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Meeting Agenda

Thursday, July 27th, 2017

Time	Event	Location
1:30 pm	Registration	Salon Lobby
2:45 pm	Break	
3:00 pm	Leadership Speaker	Cabana
4:15 pm	Break	
4:45 pm	Poster Session	Salon C
6:15 pm	Banquet	Salon A & B

Leadership Speaker

Rafael E. Luna, PhD

*Executive Director of the National Mentoring Network, Principal Investigator
Administrative Core Boston College*



As the Executive Director of the National Research Mentoring Network and the Principal Investigator of the Administrative Core of NRMN located at Boston College, Dr. Rafael E. Luna utilizes data analytics to strategically grow NRMN and effectively reach all 50 states, including Hawaii, Alaska & Puerto Rico.

NRMN is funded by the National Institute of Health (NIH) and is a nationwide consortium to enhance the training and career development of individuals from diverse backgrounds, communities, and cultures who are pursuing biomedical, behavioral, clinical, and social science research careers (collectively termed biomedical research careers), through enhanced networking and mentorship experiences. The primary goal of NRMN is to enhance the participation in the biomedical workforce of individuals from groups identified as nationally underrepresented in the biomedical, clinical, behavioral, and social sciences research enterprise.

Dr. Luna earned his bachelor's degree in Biological Sciences from Southern University in Baton Rouge, Louisiana. During his junior year at Southern, he was one of six individuals selected from a nationwide competition to participate in the inaugural Biomedical Research Training Program at the National Institutes of Health, which ignited a passion for biomedical research. During his senior year at Southern, he was named as a Howard Hughes Medical Institute Scholar through Louisiana State University (LSU) and began doing molecular biology research. Dr. Luna thoroughly enjoyed his research experience, as he subsequently earned his doctorate in Biological Sciences at LSU. In 2015, Dr. Luna was named as one of six Keystone Symposia Fellows in Molecular and Cellular Biology.

Dr. Luna performed his post-doctorate research at Harvard Medical School, which centered on elucidating the sequence of protein-protein interactions leading to the decoding of the initial start codons of messenger RNAs. Dr. Luna held the position of Instructor in the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. He also held the role as Program Director for Senior Faculty Promotions in the Office for Faculty Affairs at Harvard Medical School.

In addition to doing biomedical research, Dr. Luna is the author of the book, *The Art of Scientific Storytelling*. He is a dynamic speaker and has taught his Scientific Storytelling method throughout the United States and Europe, e.g. Harvard Medical School, Harvard University, Massachusetts Institute of Technology, MIT-Koch Institute for Integrative Cancer, Wyss Institute at Harvard, Cervantes Institute- History Department at Harvard University, Children's Hospital-Boston, Brigham & Women's Hospital, Boston University Medical School, Dana- Farber Cancer Institute, University of Bergen (Norway), Saarland University (Germany), University of Graz (Austria), London School of Economics (England) and many more. Dr. Luna also believes in giving back to the community by serving as a little league coach for 10 years in inner-city Boston and four years ago was elected as the President of Mission Hill Little League.

Conference Abstracts

Mississippi INBRE Service Scholars (MISS)

S.1 **“The Effect of Sexual Education on Condom Usage in Teenagers, Ages 12-19 in Hinds Co.”**

Aleah Allen¹, Deja Abdul- Haqq², Miranda Joiner²

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According to the Centers for Disease Control and Prevention (CDC), student condom usage peaked and stalled at 60% a decade ago. In Mississippi, Hinds County ranks 48th in teenage pregnancy and 14th in STDs which reflects lack of condom usage among Mississippi youth. The lack of condom usage among teenagers age 12-19 is attributed the lack of sexual health education. The purpose of this research is to determine if targeted sexual education effects condom usages among African American teenagers in Hinds County. Theoretically, sexual health education increases self- reported condom usage. This research utilized secondary data analysis using findings from My Brother’s Keeper, Inc’s. Community Reach project that aims to reduce teenage pregnancy rates via comprehensive reproductive health training. Results from self-reported surveys completed by 162 teenagers were extrapolated from training pre- and post- test that gauged sexual health knowledge change. Results indicated that there was a 30% overall score increase in the knowledge. More importantly, there was a 41.8% increase in participants who felt that using condoms would be good for them in the next three months. Further, there was a 44.6% increase in participants who reported they would try to get their sexual partner to use condoms. In conclusion, sexual health education has a positive impact on attitudes on condom usage on teenagers in Hinds County. This study suggests that there should be greater access to sexual trainings opportunities for this priority population.

S.2 **“The Effects of Funding and Investments on HIV Testing and Prevention”**

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According to the Center for Disease Control and Prevention (CDC), Mississippi had the 9th highest rate of new HIV diagnoses in the nation in 2013. More specifically Jackson, Mississippi was ranked 4th among metropolitan statistical areas (MSA). A recent consumer survey was conducted by the Mississippi Health Department STD/HIV Office (MHD) to quantify HIV incidence, barriers, and most importantly develop strategies to prevent new HIV infections, increase access to care, improve health outcomes and reduce HIV-related disparities. The MHD survey showed 43.5% of people living with HIV in Mississippi were between the ages of 25-44 and 48.9 % between the ages of 45-64 were living in the Jackson MSA. Out all the people living with HIV (PLWH), 38% of PLWH reside in Jackson, more than half are living in rural areas that are not well served by medical providers and lack transportation networks. Although Mississippi does an excellent job with stretching limited resources, this epidemic calls for the need of proper funding, allocation of services and accessible clinics. The MDH STD/HIV Office is critically understaffed and undertrained about providing HIV/STD care due to lack of competitive salaries and lack of salary raises for all state employees in Mississippi in the past 7 years, creating the need for grassroots movements and organizations. The current model of funding for HIV/STD appears unstable, and within the additional cuts to the 2017-18 budgets, it is likely that Mississippi will continue to struggle with preventing, treating and caring for HIV in the foreseeable future.

S.3 **“The Association Between Condom Perception and Condom Usage Among MSM”**

Haley Bender¹, Dr. DeMarc Hickson², Deja Abdul-Haqq²

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According to Centers for Disease Control and Prevention (CDC), men who have sex with men (MSM), report the highest rates of HIV transmission. Condoms have been a staple method of HIV/STI transmission for decades. However, “condomless sex” has become a standard phenomenon. The purpose of this research is to determine how MSM perceive condom usage and if that perception reduces the probability of condom usage. Theoretically, there is a linkage between the perception of condom usage and the actual use of condoms. A secondary data analysis was conducted using results from the Minority AIDS Research Initiative (MARI) study conducted by My Brother’s Keeper (MBK) Center for Research, Evaluation and Environmental and Policy Change. During the MARI study, a series of questions were posed with select MSMs (208) regarding condom usage with casual partners. The results reflected a notable number of the study participants used condoms with casual partners. Of participants reporting no condom usage, 67% of them reported dislike of condoms. Moreover, of those reporting no use due to their dislike, 100% of their partners reported dislike and non-use as well. Thus, this research supports the concept of perception affecting condom usage. It is suggested that MSM communities be offered access to alternative HIV prevention options – like PrEP – in conjunction with more education and support regarding condom usage.

S.4 **“Not Really the Way You Pictured Your First Crib, Huh?”**

Sahtiah Cage¹, Jayla Collins², Krystal Phillips³

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Center for Disease Control and Prevention (CDC) suggests teen pregnancy and childbearing brings substantial social and economic costs such as lower academic achievement, increased health issues, and poverty. Also contributing to an increase in global finances, childbirth accounted for at least \$9.4 billion in costs to U.S. taxpayers for increased health care, foster care, and lost tax revenue due to lower educational attainment and income among teen mothers in 2010. In 2015, a total of 229,715 babies were born to women aged 15-19 years, having a substantially higher teen pregnancy rate in the U.S. among Hispanics and Non-Hispanic Blacks. Though, from the peak year 1990 to 2015 there has been less than 34.8 births per 1,000 girls in Mississippi. It was reported there has been a major decrease in teen birth rates from 81 percent to less than 50 percent in the state of Mississippi. The aim of this study is to determine whether informative teen pregnancy interventions are effective in promoting contraceptive use among teens aged 13-18. Survey results showed there was a significant increase in the number of individuals who found sexual classes beneficial ($p=0.017$) and those who decided to use a condom within the next three months ($p=0.036$). However, responses on condom usage, STDs, and pregnancy conception significantly decreased between surveys. Possible sources of error included decreased attention span and unclear interpretation of presented questions. At the conclusion of this study, it was determined that the data supports the hypothesis of increased awareness on teen pregnancy and contraceptive use amongst teens as effective means in preventing teen childbirth. A limitation of the study would include the small sample size which is not an attributable representation of the entire population of teens in Mississippi. However, this study suggests that increased awareness of STDs and contraceptive use can decrease childbirth rates amongst teens. Promoting healthy sexual behaviors and providing additional resources for teen pregnancy prevention as well as beneficial alternatives to those who may have become pregnant can increase positive outcomes within these individuals.

S.5 **“Cervical Cancer Risk Factors for Women with HIV: Recommendations to Decrease Mortality”**

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The number of deaths from cervical cancer has decreased over the last 40 years; however, women with HIV have a six-fold greater chance of being diagnosed with invasive cervical cancer. Lower CD4 counts create a greater risk; hence, the chance of cancers is increased in individuals who have HIV. In the clinical latency stage, the body is still at a greater risk of developing cervical cancer than a woman living without HIV. According to the Center for AIDS Information and Advocacy, rates of Cervical Intraepithelial Neoplasia (precancerous abnormality, CIN) in women with HIV versus without HIV were 14.3% versus 4.3% for CIN1, 11.3% versus 3.6% for CIN2, and 6.8% versus 2.5% for CIN3 or worse demonstrating a disparity in HIV positive women. For HIV positive women, a bi-annual pap smear test along with regular STD testing is recommended to detect abnormal changes in the cervix and distinguish precancerous growths. Antiretroviral drugs, HPV vaccines, and protection can decrease risks. However, radiotherapy and chemotherapy are not available in many resource-limited settings for reasons of cost and limited health infrastructure thus higher rates among rural women (40%), women living in poverty (43%), and women aged 50 to 64 (47%). Therefore, easier access to healthcare would significantly decrease the risks of HIV positive women being diagnosed with cervical cancer. Thus, it is crucial to have easy access to health care. Lower cost for STD testing, bi-annual pap smears, HPV vaccines and antiretroviral therapy will decrease rates by increasing the overall health of the individual.

S.6 **“Reducing Adolescent Obesity Through a High School Intervention Program in Mississippi”**

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While the American Medical Association classified obesity as a disease in 2013, it is still a condition that is considered preventable. Being physically active and nutritionally aware are lifestyle components that aid in preventing the disease; however, these components must be carried out habitually. It can be difficult for adults to form healthy habits, but adolescents are still forming habits daily. If high school students are exposed to such lifestyles through their schools, obesity rates could decrease and also prevent the development of obesity in adulthood. According to stateofobesity.org, Mississippi has the highest rate of obesity among high school students at 18.9% and another 17.1% of students classified as overweight. With the Mississippi Department of Education accounting for over 130,000 enrolled in high schools, these students need to incorporate healthy habits into their lifestyle. With the program, each student would have the option of eating from a fresh fruit and vegetable bar coupled with daily walks with their classes. The intervention would be school wide and incorporated into the everyday lives of teachers and students. Every student in the school would be weighed twice a year while the number of students choosing to eat from the fresh bar would be documented by lunch registers. With the CDC publishing guidelines that identify school policies and practices most likely to be effective in promoting lifelong physical activity and healthy eating, this in-school intervention could form healthy habits and thus reduce adolescent and then adult obesity rates.

