

MCNAIR SCHOLARS RESEARCH JOURNAL 2017





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Please join the McNair Staff in congratulating the Scholars on their contributions to the advancement of knowledge in their respective fields of interest.

GO WARRIORS!!!!!

The Wayne State University McNair Scholars Staff

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CAN TRAUMATIC STRESS EXPOSURE IN ADOLESCENTS CREATE FEAR LEARNING DURING ADULTHOOD?

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Major: Public Health

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ABSTRACT

Much research has been done that focuses on the effect that stress has on individuals, with adults as the focal point of this research and very few investigations of the effects of stress during adolescence. Utilizing a rat model, an experiment was conducted to determine the effects of traumatic stress exposure during adolescence on anxiety, learning/memory, and fear-related behaviors in adulthood. The purpose of the experiment was to develop a translational model to further the research in humans that shows that children that are exposed to trauma at an early stage in life are more vulnerable to developing PTSD in adulthood. To conduct the testing, a single prolonged stress (SPS) rat model of PTSD was utilized. Single prolonged stress consists of four stages. First the rats, in this case (male Sprague Dawley rats,) underwent a 2 hour restraint. Second, they underwent forced group swim for 20 minutes. Third, they were then exposed to ether until loss of consciousness. The final step was an undisturbed period that occurred over a period of days, which has been shown to be necessary for the development of PTSD-like behaviors in this model in adult rats. For the results of the experiment we hypothesized that introducing trauma in early adolescence would increase anxiety like behaviors and enhance fear learning during adulthood. Instead our results displayed that SPS exposure during adolescence did not have a significant effect on anxiety during adolescence or adulthood but displayed abnormal fear learning as adults. Rats exposed to SPS during adolescence displayed altered fear learning and extinguished fear association faster than rats not exposed to trauma. These data suggest that a single traumatic experience at an early age may change fear learning strategies and influence the way young individuals deal with trauma in adulthood.

INTRODUCTION

The American Psychiatric Association (APA) defines post-traumatic stress disorder (PTSD) as a history of exposure to traumatic events which meets specific symptoms that are categorized into four clusters (APA, 2013). Which includes intrusion, avoidance, negative alterations in cognitions and moods, and alterations in arousal and reactivity (APA, 2013). PTSD was initially introduced in 1980, when the APA acknowledged PTSD as a serious mental disorder (Friedman 2013). Although controversial when first introduced, the PTSD diagnosis has been able to fill an important gap in psychiatric theory and practice. From a historical prospect this change allotted room to view the disorder as dealing more with means that are on the outside of an individual rather than marking the individual to be simply weak (Friedman, 2013). PTSD is a very complex disorder, so research is extremely valuable in understanding the problem and the effects it has on the health of humans and their relationships. Additional research will assist in developing and testing treatments to mend the problem and to help prevent the problem from occurring initially.

In the past years much research has been done to understand the way adults handle stress and the persistent negative effects it caused throughout their life. Research has shown that negative experiences during adolescence increase the risk of psychiatric disorders in adulthood, social defeat may produces a delayed

impairment in cognitive flexibility during adulthood, and the social condition following stress appears to play an important intermediary role in the development of these cognitive deficits (Zhang, 2016). Children handle stress differently from adults and somethings that occur when an individual was younger may seem to be forgotten as the years progress, but sometimes it is ingrained in their memory producing characteristics or attributes when they become an adult. For an example, a child raised in a household where yelling and domestic abuse were the norm may steer away from loud yelling and abusive situations, whereas another individual without a history of trauma as a child may have the tools to deal with stressful situations and not be affected as much.

The experiment at hand focuses on that question, does trauma induced early in life affect behavior in adults.

LITERATURE REVIEW

Merriam Webster's dictionary defines trauma as a very difficult or unpleasant experience that causes someone to have a mental or emotional problem lasting for a long time. A life changing situation that can at times alter an individual's perspective is something to take serious. No two people view or experience a situation the exact same way, so a situation that may cause manageable stress to one individual may induce a life changing mental disorder for the next person.

Trauma in adulthood is very important due to the fact that it decreases the number of individuals that are functioning in society (Millikan, 2014). Furthermore, it has a detrimental cost to society with high financial and social consequences from the significantly raised rates of hospitalization, suicide attempts and alcohol abuse (Aderka, 2013).

Prolonged trauma then produces Post Traumatic Stress Disorder (PTSD). The National Institute of Mental Health goes a bit further to define the disorder as a condition of persistent mental and emotional stress occurring as a result of injury or severe psychological shock typically involving disturbance of sleep and a constant vivid recall of the experience, with dulled responses to others and to the outside world.(NIH, 2016). The study of PTSD is very important because despite the significance of this problem, to date, the mental health needs of many Americans have been neglected (Young, 2016). Some theories state that the lack of support for mental health goes back to the uncertainty and considerable debates around how to best provide early psychological intervention for traumatized children and adults (Haqq, 2016). Basically, stating that the lack of knowledge causes society to push important, life threatening situations under a rug. The design of a key study, which used a randomized controlled trial design, included children (aged 1-6 years) who were screened as at risk for PTSD 1 week after an unintentional injury and their parents, and the children were given either (1) CARE intervention or (2) treatment as usual (Haqq, 2016).

The experiment exemplified how prevention is better than treatment with the intentions that the outcome of the research would lead to additional scientific contributions in assisting early intervention for adolescence. Intrusive memories are common following a traumatic event and among the hallmark symptoms of posttraumatic stress disorder (PTSD) (Kliem, 2013). In a recent study, the authors decided to take a closer look at PTSD as a whole, defining what they coined as the hallmark symptom. In their experiment, they used an ecological momentary approach and indexed intrusive memories in trauma survivors with and without PTSD using electronic diaries. Forty-six trauma survivors completed daily diaries for 7 consecutive days recording a total of 294 intrusions (Kliem, 2013). Their results showed participants with PTSD experienced an insignificant amount of more intrusions than those without PTSD, but experienced them with more "here and now quality," and responded with more fear, helplessness, anger, and shame than those without PTSD. Individuals with PTSD experienced diary-prompted voluntary trauma memories with the same sense of now-ness and vividness as

involuntary intrusive trauma memories. (Kliem, 2016) Their study assisted with taking a deeper look into actually understanding every day experiences of an individual that survived trauma, which ultimately offers future clinical treatment.

Negative social experiences during adolescence increase the risk of psychiatric disorders in adulthood (Zhang, 2016). In this published set of experiments mice were exposed to social defeat in their adolescent years and expressed social-avoidance behaviors in adulthood (Golden, 2011). This study showed how continuous stress in adolescents through social stressors can affect the mice later on in life. After going through the ten days of social defeat, 6 weeks later their findings suggested that the effects of social defeat on emotion and cognitive function are differentially affected by the developmental stage and social condition (Zhang, 2016). In other words the effect of trauma on the emotional and social level affects individuals differently depending on their developmental stage and social conditions. This study continues to peer into the idea that trauma exposure in early adolescents may have a more severe effect than trauma induced at a later stage.

Repeated social defeat of adolescent male rats resulted in adult mesocortical dopamine hypofunction, impaired working memory, and increased contextual anxiety-like behavior (Novick, 2016). These findings suggest that prolonged trauma during adolescence can modify the way the brain functions, how memory works, and cause increased anxiety. Within this study the experimenters observed the role of glutamate in dopamine regulation, focusing on potential changes to N-methyl-D-aspartic acid (NMDA) receptors, in cognition, fear learning and anxiety-like behaviors following adolescent social defeat. Their study focused on the NMDA receptors because these receptors control the synaptic plasticity and memory functions (Blanke, 2009). The results of their studies showed that adolescent social defeat alternated NMDA receptors leading to dysfunctional glutamatergic system, which caused with mesocortical dopamine deficits, leading to long-term behavioral consequences as a result of social stressors during later development (Novick, 2016).

Surprisingly, there are some studies that suggest that trauma introduced at a young age may be able to produce a resilience to fear. For example, one study took the perspective of childhood bullying, hypothesizing that if children are bullied at a young age, they become resilient to stress during adulthood. In their study the authors initially hypothesized that Juveniles are particularly sensitive to stressors in their environment due to the relatively late maturation of brain areas that are targeted by stress such as the prefrontal cortex and hippocampus (Buwalda, 2013). This would display that trauma induced at an early age would then affect the way that the rats learn fear which later resurfaces as a negative anxiety like behavior in adulthood. Their results were opposite of what they actually predicted. They coined it as a mismatch hypothesis, the final consequence of childhood adversity depends on how well the early life environment matches the challenges in later life. Socially stressed adolescents are rather resilient to the lasting behavioral and physiological effects of the stress exposure if they are socially housed afterward and have the ability to recover (Buwalda, 2013).

The numerous amount of literature that's included shows some previous studies that have been collected that represents some background information pertaining to this field. With the help of the published articles we are then able to do some brainstorming and draw some conclusions together to distinguish if we can add to the findings to discern what stress at an early age can actually do later down the line.

METHODOLOGY

All methods were approved by the Wayne State University Institutional Care and Use Committee and personnel were trained and certified before studies began. The subjects were 20 male Sprague Dawley rats weighing 62-74 grams which corresponds to the age of the rats, the rats originated from Charles River Laboratories. They were housed in groups of five in the vivarium of Scott Hall at Wayne State University. Rats were housed under

a reversed light/dark cycle (lights on at 6pm, lights off at 6am) and allowed to acclimate for 6 days. After the acclimation process rats were subjected to SPS, which consists of restraint, forced group swim, ether exposure, and incubation.

SINGLE PROLONGED STRESS

The first stage in SPS began with the restraint. In this step the rats are restrained for two hours. This consists of being placed into tubes that does not allow them to turn around and restricts movement. This phase of the single prolonged stress is known as a physical stressor. The second stage is the forced group swim. Immediately after being released from restraint, rats are placed in a large tub of room temperature water in groups of 6-8. They are then made to swim for 20 minutes. This is known as a psychosocial stressor. Following the group swim is the ether inhalation. Here, a small enclosed space was filled with ether, and then the rats were placed inside. Placing the rats within the ether-filled enclosed space causes for them to lose consciousness which activates the hypothalamic pituitary adrenal (HPA) axis. (Zhang) This is known as a pharmacological stressor.

TIMELINE OF BEHAVIORAL TESTING

Following SPS or control exposure which began at post-natal 31 (P31-days from birth), which consisted of brief handling instead of stress treatment, a series of behavioral tests were conducted to determine the effects of stress during adolescence and adulthood. Relative to this process was the novel object recognition (P41) that was measured during adolescence which included an open field test. During the adolescent years the Light/Dark test (P42) was also conducted. Once that stage is over, the rats then went through an Elevated Plus maze (P62) which takes place during adulthood and then from P63-P65 the rats then go through a fear conditioning stage. The fear conditioning period is the stage where the rats learn to predict.

