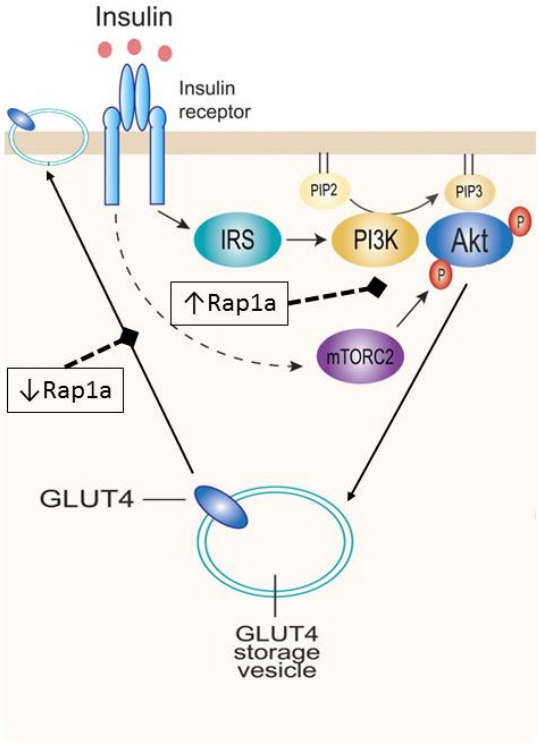

Figure 1. Representative signaling mechanism of insulin signaling and GLUT-4 translocation through PI3K/AKT/mTOR(Complex) signaling cascade.

pathway resulting in insulin insensitivity and decreased glucose uptake (Figure 1). Additionally, previous studies performed by our laboratory has demonstrated that Rap1a is significantly elevated in type 2 diabetes. The purpose of this study was to identify a role for Rap1a in the PI3K/AKT/mTOR pathway. Therefore, we hypothesized that knocking out Rap1a expression would prevent PI3K/AKT/mTOR signal depression to attenuate diabetes onset or progression.

The goal of this study is to define a functional link between increased Rap1a signaling cascade and changes in GLUT-4 translocation/recycling. We propose that knocking out Rap1a would prevent insulin receptor dysfunction and intracellular uncoupling of PI3K/AKT/mTOR pathway from insulin signaling. Alternatively, observational and Western data, presented below, show Rap1a ablation may result in a loss of required signaling to prevent GLUT-4 translocation/recycling.

MATERIALS AND METHODS

Animal model**:** Rap1a knockout mice (Rap1a-/-) were used as an animal model for this project. Rap1a-/- mice contain a mutation in the exon 4 in which an antibiotic resistance box was inserted. A high fat-high caloric Western diet was fed to the mice starting at 4 weeks of age and maintained on the diet until 16 weeks of age. This diet was used to promote obesity and type 2 diabetes in nondiabetic (Rap1a-/- and Rap1a+/+) in order to determine an *in vivo* role for Rap1a in GLUT4 translocation to promote systemic glucose uptake. The effects of high fat-high caloric feeding on both expression and translocation of GLUT4 will be determined in experimental animals fed a Western diet for 12 weeks. It has been well documented that upon high fat feeding, C57BL/6J mice will develop obesity, hyperinsulinemia, and hyperglycemia when compared to C57BL/6J mice fed a control diet. All studies are approved by MSU IACUC, protocol number 14-046.

Western blot analysis: Lysates were made from the muscle tissue and western blot analysis will be performed with the following antibodies: Rap1a, IRS-1, GLUT-4, PI3K, AKT, and mTOR. GAPDH was used a loading control. Densitometric analysis performed with NIH image software.

Statistical analyses: Repeated measures analysis of variance (ANOVA) will be used to compare Western data for the genetic groups. When significant difference ($p<0.05$) is found, appropriate pairwise comparison methods will be used for inter-group comparison. For signaling studies, power calculations indicate n=4-5 individual tissue lysates will be required per group to achieve power of at least 95% for testing all pair-wise group comparisons using significance of $p<0.05$.

RESULTS

The data for this project proposal fellowship is from current research being conducted in the Stewart lab. The data shown will be presented at both the Biological Sciences Undergraduate Research Program (BURP) and the Honors Undergraduate Research Program (HURP). This data should be considered confidential and not be disseminated without prior approval from the project's sponsoring investigator.

Observational data revealed, when Rap1a gene deletion (Rap1a-/-) was bred into a diabetic mouse strain, Rap1a -/- diabetic mice did not present phenotypic

characteristics of the model (i.e., slight obesity and higher than normal post prandial hyperglycemia) (Figure 2). In addition, genotypic diabetic db/db Rap-/- mice died between 4-5 weeks of age. The time of death coincides with the age in which these animals begin to present prediabetic characteristics, such as hyperglycemia and obesity. Therefore, another approach was taken to make the Rap1a-/- and Rap1a +/+ diabetic. The mice were fed for 12-weeks a 60% high fat-high Western diet from 4-16 weeks of age to make the mice diabetic or prediabetic.

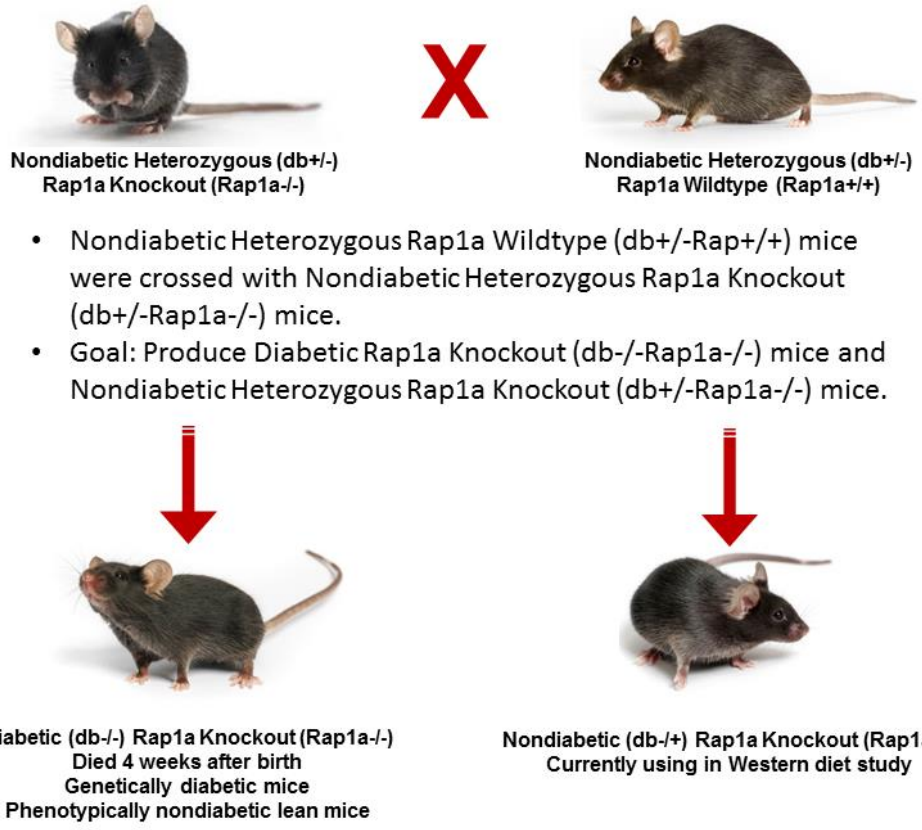

Figure 2. Representative schematic of observational findings when diabetic db/db Rap1a-/- mice were being created. Time of death of the db/db Rap1a-/- mice coincided with onset of prediabetes.

Cardiac muscle samples were harvested for proteins to determine differences in components of the insulin receptor signaling cascade, PI3K/AKT/mTOR pathway, and GLUT-4 phosphorylation. Data presented herein represent protein measurements observed in the heart tissue of animals fed both high fat diets and normal diets.

<u>Rap1a protein expression was significantly elevated in type 2 diabetes heart tissue (Figure 3)</u>: Rap1a protein expression measured by Western blot analysis was significantly increased in diabetic db/db

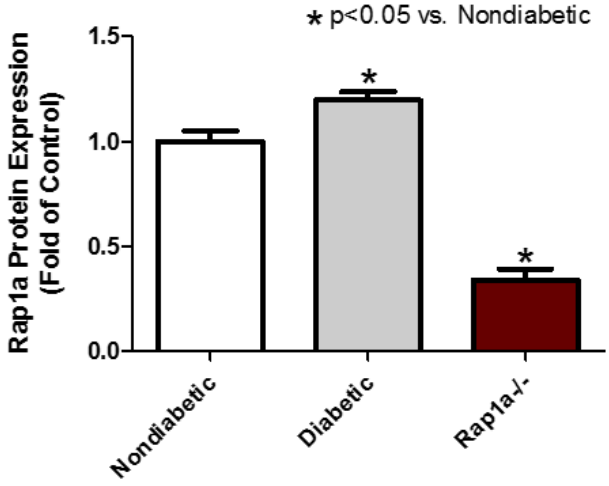

Figure 3. Rap1a protein expression was significantly increased in diabetic (db/db) heart tissue and significantly decreased in Rap1a knockout (Rap1a-/-) heart tissue. Polyclonal Rap1a antibodies were used that can cross react with Rap1b. *p<0.05 vs. Nondiabetic (db+/-) One-way ANOVA with Tukey's post hoc analysis. Data represent an n-value of 3-4 mouse hearts/group.

hearts. While Rap1a protein expression was significantly decreased in Rap1a -/- mouse hearts. Rap1a and Rap1b share a near 95% homology, yet are phenotypically different [8]. Therefore, there will be a consistent margin of antibody overlap when using commercially available polyclonal antibodies. These results were observed in normal fed animals.