S.7 **“Are People Living with HIV More Likely to Suffer from Depression Than People Living Without HIV”**

Ilivia Jackson¹, Tonya Adams²

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In society, the negative stigma surrounding HIV can create a world of isolation. Those who suffer from it are likely to deal with feelings of rejection and deepening sadness. Depression is a mental health disorder characterized by a persistently depressed mood or loss of interest in activities, causing significant impairment in daily life. HIV is a virus that is spread through certain body fluids that attacks the body's immune system. According to the World Health Organization (WHO), 350 million people worldwide suffer from depression. There are 70 million people around the world living with HIV and 47% are suffering from depression. It can cause the affected person to suffer greatly and function poorly at work, school and in the family. The purpose of this project is to show that people living with HIV do not suffer more with depression than people living without HIV. The Hypothesis was tested using My Brother's Keeper (MBK) Shield Intervention 2016 questionnaire survey that was given to people living with HIV and to people who were HIV negative. The result shows, 26.1% of people living with HIV stated that they are rarely or never felt depressed; while, 35.9% felt depressed. The result of people living without HIV, 73.9% rarely or never felt depressed; while, 64.1% felt depressed. As a collective, the stigma that people living with HIV suffer more from depression than people living without HIV should cease and focus more on those who actually need help with depression.

S.8 **“Investigate the Socio-Economic Factors of Birth Defects Among Children in Mississippi”**

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Purpose: The purpose of the study was to investigate the association between the prevalence of birth defects in Mississippi and social determinants of health (income, education, poverty level, and access to healthcare). The hypothesis is that socio-economic status contributes significantly to the high prevalence of birth defects in Mississippi.

Method: A comparative analysis was conducted to examine demographic data for Mississippi counties with the highest and lowest rates of birth defects. Data was collected from the CDC, U.S. Census Bureau, Mississippi Department of Health, and March of Dimes. SPSS was used to analyze the data.

Results: The Pearson Correlation Analysis showed a positive correlation between poverty levels and prevalence of birth defects (.667) indicating that increased poverty levels contribute significantly to increased birth defects. The data is also a strong negative correlation between median household incomes and prevalence of birth defects (-.720). This means that as the household income increase, the prevalence of birth defects will decrease. Results of the comparative analysis revealed the counties with the highest prevalence of birth defects in Mississippi were Neshoba, Newton, Jasper, Lauderdale, and Jones (avg. 452 per 10,000 live births) The counties with the lowest prevalence of birth defects were Jackson, George, Marshall, Desota, and Hancock (avg. 80 per 10,000 live births).

Conclusion: The study revealed that socio-economic status significantly correlated with high prevalence of birth defects in Mississippi. Reducing economic barriers to screenings may result in a considerable decrease in birth defects in underserved communities.

S.9 **“Body II Body: A Reproductive Health Awareness Campaign for Adolescent Students Ages 13-18”**

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According to the CDC, in 2014 nearly half of the 20 million people diagnosed with HIV and STDs were ages 15-24. In 2014, Mississippi ranked fourth in HIV, fourth in Chlamydia, and third in Gonorrhea. These infections were highest among individuals ages 15-29. Many young people engage in sexual risk behaviors resulting in unintended health outcomes such as HIV/STDs infection. Because of various factors, many young people, especially adolescents, lack knowledge about HIV/STDs infection. The *Body II Body* (B2B) campaign aims to educate adolescents ages 13-18 about the contraction and prevention of HIV/STDs through a community outreach approach (i.e. educational sessions) in urban and rural areas. At two educational sessions (one urban – Jackson, MS, one rural – Port Gibson), adolescents received information about HIV/AIDS and STDs. Each adolescent was given a pre- and post-test to assess their level of knowledge before and after the session, and completed a course satisfaction survey with the added benefit of a resource guide. In the rural area, results showed a 1.83 difference in pre- and post-test assessments yielding a 12.2% change in knowledge. In the urban area, results showed a 2.80 difference yielding an 18.7% change. Even though the urban area had a larger increase in knowledge, adolescents in rural areas scored higher on their pre-tests, which signifies that adolescents in rural areas had more prior knowledge than those in urbanized areas. Jackson is an area with more services and outreach teams. Therefore, public health workers conduct more outreach and educational sessions in rural areas.

S.10 **“Assessing the Effectiveness of Many Men, Many Voices (3MV) Intervention in the Mississippi Gulf Coast Region”**

Thuy Phuong Le¹, Zedekiah Toy², Telvin Harrington³, Joseph Lindsey⁴

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The Mississippi Gulf Coast region is the second highest district in Mississippi in regards to number of new cases of HIV each year. Among those infected with HIV, the prevalence of HIV is disproportionately high for African-American men who have sex with men (MSM). The purpose of this study is to determine the effectiveness of an HIV Prevention intervention with Black MSM in this region. We hypothesize that a HIV prevention intervention would be effective in motivating participants to reduce risky sexual behaviors and increase their use of condoms. Many Men, Many Voices (3MV) is an evidence-based intervention program that targets African-American MSM to prevent STDs and HIV through 7 interactive group sessions. The 3MV program was implemented in the Mississippi Gulf Coast in 10 waves with a total of 95 participants from February 2016 to May 2017. The analysis included pre- and post- 3MV intervention survey responses. Results show increased community connectedness to the Black gay community, higher confidence levels in getting a sexual partner to use a condom, improved attitudes of using a condom for dominant and receptive partners, and increased confidence in their skill to use a condom efficiently. It is concluded that 3MV was not only effective in encouraging participants to reduce risky sexual behaviors and increase their use of condoms but as well as reducing social and cultural tensions with increased community connectedness. The proven effectiveness of this study supports the use of 3MV as an HIV prevention intervention for Black MSM.

S.11 **“The Affect of Farmer’s Markets on Both Urban and Rural Communities”**

Kyla Lewis¹, Deja Abdul-Haqq²

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The purpose of my project is to show the priorities of urban and rural communities related to farmers markets. The secondary data analysis includes findings collected from farmers market participants in Hinds County and Natchez/Wilkinson County. The hypothesis of this analysis is that because of the differences in location and demographics, the priorities related to farmers markets would be different. In both Jackson and the Southwest Delta region, most of the people that visited these markets were women, and the main reason for visiting the market, amongst men and women, was to support local farmers (Jackson: 53%, Natchez: 80%). In Hinds county, 88% of the people that visited the market were African American, and 82% of people that visited the market in Natchez/Wilkinson counties were Caucasian. With these different demographics, findings still show that these counties had many similar market priorities. These include shopping at grocery stores outside of the farmers market and agreeing that the produce in the grocery stores are about the same price as those at the markets. In the counties the farmers market visited, the citizens had intentions of consuming more fruits and vegetables in the future.

S.12 **“The "Haves" and "Have Nots": An Analysis of the Impact Socioeconomic Status has on Teen Pregnancy Rates in Central MS”**

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³*My Brother’s Keeper, Inc., Jackson, MS*

Mississippi has one of the highest rates of teen pregnancy in the United States. According to recent statistics, Mississippi ranks 3rd in teen birth rates among girls ages 15-19 (34.8 per 1,000 girls). This research will examine the impact socio-economic factors have on the rates of teen pregnancy in Madison, Rankin, and Hinds counties. To analyze socioeconomic factors and teen pregnancy in these counties, statistics from the Mississippi State Department of Health and County Health Ranking documents were utilized. Hinds county ranked the highest in teen pregnancy among the tri-county area followed by Rankin county in the year 2016. As of 2016, Hinds county teen pregnancy rate was 52 per 1,000 females ages 15-19 compared to Rankin and Madison counties whose teen pregnancy rates were 41 and 33 per 1,000 females ages 15-19. Hinds county median household income is \$37,324, whereas Rankin county median income is \$58,801 and Madison county median income is \$64,376. Hinds county had a graduation rate of 70% compared to Madison and Rankin counties graduation rates were 83% and 85% as of 2016. Research data reveals the counties with low socio-economic status have higher teen pregnancy rates and counties with higher socio-economic status have lower rates of teen pregnancy, which clearly suggests that socio-economic status has a direct impact on teen pregnancy rates.

Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

S.13 **“Does the Mental and Emotional Impact of Stress Cause Psychological Consequences?”**

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The aim of this study is to determine how many individuals are affected by psychological consequences of mental health. Mental Health includes the emotional, psychological, and social well-being. Mental health is more than just being incapable of managing individual’s daily lives. There are many different mental illnesses, including depression, schizophrenia, attention deficit hyperactivity disorder (ADHD), autism, and obsessive-compulsive disorder. Each illness alters a person's thoughts, feelings, and/or behaviors in

distinct ways. Even though there are many different illnesses there are treatments to help. Data was collected from Web D-Intellectual Disability Information and The National Center for Biotechnology Information-Morbidity of Psychiatric Disorder. Additionally, graph results were done based on various mental health questions. Data was also collected from Open Arms Healthcare Center. There were several questions issued to The Becoming a Healthier you participants based on their mental well-being. Questionnaires were issued to the participants during the patient registration. Data was randomly selected from 50 men and 50 women ranging from ages 18 through 55. The participants were randomly selected from the month of June 1st through July 1st. In conclusion, we have studied that mental health affects the everyday life of individuals. It affects the emotional impact of the stress of the overall well-being and it also causes psychological consequences. Based on the data the no percentages on depression were higher than yes percentages. The data also shows that more individuals have attempted suicide before. It also shows that attempted suicide in the African American race was higher than the Caucasian race.

S.14 **“Perception of PrEP Amongst African American Women in the Jackson, MS MSA”**

TaQuane’ Sanders¹, Deja Abdul-Haqq²

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²*My Brother’s Keeper, Inc., Jackson, MS*

Women make up nearly half of those infected by HIV globally and 20% of new infections in the US, demonstrating a need to optimize HIV prevention options in this population. Pre-exposure prophylaxis (PrEP) is a proven HIV prevention strategy, but PrEP knowledge remains low among women though clinical trial data demonstrates effectiveness of PrEP for reduction of HIV acquisition among women when used consistently. However, social, economic, and behavioral factors and lack of access to and knowledge about PrEP can contribute to HIV transmission. The purpose of this study is to enhance the body of scientific knowledge regarding PrEP and the low percentage of African American women that know about it. A concise survey was conducted for the study. Survey items assessed demographics, HIV status, sexual practices, and prior knowledge of PrEP. Following completion of the survey, all participants were offered PrEP information. Respondents were all African American women (n=73). Respondents were 87.1% heterosexual. Though a notable number of participants reported being sexually active, only 31% had previously heard of PrEP. The majority of respondents (71.8%) accepted information about PrEP after completing the survey and 80.3% expressed that they would encourage their sexual partners to use PrEP. Thus, study findings support the theory that few African American women know about PrEP and more African American woman would consider it for themselves and their partners after PrEP education. Furthermore, it is suggested that PrEP messaging should target African American women to possibly reduce HIV exposure in this disproportionately affected population.

S.15 **“Is Your Friendzone Beneficial? The Influence of Social Support on Body Image, Optimism, and Sexual Practices”**

Xavier Short¹, Kambria Badgett^{2,3}, Obie McNair, MPH³

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African-American men who have sex with men (AAMSM) are usually the targeted group associated with HIV prevention since the 1990s. AAMSM are more at risk to contract the virus and this increases for those between the age of 13-24. Social networks, and their input on practicing safe sex, shape the decisions made by the individuals during the time of having condom-less anal intercourse. For this project, we analyzed effects of optimism, body image, and social support to see if it plays a role in the lack of condom usage within AAMSM. For this cross-sectional analysis, we included data from 333 AAMSM participants enrolled in The MARI Study. The analysis consisted of descriptive statistics and Pearson’s and Spearman correlations to determine associations. The variables included in our analysis focused on areas of sexual behavior, optimism, social support, and body image. The results showed a heavy influence an inner social

support system has regarding sexual engagement, body image, and optimism. We discovered a positive correlation between opinions regarding condom use and casual sex partners. There is also a positive correlation between negative perceptions of body image and social support. Lastly, we found a positive correlation between optimism and inner social support networks. The effects of social support, optimism, and body image, are all intertwined and extremely dependent on each other. We discovered that decisions made by the individual's personal social circles influenced the choice to not practice safe sex. Future research should examine the variables relationships with social support more deeply.