NOVEL OBJECT RECOGNITION

After SPS, the rat is then put through a novel object recognition paradigm to test recognition memory. Here there are three stages each lasting for 5 minutes. In the first stage the rat is placed in an empty box and is allotted the opportunity to explore the empty space. In the second stage the rat is placed in the same box with two identical objects and is left alone to explore. Then we place the rat in the box with one item from the previous phase and one new object (novel object). If the rat explores the new object more it shows its short term memory is working because it remembers the familiar object, and will therefore not explore it as much.

LIGHT/DARK BOX TEST

Following the novel object recognition test is the Light/Dark Box test. Here the rat is placed in a box with two compartments. The light side has white walls and is left uncovered, and the dark side has black walls and is covered with a lid. In this test, the dependent measure is the time in light and dark side of the box, and a more anxious rat will stay longer in the dark side of the box.

ELEVATED PLUS MAZE

The elevated plus maze is a cross shaped maze that is elevated above the ground and has four arms, two of which have walls on their side (closed arms) and two arms that do not have closed sides (open arms). The dependent variable in this test is the amount of time spent in either the closed or the open arm, and the more the rat remains in the closed arms, the more anxious it is considered.

FEAR CONDITIONING

The final stage of testing is the fear conditioning part. Fear conditioning was used to determine if SPS during adolescence had long-lasting effects on fear learning. Conditioning phase the rats were shocked and a tone was paired with each shock to form an association. Rats were then exposed to the tone again in the absence of the shock and the amount of time spent freezing ("deer in the headlights") was measured. The state of freezing was used to as a measure of fear related response.



RESULTS

OPEN FIELD







NOVELTY OBJECT RECOGNITION



LIGHT/DARK BOX



ELEVATED PLUS MAZE



FEAR LEARNING BEHAVIORS (I.E. BEHAVIORS DURING ACQUISITION OF FEAR CONDITIONING)



In figure 1, the results of Open field testing is shown Rats were free to explore a large open area to measure anxiety-like behavior. This measurement was also part of the Novel Object testing and considered the habituation phase of that test. Each subject was placed in the same corner, designated the placement area, each time. Thigmotaxis or "wall-hugging" behavior was similar between groups, suggesting anxiety levels of the SPS and control rats were no different. The activity in open field between the control and SPS rats were not significantly different. However, SPS-exposed rats spent more time in the placement area than control rats (p<0.05). This could be representative of behavioral inflexibility or another form of anxiety.

In figure 2, Novelty Object recognition data are shown. In this test, rats were placed in the same box from Open field with two identical objects in T1, and in T2 an object from T1 and a novel object are in the area. This test is used to measure short-term memory and recognition. While both control and SPS-exposed rats showed a preference for the novel object during memory recognition phase (T2), we did not see a difference between groups. In other words, traumatic stress exposure in adolescent does not affect short term object recognition memory in later adolescence.

In figure 3, the anxiety-like behavior measure by the Light/Dark Box is shown. Rats were free to roam in a two chamber apparatus. One chamber was covered with a lid with a black floor and black walls, while the other was not covered and had white floor and walls. This measures anxiety-like behaviors. There was no significant difference between the amount of time SPS-exposed and control rats spent in the light area, as well as in the number of transitions they made. This suggested traumatic stress exposure during does not affect anxiety-like behaviors in later adolescence.

In figure 5 Elevated plus Maze data are shown. Rats were placed in an elevated cross shaped apparatus comprised of two closed arms (with walls) and two open arms (without walls) This is meant to measure anxiety-like behavior. The amount of time SPS-exposed and control rats spent in the open arms were not significantly

different, nor were the amount of entries made by the subjects. This shows that traumatic stress exposure during adolescence does not affect anxiety-like behavior in adulthood.

In figure 4, behaviors during acquisition of fear conditioning are shown. (Fear learning behavior) Results show that scanning and the bout of scans were significantly higher in rats exposed to SPS during adolescence, suggesting that the rats had exhibited a different type of behavior compared to control, which showed increases in freezing behavior during this time. The data shown for grooming and rearing were not significantly different between the SPS and the control rats during acquisition. All animals showed similar levels of freezing behavior during fear memory retention testing. Collectively these data suggest that the traumatic stress exposure during adolescence may create an alternative fear-related learning strategy that is used as adults to properly acquire fear memories.

DISCUSSION

The rats that were exposed to traumatic stress during adolescence consistently showed little change in anxietylike behavior either during adolescence or adulthood. The only significant difference was in the Open Field behavioral test, where SPS-exposed rats frequented the area where they started the test (the placement area) more than the control rats. This could be due to the fact of behavioral inflexibility or another form of anxiety. In addition to this, the SPS exposed rats were less responsive when in the presence of a threat, such as during the shock in the Fear conditioning test. This suggests that being exposed to high levels of stress during adolescence may lead to the subject developing resilience to exaggerated fear learning later in life. This finding is consistent with the study done by Bulwada and colleagues, where it was suggested that adolescent social stress may not necessarily lead to a compromised adaptive capacity during adulthood.

Viewing it at as in the case of humans, for an example, if an individual at a young age lived in home where their parents argued all the time and domestic violence was involved for many years that experience as a young person may create a resilience to that type of environment. From early adolescence that sort of activity has been ingrained in their memory, so I would not see why when they are faced with an environment as such that they would freeze up or fear it. Another example would be with the rats that were involved in this study. Alternative to our original hypothesis, it could be hypothesized that the single prolonged stress during adolescence that the rats underwent may have made them less reactive to stressful or aversive stimuli as adults. In other words, trauma during adolescence may infer a resilience to conditioned fear and stress during adulthood.

There are some limitations to the experiments we've conducted. For example, the aims of this experiment such as introducing fear and then planning to discover that the individuals learn the fear may be flawed in the aspect of every individual may not fear the same thing. Also what we coin as fear or anxiety like activities in animals may not be what we mean in humans. Reactions to trauma may not be similar among all people either. For an example if two people are involved in a car accident and one is diagnosed with PTSD with the other is not, it may be because their biological response to stress is innately different. I also think that this study go further if scientists focused on what in the brain triggers PTSD for one person but not in another person after undergoing a similar traumatic event.

In conclusion we saw that trauma experienced during adolescent years had little to no significant effect on the rats and instead of creating anxiety like behaviors within the rats while actually creating a resilience to fear based behaviors in adulthood. Which proves our hypothesis to be incorrect, which allowed more questions to arise for me. Pertaining to humans, it made me consider if introducing trauma to humans at an early age may be good for them in the sense that they may learn how to overcome their fears. Also, taking it further for me

to question what in the brains of humans decide that they will fear one significant thing and not the other. Also what causes one person to be afraid of one thing and another individual not to be afraid of the same thing? For future endeavors, I am very aware that another testing of this experiment is already underway to see if the experiment is flawed or if the results are definite.

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DEVELOPMENT OF CHEMICAL TOOLS TO PROBE BACTERIAL KINASES

By Monika Franco Major: Biochemistry Mentor: Mary Pflum, PhD, Department of Chemistry

ABSTRACT

Cellular signaling controls all aspects of life, which is true in both eukaryotes and prokaryotes. Kinases are the catalyst for many cellular signaling reactions. Although many tools are available to probe the kinase activity in eukaryotes, methods to study prokaryotic kinases are limited. The Pflum lab has developed tools with ATP analogs that allow for kinase substrate identification in eukaryotic lysates. The focus of this project is the use of the ATP analog, ATP-biotin, to explore bacterial kinases in two prokaryotic cells: Escherichia coli and Myxococcus xanthus.

INTRODUCTION

Cellular signaling is arguably the most important process in life, and critical enzymes in signaling are kinases (Deng 2012). Kinases catalyze phosphorylation reactions by transferring a phosphate group from ATP (adenosine 5'-triphosphate) to cellular proteins (Figure 1A-D, Figure 2A) (Seifert 2002). The two well-known



eukaryotic protein kinases are Serine/Threonine and tyrosine kinases (Figure 1A-C) (Leonard 1998). Though some prokaryotes are known to also contain Serine/Threonine and Tyrosine kinases, they largely contain a different type of kinase, called Histidine Kinases (Figure 1D) (West 2001). Histidine kinases are different from Serine/Threonine and Tyrosine Kinases in that they are a part of a two component system containing a histidine core and response regulating proteins (Stock 2000). Serine/Threonine and Tyrosine Kinases work by using ATP as a co-substrate to phosphorylate the hydroxyl group of the respective protein (Figure 1A-C). On the other hand, the two component system has three different phosphorylation reactions (Figure 1D), involving the histidine kinases and the regulating proteins, where histidine is first phosphorylated to start the cascade of reactions (Stock 2000). Although there are many tools to study eukaryotic kinases, methods to study prokaryote kinases are limited (Leonard 1998). For

Figure 1: Kinase adds a phosphoryl group to the Hydroxyl group of Serine.(A) Threonine. (B) Tyrosine (C) Histidine doesn't have a free hydroxyl group but the nitrogen has the ability to accept a phosphoryl group. (D)



Figure 2 : The protein substrate is phosphorylated an ATP analog, such as ATP-biotin (B), to label the protein with a phosphoryl group by natural ATP (X=O-) or a phosphoryl biotin group. (A) (Green 2007) many years prokaryotes were thought to be absent of protein kinases (Wang and Koshland 1978).

The first protein kinase was discovered in Escherichia coli in 1969 (Kuo and Greengard 1969). Salmonella typhimurium also showed evidence for protein kinase activity (Wang and Koshland 1978.) As more bacterium have been discovered (Munoz-Dorado 1991, Wylie1988, Deutscher & Saier 1983) and the awareness that prokaryotes have protein kinase ability has become obvious (Mijakovic & Macek 2012, Deutscher & Saier 2006, Zhang 1996), so has the need for tools to identify and characterize prokaryotic kinases (Mijakovic & Macek 2012.)