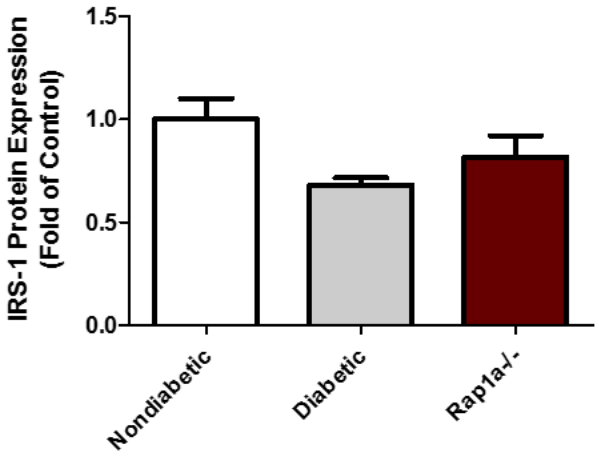

Figure 4. IRS-1 protein expression was not significantly changed between groups. Data represent an n-value of 3-4 mouse hearts/group.

Rap1a knockouts did not have altered IRS-1 protein expression (Figure 4): Hearts from Rap1a -/- mice did not have altered IRS-1 protein expression as measured by Western blot analysis. IRS-1 levels were decreased in diabetic db/db mouse hearts as one would expect at the prediabetes time points. Further studies will be performed to determine is IRS-1 phosphorylation patterns were altered. These results were observed in normal fed animals.

Rap1a knockouts did not have altered total GLUT-4 protein expression (Figure 5); however, phosphorylated GLUT-4 levels were significantly decreased (Figure 6): In these studies hearts from Rap1a -/- mice did not have altered total GLUT-4 transporter levels as measured by Western blot analysis. Thus, indicating GLUT-4 translation was not affect by knocking out the Rap1a gene. Conversely, phosphorylated levels of GLUT-4 were significantly decreased which depict an uncoupling event has occurred in the insulin receptor. GLUT-4 transporters in the db/db mice appear to be only slightly elevated above control levels. This observation should be expected of diabetic animals in the prediabetes stage of the disease [9]. Interesting, at this stage the insulin signaling-GLUT-4 transporter cascade is recoverable when moderate exercise is performed. These results were observed in normal fed animals.

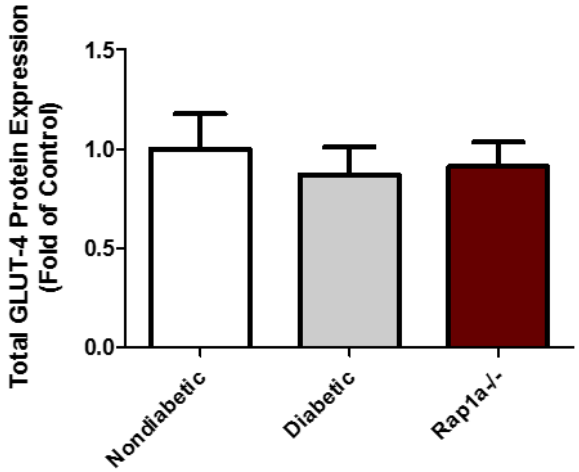

Figure 5. Total-GLUT-4 protein expression was not significantly changed between groups. Data represent an n-value of 3-4 mouse hearts/group.

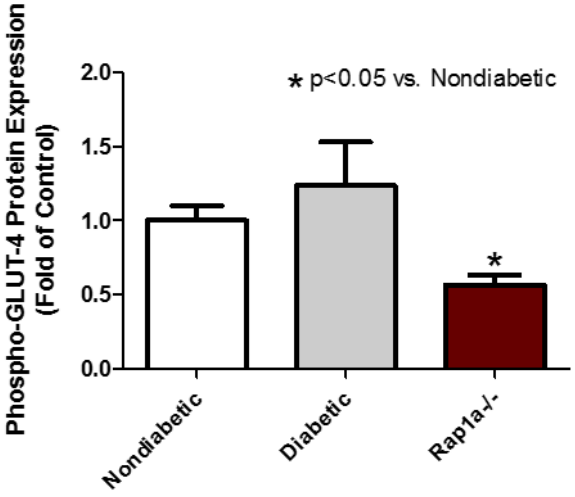

Figure 6. Phosphorylated GLUT-4 protein expression was significantly decreased in Rap1a knockout (Rap1a-/-) heart tissue. Representing decreased GLUT-4 translocation to cell membrane. *p<0.05 vs. Nondiabetic (db+/-) One-way ANOVA with Tukey's post hoc analysis. Data represent an n-value of 3-4 mouse hearts/group.

High fat diet altered Rap1a heterozygote physiological parameters (Figure 7). After 12-weeks of high fat diet (HF) diet Rap1a+/- mice had significantly elevated A) heart weights, B) body weights, and C) blood glucose levels similar to that of their high fat fed wild-type (WT) counterparts.

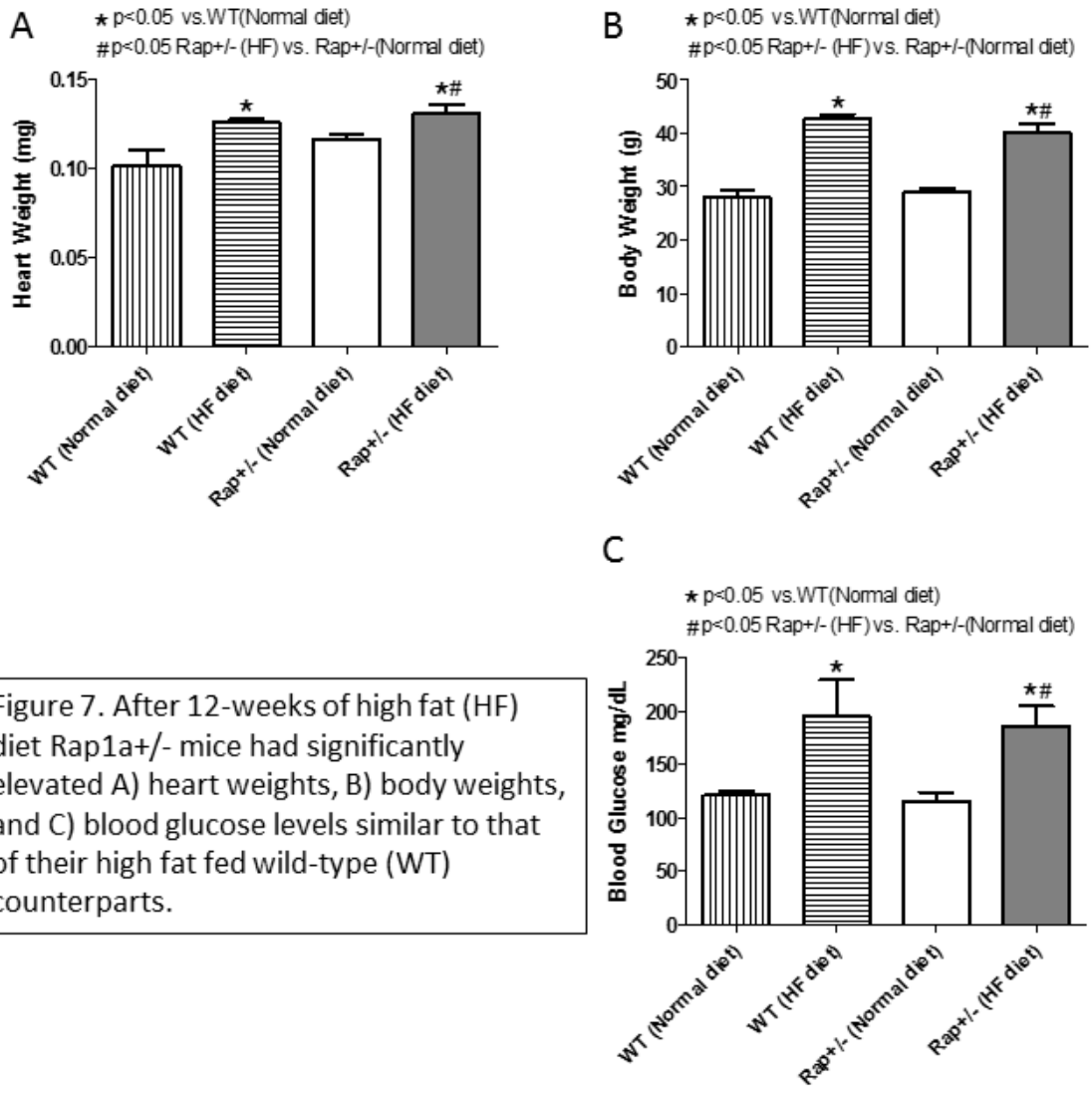

Figure 7. After 12-weeks of high fat (HF) diet Rap1a+/- mice had significantly elevated A) heart weights, B) body weights, and C) blood glucose levels similar to that of their high fat fed wild-type (WT) counterparts.