S.16 **“How Do Mental Health and Coming Out Correlate with Men Who Have Sex with Men?”**

Deshara Thomas¹, Maranda Joiner², Deja Abdul-Haqq²

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The purpose of my project is to show how coming out correlates with the mental health of men who have sex with men after they come out to their families. Depression and anxiety both correlate with coming out. Some men who have come out to their families are not met with positive reactions, which can cause depression and anxiety. In a My Brother's Keeper study conducted in 2016, most men who have sex with men had come out to their families. 54% of men had come out to their families, 25% were somewhat open about their sexuality with their families, and 20% had not told any of their family members about their sexuality. Men who come out to their families may experience homophobia; and in extreme cases, violence. The aim of this study is to show that coming out does not always lessen depression and anxiety. There is a correlation between coming out and mental health, especially with men who have sex with men. Although the correlation is not statistically significant, there is a strong association with mental health and coming out with men who have sex with men. Of the 54% of men who had come out to their families, 18% experienced moderate depression. In closing, mental health issues such as, but not limited to, depression and anxiety in men who have sex with men who come out do correlate.

S.17 **“The Association Between Patient-Reported Exposure and Rectal Chlamydia and Gonorrhea in Young Black MSM”**

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African Americans are disproportionately affected by human immunodeficiency virus (HIV) than any other race. Among African Americans, young males who have sex with men (MSM) are an extremely high risk population for HIV transmission¹. The rates of STIs, more specifically, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) ranked fifth and third in the state of Mississippi, respectively². The Centers for Disease Control (CDC) recommends routine urethral, pharyngeal, and rectal screenings to combat dual epidemics as CT and GC often present as co-infections.³ The objective of this study was to conduct a secondary analysis of the association between patient-reported exposure with the incidence of rectal GC and CT infections in young black MSM (YBMSM) from two urban STD clinics in Jackson, MS. The sample (N= 609) included YBMSM ages 15-29. The mean age of this group was 22.6 years and 27.4% were HIV positive. Over half (60.2%) had some college education and were currently employed (56.6%). Of the participant population that reported to only engage in receptive anal sex (RAS), 30.6% (99% CI) presented with CT/GC infections regardless of consistency of condom use. Overall, CT/GC infection rates in this population were high. A large number of infections were identified at anatomic sites not consistent with reported exposure in the past 90 days. These findings may implicate that clinicians should consider screening individuals for CT/GC at all anatomical sites regardless of reported exposure.

Mississippi INBRE Research Scholars (MIRS)

R.1 **“The Impact of AGE-RAGE Signaling on Mitochondrial Function in an Aging Mouse Model”**

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Over time, elevated blood glucose levels lead to the accumulation of advanced glycation-end products, or AGEs. These non-enzymatic modifications of proteins and lipids contribute to cellular dysfunction. Of particular importance to the heart, the covalent modification of extracellular matrix proteins reduces their overall elasticity contributing to myocardial dysfunction. In addition, extracellular AGEs are recognized by RAGE, a transmembrane receptor whose activation leads to increased RAGE expression, further contributing to cellular stress. Observational data from our labs suggest that mice harboring a deletion of RAGE (R-/-) have a longer lifespan than their wild type (WT) littermates. Microarray data found that these mice had elevated transcript levels of genes involved in mitochondrial biogenesis. Given the association between cellular aging and mitochondrial function, the aim of this project is to compare the levels of mitochondrial and stress-related proteins in heart tissue isolated from WT and R-/- mice. Based on our preliminary data, we hypothesize that knocking out RAGE will stop downstream signaling activities resulting in higher mitochondrial activity relative to wild type littermates. To test this hypothesis, total RNA from heart tissue will be isolated using TRIzol for use in real-time qPCR to identify differences in mRNA transcript levels for select mitochondrial proteins. In parallel, samples will be processed for protein extraction for Western blot analysis using antibodies directed against mitochondrial proteins as well as proteins known to be involved in cellular stress. Identifying differences between WT and R-/- may shed light on a potential role for AGE/RAGE signaling in organismal aging.

This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) under grant number P20GM103476.

R.2 **“Histomorphometric Analysis of the Kidney of Ovariectomized Rats Following the Release of an NPY -1 Receptor Antagonist”**

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Neuropeptide Y (NPY) is a 36-amino acid neuropeptide that acts as a neurotransmitter in the brain and in the autonomic nervous system of humans. In the autonomic system it is produced mainly by neurons of the sympathetic nervous system and can activate at least 5 subtypes of receptors (Y1-Y5). Innervation of the Y1 receptor induces vasoconstriction while Y2 reduces the constriction. Both Y1 and Y2 receptors have been found in heart and renal tissue. Recently, NPY has been shown to increase during stress and in the hypothalamus in post-menopausal women. The goal of our study was to evaluate the kidney of ovariectomized rats and determine the role of the NPY 1 receptor in the kidney by using sustained delivery of a selective Y1 receptor antagonist. Twenty female Sprague Dawley rats were divided into four equal groups (intact control, OVX control, SHAM (non-drug loaded TCP capsule), and TCP+NPY-1RA (implants containing 5 mg of NPY receptor antagonist). Body weights, organ weights, and kidney tissues (glomeruli length, width and area) were measured, evaluated, and the results were compared for statistical differences. Overall, body weight and kidney wet weights were increased in SHAM and OVX control animals when compared to intact control animals, for the duration of the study. At two weeks there were no significant differences in glomerular area between intact control and treatment groups. After 4 weeks the animals in the OVX and SHAM groups had body weights and glomerular areas which

were statistically larger than NPY-1RA treated or intact control. After 8 weeks OVX and SHAM animals had body weights that were larger than intact control and NPY-1RA but the changes seen at the glomeruli were no longer evident. Whereas in the glomerular area in the NPY-1RA treated animals were statistically smaller than all treatment and intact control animals. Conclusion: NPY-1RA treatment was capable of reversing OVX associated weight gain for the duration of the study. NPY-1RA resulted in a time dependent decrease in glomerular cross sectional area which may result in modulation of glomerular filtration rate, and additional studies are need to determine if the changes are due to changes in intra-renal hemodynamics.

R.3 **“Early Life REM Sleep Deprivation Affects Behavior in Young Rats”**

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The purpose of this current experiment is to investigate the effects of rapid eye movement (REM) sleep deprivation on the behavior of young rats. During early life development there are critical periods (CPs) during which the nervous system is sensitive to environmental changes. Specifically, these environmental stimuli can affect the organism's function and behavior later in life. Recent research has showed that during these CPs of early life development, REM sleep disturbances can create vulnerabilities to neuropathologies in juveniles and young adults. Additionally, these disturbances can cause reductions in hippocampal synaptic plasticity, which is the ability of synapses to strengthen or weaken over time. Our research aims to examine how early life REM sleep deprivation (ERD) can affect social development and related behavior. This will be tested using two procedures, an Open Field Test and Juvenile Play Behavior observations. Both of these procedures allow for comparisons to be made between ERD rats and their control counterparts. In each procedure, specific behaviors are looked at that would indicate anxiety or nervous behavior, both of which are associated with symptoms often seen in depressed patients and may result from altered brain maturation. Given the known effects of REM sleep deprivation on brain maturation, we expect the ERD rats to display these behaviors at a higher rate than their control counterparts.

R.4 **“Does Hypoxia and/or Endothelin affect CYR61 protein expression in smooth muscle cells derived from Uterine Fibroids?”**

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Studies have identified imbalances in angiogenic factors such as cysteine -rich protein 61 (Cyr61) in women with uterine fibroids. Low oxygen conditions, such as hypoxia, have been shown as a possible mechanism to increase endothelin-1 and potentially lead to aberrant expression of Cyr61. We hypothesize that hypoxia decreases CYR61 expression via activation of the ETA receptor. Under an IRB approved study fibroid and myometrium tissues were excised from the uterus of women undergoing hysterectomies for uterine fibroids (n=7). Tissue was washed in phosphate-buffered saline and digested in a collagenase solution for 6hrs. The resultant smooth muscle cells were grown until confluent under normal atmospheric conditions at 37°C before, prior to trypsinization and seeding at 50,000 cells/well. Cells were cultured under normoxia (6% oxygen) or hypoxia (1% oxygen) for a period of 24hrs with/without 1µM of the ETA receptor antagonist; ABT-627. Cell culture supernatant was collected for ELISA (CYR61; RnD Systems) and SMCs were harvested for western blot (CYR61; Santa Cruz). Results were analyzed with ANOVA and paired t test. There was not a significant difference in CYR61 secretion between mSMCs and fSMCs exposed to hypoxia (p=0.823). CYR61 secretion was significantly decreased in fSMCs exposed to hypoxia compared to normoxic fSMCs (p=0.03), similar results were seen with mSMCs (p=0.03). Blockade of the ETA receptor prevented a hypoxia-induced decrease of CYR61 secretion in

fSMCs (p=0.521) and in mSMCs (p=0.339). These results suggest that hypoxia decreases CYR61 secretion from SMCs and also that blockade of the ETA receptor prevents these decreases.

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R.5 **“Expression of *Streptococcus pneumoniae* Surface Proteins for identification of Host Cell Ligands”**

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Streptococcus pneumoniae is a Gram-positive colonizer of the human nasopharynx, sometimes causing sinusitis and otitis media or invasive infections such as pneumoniae, bacteremia, and meningitis. Colonization is made possible by the binding of bacterial surface proteins to host cell receptors on the epithelium. Our lab has previously identified that the zinc-binding surface protein AdcAll plays a role in colonization by *S. pneumoniae*. Therefore, we hypothesized that AdcAll and related zinc-binding proteins PhtD and PhtE could interact with specific host cell proteins on the epithelium. To test our hypothesis, we used a staphylococcal expression system to produce each protein fused to a staphylococcal Hla signal sequence for secretion into the medium for purification. We utilized splicing by overlap extension (SOEing) PCR to fuse the genes to the Hla signal sequence. Once fused, the PCR products were cloned into plasmid PMin166 transformed into *S. aureus*. Recombinant proteins, expressed by *S. aureus* were purified by affinity chromatography and will be used for far-western blotting with the potential pneumococcal adhesions. Characterizing such interactions will be an important first step in developing vaccines or drugs that block colonization.

Acknowledgement: This work was funded by an Institutional Development Department Award (IDeA) from the NIGMS under grant number P20GM103476.

R.6 **“Neuroprotective Effects of Estradiol and Genistein in Zebra Finch Cerebellum”**

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Estradiol (E₂) and aromatase are associated with neuroprotective and neurogenerative effects in vertebrates. Zebra finches are frequently used in such studies because the avian brain is highly steroidogenic and plastic. The cerebellum is thought to have low plasticity. However, it is highly steroidogenic and has many estrogen β receptors (ERβ). In zebra finches, we have found that constitutively low aromatase, an enzyme that synthesizes testosterone into E₂, is upregulated by cerebellar lesions and that these lesions impair cognitive and motor functions. Furthermore, exogenous E₂ improves cognition post-lesions while the aromatase inhibitor, letrozole, reduces E₂-induced recovery of cognitive and motor functions. It is unlikely that the effects of E₂ and aromatase are merely correlated. Instead, E₂ and aromatase most likely have independent roles in cerebellar neuroprotection via neuronal-ERβ and aromatase-induced reactive glia. The phytoestrogen, genistein, may play a role in cerebellar neuroprotection, as it acts potently on ERβ. Additionally, genistein is known to have fewer negative effects on male reproduction and, for some cancers, is reparative rather than inductive like E₂. In our study, male zebra finches received E₂ (500 mg) and genistein (1000 mg) or the vehicle (silastic adhesive) via subcutaneous implant for 12 days, followed by unilateral puncture lesions to the cerebellum accompanied by intracerebellar injections of letrozole or saline. Birds were sacrificed 48 hours later. Birds were weighed pre-implant, at implant, 5 days post-implants, at lesion, and at sacrifice. Brains, serum, and testes were collected. Lesion sites will be stained with TUNEL and Fluoro Jade B to label apoptotic and degenerating cells, respectively. The quantity of dying cells surrounding the lesion will be compared treatments effects on neuroprotection. Testis mass and volume, and sperm maturation will be examined to determine if E₂ is related to size and activity reduction in testes. We will use HPLC to determine estrogen, testosterone, and genistein levels in serum. If E₂ and aromatase have additive effects in neuroprotection, cell death will be highest in the silastic+letrozole group, and smallest in the E₂+Saline

group. As expected, intracerebral aromatase had no effect on testis or body mass. E₂ but not genistein, reduced testis mass. Unexpectedly, birds treated with E₂ and silastic lost weight across the study, but genistein birds did not. Results thus far suggest implants were effective at time of sacrifice.

Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the national Institutes of Health (NIH) under grant number P20GM103476.

R.7 **“Molecular Characterization of α -D-Galactosidase in the Lone-Star Tick, *Amblyomma americanum*”**

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Mammalian food allergies is an emergent allergy, increasingly widespread in tick endemic areas in the United States of America, and also worldwide where ticks are endemic. Intriguingly, research has shown that tick bites, specifically by the lone-star tick (*Amblyomma americanum*), might be solely responsible for causing an increase in IgE-mediated allergic reactions to galactose- α -1,3-galactose (α -gal) with the southern and eastern US. The objective of this study is to functionally characterize the lone-star tick α -D-Galactosidase enzyme and assess its role in α -Gal synthesis. In our previous study (Karim and Ribeiro 2015), α -D-galactosidase was identified, which cleaves terminal α -linked galactose sugars from larger glycan structures. This enzyme is part of a pathway that can lead to the production of UDP-galactose, which is necessary for the synthesis of precursor glycan structures that can be capped with galactose. Therefore, we hypothesized that silencing of α -D-Galactosidase by RNA interference approach will impair the tick's ability to synthesize α -gal, and change the overall saliva glycan profile. The time and tissue-dependent transcriptional expression of the α -D-Galactosidase revealed the highest in unfed tissues and during early attachment on the host. Further, silencing of the target gene will provide the clear picture of its functional role in tick's ability to synthesize α -gal, and hematophagy.

R.8 **Histomorphometric Analysis of the Kidney Glomeruli of Ovariectomized Rats Following Sustained Delivery of Estrogen Replacement Therapy**

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Recent research has shown that older women who are taking hormone replacement therapy containing estrogen (E-HRT) had increased blood pressure. In addition, E-HRT is associated with an increase in the formation of kidney stones. Because kidney stones and blood pressure are influenced by a variety of lifestyle and other related health factors, the true impact of E-HRT on kidney ultrastructural changes has not been fully determined. The goal of our study was to evaluate the kidney of ovariectomized (OVX) rats and determine the role of E-HRT at 2, 4 and 8 weeks' post-implantation of a tricalcium phosphate (TCP) delivery device containing estrogen. Twenty female Sprague Dawley rats were divided into four equal groups (intact control, OVX control, SHAM (non-drug loaded TCP capsule), and TCP+E (implants containing 5 mg of estrogen benzoate)). Body weights, organ weights, and kidney tissues (glomeruli length, width and area) were measured, evaluated, and the results were compared for statistical differences.

RESULTS: Overall, body weight and kidney wet weights were increased in SHAM and OVX control animals when compared to intact control animals, for the duration of the study. There was a significant ultrastructural change in kidney size in the OVX control and SHAM animals compared to the intact control. At two-weeks, there was a significant increase in the area of kidney, and by 8 weeks the area of the kidney was significantly reduced compared to intact control animals. There also was a statistically significant increase in glomerular area, length, and width. Significant effects of TCP + E were seen at the 4-week time point. Sustained delivery of estrogen reversed weight gain and the kidney changes seen in the OVX control and SHAM treated animals. Removal of the ovaries results in loss of estrogen and increases in

body weight, kidney wet weight, and overall kidney size, with substantial changes in the glomeruli which were reversed by HRT.

R.9 **“Investigation of the Effects of the Novel Kappa Agonist, Nalfurafine, on the Abuse-Related and Antinociceptive Effects of Oxycodone”**

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The consistent rise of prescription opioid related mortality in the United States has led to the search for new formulations of clinical analgesics that will deter abuse. One approach for abuse deterrent formulations is to combine traditional opioid analgesics with other compounds that attenuate the euphoric effects. One class of compounds, kappa agonists, have been shown to produce this effect. *Objectives:* We hypothesized that nalfurafine, a novel kappa agonist, would be able to lower the abuse liability of oxycodone without reducing the therapeutic effects of the drug. Furthermore, we hypothesized the antinociceptive effects would vary as a function of sex.

Methods: A progressive reinforcement ratio was used to allow male subjects to intravenously self-administer oxycodone alone, nalfurafine alone, and varied mixtures of the two in order to determine the effect of nalfurafine on the reinforcing effects of oxycodone. Next, the same subject group was tested for antinociception with oxycodone and nalfurafine, either alone or as mixtures. Subsequently, male and female rats were compared with antinociception induced by oxycodone and nalfurafine, alone and combined. Adding nalfurafine to oxycodone decreased oxycodone self-administration in a dose-dependent manner and enhanced oxycodone-induced antinociception. A subsequent test revealed no sex differences in the antinociceptive effects of nalfurafine and oxycodone when administered separately or as mixtures. These findings confirm the possible utility of a mu/kappa agonist combination in the clinical treatment of pain without the unintended consequence of addiction traditionally associated with opioid analgesics.

R.10 **“Torque Properties of Porcine Patellar Tendon”**

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As a result of athletic injuries, the anterior cruciate ligament (ACL) is commonly replaced by the central third of the patella tendon (PT). The ACL failure mechanism is complex; however, research has shown that its failure is commonly associated with a torsional stress state. Research studies suggest that the PT, when compared to the hamstring replacement of ACL and allografts, is a more effective replacement for the ACL. The next step is to quantify the torque properties of the PT and determine if its properties are close to that of the ACL. For this study, porcine PTs were subjected to a torque stress state with a customized device developed in-house. The PT was attached to the patella bone at its proximal end and tibia and fibula at the distal end. Both the patellar bone and tibia/fibula were cemented into separate blocks and then loaded onto the torque device. One end of the device was connected to the gear, which turned the potted bone while the other potted bone remained stationary. This motion allowed for capturing tendon torque. Experimental data showed an increasing linear trend as is also shown in the typical stress-strain response of tendon in tension. These current results suggest that the novel test setup works properly, the recorded data was from the tendon only, and the tendon can withstand torque. Future testing, including testing to failure, will allow for comparisons to the ACL torque failure rates that have already been observed through prior research studies.

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- R.11 **“In Vitro Effects of Doxorubicin and ELP Bound Doxorubicin on Cell Proliferation and Cell Uptake”**
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Doxorubicin (Dox) is a chemotherapeutic drug used to treat breast cancer and many other types of cancer, but for this project I only focused on the breast cancer cell line MCF7 and the multi drug resistant cell line NCI/ADR. Using those two cell lines, I focused on the in vitro effects of free doxorubicin and Elastin-like polypeptide (ELP) bound doxorubicin on cell proliferation and cell uptake. ELP bound Dox is a way to deliver Dox to the cells by lowering the chances of cardiotoxicity and myelosuppression. I focused on analyzing two ELP bound dox constructs, SynB1-GFLG-NcDox and SynB1-GGC-cDox. From this research, free dox appears to have more of an effect on breast cancer cell lines than ELP bound dox. To test my hypothesis, I used flow cytometry to measure the fluorescence given off by the drug in breast cancer cells, and I used an MTT assay to measure the rate at which metabolic events lead to apoptosis or necrosis. The MCF7 cell line had more fluorescence than the NCI/ADR cell line when treated with free dox because the MCF7 cell line is more sensitive than the multi-drug resistant cell line NCI/ADR. In cell proliferation, NCI/ADR's rate of survival was greater compared to MCF7 while using free dox. MCF7's cell lines are sensitive while ADR's are multi-drug resistant, and the data collected matched my expectations. Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

- R.12 **“Virus Capsid Based Metal Complexes for Cancer Chemotherapy”**

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Despite the many cancer drugs on the market, there is still a need for safe and effective methods of killing cancer cells. Cancer drugs that kill malignant cells unfortunately kill normal host cells as well. Cisplatin is limited in usefulness, due to its level of toxicity. We aim to develop a technique that allows toxic cargo, platinum ions, to target malignant cells without affecting normal host cells. Our aim is to use non-replicating virus-like particles designed so that toxic cargo is carried by the viral capsid protein. Our protein carrier, Cp149, is derived from hepatitis B Virus (HBV) and is expressed in *E. coli*. To purify Cp149, it is run over a series of columns that separate based on molecular size. To test whether the Cp149 protein is able to carry toxic cargo, we will incubate Cp149 with a platinum donor, K₂PtCl₄. We then run a series of column fractionation experiments that will allow the protein- platinum mixture to pass through. We will then test for the presence of platinum in protein- containing fractions to determine whether the platinum has bound to the protein. Cp149 expressed in *E. coli* is easily manipulated and may provide a cost effective therapy. Ongoing research by others in our laboratory is -aimed at finding methods to specifically target these platinum-containing complexes to cancer cells.

R.13 **"The Effect of Growth Factors VEGF 121 and PLGF on Trophoblast Cells"**

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Trophoblast cells migrate, invade and remodel maternal uterine spiral arteries. Inadequate trophoblast remodeling of the spiral arteries occurs in preeclampsia, which is a pregnancy specific hypertension. Vascular Endothelial Growth Factor (VEGF) is released from trophoblasts and signals angiogenesis and remodeling of the maternal spiral arteries. Free VEGF is reduced in pre-eclampsia and may contribute to changes in placental angiogenesis. Our laboratory has previously shown that β ENaC is required for normal trophoblast migration. However, the importance of VEGF on trophoblast expression of β ENaC is unknown. Therefore, the goal of this study was to determine the effect of VEGF on β ENaC expression in trophoblast Cells. BeWo cells were used as a trophoblast cell model. Cells were plated in 100 mm dishes and treated with VEGF₁₂₁ (0.5 or 5.0 ng/ml) for 48 hours. Cells were lysed in standard RIPA buffer and centrifuged to obtain a membrane-associated insoluble pellet. Proteins were separated using SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed with rabbit anti β ENaC (1:2000), and mouse-anti β actin (1;10,000), followed by donkey-anti rabbit 680 and mouse 800. Antibody labeling was visualized and quantitated on an Odyssey Infrared Scanner. Expression of β ENaC was enhanced by VEGF₁₂₁ treatment at both concentrations. These raise the possibility that altered growth factor expression in preeclampsia may regulate migration and angiogenesis by altering β ENaC expression.

Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

R.14 **"Characterizing the Stress-Response Protein *DDR48* in the Pathogenic, Dimorphic, Fungus *Histoplasma capsulatum*"**

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Histoplasma capsulatum (*Hc*) is a systemic, dimorphic, fungal pathogen. *Hc* grows as a multicellular mold at environmental temperatures (25°C) whereas, upon inhalation into a human or other mammalian host (37°C), it transforms into a unicellular, pathogenic yeast. Our research aims to elucidate the numerous stress response pathways (e.g., oxidative stress and DNA damage) that *Hc* utilizes to survive in the ever-changing environment. Specifically, we are characterizing the DNA damage-responsive protein *DDR48*, an *Hc* homolog sharing sequence similarity to *C. albicans* *DDR48p*. Previously in our lab an allelic replacement deletion-mutant was generated (*ddr48Δ*) to elucidate the function of *HcDDR48*. *HcDDR48* is required for resistance to common oxidizers such as paraquat and diquat. RNAseq analysis of the deletion mutant showed that most down-regulated genes are involved in cell signaling of oxidative stress and DNA damage response. Interestingly, many genes that were up-regulated in the deletion mutant are involved in cysteine/sulfur metabolism as well as oxidative stress response and carbohydrate/nitrogen metabolism. Research is ongoing to identify key players from each of these response categories.

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R.15 **“Intraarticular Drug Therapy with Genipin and Punicalagin”**

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Intraarticular drug therapy will study the effects of genipin and punicalagin as a precursor to developing an injection that will modify osteoarthritis by strengthening and protecting the cartilage from degradation. Genipin will be used as a crosslinking agent and the punicalagin will provide anti-inflammatory and protective properties to the cartilage. The use of these drugs in combination will hopefully result in additive benefits to the articular cartilage extracellular matrix. Genipin and punicalagin will be tested individually with four different tests. Genipin and punicalagin will also be tested in combination to study how the two drugs interact. Mechanical testing will determine how these concentrations will effect the articular cartilage surface through friction and compression testing. Other tests will study how the treated cartilage will react to the enzymatic breakdown caused by osteoarthritis and also how many free amino groups are released from each sample. The enzyme collagenase will be used to mimic osteoarthritic breakdown of the articular cartilage extracellular matrix. Once treated with genipin and punicalagin, samples will be introduced to this enzyme to determine how effective the treatments are at reducing breakdown. The final test reads how many free amino groups there are being released by each sample. If the sample is sufficiently crosslinked by the use of the treatments, there should be fewer free amino groups. In combination, the drugs have shown significant results during collagenase testing. At this point in the study, treated cartilage has withstood the effects of the enzyme dramatically more than the untreated. Genipin has shown progressively positive results with each increased concentration, and punicalagin is expected to have similar results. The hope is to be able to use this information to develop an injection that can drastically impact osteoarthritis in the short time frame the drug interacts with the cartilage.