The ability to identify and characterize prokaryotic kinase activity will lead to a more in-depth understanding of how bacterial cell environments work and how cell signaling contributes to those environments. There is currently research testing the use of kinases to combat antibiotic resistance (Correia 2006, Wehenkel 2008). For example, the kinases of M. tuberculosis were inhibited as a method to fight antibiotic resistance (Wehenkel 2008). In E. coli a serine/threonine kinase called HipA was discovered to be crucial for fighting antibiotic resistance and drug tolerance (Correia 2006). Kinase phosphorylation reactions drive many important reactions in the human body, but over activity can lead to ailments, such as cancer (Gross 2015). Kinase inhibition has become very prominent recently, especially in regards to anticancer therapeutics (Gross 2015, Zhang 2009). Kinase inhibition is the process of blocking a kinases ability to catalyze phosphorylation reactions by means of an inhibitor or through denaturation. One method, heat denaturation, is a process of inactivating the kinases using heat, while still preserving the other proteins. Staurosporine is a known small molecule

serine/threonine and tyrosine kinase inhibitor used in eukaryotic kinase studies. There are a limited number of tools available for inhibiting Histidine kinases, which is another possibly way to combat antibiotic resistance (Stock 2001). Understanding kinase activity will lead to future discovery in prokaryotic studies.

Kinases use the substrate ATP to phosphorylate serine, threonine, and tyrosine on the protein (Figure2A). Pflum lab has shown that kinases can also accept gamma modified ATP analogs, such as ATP-biotin (Figure 2B) to label proteins. Labeling of phosphoproteins with different functional groups, such as biotin, enables the study of phosphorylation.

The ATP analog used in this project is ATP-biotin (Figure 2B). The synthesis and use of ATP-biotin for phosphoproteomics was previously published in Pflum lab (Senevirathne, 2013), but ATP-biotin has only been used with kinases in mammalian lysates. This project explores the possibility of using this technology in a bacterial environment, in collaboration with Dr. Higgs from the Department of Biology at Wayne State University. If the Pflum lab strategy can be used with bacteria, it will enable the study of bacterial kinases.

The bacteria that was tested in these experiments were Myxococcus xanthus and Escherichia coli. Myxococcus xanthus is a gram negative bacterium that was first discovered to contain serine/threonine protein kinases in 1991 (Muñoz-Dorado 1991). E. coli is known to have the common prokaryotic histidine kinases (Rowland 2004). Recently, it has been discovered that E. coli contains a serine kinase, Hip A (Correia, 2006). Here we tested if the analog ATP-biotin would work with the kinase activity in bacterial lysates from E. coli and M. xanthus. Incubating lysates with no ATP-biotin was used as a negative control to test the validity of the experiment. ATP-biotin is expected to label kinase substrates in bacteria since it has already shown to work with serine/threonine protein kinases in eukaryotes. With the results obtained to date, ATP-biotin is working with kinases in bacteria, although it is unclear if histidine or serine kinases are involved. The results of the experiment were promising and show that ATP-biotin might facilitate the study of signaling in E. coli and M. xanthus.

RESULTS

ATP-BIOTIN SYNTHESIS

ATP-biotin synthesis was successful. By following the procedure previously established in an earlier publication (Figure 3), ATP-biotin was synthesized and used for these experiments (Senevirathne 2013).





KINASE-CATALYZED PHOSPHORYLATION AND BIOTINYLATION

An exploratory experiment was carried out to see if kinases in bacterial lysates could label substrates with ATP-biotin. Lysates from either E. coli or M. xanthus were incubated with ATP-biotin before separating proteins by SDS-PAGE(Sodium Dodecyl Sulfate -Polyacrylamide Gel Electrophoresis) and visualizing total proteins with Sypro Ruby stain and biotinylated proteins with streptavidin Cy5 dye. Biotin labeling was observed with E. coli lysates in the presence of ATP-biotin (Figure 4A, lane 1). Biotin labeling was also shown in M. xanthus lysates (Figure 4A, lane 3). The sypro ruby image (Figure 4B) shows equal protein amounts in each respective reaction. The lack of signaling in the reaction without ATP-biotin (Figure 4A, lanes 2 & 4) is consequently not due to protein loss or protein loading difference. It is surprising that M. xanthus did not label as well as E. coli (compare Figure 4A lanes 1 to 3). M. xanthus is known to have more Serine/threonine kinases than E. coli. The results indicate that both E. coli and M. xanthus are compatible with ATP-biotin.



ATP-

biotin	+	-	+	-
E. coli	+	+	-	-
M. xan	-	-	+	+

Figure 4A (top): Membrane treated with SA-Cy5.

Figure 4B (bottom): Syrpo Ruby Gel E. coli or M. xanthus lysates were incubated with ATP –biotin and as a control lysates were incubated with ATP-biotin.

KINASE-CATALYZED PHOSPHORYLATION AND BIOTINYLATION

The labeling in the first experiment was faint, so another exploratory trial was run to see if an increase concentration of ATP-biotin could label more effectively. This experiment was set up as previously mentioned except the amount of ATP biotin was increased by 50%. In addition, mammalian HeLa lysate control was added to ensure the quality of ATPbiotin. The use of ATP-biotin with HeLa lysates was previously published (Senevirathne & Pflum, 2013). The experiment was successful because there are clear biotinylated bands with every lysates incubated with ATP-biotin (Figure 5A, lanes 1, 3, and 5). There are many clear dark bands expected with HeLa lysates (Figure 5A, lane 1). E. coli lysates incubated with ATP-biotin also produced dark biotinylated protein bands (Figure 5A, lane 3). M. xanthus produced predominantly one dark band at the 95kD and two lighter bands towards the 35 kD range (Figure 5A, lane 5). As a control the lysate were stained with Sypro Ruby Stain (Figure 5B) and dark signaling was indicative that all reaction contained proteins. Lysates were incubated without ATP-biotin as a control and no labeling was observed (Figure 5A, lanes 2, 4, and 6).



ATP-

biotin +	-	+	-	+ -	
Hela +	+	-	-		
E.coli -	-	+	+		
M. xan -	-	-	-	+ +	

Figure 5A (Top): Membrane treated with SA-Cy5

Figure 5B (Bottom): Sypro Ruby Gel E. coli or M. xanthus incubated with ATPbiotin at 50% higher concentration than previous experiment. Lysates were incubated without ATP-biotin as a control. HeLa lysates were incubated with ATPbiotin to test the quality of the ATP-biotin.

INHIBITOR STUDIES IN BACTERIAL LYSATES

In order to show that biotin labeling is kinase dependence, ATP labeling was carried out in the presence of kinase inhibitor Staurosporine (STSP) and heat denaturated lysates. To heat denature the kinases in E. coli lysates, the reaction was heated to 95°C for 5 mins and then incubated with ATP-biotin (Figure 6A, lane 1). To inactivate the kinases in M. xanthus, the lysates were incubated with the small molecule inhibitor staurosporine (Figure 6A, lane 3) before adding ATP-biotin. With E. coli lysates, the reaction after heating (Figure 6A, lane 1) showed stronger biotin labeling than untreated reaction (Figure 6A, lane 2), which is unexpected. Unfortunately, the minimal labeling that was observed with E. coli lysates suggests that the reaction needs to be optimized. With the M. xanthus lysates, the reaction after incubation with the staurosporine inhibitor showed a decrease in biotin labeling (Figure 6A, lane 3) in comparison to untreated lysates (Figure 6A, lane 4). The dependence of labeling on inhibitor treatment indicated that the reaction is kinase catalyzed with serine/threonine and tyrosine kinases. As a control, proteins were present in each lane of the respective lysates (Figure 6B), which indicated equal protein loading. This experiment was successful and suggests that ATPbiotin is compatible with of bacterial kinases.



	1	2		3	4
ATP-					
biotin	+	+	+	+	
E. coli	+	+	-	-	
M. xan	-	-	+	+	
Heat					
Denatur	e +	-	-	-	
STSP	-	-	+	-	

Figure 6A (top): Membrane treated with SA-Cy5

Figure 6B (bottom): Spryo Ruby Gel E. coli or M. xanthus lysates were incubated with ATP-biotin. M. xanthus lysates were incubated with small molecule kinase inhibitor, staurosporine. E. coli lysates were heat denatured before adding ATP-biotin.

CONCLUSIONS

The tools for kinase characterization in prokaryotes is especially lacking. In these exploratory experiments we have learned that ATP-biotin reacts with the kinases in bacterial lysates. Though this research is in the exploratory stage, the results are promising. Successful labeling was observed with kinases from both E. coli and M. xanthus. What is very intriguing is that E. coli showed better labeling that the M. xanthis (Figures 4A, lanes 1 and 3, Figure 5A, lanes 3 and 5, and Figure 6A, lanes 2 and 4). This is especially curious because M. xanthus has more Ser/Thr and Try kinases than E. coli. With M. xanthus lysates, two clear bands at the 35kD were observed, which shows some continuity in these experiments (Figure 4A lane 3, figure 5A lane 5 and Figure 6A lane 4). However, the relatively small number of biotinylated bands in M. xanthus could be an indication that the conditions for the lysates. This research is new, exciting, and promising at this exploratory stage.

Inhibition experiments were partially successful, but further research needs to be done. The goal of these inhibition experiments will be to assure that the ATP-biotin reaction is kinase dependent, and not just a chemical, nonezymatic reaction. Depending on the results of these experiments, we can then conclude the validity of the use of ATP-Biotin as a tool to detect kinase substrate pairs in prokaryotes. For now, the current results are exciting and indicate a strong possibility that we have discovered a new tool for prokaryotic research. If this is true, the field of prokaryotic will be able to better understand cell signaling and may even be able to use this tool in studies that deal with antibiotics, including antibiotic resistance.

EXPERIMENTAL SECTION

ATP-BIOTIN SYNTHESIS

The synthesis of ATP-biotin was published elsewhere (Senevirathne & Pflum, 2013).

KINASE-CATALYZED PHOSPHORYLATION AND BIOTINYLATION WITH E. COLI AND M. XANTHUS

Lysates of M. Xanthus and E. coli were grown and lysed in the Higgs lab. Phosphorylation and biotinylation reactions were performed by incubating E. coli (100 μ g) or M. xanthus (100 μ g) with commercial PK (protein kinase) buffer (50mM Tris-HCl, 10mM MgCl₂, 0.1mM EDTA, 2mM DTT, 0.01% Brij 35, pH 7.5), and ATP-biotin (8mM). Water was added first, then PK buffer, E. coli or M. Xanthus lysates afterwards and finally ATP-biotin. The final volume of each reaction was 25 μ L. A second experiment was run that included the same conditions and buffer, but an increase of ATP-biotin concentration (12mM) and an increased M. xanthus concentration (160 μ g). The M. xanthus (160 μ g) lysate concentration increase caused the final volume to be 30 μ L. As a control, lysates were incubated without ATP-biotin. The mixtures were then incubated at 31°C for 2 hours without shaking. After the 2-hour incubation period, proteins in the reaction were separated by SDS-PAGE.