High fat diet did not promote diabetic remodeling in Rap1a heterozygote mice (Figure 8). After 12-weeks of high fat (HF) diet Rap1a+/- mice had similar levels of A) Rap1a, B) RAGE, and C) smooth muscle actin protein expression levels similar to that of their normal diet fed wild-type (WT) and Rap1a+/- mouse counterparts. From this data it would appear that Rap1a+/- hearts are spared diabetic remodeling compared to high fat diet fed mice.

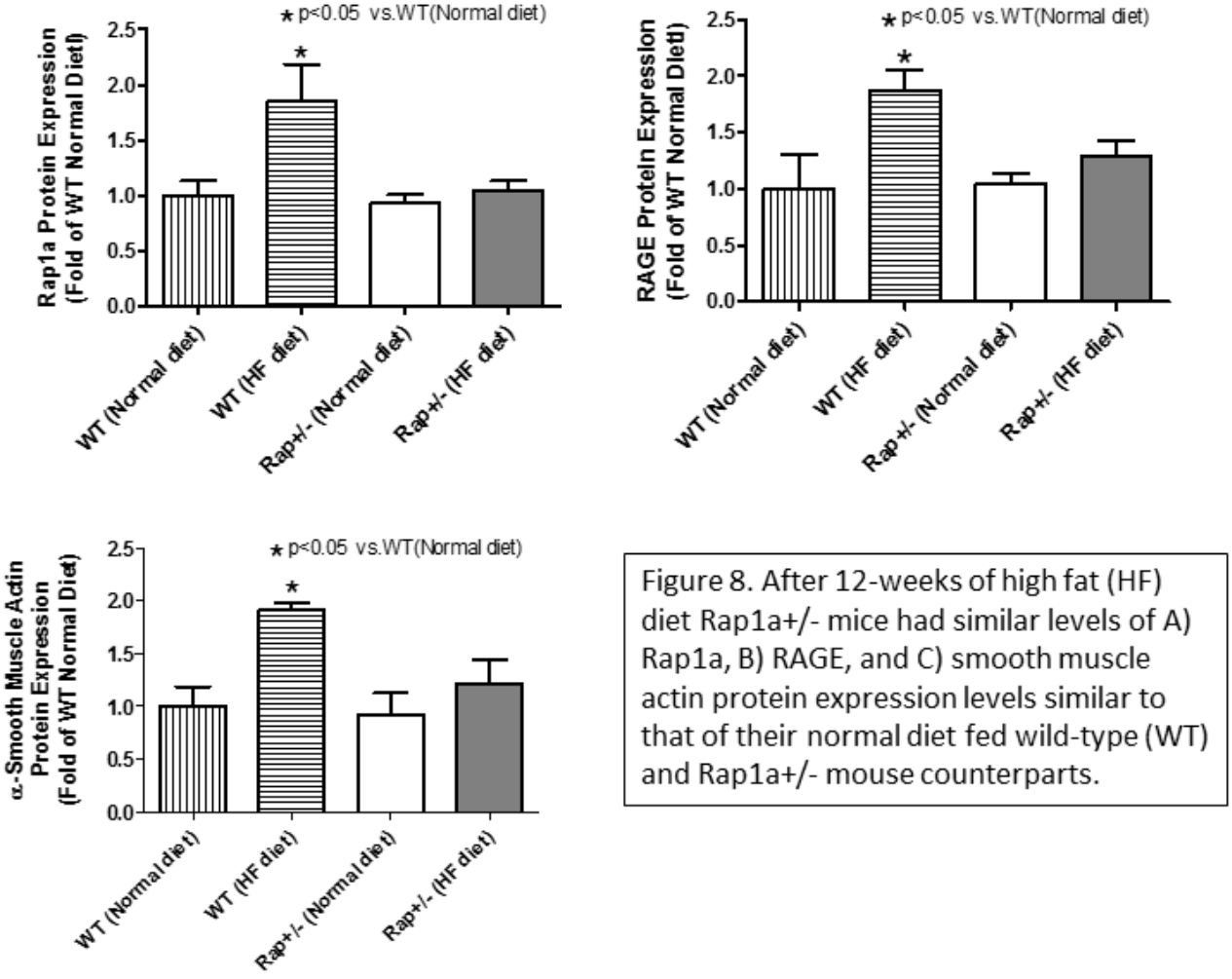

Figure 8. After 12-weeks of high fat (HF) diet Rap1a+/- mice had similar levels of A) Rap1a, B) RAGE, and C) smooth muscle actin protein expression levels similar to that of their normal diet fed wild-type (WT) and Rap1a+/- mouse counterparts.

Summary: The data could provide an explanation to mortality observed at the prediabetic stages of diabetic db/db Rap1a knockout mice. The rapid initial increases of blood glucose levels in diabetic db/db Rap1a -/- mice could not be overcome due to dysfunctional insulin-GLUT-4 transporter signaling. Thus, resulting in death. Our data also demonstrates that knocking out Rap1a GTPase affects GLUT-4 phosphorylation, may alter either transporter translocation to the cell membrane and/or intracellular transporter recycling. Conversely, significant increases in Rap1a protein expression may also negatively impact the insulin-GLUT-4 signaling pathway. Lastly, we observed that loss of Rap1a allele resulted in a cardio-protective response that prevented diabetic remodeling.

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Name: Leonard, Will

Faculty Advisor: Dr. Hugh Medal

Project Title: *A Framework for Evaluating System Architectures in Critical Infrastructure Systems*

The awarding of funding for research work for the summer of 2016 required a commitment to create a framework for insider threat analysts to use when evaluating system architectures for critical infrastructure systems. The work was expected to determine which employee roles are the most important to protect against insider threats.

The National Cybersecurity and Communications Integration Center (NCCIC) defines an insider threat as "a current or former employee, contractor, or other business partner who has or had authorized access to an organization's network, system, or data and intentionally misused that access to negatively affect the confidentiality, integrity, or availability of the organization's information or information systems" (2014). Based in part on the NCCIC's definition, insiders were defined for the purpose of analysis as employees, contractors, and the like that have a contractual relationship with critical infrastructure facilities (e.g., electrical transmission and distribution facilities).

To recognize the nature of insider threats, it was necessary to consider the particular threats posed by insiders by virtue of their human characteristics. For example, a human view of critical infrastructure facilities' employees was established. To do so, first, areas of potential interest were identified in a list. The list included such aspects related to humans as personnel roles, social networking among the employees, and human-specific tasks and interactions. Next, the human attributes were investigated in the context of a critical infrastructure facility – for example, the human-specific tasks and interactions among employees at electrical distribution

facilities were subject to examination. This part of the process involved looking at both digital and paper resources in an effort to discover records showing the desired data.

Finally, after the compilation of such data, the relationships between and the activities among insiders at a critical infrastructure facility were perused and analyzed repeatedly. Significant information put together as a result of this process included concept maps linking employees with various job responsibilities and hierarchical decision-making diagrams, together with a detailed discussion of more than ten pages in length. These items and the analytical report are hoped to be invaluable for the function of serving as a framework for insider threat analysts to use when evaluating system architectures for critical infrastructure systems.

National Cybersecurity and Communications Integration Center. (2014, May 2). Combating the Insider Threat. Retrieved April 13, 2016, from https://www.us-cert.gov/sites/default/files/publications/Combating%20the%20Insider%20Threat_0.pdf

Name: Majors, Katelyn

Faculty Advisor: Dr. E. Samuel Winer

Project Title: *Investigating Depressive Symptoms Overtime*

This summer, a portion of the time I spent in the lab was used to organize the entirety of the protocol used to guide the efforts of all personnel who are a part of Dr. Winer's Emotional Processes and Experimental Psychopathology Laboratory. Amy Wallace designated specific tasks for me to complete in an effort to streamline the protocol into easily accessible word documents that all individuals who are a part of the lab, experienced or not, can reference in the future. The biggest takeaway for me in my efforts to help organize lab protocol was a renewed understanding of lab procedures and how to properly complete all tasks related to research protocol. Specifically, I became very familiar with the protocol used to distribute research recruitment materials to Mississippi State's campus and the surrounding community. This familiarity provided the opportunity for me to step into a leadership role by leading recruitment efforts in the fall semester of lab activities. These efforts included printing and trimming of paper napkin holders and flyers as well as delineating location assignments to other undergraduates in the lab. This opportunity was crucial in fostering my leadership skills, which I have identified as an area that I wish to work on developing in preparation for my future endeavors.