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R.16 **“Egg Laying Male Has Androgynous Song System and Plumage Coloration”**

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Zebra finches (*Taeniopygia guttata*) are oscine Passerine songbirds that have sexually dimorphic plumage, song nuclei, and behavior. The roles of chromosomes, hormones, and genes on sexual differentiation are not completely understood. A gynandromorph in our aviary had male plumage, a male partner, and produced viable offspring. Mate preference tests revealed lower preference for the gynandromorph lineage than controls, suggesting some traits that made them unattractive to other birds. Gynandromorph lineage males had greater same-sex preference than control males. Male zebra finches are homozygous ZZ, and females are heterozygous ZW. All sampled gynandromorph tissues, including sexually dimorphic plumage regions, had ZW female chromosomes. Zebra finch males sing and females do not, and the regions associated with song learning and production are larger in males than in females. We measured the volume, cell size, and cell number of sexually dimorphic song nuclei in our gynandromorph to compare to males and females. Additionally, color spectra were analyzed to determine if the gynandromorph or its progeny had plumage differences, including those in the ultraviolet spectrum, which would be undetectable by the human eye. Video analysis of the gynandromorph and its mate has shown that unlike other females, the gynandromorph did sing. Additional analysis is being done to observe nesting behavior of the gynandromorph and its mate.

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R.17 **“Evaluation of Bone Marrow and Subcutaneous Fat in Ovariectomized Rats Following Sustained Delivery of Estrogen or NPY – 1Receptor Antagonist”**

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Hormones such as estrogen, leptin, and NPY are known to influence body fat distribution and adipocyte differentiation. The aforementioned hormones are essential for regulating appetite and are responsible for energy balance. The changes in these hormones at menopause and their influence on increased body fat is not clear. Our aim was to characterize these three hormone levels and evaluate the characteristics of size and distribution of the fat in the bone marrow and subcutaneous tissue surrounding a sham, estrogen, or NPY-1 receptor antagonist tricalcium phosphate (TCP) drug delivery device. Forty female Sprague Dawley rats were divided into five equal groups. Animals in group 1 served as ovariectomized control, Animals in Groups 2-4 received a non-drug loaded TCP capsule (SHAM), or an implant containing 5 mg of either estrogen benzoate or a NPY-1Receptor antagonist for 4 weeks. Animals in Group 5 were naïve females with ovaries intact. Body weights, blood, and tissues were collected and the analysis of estrogen, leptin, NPY, and fat distribution was determined and compared to group 1 animals. Animals in the OVX and SHAM groups had body weights which were significantly higher than naïve control. Animals in the estrogen and NPY-1RA treated groups had body weights which were significantly lower than OVX and Sham animals but still higher than naïve control after 4 weeks. Serum levels of leptin and NPY were both elevated in OVX, Sham, and NPY-1RA animals and estrogen was barely detectable. Estrogen administration returned serum levels of NPY, leptin, and estrogen comparable to values obtained for naïve control animals. Estrogen had the most significant effects on the size and number of fat droplets in both the subcutaneous tissue and bone marrow when compared to the other treatment groups and the OVX and intact control animals. The size of the adipocyte was substantially smaller. The adipocytes in sham and OVX groups were larger than those seen in estrogen, and smaller than those obtained in NPY-1RA treated animals. NPY-1RA returned the size and distribution of the adipocytes to control intact values. Overall, estrogen possibly had effects at the adipocyte and HPA axis whereas, NPY-1RA had appeared to have direct effects on the adipocyte peripherally.

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R.18 **“Investigating a Role for the Retrograde Signaling Protein, Rtg2p, in its own Transcription”**

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In eukaryotic cells, retrograde signaling is used to communicate organellar dysfunction to the nucleus which leads to altered target gene expression to accommodate specific dysfunctions. In the yeast, *S. cerevisiae*, the retrograde signaling protein Rtg2p has been shown to be a key cytosolic sensor for detecting mitochondrial dysfunction. Recent work in the lab identified a single amino acid deletion in Rtg2p that results in defects in retrograde signaling (Jiang, J., unpublished data). Western blot analysis showed decreased Rtg2p steady-state levels possibly due to enhanced protein turnover. Interestingly, qPCR analysis found no detectable levels of *RTG2* mRNA for this mutant. As Rtg2p has been identified as a member of the SLIK (SAGA-like) transcriptional complex, this finding suggests that Rtg2p may have a role in influencing its own transcription. To investigate this, we used restriction-based cloning to replace the *RTG2* promoter with the constitutive GPD promoter in plasmids containing the wild type and mutant versions of *RTG2*. Once confirmed by restriction analysis, these plasmids were introduced into three yeast strains to test for Rtg2 protein levels by western blot analysis, activation of retrograde signaling using a yeast colony color assay, and *RTG2* mRNA levels by qPCR analysis. If Rtg2p is involved in transcriptional self-activation, then promoter replacement should remove this regulatory step and return mRNA transcript levels for the mutant *RTG2* to that of wild type. Ultimately, results from these assays will be

used to broaden our understanding of the many biological roles carried out by the mitochondrial retrograde sensor, Rtg2p.

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R.19 **“Role of *msaABCR* Operon in *Staphylococcus epidermidis* Biofilm Development”**

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Staphylococcus epidermidis is an important nosocomial pathogen that causes infections primarily related to those of indwelling medical devices. Their ability to form biofilm and evade host immune defense systems allow for this opportunistic pathogen their increased survivability and antibiotic resistance. A newly described operon, *msaABCR* operon has recently been linked to biofilm development and virulence regulation in *Staphylococcus aureus*. While *msaA*, *msaC*, and *msaR* are thought to be regulatory genes, MsaB is the only putative protein transcribed from this operon. Bioinformatics analysis showed a good homology between the MsaB in *S. aureus* and *S. epidermidis*. Therefore, we hypothesized that *msaABCR* operon will play a similar role in the regulation of biofilm development in *S. epidermidis* strains. To study this, we deleted *msaABCR* operon gene from *S. epidermidis* RP62A strains. We performed in-vitro microtiter based biofilm assay. We observed that the *msaABCR* deletion mutant is defective in late stages of biofilm, but not the initial biofilm stages of biofilm development. The mechanism of biofilm development between the two species is different. RP62A develops PNAG-mediated biofilm, whereas, *S. aureus* develops biofilm independent of PNAG. In *S. aureus*, *msaABCR* has been shown to regulate biofilm development via extracellular proteases, however, in RP62A, deletion of *msaABCR* operon does not have any effect on the regulation of extracellular proteases. In future, we seek to define the mechanism of biofilm regulation via *msaABCR* operon in *S. epidermidis* RP62A. The findings may be therapeutically relevant to control the biofilm-associated infections in both *S. aureus* and *S. epidermidis* strains.

R.20 **“Investigating the Functional Role of β -1,4-Galactosyltransferase in the Lone-Star Tick, *Amblyomma americanum*”**

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The lone-star tick (*Amblyomma americanum*) bites have been implicated in a rare allergy to red meat. This allergy is caused by an Immunoglobulin E (IgE) sensitization to the carbohydrate galactose- α -1,3-galactose (α -gal) found in most mammals, and absent in humans/ old world primates. The aim of this study is to assess the functional role of tick's β -1,4-galactosyltransferase (β -1,4GT) in the synthesise of α -gal in the lone-star ticks. In our previous study (Karim and Ribeiro 2015), β -1,4-galactosyltransferase was identified, which attaches a free galactose sugar and transfer it to a glycan structure. Therefore, we hypothesized that β -1,4-galactosyltransferase plays a key role in α -gal synthesis responsible for red meat allergy. The temporal and spatial transcriptional expression provide an insight into the bloodmeal-induced 2 to 9 fold up-regulation throughout the bloodmeal. Additionally, the knockdown of target gene by RNA interference approach will provide an insight into the functional role of target gene into α -gal synthesis and impact on tick's ability to blood feed. These results provide a platform for future research investigating the occurrence of α -gal in the Lone-Star tick.

R.21 **"Neurobehavioral Impairment in Juvenile Rat Exposed to Reduced Uterine Perfusion During Late Gestation"**

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Adverse events during pregnancy are associated with negative effects on newborn development, with substantial perinatal mortality and morbidity. Little is known about the longer-term psychosocial sequelae following pregnancy complications. This study aimed to explore the behavior of juvenile rat offspring exposed to reduced uterine perfusion during gestation. We hypothesized that the exposure to reduced uterine perfusion is associated with increases in oxidative stress and dysfunctional neurobehavioral response in juvenile rat's offspring. To test our hypothesis, we performed the reduced uterine perfusion (RUP) surgery in pregnant rats at 14 days of gestation, and sham surgery in controls. Birth weight was measured within 12 hours post-natal. Motor and behavioral skills were tested in offspring at post-natal day 21 (P21), as well as measurement of oxidative stress markers. RUP-exposed offspring weight shows significant reductions at birth and at P21 compared to controls. Cliff avoidance response and beam walking latency time were significantly delayed in RUP-exposed offspring compare to controls ($P < 0.05$) (Cliff Male: 5.29 ± 0.9 VS 13 ± 1 ; Female: 4.6 ± 0.9 VS 13.83 ± 1.7 . Beam walking: Male 8.43 ± 0.57 VS 17.50 ± 2.25 ; Female 7.5 ± 0.83 VS 12.17 ± 0.48). Markers for oxidative stress were significantly increased in RUP-exposed offspring compared to controls. ($P < 0.05$) (T-Bars 13.586 ± 1.31 VS 27.18 ± 1.78 ; Superoxide Anion 10076.3 ± 519.99 VS 21147.4 ± 2361.63). These results suggest that exposure to RUP during gestation is associated with increased oxidative stress and neurobehavioral impairment in juvenile rat offspring.

R.22 **"The Effect of Growth Factors VEGF 121 and PLGF on Vascular Smooth Muscle Cells"**

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Preeclampsia is a pregnancy specific condition that is characterized by new onset hypertension. Abnormal trophoblast migration and spiral artery remodeling is thought to contribute to the etiology of pre-eclampsia. Growth factors such as Vascular Endothelial Growth Factor (VEGF) are released from trophoblasts and signal placental maturation and angiogenesis. Free VEGF is reduced in pre-eclampsia and may contribute to cellular migration. Our laboratory has previously demonstrated that the beta Epithelial Sodium Channel protein (β ENaC) is required for migration of trophoblasts and vascular smooth muscle cells (VSMC). We sought to determine the effect of VEGF₁₂₁ on β ENaC expression in VSMCs. A10 cells were used as a model for VSMCs. Cells were plated in 100mm dishes and were treated with growth factors VEGF₁₂₁ (0.5 or 5.0 ng/ml) for 48 hours. Cells were lysed in standard RIPA buffer and centrifuged to obtain a membrane-associated insoluble pellet. Proteins were separated using SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed with rabbit anti β ENaC (1:2000), and mouse-anti β actin (1;10,000), followed by donkey-anti rabbit 680 and mouse 800. Antibody labeling was visualized and quantitated on an Odyssey Infrared Scanner. Expression of β ENaC was enhanced by VEGF₁₂₁ treatment at both concentrations. These raise the possibility that altered growth factor expression in preeclampsia may regulate migration and angiogenesis by altering β ENaC expression.