KINASES-CATALYZED PHOSPHORYLATION AND BIOTINYLATION WITH HELA LYSATE

PK buffer, HeLa lysates (100 μ g) and ATP-biotin (12mM) were incubated together at a final volume of 25 μ L. The lysates were incubated for two hours at 31°C without shaking. Afterwards, the proteins were separated by SDS-PAGE.

KINASE INACTIVATION

Phosphorylation and biotinylation inhibition reactions were performed by first resuspending E. coli (100µg) or M. xanthus (160µg) lysates in commercial PK Buffer. To generate heat denatured E. coli lysates, the lysate mixture was heated at 95°C for five minutes to denature the kinases before ATP-biotin (2mM) addition. For the inhibition reaction in M. xanthus, staurosporine (1µM) was preincubated for 1 hr at 37°C with the lysates before ATP-biotin (2mM) was added and incubated. The final volume the E. coli reaction was 25µL, while M. xanthus final reaction volume was 30µL As described earlier, reactions were then incubated at 31°C for 2 hours without shaking. After the 2-hour incubation period the reaction the proteins in the reaction were separated through an SDS-PAGE.

SDS-PAGE PROTOCOL

The SDS-PAGE protocol from Sino Biological was followed (Sino Biological). The SDS-PAGE was composed of 10% acrylamide/bisacrylamide (37.5:1). The separating layer consisted of acrylamide (10%), Tris-HCl (1.5M, pH8.8), SDS (10%), APS (ammonium persulfate)(10%), and N,N,N',N'-tetramethylethylene-diamine (TEMED)(0.05%). The stacking layer consisted of acrylamide (10%), Tris-HCL (0.5M, pH 6.5), SDS (10%), APS (10%), and TEMED (0.05%). Once biotinylation reactions were complete, a loading dye (10% w/v SDS , 10 mM beta-mercapto-ethanol, 20 % v/v Glycerol, 0.2 MTris-HCl, pH 6.8, 0.05% w/v Bromophenolblue), which was added to facilitate the denaturation of the proteins. The samples were heated at 95°C for one minute and spun down for 1 min at 6RPM in a centrifuge. Sample were then loaded onto a prepared gel and run usuing electrophoresis apparatus (Biorad) using running buffer (Tris-HCl (25mM), Glycine(200mM), and SDS (0.1%), diluted to 1000mL).

ELECTROPHORESIS

Electrophoresis protocol from Mahmood (2012) was followed. The gel was placed in electrophoresis apparatus (Biorad) containing running buffer Electrophoresis was performed at 110V for 10 min to pass through the stacking layer and then 200V for 60 min or until the proteins pass through the separating layer completely. After samples were completely separated, one gel was stained by Sypro Ruby and the other was stained with Streptavidin Cy5.

SYPRO RUBY STAIN

Sypro Ruby staining protocol was obtained from Thermo Fischer. The gel was incubated in destaining solution (methanol (%50), Acetic acid (%7)) for 1-2 hrs and then SYPRO® ruby gel stain (approximately 30mL) overnight. SYPRO® ruby gel stain was reused and stored at 4°C. Before scanning the gel with Typhoon FA 7000 from GE Healthcare Life Sciences, the gel was washed with de-staining solution (methanol (%10), acetic acid (%7)) for half an hour. Protein bands in the gel were visualized using the Sypro Ruby parameters from Typhoon FLA 7000.

STREPTAVINDIN-CY5 STAINING

Membrane staining followed the protocol from Mahmood (2012). A polyvinylidene fluoride (PVDF) membrane was cut to the dimensions of the PAGE gel and rinsed one time with methanol (5mL), and then

water (5mL), before incubating with transfer buffer (10ml or enough to completely cover the membrane).

Transfer buffer consisted of tris Base (25mM), glycine (190 mM), and methanol (%20) in 1L of water. Gel was rinsed with water and incubated in transfer buffer before electrotransfer. Filters were cut to fit the sponges on the grid that will "sandwich" the gel and membrane together. Filter and grid apparatus were soaked in transfer buffer before "sandwich" was made. For the electrotransfer transfer a "sandwich" was created with the gel and membrane in between two filters all contained in support grid. This grid was then placed into an electrode (black to black) and placed in a tank (Biorad). The tank was then filled with the remaining transfer buffer. An ice pack was placed in the tank with the electrode, and the tank was covered in ice. The transfer was then run at 90v for 120 min. Once the membrane was transferred it was blocked over night in PBST (Phosphate Buffered Saline Tween) (Na_2HPO (8mM), NaCl (150mM), KH₂PO₄ (2mM), KCl (3mM), Tween (0.5%)) containing 5% w/v milk (10mL total volume). SA-Cy5 (7ul) in PBST containing 4% w/v milk (15mL total volume) was added to the membrane and incubated for an hour, while rocking. The membrane was rinsed once with PBST (35mL) and three times with PBS (Phosphate Buffered Saline) (Na_2HPO (8mM), NaCl(150mM), KH_2PO_4 (2mM), KCl (3mM)) (10mL). Then signaling was read using a typhoon scanning instrument, under Cy-5 parameters.

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EATING THE ELEPHANT ONE BITE AT A TIME: AN EXAMINATION OF ETHNIC IDENTITY, EATING BEHAVIORS AND PHYSICAL ACTIVITY

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ABSTRACT

OBJECTIVE: The purpose of the secondary analysis is to describe the relationship among the variables of ethnic identity, physical activity and eating behaviors in African American Adolescents ages 15 – 17 years of age.

METHODS: 145 African American adolescents were recruited from community organizations throughout the Metropolitan Detroit area. Phinney's Multigroup Ethnic Identity Measure (MEIM) was used to measure ethnic identity. Schlundt's eating behavior pattern questionnaire (EBPQ) was used to measure eating behaviors. Kowalski's physical activity questionnaire (PAQ) was used to measure physical activity. Utilizing SPSS version 19.0, a descriptive correlational design was implemented.

RESULTS: In the findings of this study, it was determined that the more exercise that the African American adolescents engaged in, the less likely they were to skip a meal and experience emotional eating. Furthermore, in examining the relationship between eating behaviors and ethnic identity, African American adolescent who meal skip were more likely to affiliate with groups outside of the their ethnic group. Also, it was discovered that -participants who had other group affiliations also participated more frequently in basketball. Lastly, ethnic achievement was a significant factor in how often adolescents partook in physical activity.

CONCLUSION: The initial findings from the current study contribute to health care and society by beginning to address the gaps in the literature that focus on the African American adolescent, particularly with regard to obesity and weight-related behaviors. It is essential to inquire about the cultural influences of eating and physical activity as they relate to the adolescent's weight status, especially if they are obese or overweight.

INTRODUCTION

Obesity is particularly problematic in African Americans, especially adolescents. African American adolescents consume foods high in fat and calories, and they are less physically active than their white counterparts, (Perlman & Seidman, 1996; Vertinsky, 1998). The culmination of eating and physical activity factors could be the beginning point of the obesity disparity experienced. Many overweight and obese African American adolescents have impaired glucose tolerance and type 2 diabetes, and they show early signs of cardiovascular risk and insulin resistance syndrome, also referred to as metabolic syndrome or syndrome X. The prevalence of these diseases result in increased morbidity and mortality rates in African American Adolescents.

Additionally, there is some evidence that obesity in African American adolescents has unique cultural features that stem from eating behaviors, physical activity and their ethnic identity. Ethnic identity may be a factor that further influences physical activity and eating behaviors in African American adolescents, but

there is very little evidence on this relation. Studies have shown that there is a higher level of cultural tolerance for obesity in the African American community as opposed to their Caucasian counterparts,

(Baskin, Dulin-Keita, Thind, & Godsey, 2015). The cultural tolerance surrounding ethnic norms associated with eating and physical activity, presents problems for public health interventions that advocate engagement in healthy eating and physical activity.

STUDY PURPOSE

The purpose of this secondary analysis is to describe the relationship among the variables of ethnic identity, physical activity and eating behaviors in African American Adolescents ages 15 - 17 years. The examination of the relationship among these variables will help inform culturally sensitive and developmentally appropriate health interventions toward eliminating this disparity in African American adolescents.

RESEARCH QUESTIONS AND HYPOTHESES

Research Question #1: What is the influence of ethnic identity on eating behaviors in African

American Adolescents?

Hypothesis 1: There will be a positive correlation between ethnic identity and eating

behaviors in African American Adolescents.

Research Question #2: What is the influence of ethnic identity on physical activity in African

American Adolescents?

Hypothesis 2: There will be a positive correlation between ethnic identity and eating

behaviors in African American Adolescents.

Research Question #3: What is the relationship between physical activity and eating

behaviors in African American adolescents?

Hypothesis 3: There will be correlations demonstrated between physical activity

and different eating behaviors in African American adolescents.

THEORETICAL FRAMEWORK

Allen and Allen's (1986) concepts of sociocultural factors, transmission of sociocultural norms, weightrelated behaviors and cognitions, and their relationships will continue to provide the basis for the secondary analysis. Their sociocultural approach to social ecology differs from an exclusive focus on the individual; rather, their focus is on the individual within his or her sociocultural environment. Because the individual's social culture has strong norms for health behaviors, it is essential to understand the power of these norms to fully understand the individual's health behaviors and ultimately his or her health.

LITERATURE REVIEW

The goal of this secondary analysis was to extend the knowledge of weight related behaviors and cognitions (physical activity and eating behaviors) among African American adolescents as they identify with their ethnic identity. The following literature review provides an overview of relevant research related to the study variables published between 2011 through 2015.

ETHNIC IDENTITY

Ethnic identity is one's sense of importance and belonging to an ethnic group and the part of one's thinking, perception, feelings, and behavior that is due to ethnic group membership on an individual, intragroup, and intergroup level (Phinney & Rosenthal, 1992; Scottham & Sellers, 2008). There is a dearth of literature present regarding ethnic identity and its relation to eating behaviors and physical activity in African American adolescents. One of the only studies ascertained was published in 2008. Granberg, Simons, Gibbons, and Melby (2008) found that in a study of African American girls ages 12 to 14, there was no significant relationship between ethnic identity and weight status discovered. It is suspected that the finding was present as a result of the age range for assessing ethnic identity may have been too young to examine a correlation between identity and weight status.

PHYSICAL ACTIVITY

Physical activity is defined as bodily movement produced by skeletal muscles resulting in energy expenditure (Bailey, 2006). There are many influences on physical activity in African American adolescents. Based on current literature, many influential factors have their underpinnings in the social, economical, and environmental realms of the adolescent's realities. Within social settings, Baskin, Dulin-Keita, Thind, and Godsey (2015) found that there were different factors influencing physical activity in African American Adolescents. Factors influencing physical activity included pressure from social networks, serving as a role model for younger children and competition among their peer groups. Additionally, Bonhert, Burdette, Dugas, Travers, Randall, Richards, and Luke (2013) stated that peers have a strong influence within the African American adolescent community. These adolescents were more likely to engage in sedentary activities while with their parents in contrary to their peer groups.