To culminate the efforts of my summer in the Emotional Processes and Experimental Psychopathology Laboratory, the poster that I worked on with Dr. Winer was submitted to the International Convention of Psychological Science was accepted. Amy Wallace and I were co-authors on this poster, and this was my first submission acceptance. The study examined if an implicit measure of emotion, specifically an implicit measure of happiness, could predict self-reported fear of happiness when moderated by response time. Individuals who responded quicker and who showed less endorsement of happiness on the implicit measure were more likely to

express a greater fear of happiness. My contributions to this effort were mainly focused on working with the research team on data analysis and helping to write the poster. The guidance I received from both Dr. Winer and Gage Jordan, a graduate student and first author on the poster submission, about data analysis will be invaluable for me in my future in graduate school. Together with Amy Wallace, I will be presenting this poster that has been developed in the efforts to investigate the speculated relationship between an implicit measure of happiness and self-reported fear of happiness at the Spring 2017 Undergraduate Research Symposium in April.

Name: Moore, Sabrina
Major: Microbiology and Chemistry
Faculty Advisor: Dr. Thronton

Project Title: *Effect of Proteases on the Virulence of Streptococcus pneumoniae*

Background and Purpose:

Streptococcus pneumoniae (pneumococcus) is the leading cause of community acquired pneumonia and acute bacterial otitis, affecting millions of people worldwide. The primary virulence factor expressed by all clinically isolated strains of pneumococcus is pneumolysin (PLY), a pore forming toxin with cytolytic, complement-activating, and immunomodulatory functions. PLY is involved in replication in the lungs and bloodstream, and contributes to invasion from the lungs to the blood. Much is known about the toxin, but crucial questions regarding its functionality still remain. It is known that the toxin initially oligomerizes to form a pre-pore in host cell membranes prior to undergoing a conformational change resulting in membrane insertion and pore formation. However, it is unknown if external factors such as protease cleavage can effect this process. Preliminary data from our lab indicates that protease cleavage enhances toxicity. Our hypothesis is that cellular effects of PLY are enhanced by protease cleavage and impact pathogenesis. The goal of this project is to identify the mechanism by which host proteases increase the virulence of pneumococcus. This will add an additional target for future therapeutics and increase our understanding of pneumococcal pathogenesis.

Significance:

Morbidity and mortality associated with pneumococcal disease is significant, resulting in $3 billion in direct medical costs per year in the United States. The economic burden is far overshadowed by the fact that more than 800,000 children under the age of five succumb to pneumococcal disease each year, primarily in developing countries. PLY is involved in nearly every aspect of pneumococcal disease, and is additionally an outstanding vaccine candidate due to its prevalence in all strains of pneumococcus. Because of this, it is necessary to understand possible changes that may affect PLY's functionality *in vivo*. A critical barrier to the development of strategies to prevent invasive disease is that we still know little about the molecular mechanisms that affect PLY's activity during pneumococcal infections. Our contribution toward this goal is to characterize how the activity of PLY is affected by host proteases. This is significant because it expands upon the basic mechanism of PLY-induced cytolysis and demonstrates that alternatives to this mechanism have important ramifications upon the infectious process and could be targeted either immunologically or pharmacologically. This research is innovative because it demonstrates for the first time that a cholesterol-dependent cytolysin's activity can be enhanced by proteolytic cleavage, a finding that will dramatically shift our current understanding of the mechanism of such toxins. These findings will allow for development of new strategies for the treatment or prevention of disease.

Progress:

A variety of flow cytometry experiments were conducted that confirmed that trypsin intensifies the effect of pneumolysin when it is added after human epithelial cells are exposed to pneumolysin. Our current hypothesis is that the trypsin cleaves the pneumolysin once it binds to the epithelial cell. Then, the cleaved pneumolysin is somehow more efficient at forming pores in the cell membrane. Several western blot experiments were done in attempt to confirm this hypothesis. While one blot confirmed that the cleavage was occurring, there was difficulty replicating it due to the low concentrations of pneumolysin required. Much of the summer was spent attempting to optimize this experiment.

Other proteases were also tested to determine if they had a similar effect on pneumolysin effectiveness. Human airway trypsin, a protease similar to trypsin that is found in the part of the airway pneumococcus inhabits, was found to have minimal effect on cell death. Preliminary experiments to determine the effect of human neutrophils showed minimal contribution to cell death, though more work is needed to confirm this. Finally, we attempted to purify listeriolysin to determine its effect on cell death. Unfortunately, we could not successfully isolate a functional protein. We are continuing to work on this issue.

Future Directions:

Going forward, we plan to further investigate the role of neutrophil proteases on pneumolysin activity. We plan to determine if varying the concentration of the proteases or increasing the incubation time will affect the activity. Additionally, we would like to purify a functional form of listeriolysin and determine if trypsin interacts with listeriolysin similarly to how it interacts with pneumolysin. This would be a more biologically relevant combination because trypsin and listeriolysin are both found in the same area.

Acknowledgements

This project was supported by Mississippi State University's Shackouls Honors Summer Undergraduate Research Fellowship program.

Name: Moseley, Max
Faculty Advisor: Dr. Philip Poe

Project Title: *Acculturation and Norm Perceptions: Influences on Hispanic Women's Intentions to Have a Mammogram / Beliefs about Exercise among Hispanic Men*

For summer 2016, I worked under the guidance of Dr. Philip Poe in the Department of Communication. Our project aimed to deliver two studies concerning perceptions of health behavior among Hispanics. The first study was to utilize the Theory of Planned Behavior to measure intention to have a mammogram among a sample of Hispanic women, and the second study was to utilize the Health Belief Model to determine the likelihood of engagement in cardiovascular exercise among a sample of Hispanic men. The studies would also measure level of acculturation and attitudes toward mammography and exercise, respectively, and would identify perceptions of subjective and injunctive norms for mammography and exercise, respectively.

Over the summer, while waiting to receive the data to conduct our analysis, Dr. Poe and I directed our efforts toward a variety of projects Dr. Poe had in various stages of completion. First, we completed a structural rewrite of our paper ""She's a Little Different": Autism-Spectrum Disorders in Primetime Television Dramas" in order to submit the paper to the *Journal of Communication Inquiry*. After we completed and submitted our paper, Dr. Poe gave me the transcripts for five focus group sessions of college freshmen—three groups of females and two groups of males—discussing fruit and vegetable consumption on college campuses. I analyzed the transcripts to find underlying trends and commonalities among the students' statements and to find representative quotes for each trend. I then gave my notes to Dr. Poe, who

adapted my notes and produced a paper called ""I Know Nothing": Perceived Barriers to Fruit and Vegetable Consumption Among College Freshmen." That paper has been submitted to the journal *Qualitative Health Research*.

Dr. Poe and I also updated a draft of his paper "Travel Journalism as Global Media Discourse: Constructions of the "Other" in Travel Writing," which was submitted and recently accepted into the Intercultural Communication Division of the Southern States Communication Association's 87th Annual Convention, to be held in Greenville, South Carolina, in April 2017.

With the funding from the Honors Undergraduate Research Fellowship, I have been able to help Dr. Poe complete his projects and submit them to conferences and journals. These submissions and acceptances could help promote the name of Mississippi State University and the research our professors do here. While we have not yet made as much progress on our intended study, we have used the Shackouls Honors College's funding to produce multiple papers and conference submissions. Dr. Poe and I are grateful to the Honors College for allowing us this opportunity.

Name: Rushing, Ben
Faculty Advisor: James A. Stewart, Jr., Ph.D.