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R.23 **“Recombinant Expression and Purification of Wild-type Amyloid- β 42 in *E. Coli*”**

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Aggregates of the amyloid- β (A β) peptide are the primary component of the proteinaceous plaques found in the brains of Alzheimer’s disease (AD) patients. Understanding the molecular mechanisms of A β aggregation is paramount to developing new treatment strategies for AD. In order to study A β in detail, a significant amount of highly pure peptide is needed. Synthetic A β can be purchased from peptide synthesis facilities; however, this procedure is expensive and requires specialized instrumentation. Thus the purpose of my research is to optimize a previously described protocol (Walsh et. al. 2009, FEBS Journal) for the expression and purification of wild-type (WT) A β 1-42 from *Escherichia coli* (*E. Coli*). To execute the protocol, sequence-verified PetSac plasmids harboring the A β sequence were used to induce expression in *E. coli* cells. As previously reported, A β was found inside inclusion bodies, which were solubilized in urea before further purification using anion-exchange chromatography. Lastly, size exclusion chromatography (SEC) was performed to obtain pure WT A β monomer. The various fractions from SEC were characterized using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and SDS-PAGE with western blotting. The results support that certain fractions do contain monomeric A β ; however, a majority of the protein was found to either be truncated at residue 19 or in an aggregated form. To further optimize this protocol, future experiments will focus on lyophilizing the sample into smaller aliquots to prevent aggregation and adding a protease inhibitor in hopes of obtaining higher yields of full-length monomeric A β .

R.24 **“An Assessment on the Efficacy of TRV 130 as a Thermal Antinociceptive Agent”**

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Rationale Traditional mu opioid receptor agonists are notorious for producing euphoria and inducing tolerance to their analgesic benefits. Previous studies examined the analgesic potency and reduced side effects of TRV130 compared to morphine; the current investigation expands this comparative study by testing the antinociceptive effects of oxycodone and TRV130.

Objective This study examines the analgesic potential of TRV130, a mu opioid receptor agonist that exhibits a bias for G protein-mediated pathways, compared to oxycodone, a non-preferential MOR agonist. Integrating male and female subjects allows an investigation into understanding sex as a biological variable in the perception of pain effected by TRV130.

Methods After subcutaneous administration of oxycodone and TRV130, animals were placed on a hotplate and latency to exhibit nociceptive behavior was recorded. Constructing a cumulative dose- effect function for both MOR agonists allowed for a direct comparison of their therapeutic capabilities.

Results Compared to oxycodone, TRV130 yielded maximum possible effect at slightly lower doses. Additionally, TRV130 proved equipotent in male and female subjects.

Conclusion The results suggest that TRV130 not only effectuates antinociception in a sex-independent manner, but also proves to be a more potent analgesic than oxycodone.

R.25 **“MHC Class I Polypeptide-Related Sequence A (MICA) as a Biological Determinant of Race Disparities in Prostate Cancer”**

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African American men (AAM) have higher incidence and mortality of Prostate Cancer (PCa), relative to Caucasian American men (CAM), however these disparities are poorly understood. Utilizing a racially diverse tissue microarray set, expression of MICA, a surface protein involved in tumor immune-evasion, was higher in tumors from CAM compared to AAM. To further understand the biological basis of racial disparities in PCa, an in-vitro system for analysis of MICA expression was set-up. LNCaP and E006AA cells, derived from CAM and AAM respectively, were assessed for the fraction of cells expressing MICA on surface. Flow cytometry analysis showed 67% cells expressing surface MICA in LNCaP, against 18% in E006AA. Following treatment with bortezomib, a FDA-approved proteasome inhibitor, the fraction of LNCaP cells expressing surface MICA increased 1.3-fold ($p < 0.01$). More so, bortezomib-treated E006AA cells expressing MICA yielded a 2.3-fold increase ($p < 0.001$). Soluble MICA (sMICA) release rate was assessed by ELISA in culture supernatants. Baseline release rate of sMICA was 54pg/ml/ 10^6 cells for LNCaP and 5pg/ml/ 10^6 cells in E006AA cells. Relative to baseline, sMICA release rate dropped 2-fold with bortezomib in LNCaP cells to 22pg/ml/ 10^6 cells ($p < 0.01$). However, sMICA release rate in E006AA rose to 531pg/ml/ 10^6 cells ($p < 0.0001$), more than 100-fold increase. Our results show race-specific expression of MICA in PCa patients and cell lines. Our next goal is to perform cytolytic assays in NK cells co-cultured with racially diverse PCa cells. This will allow us to address the functional relevance of our findings, and eventually propose MICA as a race-specific factor of tumor-immuno-evasion.

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R.26 **“Bone Density of Patients in the Jackson Heart Study”**

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Osteoporosis is a condition that is characterized by the bones of the patient having much less density than it should. This can cause the patient to be much more susceptible to a break or fracture as the bone is much weaker than it should be. The objective of the project was to find causes of osteoporosis in a wide group of people in order to further help prevent and treat the condition. For this IRB-approved HIPAA-compliant retrospective study, quantitative CT bone density measurements were taken of a sample of over 2800 different African Americans of both genders and all ages in the Jackson Heart Study. The measurement was the density of the lumbar of the patient (specifically the average of L3 and L4). Although the results have not been able to be fully analyzed yet, initial analysis showed that 176 patients (6.13%) had osteoporosis, 574 patients (19.99%) had osteopenia (weak bone density but not yet osteoporotic), and 2122 patients (73.89%) had good bone density. With all patients considered, the average bone density was found to be 149.64 milligrams per cubic centimeter and the median bone density was found to be 148.93 mg/cm³. Both of these values are considered to be good and healthy bone densities. In conclusion, a majority of the patients measured did have good bone density, but these results also need to be further analyzed to find out why some patients had very low density. This can greatly help in both preventing and treating the condition of osteoporosis.

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R.27 **“Immunolocalization of Selenoproteins in the Gulf-Coast Tick (*Amblyomma maculatum*) Tissues”**

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The gulf-coast tick (*Amblyomma maculatum*) is competent for a variety of microbes, including *Rickettsia parkeri*, a causative agent of Spotted Fever Rickettsiosis. Ticks experience a variety of oxidative stress conditions while on and off the vertebrate host. To counteract the deleterious effects of reactive oxygen species, ticks have many antioxidant molecules in their repertoire, including a robust selenoproteome system (Karim et al., 2011). These selenoproteins play significant biological role in mitigating the elevated levels of ROS by detoxifying and reducing free radicals that generates within the tick host upon blood digestion. The objective of this study is to determine the expression and localization of select selenoproteins in tick tissues by immunoblotting and immunofluorescence approach. We hypothesize that the expression of selenoproteins increases upon blood feeding on the vertebrate host. To test the hypothesis, unfed and partially blood fed tick tissues (midgut and salivary glands) are used to determine the expression of selenophosphate synthetase 2 (SPS2), and selenocysteine binding protein 2 (SBP2). Western blotting demonstrated the expression of SPS2 and SBP2 in both midgut and salivary glands. Additionally, confocal imaging showed the distinct localization of selenoproteins within the salivary acini. Our results support the up-regulation of selenoproteins upon tick's blood feeding, and unfolds a new avenue of research to study the protein expression in pathogen-infected tick tissues.

R.28 **“Dysfunctional Sensorimotor Response in Neonatal Rats Exposed to Reduced Uterine Perfusion”**

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Reduced uterine perfusion refers to a condition in which there is a reduction in the blood flow to the fetuses. This condition is often associated with intrauterine growth restriction and low birth weight in the offspring. The effects of exposure to reduced uterine perfusion on newborn sensorimotor response is not well studied or understood. We hypothesized that the exposure to reduced uterine perfusion induces increases in oxidative stress markers and dysfunctional sensorimotor response in neonatal rats. To test our hypothesis, we performed the reduced uterine perfusion (RUP) surgery in pregnant rats at 14 days of gestation. Motor skills were tested on post-natal day 8 (P8) along with measurement of oxidative stress markers. Offspring expose to RUP shows significant reductions in birth weight compared to controls. Growth pattern was also delayed in offspring expose to RUP, with significant differences compared to controls (P<0.05). Righting reflex and sensorimotor response were significantly delayed in RUP compared to controls. (P<0.05) Markers for oxidative stress were significantly increased in RUP compared to the controls. (P<0.05). This finding suggests that IUGR induced by RUP result in increases in oxidative stress and delay in neurodevelopment in newborn rat offspring.

R.29 **“Preclinical Assessment of Garlic in the Management of Acute Promyelocytic Leukemia”**

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Garlic supplementation in diet has been shown to be beneficial to cancer patients. Recently, its pharmacological role in the prevention and treatment of cancer has received increasing attention. However, the mechanisms by which garlic extract induces cytotoxic effects in cancer cells remain largely unknown. The present study was designed to use HL-60 cells as a test model to determine whether garlic treatment induced toxicity to human leukemia cells is mediated through oxidative stress. Human leukemia (HL-60) cells were treated with different concentrations of garlic extract for 24 hr. Live and dead

cells was determined by trypan blue exclusion test and microscopic imaging. The role of oxidative stress in garlic toxicity was assessed by lipid peroxidation, glutathione peroxidase (GPx) and catalase (Cat) assays, respectively. Oxidative stress biomarkers showed significant increase ($p < 0.05$) of malondialdehyde levels on one hand and gradual decrease of antioxidant enzyme activity (GPx & Cat) on the other hand with increasing garlic doses. Taken together, finding from the present study demonstrates that at therapeutic concentrations, garlic treatment induced cytotoxic effects through oxidative in HL-60 cells

Keywords: Garlic, HL-60 cells, trypan blue Test, oxidative stress, microscopic imaging

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R.30 **“Astrocyte and Microglia Morphologic Changes in the Nucleus of the Solitary Tract in a Rat Model of Thoracic Level Spinal Contusion”**

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Injury to the spinal cord results in long-term, debilitating sequelae to individuals. Spinal cord injured patients have increased risk for the development of metabolic disease (i.e. obesity, diabetes, dyslipidemia, and cardiovascular disease) which could further hinder the effectiveness of treatments to rehabilitate the cord and improve quality of life long-term. The initial spinal cord injury is marked by profound inflammatory changes in the area of the trauma as well as systemic changes in immune mediators.

In the present study, we sought to determine whether microglial and astrocytic morphologic changes exist in the brainstem, particularly the nucleus of the solitary tract, which is strongly involved in the control of food intake.

Adult, male, Long Evans rats received either thoracic level contusion of the spinal cord or sham laminectomy and then were allowed to recover on normal rat chow for 4 weeks. Body weight, food intake and adiposity was measured. Brainstems were harvested after 4 weeks of recovery, and processed for immunohistochemical staining for microglia (Iba-1) and astrocytes (GFAP). Furthermore, in a separate cohort, we extracted mRNA from microdissected brainstem sections. Preliminarily, we see no mRNA changes in expression of *aif* (a marker of microglia). Taken together, the data suggests that the NTS may be protected from immune perturbations in the cord and in circulation after the first weeks of recovery from thoracic spinal contusion.

R.31 **“Understanding Antibiotic Induced *Staphylococcus aureus* Persister Cells”**

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The rise in antibiotic tolerance and mutations within infectious microorganisms continues to challenge the scientific community and presents a potential danger to humans. Within a bacterial population, persisters are dormant, yet genetically variants which manifest stochastically and are exceedingly antibiotic tolerant. Recent research has proven persisters' ability to maintain their metabolically quiescent states allows persisters to withstand bactericidal antibiotics and robustly proliferate in opportunistic environments. However, the mechanisms which fosters persisters' dormancy is not completely understood. Our research aims to eliminate the microbial targets required to maintain a metabolically quiescent state within antibiotic induced *Staphylococcus aureus* persister cell lines. Through antibiotic treatments and a time sensitive serial dilution method, we are able to select for persisters within *Staphylococcus aureus*. Yet, our research is on going and no result have been generated.