From an economic and environmental perspective, Perkins and Partidge (2014) found that cost was a factor related to physical activity participation within the African American adolescent community while Siceloff, Coulon, and Wilson (2011) found that neighborhood walkability was indirectly related to physical activity in African Americans living in low income communities. Residents living in underserved communities may not be afforded adequate opportunities to engage in physical activity.

EATING BEHAVIORS

For the purpose of this secondary analysis, dietary intake was expressed as eating behaviors. Eating behaviors are the ways in which humans use food in addition to the sociocultural affiliation with food: how food is obtained and stored, how it is prepared, how it is served and to whom, and how it is consumed (Bailey, 2006). Behaviors for the purpose of this secondary analysis include low fat eating, emotional eating,

haphazard meal planning, sweets intake, meal skipping and cultural eating. There was very little literature found on haphazard meal planning or sweets intake in the African American adolescent between 2011 – 2015; however, there was published evidence describing emotional eating, low fat eating, meal skipping, and cultural eating in this population. Emotional eating was described in a study by Olvera et al (2013) who found that girls ages 9-14 years who have experienced weight related teasing by peers were more likely to emotional eat. Girls who experienced weight related teasing by parents were more likely to have weight concerns and display eating problems. Meal skipping was addressed by Schembre et al (2013) who found eating breakfast less frequently was associated with less time spent being physical active. Eating breakfast less frequently was also associated with a higher percentage of body fat. Culturally, Christiansen et al (2013), found that low-income African American adolescents participating in the study reported that their family and neighborhood influenced their eating habits. Their peers had a lesser effect on their eating habits. Additionally, Bohnert et al (2013), found that urban, low income adolescents who spent more time with their parents consumed foods higher in fat. This is suspected to be the result or the parents' choice in food, rather than that of the adolescent's.

METHODOLOGY

This section addresses the methodological procedures that were implemented in the secondary analysis. Research design, sample, recruitment, instrumentation, and statistical analysis procedures are discussed.

DESIGN

Different from other studies that have only investigated predictors of obesity in African American Adolescents. This study however was a correlational design allowed investigators to describe the phenomenon of obesity in African American Adolescents while examining the relationship among the variables ethnic identity, physical activity, and eating behaviors.

SAMPLE

The sample size consisted of 144 African American Adolescents ages 15-17. There have been substantial amounts of documentation on the developmental and sociocultural changes that occur with African American Adolescents (Erikson, 1968; Garcia-Coll, et al., 1996; Quintana, 2007). During Adolescence, which occurs between the years of 12-21, it is believed to be a period of marked change. Middle adolescents, ages 15 through 17 years' experience significant amounts of identity formation that include body image development and the emergence of ethnic identity.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria included the following attributes: 1) Self-identified Non – Hispanic African American 2) 15 through 17 years of age 3)Resident of inner city Detroit or its surrounding suburbs 4) Enrolled in a local high school. Exclusion criteria included the following attribute: 1) Lack of ability to speak or understand English.

RECRUITMENT

Participants were recruited from a population that attended community out-reach clinics, community organizations, churches, and through professional networks (e.g., sorority and professional organizations) throughout inner city Detroit and surrounding areas. Parental consent and adolescent assent was obtained prior to enrollment in the study. IRB approval was obtained from the respective academic institution.

DATA COLLECTION

The principal investigator explained the purpose of the study using a written script to adolescents who met the inclusion criteria. Adolescents wishing to participate were instructed to return the informed consent form completed by their legal (Appendix B) along with the adolescent assent form prior to the start of the data collection. Demographic data that were obtained from the participants and parents included gender, age, highest level of education, zip code, crossroads of neighborhood, race/ethnicity, parental marital status, highest level of familial education, and yearly household income. Data collection was conducted in a setting familiar to each of the participants, depending upon the location of solicitation. For the adolescents who were recruited from the primary care clinic, data collection was completed in an area designated by the office manager. In the church setting, data collection was conducted in the fellowship hall of their church or other designated area; for the adolescents who were recruited from a community organization, data collection was conducted in a classroom of the organization's central building or other designated area. For the participants obtained through social networks, data were collected at a mutually agreed upon location where the participant and principal investigator were comfortable.

INSTRUMENTATION

Jean Phinney's Multi-group Ethnic Identity Measures (MEIM) (Phinney, 1992) was a questionnaire that was design to assess an adolescent's degree of identification with their ethnic group, despite the unique characteristics of the group. The 20-item survey instrument consisted of a 4-point Likert-type scale that was utilized to study correlates of ethnic identity across diverse samples, as group identity is common to all humans (Phinney, 1996). Ethnic identity was measured by five factors that included self-identification and ethnicity, ethnic behaviors and practices, affirmation and belonging, ethnic identity to help identify the label that the participant used for himself or herself. Self-identification is a vital condition for the development of ethnic identity and therefore had to be separated from ethnicity to prevent confounding ethnic identity with ethnicity. Eating behaviors and practices focuses on two aspects of practices, which include involvement in social activities with one's group, and participation is cultural traditions (Phinney, 1992). Affirmation and belonging was assessed by how one esteems their background and group membership. Ethnic identity Achievement was measured by how secure one has developed in their identity formation process that is at an optimal level. Attitudes towards other groups was an assessment the participants attitudes and interactions with others outside of their ethnic group.

Kent Kowalski's Physical Activity Questionnaire for Adolescents (PAQ-A) (Kowalski, 2004) was a questionnaire that was designed to assess the amount of physical activity that a particular adolescent engages in, through a 7 day recall of physical activity experienced. Specific activity participation (i. e., dance, football, etc.) as well as weekend, after school, evening, physical education, and lunch activity levels are determined by a 5-point Likert-type scale. Items 1 to 8 are scored 1 to 5, yielding a composite score of 8 to 40, with higher scores indicating greater physical activity.

David Schlundt's Eating Behavior Pattern Questionnaire (EBPQ) (Schlundt, 2002) was a 51 item questionnaire that was designed to predict the amount of fat intake for African American Adolescents. Eating behaviors was observed by six factors, which included low fat eating, emotional eating, snacking on sweets, cultural/lifestyles behaviors, haphazard planning, and meal skipping. Low fat eating is how cognizant a participant is of the amount of low calorie and low fat foods that they consume. Emotional eating is how

often a participant eats in response to their emotions. Snacking on sweets measured how often a participant consumes sugary snacks. Cultural/Lifestyle behaviors measured the role of food in a participant's everyday life. Haphazard planning measures the participant's organization and scheduling of meals. Meal skipping measures the frequency of substitution methods of meals.

STATISTICAL ANALYSIS

SPSS Version 19.0 statistical package was used to understand and summarize the data, descriptive statistics performed. Specifically, Pearson's product moment correlations and were performed to determine the relationships among the study variables.

RESULTS

SAMPLE CHARACTERISTICS

Of the 145 respondents who completed the study, 140 or 96.6% identified as African American, four or 2.8% identified as Mixed, and one or 0.7% identified as Hispanic/Latino. Eighty-eight or 60.7% were female and fifty-seven or 39.3% were male. The respondent's ages ranged from 15 to 17 years; 48 or 33.1% were 15 years old, 53 or 36.3% were 16 years old, and 44 or 30.3% were 17 years old. One hundred and sixteen or 79.5% of the respondents were from inner city Detroit and 28 or 19.2% of the respondents were from metropolitan Detroit. Ninety two or 63.4% respondents qualified for free lunch but 53 or 36.6% did not qualify. Sixty-eight or 47.2% of the respondents were members of sports teams while 76 or 52.8% of the respondents were non-members of sports teams.

RELATION AMONG RESEARCH QUESTIONS, HYPOTHESES, AND STUDY VARIABLES

The results of the secondary analysis will be discussed in this section within the context of the research questions and hypotheses.

Research Question #1: What is the influence of ethnic identity on eating behaviors in African American Adolescents?

Hypothesis 1: There will be a positive correlation between ethnic identity and eating behaviors in African American Adolescents.

Results 1a: There were positive correlations between ethnic identity and eating behaviors in African American adolescents. Statistically significant relations include:

Low fat eating and MEIM total score (r = .188, p = .023)

Low fat eating and ethnic identity achievement (r = .287, p = .000)

Cultural eating and MEIM total score (r = .254, p = .002)

Cultural eating and ethnic affirmation/beliefs (r = .235, p = .004)

Cultural eating and ethnic behaviors (r = .256, p = .002)

Research Question #2: What is the influence of ethnic identity on physical activity in African American Adolescents?

Hypothesis 2: There will be a positive correlation between ethnic identity and physical activity in African American Adolescents.

Results 2a: There were positive correlations between ethnic identity and physical activity in African American adolescents. Statistically significant relations include:

Ethnic behaviors and dance (r = .198, p = .017)

MEIM total score and skipping (r = .168, p = .043)

Ethnic identity achievement and skipping (r =.211, p = .011)

Results 2b: There were negative correlations between ethnic identity and physical activity in African American adolescents. Statistically significant relations include:

Other group and skiing (r = -.311, p = .000)

MEIM total score and baseball (r = -.227, p =.006)

Affirmation and belonging and baseball (r =-.168, p = .044)

Ethnic identity achievement and baseball (r = -.215, p = .009)

Other group affiliation and baseball (r = -.196, p = .018)

Ethnic behaviors and skiing (r = -.225, p = .006)

Other group affiliation and basketball (r = -.269, p = .001)

Other group affiliation and skiing (r = -.311, p = .000)

Research Question #3: What is the relationship between physical activity and eating behaviors in African American adolescents?

Hypothesis 3: There will be correlations demonstrated between physical activity and different eating behaviors in African American adolescents.