Project Title: Age/Rage Signaling in Vascular Calcification

INTRODUCTION

Increased glucose levels are a defining feature of type 2 diabetes mellitus (T2DM) and considered to be an underlying cause of vascular complications, a dysfunction in the diabetic population. Vascular calcification is described as the hardening of the medial layer of the artery through deposition of hydroxyapatite minerals into the extracellular matrix. [1-3] This process, once thought to be passive and associated with aging, has now been demonstrated to be a tightly regulated cell mediated process. [4] During vascular calcification, bone morphogenetic protein-2 (BMP-2) activates core binding factor alpha-1 (CBFA-1, also known as RunX2), which acts as the primary transcriptional regulator for the maturation of osteoblasts in the bone. [5-7] CBFA-1 also upregulates the production of osteoblast proteins within vascular smooth muscle cells (VSMCs), which is thought to cause a phenotypic switch of VSMCs to an osteoblast-like phenotype. [8] Alkaline phosphatase (ALP) and bone sialoprotein (BSP) have been demonstrated to be early markers of osteoblast activity, while markers, such as osteopontin (OPN) and osteocalcin are upregulated late in the calcification process. [9-11] Their primary function is to enhance the formation and deposition of hydroxyapatite, which is composed of type I collagen and other non-collagenous proteins. [11] Primarily indicated in bone formation, ALP is responsible for cleaving pyrophosphate to phosphate to promote hydroxyapatite deposition and mineralization within the bone. [12] BSP is responsible for the nucleation of hydroxyapatite mineral. [11, 13, 14] Similar to ALP, OPN is also linked to hydroxyapatite deposition and can serve as a mediator of cell attachment and signaling. [15] Hydroxyapatite size and shape is mediated by osteocalcin through a vitamin K dependent mechanism. [16] Taken together, these data demonstrate the potential to promote bone formation within a living system, and researchers have utilized this knowledge of bone matrix proteins to understand the underlying mechanisms of vascular calcification and Type II diabetes.

VASCULAR CALCIFICATION AND AGE-RAGE SIGNALING

In addition to increased bone matrix protein expression in VSMCs during diabetic and calcification treatments, studies have also shown that advanced glycation end-products (AGEs) and their receptors (RAGEs) play a role in vascular calcification. [17] Type II diabetes patients have been shown to have a significantly higher concentration of AGEs than the nondiabetic population. [18-20] AGEs form over a lifetime as a result of increased circulating glucose as well as other reducing sugars, such as galactose and fructose, reacting with amino groups of proteins to form Schiff bases to either follow the polyol pathway to yield AGEs or to be degraded. [21] These glycated end products interact with RAGEs, which are transmembrane proteins that are a part of the immunoglobulin superfamily. RAGEs are upregulated in response to increased circulating AGE levels. [22] Upon AGE-RAGE binding, RAGE works through PKC-ς to trigger the downstream activation of a signaling cascade that works through p38 mitogen activated protein kinase (MAPK), transforming growth factor-β (TGF-β), and nuclear factor κB (NFκB). [23, 24] Suga et al. demonstrated that activation of the AGE-RAGE signaling in rat VSMCs reduced the expression of VSMC gene markers such as smooth muscle-myosin heavy chain (SM-MHC) and smooth muscle 22α (SM22α). [25] This down-regulation of VSMCs markers suggests the possible phenotypic switch of VSMCs

to an osteoblast-like phenotype. [8]This is supported by findings from human VSMCs (HVSMCs) where activation of RAGE increased mRNA expression and activity of ALP, a bone matrix protein, suggesting a role for RAGE signaling in vascular calcification. [25] These studies demonstrated some basic roles for RAGE in VSMC calcification through PKC- ζ signaling, increased expression of ALP, and decreased expression of VSMC gene markers. Figure 1 represents a proposed cell signaling mechanism for AGE/RAGE-mediated vascular calcification.

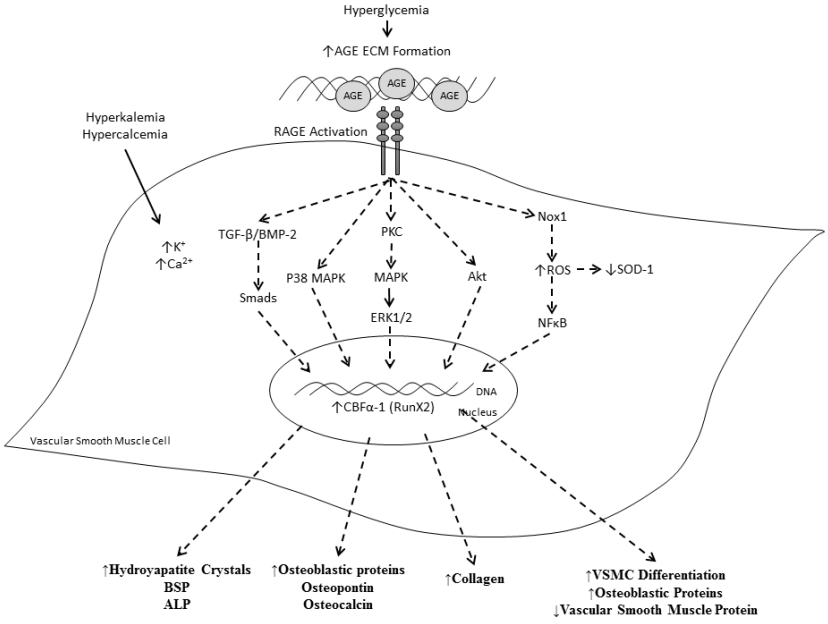

Figure 1. Representative schematic of proposed diabetes induced signaling cascade through the RAGE receptor.

HYPOTHESIS

We hypothesize that **diabetes induces increased AGE/RAGE signaling to mediate vascular calcification resulting in pronounced aortic stiffness.** The following specific aim will test the hypothesis.

The goal of this study is to define a functional link between the AGE/RAGE signaling cascade and vascular calcification. We propose that the AGE/RAGE signaling cascades will stimulate vascular calcification. Genetic ablation of RAGE will provide a loss-of-function approach to reduced vascular calcification as well as prevent VSMC phenotype switch to osteoblast-like cells.

Significance: The successful completion of this project will define molecular mechanisms involved in AGE/RAGE-dependent VSMC calcification. These studies provide unique targets for therapeutic strategies aimed at reducing hyperglycemia-mediated vascular calcification in diabetic patients.

METHODS

Animal model

Our lab uses the genetically diabetic mouse (C57BL/KsJ-db/db). The db/db mouse contains a mutation in the leptin receptor that renders it insensitive to leptin signaling. These mice develop frank hyperglycemia by 8 weeks of age, overt diabetes by 12 wks of age, and exhibit many common features of type 2 DM, including hyperlipidemia, obesity and insulin resistance. The heterozygous Db/db mice will be used as the lean control, and they cannot be distinguished morphologically or physiologically from wild type mice. The db/db mouse has been crossed with the RAGE knockout mouse (RKO). The following groups will be used for the proposed project.

Genotype	Phenotype
Db/db	Nondiabetic with RAGE
db/db	Diabetic with RAGE
Db/db^{RKO}	Nondiabetic without RAGE
db/db^{RKO}	Diabetic without RAGE

Cell Isolations

Isolated VSMC from db/db, Db/db, db/db^{RKO} and Db/db^{RKO} mouse aortas will be used to investigate the role AGE/RAGE signaling in diabetic vascular calcification. Rap1a siRNA will be used to knock-down Rap1a from both db/db and Db/db CFs. These groups will be treated with and without AGE-BSA to activate RAGEs that are present. Phenotype markers (α-smooth muscle actin), ECM regulators (AGEs, collagen I, TGF-β), signaling proteins (RAGE, PKC-ζ, ERK 1/2, p38 MAPK), and ROS markers (NFκB, NOX, SOD) will measured by western blot analysis. GAPDH will be used as a loading control. VSMCs will be isolated by mincing aortic tissue and subsequent digestion with collagenase. Fibroblasts will be maintained in DMEM containing 20% FBS. All studies will use cells at P0 for baseline characterization and at P3 for signaling experiments. The purity of the cultures (> 90-95%) will be confirmed by positive staining for the VSMC-specific marker α-smooth muscle actin and by negative staining for endothelial cell markers. Aortas from 2-4 mice used per isolation. Data from 4-5 separate isolations will be collected per group. Currently, we have data from 2 isolations from each group.

Isolated cardiac fibroblasts from non-diabetic (Db/db) and Db/db^{RKO} mouse hearts will be exposed to conditioned media from calcified VSMCs. Fibroblasts have been reported to play a role in a number of osteogenic responses in connective tissue calcification however, the exact mechanism is unclear. [26-28]Studies have proposed that the release of certain circulating factors from VSMCs such as TGF-β, osteopontin, and osteocalcin will further promote vascular calcification. Control cells from nondiabetic +/- RAGE will be exposed to conditioned media containing secreted osteogenic factors released from calcified VSMCs. The rationale for this series of experiments will be 1) to determine if there is an osteogenic response promoted in fibroblasts and 2) to determine if fibroblasts will undergo a phenotypic switch to osteoblast-like cells similar to that of VSMCs. Fibroblasts will be isolated by mincing myocardial tissue and subsequent digestion with collagenase. [29, 30] Fibroblasts will be maintained in DMEM containing 5% FBS and 10% newborn calf serum. All studies will use cells at P0 for baseline characterization and at P1 for signaling experiments. The purity of the cultures (> 90-95%) will be confirmed by positive staining for the fibroblast-specific marker DDR2 and by negative staining for endothelial cell markers. Hearts from 4 mice used per isolation. Data from 4-5 separate isolations will be collected per group.