R.32 **“Neuroprotective Effects of Estradiol, Genistein, and Aromatase on Cerebellar Lesions”**

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Brain injury induces upregulation of aromatase, which converts testosterone to estradiol (E₂). In addition, exogenous E₂ has been shown to be neuroprotective. It is unclear whether the neuroprotective roles of E₂ and aromatase are additive or independent. While the cerebellum is often thought of as a less plastic brain region, it is an excellent model for steroid related neuroplasticity as it has little constitutive aromatase, is steroidogenic, and recovery of function post-lesion is improved by both aromatase and E₂ administration. Estrogen Receptor beta, is abundant in the cerebellum and phytoestrogens, like genistein, bind mainly to ERβ, making the cerebellum an excellent model for comparing the effects of E₂ to those of genistein, which is less reproductively and carcinoginically harmful than E₂. Thus, our objectives are to examine (1) whether exogenous E₂ affects neuroprotection, (2) whether Genistein shares E₂'s neuroprotective role, and (3) whether E₂ and aromatase confer neuroprotection independently or concomitantly. We used zebra finches as models as their brains are more plastic than mammals. We quantified -5eszneurodegeneration by counting Tunel and fluro-jade stained cells and examining the amount of secondary damage surrounding lesions to the cerebellum. Male zebra finches (6-40) months of age were balanced across groups for age and weight. Groups were given subcutaneous implants containing silastic adhesive alone, E₂ (500 μg), or genistein (1000 μg), and weighed at 5 days. Seven days later, birds were weighed and unilateral puncture lesions (26g needle), counterbalanced between left and right sides, and were paired with intracerebellar injections of either letrozole or saline. Birds were sacrificed 48 hours later and serum, brains, and gonads were collected. We expect to find E₂ and genistein implants, when used without letrozole, will allow the least amount of cellular damage post lesion. We found that the higher levels of E₂ or genistein given, the testis would shrink due to the imbalance of hormone levels. We are still investigating whether E₂ and aromatase work independently. Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the national Institutes of Health (NIH) under grant number P20GM103476.

Mississippi INBRE Institutional Undergraduate Research

U.1 “Optimization of CFTR Immunoblotting”

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CFTR (Cystic Fibrosis Transmembrane-conductance Regulator) is a protein that functions as a chloride ion channel on surface of many epithelial cells. A mutated CFTR is responsible for causing the genetic disease cystic fibrosis. Immunoblotting is a commonly used biochemical technique for the detection of this protein that is present in trivial amounts especially at the endogenous levels. Goal of this study is to determine the ideal immunoblotting conditions that can provide the optimal signal for CFTR expression. We hypothesize that suitable combination of membrane, blocking agent, and concentration of methanol in transfer buffer can produce the optimal CFTR signal in immunoblotting. Epithelial cell line from human lungs (CFBE) stably transfected with wild type CFTR was treated with 5 mM sodium butyrate at 27°C for 60 hrs to up-regulate the plasma membrane CFTR expression. Cell lysates were prepared, electrophoresed, and transferred using PVDF or nitrocellulose (NC) membranes, and blocked with either gelatin, FBS, BSA, *blotto* (non-fat dry milk powder) or their mixture. Next, the best combination of membrane and blocking agent was used to determine the suitable methanol concentrations (0 to 20%) in transfer buffer for obtaining the optimal CFTR signal. Nitrocellulose membrane appeared to be more sensitive than PVDF membrane regardless of blocking agent used. Mixture of various blocking agents helped produce optimal CFTR signal over the traditionally used *blotto*. Effect of methanol on CFTR signal was found to be dependent on its concentration in transfer buffer. Blocking reagents produced synergistic effects on CFTR signal that is membrane specific. Optimum CFTR signal by immunoblotting can be obtained when proteins are transferred to NC membrane with 20% methanol in transfer buffer, and blocked with mixture of blocking reagents.

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U.2 “Identification of Molecular Mechanism of DCLK1 in Colorectal Cancer using Next Generation Sequencing”

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Colorectal cancer is the leading cause of cancer-related deaths in the United States, and the third most common cancer, affecting both men and women. The National Cancer Institute estimates 135,340 new colorectal cancer cases will be diagnosed, and 50,260 patients will die due to colorectal cancer in 2017. Accumulated mutations in the stem cells found in the crypts in the mucosa of the colorectum/colon can cause the rise of colorectal cancer. Targeting and killing the stem cells, especially the cancer stem cells only, has been a challenge to scientists with minimal success. Doublecortin like kinase 1 (DCLK1), a putative marker for gastrointestinal stem cells, has been found and might specifically label cancer stem cells. Further identifying the identity of DCLK1, its functions, and how to kill DCLK1-positive stem cells will be beneficial in our fight against colorectal cancer. The aim of this experiment is to use RNA-seq technology to determine the transcriptome change after DCLK1 overexpression. In order to achieve this goal, we established stable DCLK1 overexpression cell lines using the HCT116 cells. RNA-Seq was carried out on the Illumina NextSeq500 platform. Differentially expressed (DE) genes were evaluated by t-test [$p < 0.05$ and fold-change ± 1.5 or greater] using two methods: (1) FWER; and (2) Benjamini and Hochberg FDR (false discovery rate) which corrects for multiple comparisons. Gene networks and functional analysis were evaluated through the use of Ingenuity Pathways Analysis. We identified over 4000 DE genes in the DCLK1 over-expression cells, and several canonical pathways were modified by DCLK1 over-expression.

Our findings shed light on the molecular mechanism of DCLK1 function and will finally benefit the treatment of colorectal patients.

Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

U.3 **“Utilizing Molecular Beacons to Identify *Trichomonas vaginalis* virus 1 (TVV1) in *T. vaginalis* Cultures”**

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Trichomonas vaginalis is a flagellated protozoan parasite that infects the genitourinary tract in humans leading to trichomoniasis, the most common non-viral, sexually transmitted disease (STD) in the world with upwards of 250 million new cases each year. *T. vaginalis* infection is responsible for a range of severe complications, ranging from premature birth and increased HIV transmission, to an increased risk of cervical neoplasia and prostate cancer. Many isolates of *T. vaginalis* are consistently infected with double-stranded RNA (dsRNA) viruses, from genus *Trichomonasvirus*, family *Totiviridae*. These viruses have been shown to intensify the disease by signaling immunoinflammatory responses from human epithelial cells. Molecular beacons are dual-labeled, hairpin oligonucleotide probes, possessing a fluorophore at one end and a quencher at the other; they only fluoresce when hybridizing to a correlative target. In this study, 6-FAM-TVV1-IBQFQ specific to *Trichomonas vaginalis* virus 1 (TVV1) was used to identify the presence of TVV1 in cultures of *T. vaginalis*. To prove the effectiveness of the molecular beacon, cultures of *T. vaginalis* not possessing TVV1 were also analyzed. We found that the MB signal could be detected in living cells of *T. vaginalis* utilizing fluorescence microscopy and appeared specific to TVV1. In order to avoid false-positive fluorescence results, dead cells identified by staining with a 0.2% trypan blue solution. Moreover, using DIC and fluorescence microscopy to detect the viral genome in live cells, we observed a connected, amorphous inclusion body structure. Making use of MBs for live viral imaging is not only an effective method for visualizing TVV1 through fluorescence, but also for studying various facets of viral pathogenesis.

Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.4 **“Biochemical and Molecular Methods for the Detection of Endogenous CFTR Expression in Human Pancreatic Cells”**

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CFTR is a membrane protein that functions as a chloride ion channel on surface of many epithelial cells. Defective CFTR is the cause of genetic disease cystic fibrosis (CF). CFTR is expressed endogenously at a level that is normally difficult to detect by traditional biochemical methods such as western blotting and immunohistochemistry. In order to detect endogenous CFTR levels we first aimed to increase the sensitivity of CFTR immunoblots by altering the concentrations of SDS and methanol in the transfer buffer. SDS facilitates the removal of proteins from polyacrylamide gels (PAGE) and methanol allows their binding to nitrocellulose (NC) membrane. Endogenous (Capan-1, CFPAC) and exogenous (CFBE) CFTR expressing epithelial cell lines were treated with 5 mM SB for 60 hrs to up-regulate the CFTR expression. CFTR protein levels were determined either by western blotting or immunoprecipitation. RT-PCR was used to determine the CFTR mRNA expression. The addition of 0.015% SDS in transfer buffer enhanced the detection of exogenous but not endogenous CFTR expression levels. Endogenous CFTR expression levels, however, could be detected by immunoprecipitation with anti-CFTR antibody. At molecular level, the endogenous CFTR mRNA expression can also be detected and quantified by RT-PCR. Reliable methods for

the detection of endogenous CFTR expression may be useful in analysis of patient specimen in diagnosis of CF.

Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

U.5 **“Differential Roles of Hydroxyflavones against Nicotine Induced Stress in Human Lymphoblast Cells”**

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The polyhydroxy flavonols are ubiquitous in plant of higher genera. They are widely studied for their potential to treat or even prevent a broad spectrum of free radical mediated diseases, which include neurodegenerative disorders, cancer, atherosclerosis. Flavonoids are comprised of a common structure of diphenylpropane, C6-C3-C6, consisting of two aromatic rings (rings A and B) linked through a three carbon bridge or by a pyrone or pyrane ring (ring C) (Scheme 1). The present study is focused on two common isomeric flavonols morin and quercetin (Scheme 1) which are widely present in citrus fruits and onion respectively, against oxidative stress induced by nicotine (NIC) in TK6 cells. NIC links smoking to renal injury via increased oxidative stress. Smokers are at high risk for the development and progression of chronic kidney disease (CKD). *In vitro* studies confirmed that NIC stimulates mitochondrial ROS production, which leads to a mitochondrial depolarization-dependent injury of renal proximal tubule cells. Therefore, modalities that ameliorate smoking/nicotine exposure-associated renal oxidative stress are of high importance. To determine the beneficial effects of morin and quercetin on NIC-induced cell injury, the following experiments were carried out: human lymphoblast (TK6) cells were treated with 20 μ M morin or quercetin along with 200 μ M NIC and cell viability was determined after 24 hours. Phase contrast imaging and light scattering studies were performed on the cells. Results indicated that 20 μ M morin is more effective than quercetin. Further studies are underway.

Acknowledgement

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U.6 **“Maleimide Coupled Doxorubicin”**

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Doxorubicin is a common cancer chemotherapy drug. It is a strong DNA intercalator and targets topoisomerase II. Topoisomerase II relaxes supercoiling during transcription by inducing temporary double-strand breaks to relieve the high superhelical density. Doxorubicin stabilizes the temporary double-strand breaks, the DNA does not get resealed and thus the transcription process comes to an end. Doxorubicin is often administered intravenously, but serious side-effects make a more targeted delivery method desirable. Doxorubicin is named “The Red Devil” or “Red Death” because of the red color and those serious side effects.

ELPs (Elastin-like Polypeptides) are thermo responsive macromolecules that can be equipped with cell-penetrating peptides and allow for the attachment of compounds via cysteine-maleimide crosslinking. Consequently, compounds can be delivered by hyperthermia to target cancer cells.

Maleimide linkers can be attached to doxorubicin via a ketone and or an amine. The ketone has been connected to maleimide via a hydrazone, which is susceptible to hydrolysis. The amine is connected to maleimide via an amide linkage which has a much higher stability. Herein we present our efforts to optimize the coupling reaction and work-up, based on a literature known procedure. Separation by preparative HPLC is improving the purity of the coupling product.

This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.7 **“Correlation of Double Cortin Like Kinase 1 (DCLK1) with Colorectal Cancer”**

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Colorectal cancer is a prevalent disease. Almost 50,000 people die from colorectal cancer each year. The current treatment for colorectal cancer includes surgical removal plus chemotherapy. However, about 50% of the patients will have recurrence within 5 years. The reason for this recurrence is believed to be the existence of tumor stem cells (TSCs). TSCs have become very important in the cancer biology world because they can become potential therapeutic targets for the treatment of cancer patients. Specific stem cell markers have been identified for the stem cells in the gastrointestinal tract, and double cortin like kinase1(DCLK1) is one of them. To determine the correlation of DCLK1 with the tumor genesis of colorectal cancer, we established a DCLK1 over-expressing cell line with HCT116 cells, which is a colorectal cancer cell line. We confirmed over-expression of DCLK1 with Western Blot. To assess whether DCLK1 affected growth of HCT116 cells, we measured cell growth using MTT assay over a time course study. To evaluate whether DCLK1 is correlated with chemoresistance of colorectal cancer cells, we treated cells with 5-fluorouracil (5-Fu) at different dosages. Our results demonstrated that DCLK1 over-expressing cells do show a much higher level of DCLK1. DCLK1 over-expression inhibited growth of the cells, indicated by a lower OD value than the wild type. Wild type and DCLK1-over-expressing cells demonstrated different viability after 5-Fu treatment at different dosages. In conclusion, DCLK1 affects the cell growth of colorectal cells, and it might correlate with chemo resistance of colorectal cancer cells.