Results 3a: There were positive correlations demonstrated between physical activity and different eating behaviors in African American adolescents. Statistically significant relations include:

Physical activity and low fat eating. (r = 221, p = .007)

Skipping and low fat eating (r = .242, p = .003)

Juggling and low fat eating (r = .223, p = .008)

Tag and haphazard meal planning (r = .241, p = .004)

Result 3b: There were negative correlations demonstrated between physical activity and different eating behaviors in African American adolescents. Statistically significant relations include:

Physical activity total and emotional eating (r = -.168, p = .043) Walking and emotional eating (r = -.172, p= .042) Walking and sweets eating (r = -.219, p = .009)

Football and meal skipping (r = -.339, p = .000) Soccer and meal skipping (r = -.176, p =.033) Football and emotional eating (r = -.171, p = .040) Field hockey and cultural eating (r = -.179, p = .30) Basketball and emotional eating (r = -.203, p = .015) Basketball and meal skipping (r = -.208, p = .013) Low fat eating and ice skating (r = - .166, p = .046)

DISCUSSION

During the primary analysis of this study, ethnic identity was not found to have a statically significant correlation with eating behaviors and physical activity in African American adolescents. However, within the current secondary analysis, a closer examination of the subscales of the instrument demonstrated statistically and theoretically significant relationships. In review of the relationship between physical activity and eating behaviors, it was determined that the more exercise that the African American adolescents engaged in, the less likely they were to skip a meal and experience emotional eating. Furthermore, in examining the relationship between eating behaviors and ethnic identity, African American adolescent who meal skip were more likely to affiliate with groups outside of the their ethnic group. Also, it was discovered that -participants who had other group affiliations also participated more frequently in basketball. Lastly, ethnic achievement was a significant factor in how often adolescents partook in physical activity.

IMPLICATIONS AND CONCLUSION

This secondary analysis was the first of its kind to examine the relationships among, ethnic identity, physical activity, and eating behavior patterns in African American adolescents. Initial findings from the study will assist in helping healthcare practitioners and researchers to better understand the factors related to the behaviors and cognitions related to the obesity epidemic that affects the African American adolescent in disparate proportions. These variables are essential components to include in developmentally appropriate

and culturally relevant, targeted interventions with this population. Health care providers and researchers who work with youth can utilize the initial findings from this study to be the advocates of healthy lifestyles while reducing the obesity disparity within the African American adolescent population.

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CAN THE FOOD IN YOUR CABINET SAVE YOUR LIFE? A CASE STUDY ON FOOD AS MEDICINE IN METRO DETROIT.

By Kizzmett Littleton Major: Nutrition and Food Science Mentor: Yuson Jung, PhD, Department of Anthropology

ABSTRACT

To what extent do Detroiters consider food to be medicine? The purpose of this study is to identify the standpoint of local Detroiters on the concept of food being used medicinally to prevent and treat ailments using a qualitative approach. This is a significant research topic because more than 133 million people have chronic illnesses in the United States requiring mandated medications that more often than not only treat the symptoms but not cure the illnesses. This places a physical, mental and financial burden on millions of American families. Overall, this research will identify the general response of Detroiters as a case study. It will also challenge Detroiters to take a deeper look at the concept of "food as medicine" as a whole. Thus my research findings can also have practical implications encouraging Detroiters to do research of their own to discover whether or not food can have a healing impact.

INTRODUCTION

There has been a growing interest in the discussion of food in which healing can occur naturally through food and supplements. This research will uncover how Detroiters are responding to this notion of "food as medicine" and challenge the assumption that pharmaceutical medicine is the ultimate solution to cure ailments. In America this is a prevalent topic because over 45% of the American population, which is more than 133 million people, have at least one chronic illness. According to U.S National Center for Health Statistics, a chronic disease is defined as a disease lasting three months or longer and generally incurable. There are over 24 diseases that are deemed as "chronic" by healthcare providers. For this reason, the cost of health care is very high; more than 75% of all health care cost are due to these conditions. More often than not, these chronic diseases require mandated medication that will not cure the disease itself, but instead only treat or subdue the symptoms. This is an issue that must be addressed because chronic illnesses take a physical, mental and financial toll on Americans. This research will examine views on the cultural perception of food and its correlation to medicine and healing. By doing so, it will challenge the cultural assumptions currently made about food and medicine especially pertaining to Western societies.

Though there is a plethora of information regarding food as medicine, the question remains, to what extent people agree on this relationship and what this relationship means to their everyday practice of taking care of their health. The research is to discover the ways in which food is being portrayed in society, and how Detroiters respond to information provided in popular health and medical sources, media as well as literature. What is the current discussion on food and health in Detroit? To what extent is it widely accepted that food can heal illnesses? To what extent is food only used for dietary needs? What is the source of Detroiters' response? Using a quantitative approach, this research will use a survey method to identify what impact food discourse and practices have on the lives of ordinary Detroiters.
LITERATURE REVIEW

Using three different bodies of literature surrounding food and medicine I will compare and contrast ideas on the concept of using food to heal. I chose medical doctors, anthropologists and nutritionists because each profession had analyses written from their respective perspective. I found that the three bodies of literature had many similar thoughts about food and its benefits, but did differ on how food can or should be used. These previous studies will help me to better analyze my collected data and situate my study for further research on this topic.

MEDICAL DOCTORS

According to books written by medical doctors for popular audience, many illnesses can actually be cured through changes in diet and lifestyle. They posit that when diet and lifestyle changes occur, a person will improve in virtually every way measurable, including heart disease, decreasing of PSA (prostate specific antigen) levels, or even a change in gene expression changing (Robbins 2013). People who choose to have excellent nutrition in turn for better health are called "nutritarians," according to Dr. Joel Fuhrman, a physician who is in the forefront of public education on a micronutrient-rich diet that will end diseases. He uses the formula H=n/c (health equals nutrients divided by calories) to advocate that people are able to use micronutrients, which include vitamins, minerals and phytochemicals, to strive for better nutrition (Robbins 2013). Eating an ample amount of these micronutrients will help people to live longer, repair cellular damage, and protect against late-life diseases including dementia (Robbins 2013). Unfortunately the American diet is deficient of many micronutrients sequentially leading to illnesses such as cancer and heart disease. Dr. Fuhrman created an acronym known as G-BOMBS to help people remember foods they should be daily consuming. The acronym stands for greens-beans, onions, mushrooms, berries, and seeds. Just by constructing a person's diet, the human immune can reach an optimal high performance function. The accurate supply of sunlight, fatty acids, and micronutrients supports the immune system function and will aid in achieving excellent nutrition (Robbins 2013: 53-54).

Another natural healing process commonly discussed in literature surrounding health through food was a concept known as homeopathy. Homeopathy is a natural system of medical therapeutics that stimulate the body to heal and repair itself (Shohet 2005). It falls in between the medical and psychodynamic models of health, and gives a true holistic view of a person's health, illness and treatment (Shohet 2005). According to David Owen, a homoeopathist, this form of treatment has allowed him to answer the question of why a person has taken ill and what must be done to treat and prevent this illness from reoccurring (Shohet 2005). One suggestion offered by Dr. Steve Bratman, a physician and medical director for Prima Health, as to why people choose natural medicine is because conventional drugs don't offer the benefits some herbs and supplements can supply with fewer side effects, and in turn help people feel better (Bratman 2000). According to these medical doctors who advocate for treating food as medicine, pills may not make a person feel better, however a change in lifestyle and diet can, and that's how people like homeopaths continue to use this alternative approach with food to cure ailments. This in turn leads to a virtuous cycle of people extending their lifespan and living better (Robbins 2013). In general medical doctors conclude that vitamins and minerals in fresh food can be used to heal the body because if it has an ample about of micronutrients.

ANTHROPOLOGISTS

Anthropologists take a cultural approach when examining the topic of food as medicine. In many cultural traditions, food is considered a healing tool. One method is called food therapy or diet therapy in China (Chen 2007). The central principle of food therapy, *shi liao*, is to eat every meal or specific food item with

the intent to maintain good health, promise vitality or alleviate illness. Diet therapy, within traditional Chinese medicine, was claimed to be the first and highest form of medicine. It is established based on a system of four properties: hot, cold, wet, and dry as well as different degrees of heat, coolness, dryness, or dampness. These qualities can also be characterized as yin and yang. Yin is believed to have the effects of cooling thus lowering the body's energy level or metabolism. On the other hand, yang foods heat up or increase the metabolism. The combinations of foods with different properties has the ability to promote or even restore balance in a person's health (Chen 2007: 19-23). Furthermore, Chinese medicine can be considered to take a "holistic" approach because it joins together symptoms of illnesses to mental and social factors without referral to parts of the body, but instead with relationship to the appearance of the brain. In other words, Chinese medicine is characteristically known for looking at the psychological and pathological changes. Symptoms occurring at the same time suggest a relationship, and as the physicians of Chinese medicine, their job is to determine the exact location in the brain to which the production of these symptoms relate (Farquhar 2002).

Different cultures, including ancient Greek, Islamic, and Ayurvedic as well as Ancient Chinese have shared perspectives on the connection between food and medicine in relation to the body, its properties/humors and ideas of energy and nutrition (Chen 2007). Herbs and spices were part of a broad spectrum of beliefs surrounding the specific medicinal properties of food. Rarely was food and medicine separated, both were considered as "eating" (you are eating the medicine just as you are eating food), and food was considered medicine (Chen 2007). Ayurveda, meaning knowledge of life and longevity, is a system of medicine that is also based on humoral theories (medicine derived from the makeup of the human body). Ayurvedic medicine encompasses a variety of dietary and therapeutic categories using the qualities of energy in the body and environment. Deriving from spiritual Hindu beliefs and philosophies, Ayurveda main focus pertains to diet, lifestyle, hygiene and other routines leading to an ideal holy/healthy lifestyle. Through cultural knowledge, Ayurveda placed a deep emphasis on humors based on knowledge because the imbalance of humors resulted in diseases. As tradition developed, the focus became intent on the interaction between different humors and the quality of various types of foods (Chen 2007: 23-24).

From an anthropological perspective, one must not only look at the science part of food, but how food fits in the world every day. A cultural analysis thus can help realize how culture affects who eats what, when, where, why, and how. In many cultures, food is considered to have medicinal qualities and medicine is "eaten" in the same manner as food (Chen 2007). Nonetheless, eating and medication have become two entirely different entities. Cultural changes have even affected the way medicine is now viewed in those cultures. Similar to America, the Chinese are leaning more towards medicine in the pill form that can be bought at the local drug store. It is viewed as more convenient because the pills are considered easier to swallow and handier to take. It is however also thought to be less effective. Therefore many people still refer to traditional Chinese medical practices when searching for healing (Farquhar 2002). In traditional Chinese medical continues to play a significant role (Chen 2007). Generally anthropologists examine and analyze cultural traditions and practices holistically and consider food, diet and lifestyle as a tool for healing the mind and body.

NUTRITIONISTS

Nutritionists on the other hand look at how food can heal the body by providing nourishments thus allowing the body to heal itself. Nutrition, of course, is the main focus for nutritionists. Based on various books and articles, nutritionists posit that if the body has an efficient amount of vitamins and minerals, the body can treat and prevent even the worst of disease. Even though the amount of nutrients needed may differ, all, if

not most, nutritionists do agree nutrients play a key role in the wellness of a person. One of the best places to get those nutrients are from food. *The Healing Foods* and *Healing with Whole Foods*, for example, were two books written by nutritionists, and both books provided detailed information on different foods and their properties, how they could be used to heal the body, as well as what foods are best to eat in order to treat aliments in the body (Pitchford 2002). Overall nutritionist, similar to what medical doctors believe, if a person maintains all nutrient requirements the body will naturally be able to heal itself.