Western blot analysis

Lysates from CF cultures and conditioned media will be collected. Western blot analysis will be performed with the following antibodies: ECM regulators (AGEs, collagen I, TGF-β), signaling proteins (RAGE, PKC-ζ, ERK 1/2, p38 MAPK), and ROS markers (NFκB, NOX, SOD). GAPDH will be used as a loading control. Densitometric analysis performed with NIH image software.

Statistics

Repeated measures analysis of variance (ANOVA) will be used to compare treatment groups for protein changes. When significant difference ($p<0.05$) is found, appropriate pairwise comparison methods will be used for inter-group comparison. For all CF

experiments, ANOVA with one grouping factor, strain of mouse or treatment, will be used. For signaling studies, power calculations indicate n=4-5 cultures will be required per group to achieve power of at least 95% for testing all pair-wise group comparisons using significance of $p<0.05$.

RESULTS

The data presented for this project is from current research being conducted in the Stewart lab. The data shown will be presented at both the Biological Sciences Undergraduate Research Program (BURP) and the Honors Undergraduate Research Program (HURP). This data should be considered confidential and not be disseminated without prior approval from the project's sponsoring investigator.

Increased exposure to *in vivo* hyperglycemic conditions resulted in VSMC calcification (Figure 2)

VSMCs were isolated from Db/db and db/db mouse aortas. Cells were cultured in either euglycemic conditions (nondiabetic Db/db cells) or hyperglycemic conditions (diabetic db/db cells) until confluency. The cells were then cultured in calcification media for 7-days at which time alizarin red analysis was performed to determine the level of calcification in Db/db and db/db VSMCs. Diabetic db/db VSMCs demonstrated higher calcification levels at 7-days than nondiabetic Db/db cells. These results indicate that diabetic hyperglycemic conditions will promote vascular calcification with chronic exposure.

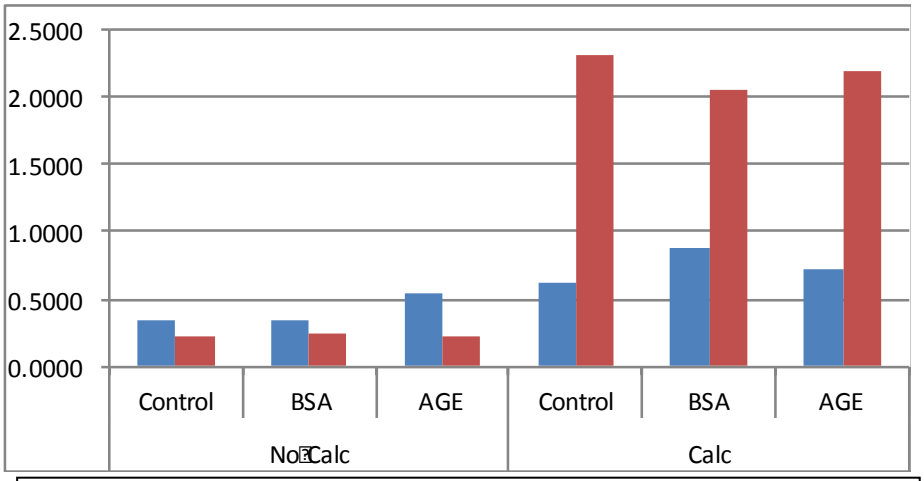

Figure 2. There was increased calcification at 7-days in db/db diabetic VSMCs (red) as compared to Db/db nondiabetic cells (blue).

Increased exposure to *in vivo* hyperglycemic conditions resulted in RAGE upregulation and loss of α-smooth muscle actin in 7-day VSMC calcification studies (Figure 3)

Isolated VSMCs from db/db diabetic mouse aortas were exposed to either calcification media or no calcification for 7-days. Immunofluorescence was performed to determine 1) if RAGE expression increased as a result of calcification and 2) if VSMC marker (α-smooth muscle actin) is lost due to changes in cell phenotype. Our data demonstrates that with calcification db/db cells RAGE expression is increased and appear to be localized to the cytoplasm of the VSMCs. In addition, there is also a loss of α-smooth muscle actin which may indicate a shift in VSMC phenotype to that of an osteoblast-like cell. Further studies will still need to be performed to determine the extent of phenotype remodeling.

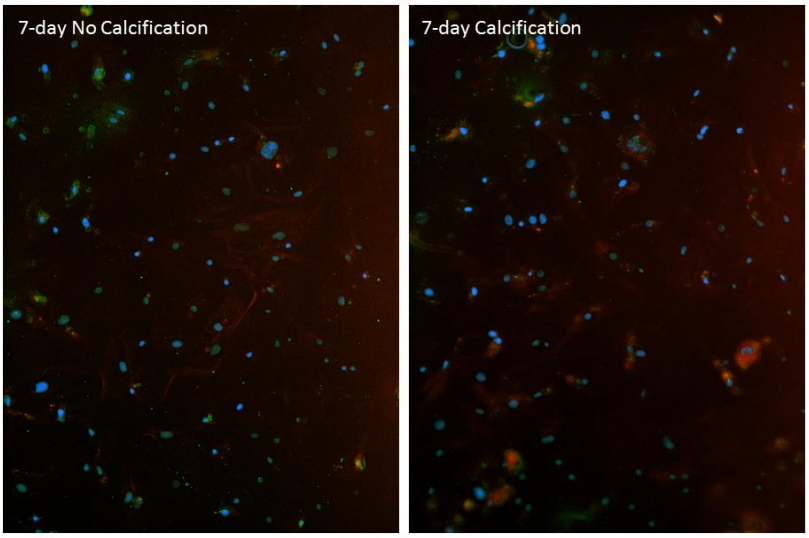

Figure 3. There was increased RAGE expression localized to the cytoplasm at 7-days in calcified db/db diabetic VSMCs (red staining) (right panel) in comparison to 7-day no calcification cells (left panel). Additionally, there was a loss of α-smooth muscle actin at 7-days in calcified db/db diabetic VSMCs (green staining). Nuclei were stained with DAPI (blue staining).

Cardiac fibroblasts exposed to conditioned calcification media results in a gain in α-smooth muscle actin (Figure 4)

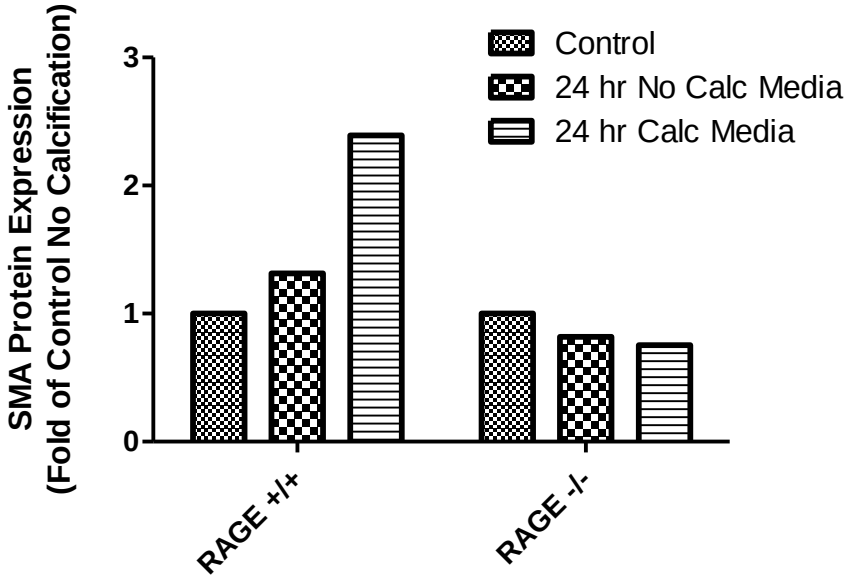

Figure 4. There was increased Smooth Muscle Actin (SMA) expression in RAGE WT cardiac fibroblasts exposed to conditioned calcification media for 24-hours. Conversely, no response was reported in RAGE KO cells.

There was increased Smooth Muscle Actin (SMA) expression in RAGE WT cardiac fibroblasts exposed to conditioned calcification media for 24-hours. Conversely, no response was reported in RAGE KO cells. This finding demonstrates RAGE may play a role in vascular calcification process. Increased SMA levels indicates “activated fibroblasts” capable of secreting excess ECM to serve as a scaffold for medial layer calcification.

SUMMARY

The preliminary data supports our overall hypothesis that diabetes induces increased AGE/RAGE signaling which will be mediated vascular calcification in the aorta. In addition there is a potential positive correlation between increased RAGE expression and changes in VSMC phenotype to that of an osteoblast-like cell. Fibroblast activation and ECM production may indicated a possible mechanism for vascular calcification.