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U.8 **“Cytoadherence of Three *Trichomonas vaginalis* MLST Genotypes”**

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Trichomoniasis is the most common nonviral sexually transmitted disease (STD) in the world. The protozoan *Trichomonas vaginalis* causes trichomoniasis. *T. vaginalis* infection can lead to a higher risk of HIV infection and adverse pregnancy outcomes. Increased cytoadherence leads to a higher infectivity in the urogenital tract. *T. vaginalis* has been divided into three MLST genotypes: GT1A, GT1B, and GT2. Here, the cytoadherence of, GT1A (strains W047, W060B, T009, W046), GT1B (strains 50167, PA, W091, T132), and GT2 (strains PRA98, T095, T182, T007, C8) were compared. Cervical (HeLa) and ectocervical (Ect 1) monolayers were exposed to aliquots of *T. vaginalis* cultures (~10⁵ parasites) and cytoadhesion was assayed using an automated cell counter (TC20 Bio-Rad). Preliminary results show that there was no significant difference between the cytoadherence of the three MLST genotypes of *T. vaginalis* and HeLa cells.

Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.9 **“An Analysis of Surface Proteins in Three Genotypes of *Trichomonas vaginalis*”**

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Trichomonas vaginalis is a flagellated protozoan parasite that colonizes the human genitourinary tract and causes trichomoniasis, the most common non-viral sexually transmitted disease in the world. Using

multilocus sequence typing (MLST), this protozoan has been classified into three genotypes, known as 1A, 1B, and 2. Previous reports have demonstrated that some strains exhibit varying cytoadherence and cytotoxicity phenotypes. *T. vaginalis* strains W060B, PA, and PRA98 (1A, 1B, and 2 respectively) were cultured and the surface proteins were isolated by biotinylation and subsequent capture on streptavidin magnetic beads. Samples were trypsinized and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Institute for Genomics, Biocomputing and Biotechnology at Mississippi State University). A total of 32 unique proteins were identified by comparison with the *T. vaginalis* G3 genome, including enolase 3 (XP_001315627.1), detected in all three samples, and an a-agglutinin attachment subunit precursor (XP_001281006.1), found in PA and PRA98.

Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.10 **“Synthetic Approaches to Aromatic Heterocycles with Various Substituents”**

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N-Methoxy substituted aromatic heterocycles are photoactivatable compounds that produce two transient reactive species upon excitation. The reactive species, a methoxy radical and a heteroaromatic radical cation, have been shown to cleave DNA. The efficiency of DNA cleavage is limited due to low ground-state association for the simple compounds based on quinoine, isoquinoline and phenanthridine structures. To increase cleaving efficiency, a DNA-binder (1,8-naphthalimide) has been synthetically attached. So far, we are limited to commercially available precursors based on the aromatic heterocycle pyridine. It is necessary to shift the absorption maximum to longer wavelengths to use more cell penetrating irradiation.

To further improve binding and cleaving efficiency, attempts to synthesize novel amino-heterocycles are undertaken. The goal is to extend the absorption maximum to longer wavelengths and have a flexible linker size connecting the heterocycle to the naphthalimide. The approach is to functionalize alkylquinolines or related heterocycles. The functionalization can then be modified by simple organic transformations. Radical bromination leads to a halogenated product that is modified by various Grignard reactions or directly substituted with potassium phthalimide. To bypass the cleavage of phthalimide, direct reaction with naphthalimide might prove to be more efficient. We are attempting to use environmentally conscious synthetic routes to minimize impact. This includes avoiding halogenated solvents for the radical bromination. Several methods have been suggested in the literature, including a photochemical flow-reactor.

This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.11 **“*Tetrahymena thermophila* Rufy is Required for Proper Contractile Vacuole Function”**

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The contractile vacuole is an organelle that regulates the osmolarity in protozoa. This organelle is essential for pumping water out of fresh water protists, such as *Paramecium* and *Tetrahymena*. While *Paramecium* and *Tetrahymena* are closely related evolutionarily and their contractile vacuoles are functionally similar, the structure of these organelles is markedly different in these organisms suggesting important adaptations that occurred after the emergence of this organelle. Despite these differences ultrastructural studies suggests that membrane tubulation, fusion, and trafficking are required for proper function of the contractile vacuole in these organisms however there are few molecular data on the genes that might regulate this process.

Here we begin to characterize a novel *Tetrahymena* protein, Rufy (RUN and FYVE domain containing protein) in the regulation of contractile vacuole function. This protein shares its domain organization

(Figure 1) with mammalian proteins Rubicon and Rufy, known to regulate membrane trafficking and autophagy. Interestingly, RUN domains were first characterized as Rab interacting domains and Rab proteins have been shown to localize to the contractile vacuole in *Tetrahymena*. Interestingly, *Tetrahymena* Rufy possesses a RING domain (not present in mammalian homologs) and this RING domain shares homology to the RING domain of E3 ubiquitin ligases, suggesting that *Tetrahymena* Rufy may interact with ubiquitin modified substrates, in addition to Rab (RUN domain) and lipids (FYVE domain). Endogenous GFP tagging experiments in our lab show that Rufy-GFP localizes to the contractile vacuole membrane. Moreover, deletion of Rufy results in reduction of contractile vacuole contraction. Future experiments are aimed at determining the role of each domain within Rufy in regulating its function as well as identifying binding partners.

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U.12 **“Optimizing Parameters for Electrospinning Drug Loaded Alginate- and Chitosan-Based Nanofibers”**

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Electrospinning natural polymers is a developing interest in the field of biomaterials. Electrospun nanofibers have been shown to facilitate tissue regeneration and emulate body tissue, making it ideal for modern wound healing dressings. Several of these water soluble natural polymers, including alginate and chitosan, show promise as drug delivery vehicles. However, many of these biopolymers are inherently charged, making the formation of nanofibers difficult. In this project, optimal parameters for electrospinning drug-loaded alginate- and chitosan-based nanofibers are being investigated. To help overcome the innate charges of the natural polymers, co-polymers such as poly(vinyl alcohol) and agarose are used at various concentrations with the natural polymers. Additional electrospinning parameters, including voltage and polymer flow rate were altered. Once optimized, these parameters coupled with release rate studies of the drug-loaded fibers will be used to create a catalog of small molecule release profiles. The cataloged profiles can be applied in the further development of biomaterials used in drug delivery and modern wound healing dressings.

This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

U.13 **“Determination of the Polarity of Bifunctional 1,8-Naphthalimides”**

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The moderate fluorescence of 1,8-Naphthalimides can be used to localize conjugated compounds in various environments. Our DNA-cleaving nitrogen onium salts are connected to 1,8-naphthalimides which should allow us to track their movement in tissues due to the variable fluorescence intensity and wavelength. 1,8-naphthalimide fluorescence is strongly influenced by solvent polarity, an ideal requirement for a fluorescence sensor system.

To obtain more information about the quenching process, intermolecular quenching experiments are employed. Quenching of N-methyl 1,8-naphthalimide with various pyridine derivatives with electron donating and withdrawing substituents shows that certain substitution positions are more efficiently quenching than others. The electron-rich N-oxides are efficient fluorescence quenchers. Interestingly, exciplex formation is observed for pyridine derivatives in acetonitrile, a rare occasion in such a polar solvent. Other deactivation mechanisms are electron-transfer quenching and energy transfer.

To mimic the membrane environment we analyzed the fluorescence of 1,8-naphthalimides in reverse micelles. AOT as an anionic surfactant in reverse micelles electrostatically attracted the cationic nitrogen onium moiety, but the fluorophore appears to localize in the hydrophobic part of the system. Further experiments in various reverse micelles need to be undertaken to verify these findings.

To confirm the results, partition coefficient experiments with all compounds were performed. Cell uptake studies with *Tetrahymena thermophila* by monitoring of the fluorescence of the 1,8-naphthalimide chromophore after exposure give insight into how well the compounds penetrate membranes in living organisms.

This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

SEPA, STEMI and Mississippi INBRE Collaboration

- A.1 **“Community Collaboration for STEM Education in Mississippi: SEPA, STEMI and INBRE”**
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The Science Teaching Excites Medical Interest (STEMI) project at the University of Mississippi Medical Center (UMMC) is funded by the Science Education Partnership Award (SEPA) program at the National Institutes of Health. STEMI, begun in 2016, engages Mississippi STEM teachers in a community of health learners and development of expertise in flipped classroom pedagogy that focuses on major Mississippi health needs of health literacy, social determinants of health and obesity. Effective July 1, 2017, the SEPA program has become an administrative component of the National Institute of General Medical Science (NIGMS), which also funds the Mississippi INBRE program. Alignment of the educational objectives of SEPA and INBRE projects has been given a high priority by directors of both SEPA and NIGMS. The STEMI project is partnering with the Mississippi INBRE project to share resources that advance and support networks for STEM teacher excellence, pedagogical innovation, and improvement of STEM education and research career enhancement. STEMI uses tested and successful techniques during intensive summer training for high school STEM teachers to develop competitive proposals for external funding for those teachers. The Mississippi INBRE project now permits high school teachers to apply for funding to support development of innovative STEM curricula. This new partnership significantly elevates high school teacher professional development, fosters implementation of cutting-edge STEM pedagogy models, and substantially extends the educational pipeline for biomedical career training into secondary schools. (This project was supported by the Office of The Director, National Institutes Of Health under Award Number R25OD020215-01A1. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.)

B.1 Examining perceptions of health in association to cardiometabolic risk factors

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The state of Mississippi is currently leading the nation in obesity prevalence. Mississippians have ranked last in living habits, body weight, and physical activity and these three factors have been linked to many of the health-related problems that Mississippians face. It is also noted that health perceptions are related to health outcomes. This study will help Mississippians understand their health status which has different from the perceived health. This will provide Mississippians better health standards to avoid developing cardio metabolic disease.

The purpose of this study is to determine whether preconceived self-perceptions of health is related to cardiovascular disease risk factors among Mississippi African Americans. This relationship shall be accessed by examining the perceptions of perceived and actual health. This data was collected from the participants of the Church Bridge Project. The program was designed to give nutrition and physical activity tips, along access to professional nutritional advice. Data measures were based on physical activity, sedentary behaviors, and eating behaviors. A baseline data on demographics, physical activity, health perception, and eating confidence surveys is collected. The participants consisted of African-Americans of ages 18 to 55 years with a BMI > 30, and resident of an underserved area. The American college of Sports Medicine Health Review and Medical History were used to determine risk factors associated with CVD risk factors.

Based on the total sample of 29 in the project, the independent two sample t-test for continuous variables and chi-square test of independent for categorical variables will be performed. The difference between the perceived health and the actual health status will be tested.

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B.2 Examining the Stages of Behavioral Change for Weight Loss and the Factors that Affect Them in African-American Young Adults in South Mississippi

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The purpose of this study is to determine if there is an association between Stages of Change for weight loss and factors such as, Income status, Education level, Social Support for Exercise and Eating habits. The Church Bridge Project was developed to offer nutrition tips, physical activity advice, group support, and access to a Nutrition Professional allowing participants different resources to improve weight loss. Individuals with a low Stage of Change for weight loss are more likely to be less physically active, thus, leading to a greater propensity of obesity. Multiple demographic factors, socio-economic factors as well as Social Support for eating and exercise habits are suspected to contribute to Stage of Change of an individual.

The above-mentioned variables of interest were collected in the Church Bridge Project and a total of 29 participants (31% females; 69% males) provided baseline responses that were examined via Chi-Square and Independent sample t-test. From the analyses, it is concluded that more educated group shows more

in action stage of change. ($\chi^2 = 4.398$, $p < 0.05$). If friends encourage the participants more, they would act more for better health ($t = -2.094$, $p < 0.05$) and consequently, increasing the Stage of Change of an individual. Similarly, if there is more social support, such as, if friends participate in the same action along with the participants ($t = -3.318$, $p < 0.05$), then they show more action for better health. Lastly, when family rewards and punishes for actions, then the participants would act more for better health ($t = -2.468$, $p < 0.05$).

It can be concluded that having a better education, participation of friends in eating and exercise habits, as well as rewards and punishments given by family increases the Stage of Change of an individual, thereby, encouraging them to be more active for better health.

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