Social media plays an important role in how nutrition is displayed and perceived. Shows like Dr. Oz, the Doctors, Dr. Axe, for instance, give viewers with an overload of information pertaining to health and food, but they don't know what effect it is having on people's lives. Nor can they assess how the information is perceived and practiced. Although the information provided in these shows may be accurate, it can be easily misinterpreted by the viewers who are not well educated in the discipline of nutrition. Other studies, like the one mentioned in *The New York Times* regarding quinoa, for example, have found that the public is confused on the health benefits of many so-called trendy health foods. Even though doctors, nutritionists, and other health professionals provide their perspectives on the nutritional values of foods, the public seems to differ in opinion substantially (Quealy 2016).

So do people accept the notion that food can be an antidote to heal illnesses? Some literature and media displays foods as a possible remedy for ailments. The question however is, to what extent do people believe this to be true? And how do they practice this treatment at home? To understand how Detroiters practice the idea of food as medicine, I learned the views of different people from the metropolitan area. This research will allow us to gain insight into the different perspectives Detroiters have, based on their own knowledge regarding food and its relation to medicine. The research was based on observing and participating at several education programs on food and nutrition in the metro Detroit area, and collecting a survey in those programs.

METHODOLOGY AND METHODS

Using two different surveys, I was able to analyze the knowledge Detroiters had on the concept of food as medicine. For this study, people participated based on their free will and were given the survey after participating in an educational class about food and nutrition. Over the period of 2 months, a total of 800 people filled out surveys from various locations including Wholes Foods Detroit, WSU Farmers Market, Eastern Market, Corktown Market, and Henry Ford Hospital. These sites were chosen because they were places in the Metropolitan Detroit area that focused on advocacy of healthy eating as well as providing information about food being used to heal the body.

Two different surveys were distributed in order to collect data. Survey 1 asked questions pertaining to the effects food had on the participant's life. The questions asked whose advice they sought out when they were sick, what roles food played in their life, and whether or not they gained any new perspective based on the information provided in the education classes on food and nutrition. Therefore, these surveys were passed out at classes attended about food and how it can be used to help heal the body. Survey 2 was created to distribute to people attending local Detroit farmers' markets. This survey asked why they had come to the market, whether or not their perception of food had changed over the course of shopping there, and their opinion of what food was used for. Survey 1 contained eight, and survey 2 consisted of five open-ended questions, allowing people the freedom to write whatever they felt. At each market I asked people if they minded filling out a quick survey for research I was doing at Wayne State University. The survey took about 5 minutes. At the markets people typically shopped with friends or family so if one person agreed to fill out a survey, then everyone with them would fill it out. In the classes I attended, I talked to the instructor before

the class asking to pass out the surveys and people who wanted would fill them out right after class was over. Overall people were pretty compliant, but when I was at the markets a lot of times people where in a rush so they wouldn't have time to fill out the survey or just did not want to participate. To my surprise, however, many others did not mind responding to the survey.

SURVEY RESULTS

The surveys used in this research were distributed to both open farmers' markets and health/nutrition classes. Surveys passed out in markets asked questions pertaining to events or circumstances of the market (see Appendix for the actual Surveys used for this research.) One of the questions asked related to how people viewed food. They were given the choice to check all that applied.



Upon gathering the information from the surveys, it can be concluded that Detroiters place a high emphasis on how food *tastes* and who they *eat it with*, but less emphasis on what food *does* to the body. Only about half of the participants responded that food could be used for the alleviation of illnesses, but 95% replied that food was used for taste. Similarly, the surveys distributed at health classes, 86% of participants responded that food brought family together. Not far from that, 84% felt that food helped with their health. For the surveys distributed throughout various classes regarding food as medicine, a similar question was asked regarding how people viewed food in their everyday lives.



Another interesting fact found from the surveys, was that 52% of participants said that they came to the market because of a close relative and 44% said that they visited the market because it was close and provided fresh foods. In Survey 1, 65% of participants said they visit their doctors when they initially get sick, less than 20% of people chose the option of homeopathy. 75% of participants, however, wrote that they had used food to treat an ailment. 53% wrote that food was used in the body to provide nutrients and 98% responded yes, they would continue to learn about the uses of foods.

DISCUSSION

Detroiters perceived food to be used to excite the taste buds as well as to meet nutritional requirements to stay alive. Although there was discussion surrounding the concept of food as medicine in the metro-Detroit area, it did not appear that many people believed and accepted the notion of food as medicine to be true. For example, the food and nutrition education classes at Whole Foods Market were taught from a holistic standpoint in which being healthy not only included maintaining a supportive and healthy diet, but also taking care of the body and mind. The classes explained the importance of organically grown foods and the consciousness one must have as to what they are putting in their bodies.

Listening to the responses of people attending the classes, it appeared that many people think changes in diet are difficult. They also seemed to be very trusting of their health care providers and felt that the medical doctors knew the best, so they did not feel compelled to question whether or not medications is the best option to cure the illnesses.

Throughout the course of this research I have found the most important reason for consuming food is to maintain good health and sustain the body. From health classes and through markets, people are becoming more educated on the idea that food can be used medicinally as opposed to just hearing of the topic. The farmers' or outdoor markets provide a place for local people to purchase weekly, fresh and organically grown produce for a good price. Even though many respondents to the survey seem to agree with the idea about food being used to treat ailments, because it is not a common discourse and practice in the United States, people seemed more hesitant and did not want to go through the process of learning how food can be used to heal.

Many Detroiters responded that a lot of information regarding food was something they learned through a close family member or friend, or something they saw on television. There was very little discussion about reading information from books or finding information about previous case studies relating to food and

medicine. At many of the places I attended, information regarding nutrition and food was always provided. In the Whole Foods classes, the instructor provided numerous books people could purchase or borrow, that discussed the information that was taught in that class. The WSU Market had a three-part-series called "Wellness for Life-Eat your Medicine" where people were able to learn about the medicinal effects food had on the body. The Eastern Market provided a table from the Michigan State University Nutrition Program with information about healthy eating options and how food can be prepared. From my surveys, it appears that people are curious to learn more about the topic of food as medicine, but not as many people actually trust food to be a treatment option. Many respondents suggested that they are taking steps to change their diet to incorporate foods that are healthier and provide a taste that they enjoy.

In conclusion, Detroiters are familiar with the concept of food being used as medicine. People are starting to take an interest and become educated in how food can be used medicinally and what foods they should or should not be eating. Generally people seem to value taste as well as company with whom they eat their food, over the use of food in the healing of the body. Through entities such as markets and nutrition and health classes people are beginning to change their diet and eat food containing higher nutritional value. From these surveys, and participation in these nutrition and health classes, I learned much of the information people obtained regarding the health benefits food could have was not through books, but things they had heard, been told by family of friends, or something they watched on television.

LIMITATIONS

One major limitation experienced throughout this study was the time constraint. There was a lot of thing to do in such a short amount of time: the study might not have been as thorough as it could have been. Also not using an IRB limited potential research subjects and collecting data based on direct interactions with them. This made it difficult to get more in-depth results concerning people's prior knowledge of the concept of foods as medicine, and why or not it was something they would practice in their own homes. Another limitation would be the time period: a lot of health classes did not continue throughout the summer. The last limitation was finding places to pass out the surveys. A lot of places that were having health related classes did not have good advertisement about the classes which made it difficult to identify such classes. When I looked up health classes in the Detroit area, rarely did I find the information posted on the internet. It was more common that the information was posted on a bulletin wall, or I heard about it through word of mouth.

FUTURE DIRECTION

In the future a more extensive study should be done as to why people value "taste" over nutrition when it comes to food and health. In addition, exploring further how and where people generally get their information regarding "food" and "medicine" respectively would also contribute to understanding the discourse and practice around the notion of "food as medicine." This will also help future researchers to figure out the most effective way to communicate the importance of thinking about food not only in terms of bodily nourishment but also in terms of healing and the larger socio-cultural contexts through which diverse food practices.

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Survey 1

My name is Kizzmett Littleton and I am a Junior currently attending Wayne State University with a major in Nutrition and Food Science. I am doing a research project sponsored by the Ronald McNair Program. For any questions, comments or concerns, you can contact me: ey3290@wayne.edu or my Research Advisor Dr. Yuson Jung: yuson.jung@wayne.edu. Thank You.

- 1. If you get sick what would you do? (Circle all that apply)
 - a. Go to the Doctor
 - b. Homeopathy
 - c. See a Nutritionist
 - d. Get advice from family or friend
 - e. Other:_____
- 2. Have you ever used food to treat an ailment? If so please provide an example.

If not, why or why not?

- 3. What other roles does food play in your life? (Circle all that apply)
 - a. Bonds Family Together
 - b. Brings Friends Together
 - c. Makes Me Feel Better
 - d. Helps With My Health
 - e. Other:_____

4.	What new thing have you learned about food today?							
5.	How has the information provided today changed your perspective, if in any way?							
6.	What is your opinion regarding what food actually does for your body?							
	Will you continue to learn more about the different uses of food?							
	a. Yes							
	b. No							
	c. I have not learned anything							
7.	What particular things would you like to learn about food?							
	a. How can it help me lose weight?							
	b. Can it help cure my cold?							
	c. Besides diet what are other uses for food?							
	d. What other meals can I make with diet restrictions?							
	e Other							

My name is Kizzmett Littleton and I am a Junior currently attending Wayne State University with a major in Nutrition and Food Science. I am doing a research project sponsored by the Ronald McNair Program. For any questions, comments or concerns you can contact me: ey3290@wayne.edu or my Research Advisor Dr. Yuson Jung: yuson.jung@wayne.edu. Thank You.

1. How did you hear about this place?

2. Why did you decide to come here?

3. Has your perception on food changed coming here? How so?

4. What in your view is food used for? (Check ALL that apply)

A. Dietary Requirements

B. Brings friends and family together

C. Alleviation of illness

D. Taste

E. Other: _____

5. Will you return here? Why or Why Not?

DIVERSITY'S INFLUENCE ON RETENTION

By Dwight McDonald Major: Public Relations Mentor: Donyale Padgett, PhD, Department of Communications

ABSTRACT

In this article, the argument is made that retention is influenced by diversity. Diversity and retention correlate in that retention rates vary depending on the factors that each student faces. The factors can be anything from financial status, home stability, or mental growth within college. The overall goal for the research team is to better understand retention at Wayne State University (WSU). The research team will be pulling from the entire campus population collect data. However, I specifically will be sampling African American males. The demographics will cover of race, first time in any college, sexual orientation, documented disability and many others. Within these groups, I want to look at what issues students potentially deal with. Some of which are: the level of personal expenses they have, their income, volunteer work, academic preparation prior to college and social integration into the campus.