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Name: Schwirian, Aumbriel
Major: Biochemistry
Faculty Advisor: Dr. Mark Welch

Project Title: *Polyandrous Mating within the Sister Isles Rock Iguana (Cyclura nubila caymanensis)*

Cyclura nubila caymanensis, otherwise known as the Sister Isles Rock Iguana (SIRI), belongs to one the most endangered reptile genera in the world (*Cyclura Spp.*). It is endemic to only two of the Cayman Islands: Cayman Brac and Little Cayman. Little Cayman has the larger population- approximately 1,200-1,500 individuals - and far less human development (Alberts, 2004). Conserving this species is critical for the island's health, as they serve a key ecological role in grazing plant life and seed dispersal (Iverson, 1979; Hartley *et al*., 2000). Hatchlings are the most vulnerable members of the population and often fall victim to predation to introduced carnivores. Due to small population size, it is possible that *Cyclura nubila caymanensis* could suffer from inbreeding depression (Frankham, Ballou, and Briscoe 2010). Small, isolated populations are particularly susceptible to inbreeding depression because deleterious alleles are more likely to be expressed with increasing relatedness among mates. Potential inbreeding avoidance strategies include non-random mating and polyandrous mating. Polyandrous mating is when a female mates with multiple males to increase the chances of some of her offspring having favorable phenotypes.

This study focused on continuing the study on the 2015 population data. Twenty clutches worth of data were investigated to answer question of polyandry in the SIRI population. The 2015 data was previously genotyped at 21 microsatellite loci. It was examined with a program called GERUD 2.0, by fitting the genotypic data to an algorithm and determining the number of minimum sires by testing combinations of 4 loci. Despite the results from GERUD 2.0 suggesting polyandry, the program was very limited in is capabilities as it could only determine the minimum number of sires for a clutch. It could not suggest parental genotypes, nor could take hatchlings of unknown clutches into account, which is particular problematic for wild caught hatchlings if they are not caught before they leave their nest. We determined to reexamine this data to get more useful analysis from it.

A small "pocket population" was also investigated in this study called the "museum" population. This population is a dense aggregation of iguanas on Little Cayman that has become partially habituated to humans. It is planned to map parent pairings and sibship relations of these hatchlings as more samples of the population become available with the data gathered in 2016.

I extracted and genotyped the microsatellite data from the last 50 hatchling samples remaining in the 2015 dataset. Nine additional loci were genotyped for all samples (over 250 individuals) and added to the 21 previously genotyped loci. The data gathered from the hatchlings was compared to the genotypic data all potential sires and all potential dams. COLONY software, which is a much more robust program than GERUDE 2.0 which allows us to create a much clearer understanding of genetic relationships in the Little Cayman population.

It allows a small amount of error, as it uses a maximum likelihood algorithm to assign potential parental genotypes, rather than trying to fit the parental genotypes to an exact algorithm, such as with the previously utilized software. Unlike GERUDE, COLONY can tell us more than just the number of sires for a given clutch. This software also allows us to acknowledge known maternal siblings and search arrays of candidate parental genotypes with differing probabilities of inclusion. Hatchlings were paired with the most likely parent couple; if COLONY could not determine a parent out of the pool of adults, then it inferred a possible parental genotype, along with the probability that an individual with that genotype was a parent of a given hatchling. The 2015 museum specimens were used to develop the methodologies used in COLONY. Results from the 2015 museum specimens suggests that 21 of the hatchlings are full siblings in multiple clutches with full sib arrays across all museum hatchlings. Additionally, potential parental pairs have been narrowed down to three to four individuals of both sexes for all 2015 hatchlings, which indicates some degree of reproductive skew.

The summer portion of this study yielded no definitive results, but analysis is ongoing. We hope to complete analysis by the end of the fall semester or the beginning of the spring semester. The methodologies used in this study may be expanded to other previously collected data across other iguana species gathered by the Welch lab (*C. carinata, C. ricordii, and I. delicatissima*) to explore the prevalence of polyandry across the iguanid tree in the future.

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Name: Smith, Ciarra
Major: Biochemistry
Faculty Advisor: Dr. Jeffrey Eells

Project Title: *Role of Nurr1 in Amphetamine Conditioned Place Preference*

The dopamine neurotransmitter is found in an abundance of neurochemical processes. Most notably are its key roles in motivation, reward and reinforcement mechanisms which make it a highly targeted molecule in the chemical pathway of drugs of abuse. One such drug of abuse, amphetamine, works to increase the amount of dopamine in the synapse by binding to the dopamine transporter (DAT) and reversing its function. The DAT protein facilitates the reuptake of extracellular dopamine into the presynaptic neuron after the initial release of the transmitter in response to an environmental stimulus. Amphetamine, therefore, increase the amount of dopamine located in the synapse resulting in a higher rate of neurotransmitter/receptor binding. This increased molecular binding induces a cognitive behavioral change in which the organism has now placed a greater significance on the relationship between the stimulus (the amphetamine drug) and the rewarding event (location of the reward).

Another player integral to the relationship between dopamine and amphetamine is the Nurr1 transcription factor. The Nurr1 protein regulates the rate of production of both DAT and the dopamine neurotransmitter. In order to study exactly how Nurr1 is influencing dopamine production, Nurr1-null heterozygous and Nurr1 homozygous mice were utilized in a conditioned place preference (CPP) test with amphetamine. It was hypothesized that rodents with a knocked-out Nurr1 gene would have a reduced response to the amphetamine in comparison to their Nurr1 homozygous counterparts. A decrease in the transcription regulator would essentially decrease the total amount of dopamine neurotransmitter available to be affected by the actions of amphetamine. By contrast, Nurr1 homozygous mice would have more of the transcriptional factor resulting in a greater instance of dopamine transcription equating to more dopamine available to be influenced by amphetamine leading to a higher association to the drug.

Over the summer of 2016, the initial phase of the project was completed. About 50 mice were given either amphetamine or saline and conditioned to expect the drug in a specific location. Based solely on time spent in the conditioned location, preliminary results suggest the Nurr1-null heterozygous mice appeared to be more affected by amphetamine than their Nurr1 homozygous counterparts. Whether these results suggest an adaption by the Nurr1-null heterozygous mice to increase their dopamine production or are due to a variation in the concentration of DAT is still unclear.

Further study is planned to try and understanding these findings through a chemical analysis of the nucleus accumbens and the ventral tegmental area. These brain sections house central dopamine producing neurons. The next step of our study plans to use immunohistochemistry and high-performance liquid chromatography to directly measure the concentration of dopamine and metabolites as well as Fos expression to elucidate how dopamine transmission is being influenced by amphetamine. An understanding of how drugs of abuse interact with dopamine transmission can then be translated into understanding other diseases caused by alterations in dopamine transmission such as Parkinson's Disease and Schizophrenia.

Name: Viger, Troy
Major: Biological Engineering
Faculty Advisor: Dr. Hyeona Lim

Project Title: *Efficient and Robust Non-local Means Denoising Methods for Biomedical Images*

Goal: The main goal of this research project is to develop efficient nonlocal-means based methods and their numerical algorithms for denoising biomedical images.

Introduction: Denoising is an important step to improve image quality and to increase the performance of image analysis. Most image denoising techniques assume an equal noise distribution across the images. When this assumption is not met, the denoising results can be unsatisfactory. This is the case of most biomedical images such as magnetic resonance images (MRI), ultrasound, and X-ray images, since these images are normally corrupted by random noise from the acquisition process. Recently developed filtering techniques, the so-called nonlocal-means (NLM) based methods can effectively deal with such biomedical images with spatially varying noise levels. The standard NLM filter uses the average of similar patches present in the image. It is very accurate in removing noise but has some computational complexity. We have studied more efficient and optimized NLM method and its associated numerical algorithm to reduce computational time of classical NLM method. The new method was applied to several clear images with synthetically added noise for validation and comparison with conventional methods. The method was then tested on real MRI biomedical images with natural noise. The details of our new findings are described in the next section.

Results and Findings: In the original NLM method, the gray value of each pixel is restored by the weighted average of the gray values of all pixels in the image. The main idea of this novel technique is based on the fact that each weight is proportional to the similarity between the local neighborhood of the pixel being processed and the neighborhood of other pixels. Therefore, images with repeated structures and similar patterns will remove noise very effectively using this technique. Although the quality of the results is state of the art, the main drawback of this method is on computational inefficiency. The high computational complexity is due to the cost of weights calculation since the whole image is searched to calculate weights for every pixel being processed. Instead of calculating weights at every pixel windows, we have set the following conditions:

(i) *Calculate weight as in the original NLM method if the mean (and variance) of main pixel neighborhood and the mean (and variance) of other pixel neighborhood are sufficiently close to each other*
(ii) *Set weight equal to zero if the conditions in (i) are not satisfied*

For more efficient computation, we created special matrices storing mean and variance of 7x7 neighborhood of each pixel, and used them to check the conditions for searching similar neighborhoods. Since this new improved technique, which we call nonlocal-means selective (NLMS) method, calculates weights for similar enough neighborhoods only, it can significantly reduce the computational time. Furthermore, the new method also improves the accuracy of the NLM method. This is due to the fact that the new method does not add weights of non-similar neighborhoods which could give negative impacts when calculating the final average. For a validation purpose, we tested our method on the following noise-free clean images: Figure 1. In order to compare the accuracy of the new method with the conventional methods, the

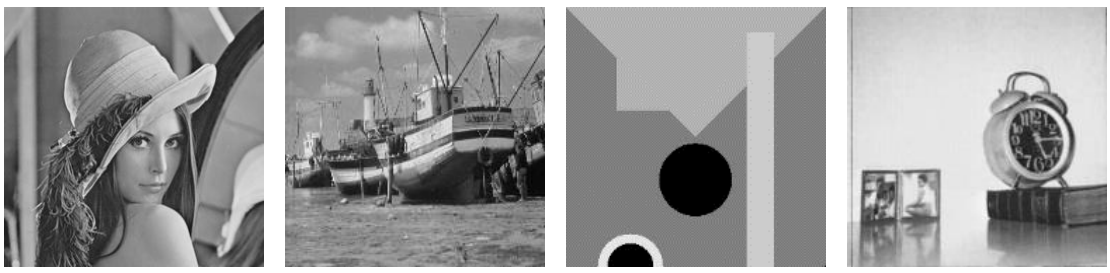

Figure 1. Original Images (Lenna, Boat, Block, Clock)

Peak Signal-to-Noise Ratio (PSNR) is defined as: $\mathrm{PSNR} = 10\ \log_{10}\left(\frac{\sum_{ij} 255^2}{\sum_{ij}(g_{ij}-u_{ij})^2}\right)$, where g is the original clean image and u is the denoised image. Note that more accurate the denoised image is, the higher the PSNR value is. Table 1

compares performances of NLMS methods and two conventional denoising methods, NLM and total variation (TV) methods. The TV method is based on minimizing the TV norm which is equivalent to solving a nonlinear elliptic partial differential equation. This is a classical novel denoising technique which reduces noise without much computation complexity. But it is much less accurate than NLM based techniques. The three methods are applied to the images in Figure 1 after synthetically adding noise at the PSNRs listed in the second column of the table. All algorithms developed for these methods are implemented in Matlab. As one can see from Table 1, the NLMS method outperforms the two

	PSNR				Time (secs.)		
	Noisy	TV	NLM	NLMS	TV	NLM	NLMS
Lenna	22.1003	28.3731	29.0487	29.4623	1.233861	170.280136	56.791578
Boat	22.1789	28.4209	28.7441	29.0047	4.820219	659.972051	262.20314
Block	22.3175	32.6271	35.5037	35.5631	1.247552	165.024191	71.01967
Clock	22.4176	28.9341	30.1621	30.5274	0.978391	165.217554	67.307156

Table 1. Performances of TV, NLM, and NLMS methods

conventional methods in PSNR for all 4 images. Although it takes more time to process the images than the TV method, NLMS takes almost three times faster than the original NLM method. Figure 2 shows the visual verification of the methods for Lenna image. In Figure 2, original clean Lenna image was contaminated by synthetically added noise (second

Figure 2. Lenna and its denoised images (TV, NLM, NLMS)

picture) and the noisy image was processed by TV, NLM, and NLMS methods (pictures 3-5). It is clearly verified that the NLM and NLMS methods produced much more accurate denoised images than the one solved by the TV method. As we have already seen in Table 1, NLMS gives more accurate result than the NLM method. Similar results can be seen in Figure 3. The Brain MRI image was naturally corrupted by noise and the three methods were again applied to denoise the image. It is clearly seen that the NLMS gives the best denoising results.

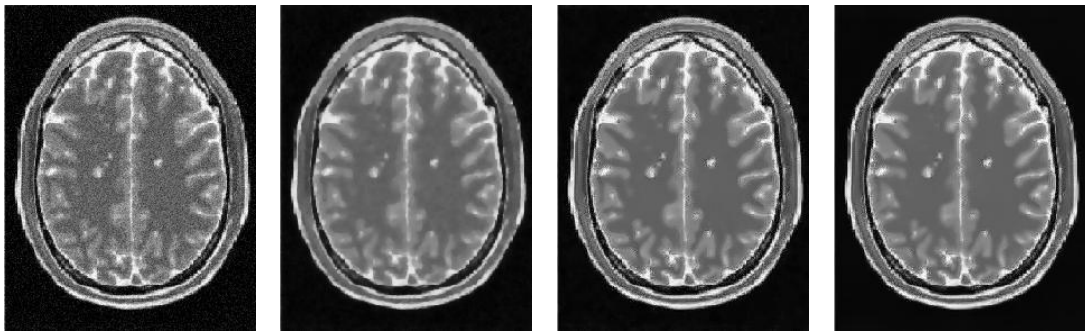

Figure 3. Brain MRI Image and denoised images using TV, NLM, NLMS (left to right)

Conclusions and Future Research: We have developed efficient and robust denoising method and its effective algorithm based on nonlocal means filtering technique. We have selectively calculated weights at pixels with similar neighborhoods by imposing special conditions. The new method significantly enhanced computational efficiency of the conventional nonlocal means method. The newly developed method was applied to several different numerical examples to verify that it outperforms conventional methods. The method is suitable for biomedical images since they often involve repeated patterns with spatially varying noise levels. We plan to further investigate more efficient and reliable denoising methods for biomedical images. When it needs to process hundreds or thousands of images, e.g. multiple 2D images produced from 3D computed tomography (CT) scan, our new method still may not be efficient. We are currently developing a way to reduce the computation time further by updating blocks of pixels instead of individual pixels.

Name: Wallace, Amy

Faculty Advisor: Dr. E. Samiel Winer

Project Title: *Protocol building and the study of reward devaluation*

This project for Ms. Wallace had two main goals. The first goal was to complete various lab protocols that further ensure the integrity of laboratory procedures. The second goal was to work with Dr. Winer, graduate student Gage Jordan, and Katelyn Majors, a current Honors Undergraduate Research Fellowship winner, to develop a research idea and submit a presentation for a conference using the data collected from a longitudinal study conducted by Dr. Winer, Jenna Kilgore, and Dr. Michael Nadorff.

Ms. Wallace developed protocols for various lab procedures with the help of Ms. Majors and undergraduate Lizzie Nichols. Protocols can be used as a reference tool if a question arises about a lab process. Also, protocols ensure that a secondary reference is available for training other undergraduate research assistants. Previous lab protocols were also updated. These lab protocols streamline the training process, increasing productivity.

In addition to developing protocol, Ms. Wallace was able to aid the research process of creating and submitting a presentation to a conference. Ms. Wallace earned third authorship on a poster submission that has been accepted to be presented at the upcoming annual ICPS convention in Austria. Ms. Wallace contributed in developing the research idea that an implicit measure could potentially be used to predict a selff report measure of happiness up to a year later and assisted with data analysis. Her work on this ICPS submission has aided in her knowledge of research.

During the project Ms. Wallace gained further knowledge about the relevance of organization in labs, as well as knowledge about grantsmanship and research. She also became more confident in her ability to conduct literature reviews and write clearly and scientifically.

Name: Welch, Bradley
Faculty Advisor: Dr. Jun Liao

Project Title: *Hydrogel Derived from Decellularized Porcine Aorta*

A. Project goal

We were able to remove all cellular contents from dissected porcine aorta using a previously discovered intact tissue decellularization procedure. The decellularized samples were then subjected to a phosphate buffered saline (PBS) wash. After thorough decellularizing and washing, the tissue samples were lyophilized and milled into an extracellular matrix (ECM) powder. In this study, we hypothesized that, if our porcine aortic ECM powder was subjected to previous non-aortic ECM hydrogel synthesis methods, our porcine aortic ECM powder would be converted into a hydrogel. However, the porcine aorta seems to possess significant biochemical differences from other previously converted ECM powders. Hence, our research was goal (1) to obtain an acellular porcine aorta, (2) to lyophilize and mill an ECM powder with a low lipid presence, (3) to successfully create a porcine aortic hydrogel using the ECM powder. The knowledge of heart ECM and of the biochemical properties of cardiac tissue is essential for understanding cardiac tissue engineering and regeneration.

B. Conclusion

Over the course of the summer, we were able to successfully meet the goals of our research plan. I am continuing this summer's project into the Fall 2016 semester and into the Spring 2017 semester. With the hope of a future publication, our new research plan includes rheology, electron microscopy, mechanical testing, cell infiltration measuring, and an *in vivo* study. Continued funding will be provided by the College of Agriculture and Life Sciences Undergraduate Research Scholars Program.