To collect this data our team will conduct a qualitative survey of varying student demographics. The data collected will show what types of students deal with certain issues more than others. More importantly, it will show how diversity has an effect on retention rates at universities like Wayne State University. This is also an analysis of what the current literature says about the topic of retention. This retention research is needed in order to give recommendations as to how we can improve the integration of students into their institutions, thus increasing the retention rates.

INTRODUCTION

You may see African American males walking across the stage on graduation day, however the retention rates for those students are still some of the lowest when compared to other student demographics. The graduation rate reported for African American males at WSU was 10 percent in 2010 Retention Advisory Committee (2013). The discrepancies in the numbers for student groups is too large. Students, regardless of cultural background, essentially are given access to the same campus resources and yet men of color still have problems completing their respective programs.

African American males do not perform well within their respective institutions because of lack of preparedness and lack of connection to the institution. This speaks to what factors affect their matriculation through college, however these only speaks for half of the problem. Lack of preparedness is an example of what is hindering the student before they got to college. There must be an analysis of what goes on after they reach the institution as well.

In his work, Reid (2013), highlights three key areas that weigh in on how African American males integrate into a particular institution. The three areas are self- efficacy, institutional integration, and persistence. Using these areas, Reid (2013) tries to better understand how the black males who do achieve success actually achieved it.

This study will look to highlight factors that influence retention before and after African American students enter an institution. By exploring the work by Reid (2013), self- efficacy, institutional integration and persistence, will be analyzed to see how they correlate to retention and retention. The work of Reid (2013) will function as key themes that will allow for expanding knowledge in the realm of retention. Gathering such information will prove valuable to universities that wish to strengthen its retention efforts. To gather this data, a qualitative survey will be administered to African American students.

LITERATURE REVIEW

Three areas of literature were used to explain what factors are relevant to African American retention and achievement. These areas are self- efficacy, persistence, and institutional integration. Each area contributes to the understanding of what is believed to influence retention. Self- Efficacy refers to an individual's belief in his or her capacity to execute behaviors necessary to produce specific performance attainments (Bandura, 1997). Essentially, students with a higher sense of belief in their own capabilities will perform better than those who do not. Self-efficacy supports that students are linked to academic achievement in that there is better management of time as well as positive views on the subject matter the students are engaging. Thus, those students who do not believe that they can achieve academically have a higher chance of not doing it.

There is record of a positive relationship between black consciousness, self-esteem and self-efficacy. Black consciousness refers to individual's beliefs or attitudes about his or her self, and own race (Okech & Harrington, 2002). The findings of their research suggest that African American male students hold higher self-esteem in areas other than academic performance. Self-esteem can have a strong effect on self-efficacy. It has been found that African Americans have high levels of self-esteem, but lower levels of efficacy. This comes from the influence of friends and family. Constantly being told they cannot do something causes lowered levels of belief. The main theory proposed by Bandura (1997), shows how belief in one's ability correlates to academic achievement.

Persistence in college refers to how students constantly pursue their degree. The literature surrounding persistence states that if students wish to be successful, they must acclimate themselves to the institution on the social and academic level (Reid 2013). Students who dive fully into what it means to be a student, are more likely to have success. Students must feel like they are a part of the campus. The academic piece involves the constant interaction with faculty members. Positive interactions with faculty have shown to produce equally positive academic outcomes. The support of the faculty helps create a sense of comfort and belonging within the university.

The other piece of persistence speaks to the influence of the other students. According to Reid (2013), students who have high levels of success, tend to have high involvement with student activities on campus. It is vital to the success of students and the continued prosperity of universities to figure out means of improving retention rates. Low retention numbers is an indicator that students are not returning to the university; three main reasons are: they are transferring to a different university, or they can no longer attend their current institution. It is important that we understanding the deeper reasoning behind these three pillars of retention.

To understand the reasoning behind the low retention rates, we must develop a better understanding of the reasons why people are forced to leave the university. This research paper will explore a sample of different factors that impact low retention. However, current research claims the biggest issue is the college environment. The campus in which a student attends can determine how that student performs at that institution.

Looking at the research conducted by Baker (2013), you can see the importance of students feeling like they belong to their respective campuses. The article highlights how the campus experience can be harmful to students, mainly on predominantly white campuses. To combat this issue, the researchers examined how social support can help students survive the campus lifestyle. The sources for student support include: support from faculty, especially when the support comes from faculty members of color. Students of color at predominantly white institutions (PWI), generally deal with feelings of discrimination and lack of campus integration. The support is classified as "personal support". It involves personal and positive interactions with faculty that are of the same ethnic background as the students. This builds a relationship that can propel students forward and help them succeed.

Having a relationship with faculty is important; however other parts of the institution are important as well. The support services that an institution provides such as counseling, learning communities, etc. are influential and impactful. Out of all the factors that affect retention the support system and connection to campus are key outliers. This further supports what was said by Reid (2013) about the interaction and presence of faculty is important to the success of the students. Faculty support is key to the success of producing positive outcomes in the area of retention.

Within the discussion of retention, inclusive excellence is an important topic tied to student success. The term is defined by Williams (2007) as changing the culture of the institution. Excellence is determined by how effectively all campus systems, structures, and processes meet the needs of all its students. Milem, Chang, & Antonio (2005) state that inclusive excellence focuses on student's intellectual and social development, as well as building a welcoming community. There is a need to measure how well Wayne State meets the needs of its students. In order to impact the field of retention, we must understand what needs to be changed as well as how to change it. As said by Williams (2007), you must start by changing the culture. If you look beneath the layers of any institution, you will see a set of values, traditions and procedures that make up how the institution operates. These things make up the culture that must be changed. However, in order to change it, you must find what are called strategic points of leverage. Every institution will have its own unique campus climate, however though the use of leverage points, there is a way to in act change to the campus culture.

To better understand the concept of leverage points, you must know what Williams (2007) means when he talks about the "two worlds" of every culture. He states the there is a visible world and an invisible world. The visible world is the surface and the invisible world is what lies beneath. The leverage point is where the two worlds meet. Thus the key to truly being inclusive is the merging of the seen administrative practices as well as the unseen campus practices and attitudes.

The first and possibly most important of the strategic leverage points discussed by Williams, is that diversity must be a campus wide concern. All campus departments and structures must be committed to achieving excellence. The matter must be discussed on a regular basis. Williams goes on to list many other points of leverage. Another salient one was the need for a chief diversity officer. By having this officer, the institution shows that it is committed to diversity and excellence. After all, a key part of inclusive excellence is making sure that all students are successful, which feeds into the diversity aspect.

The approaches that Milem, Chang, & Antonio (2005) take in terms of inclusive excellence are similar to those proposed by Williams. There are some differences though. They do believe that a supportive community that fully engages diversity is needed for student and organizational learning. In order to help students, there must be an understanding of the racial climate campus climate. Naturally, to achieve this you must bring people from different backgrounds together, however it takes more than that. The campus climate is something that as a large amount of depth to it. It must be seen as having multiple layers to it.

Overall, inclusive excellence is a concept that can truly drive the retention discussion. It encompasses everything that the university should be doing to help its students. There are external factors to consider, however in terms of university factors inclusive excellence highlights ways for institutions to increase retention and promote diversity.

METHODS

The purpose of the survey is to better understand the factors that lead to lower or higher retention rates in African American males. The approach to conduct the research was a qualitative survey. The students will belong to many student groups and come from varying backgrounds. There will be a sample of African American males used to provide a detailed look inside of how each student has dealt with college thus far in their lives.

MATERIALS

The materials used to conduct this research was a qualitative survey containing 68 questions for the participants to answer. They range from multiple choice to short response.

PROCEDURE

To gather the desired data, a qualitative survey was administered to undergraduate students at Wayne State University. The survey was made available online to students through their student accounts. The intent of a much larger project is to measure retention for the entire campus. However, for the purposes of this paper, only the African American male students will be looked at.

DATA ANALYSIS

The survey itself has questions that fall into four categories: demographic and enrollment information, sources of challenges, resources and what do you need to succeed. In each category there will be certain questions that will be looked at closely to gain more information about retention at Wayne State University.

To analyze the data, I will look at the survey results and thoroughly analyze the answers. Looking at the data I can see what students identify with what groups on campus, their race, connection to campus, and other data. Based upon the data, I can see what the students surveyed are dealing with while attending college.

I can then compare the number of students facing the same issues to the current retention rates for that particular student group. The data can be used to make recommendations to fix the issue. For multiple choice questions, the percentages for each choice were recorded. As for the short response questions, each response was read carefully to get some idea as to what influences the retention level for each individual surveyed.

RESULTS

Qualitative Survey

Demographic

The survey results were compiled and analyze based on demographic. After reviewing the data, the findings show some connection to what the literature previously argued. The participants ranged in ages 21-40 years old. All of them were residents of Detroit, MI and stayed with 15 minutes away from campus. 75 percent of the participants contribute to their educational expenses. 75 percent of them work while 100 percent of them volunteer. All participants have maintained consistent attendance at Wayne State University and plan on graduating by 2017.

Campus Inclusion and Support

100 percent of the participants reported that they have used some type of campus resource. The resources include: tutoring, counseling and student organizations. After utilizing these resources, the students have

indicated increased GPA's and feelings of inclusion. They feel as though the institution has given them the resources necessary to succeed. However, 67 percent did report that interactions with faculty and staff were "decent" at best. The same percentile was reported when asked if they felt professors would be willing to give additional help if needed. 75 percent did say they are happy with their social lives on campus and they fit into the campus in terms of peer interaction. Some of the top obstacles that were reported were finance, interpersonal interactions with Wayne State faculty and preparedness for college. Despite these factors, 100 percent the students reported that they are motivated to complete their studies at the university.

DISCUSSION

The data that was collected supports what some of the pre-existing literature says. The data supports what was said by Reid (2013). Reid stated that students must fully immerse themselves into the role of student. Those who do this are more likely to succeed. When students feel like they are a part of the campus, they are more likely to have positive outcomes. The data shows that the students who took the survey are happy with their campus social life and peer relationships. These students all said they were confident that they would complete their studies. Thus, the data has a connection to the literature in terms of peer relationships.

However, even though the data shows students are happy with their peer interactions, the same cannot be said about the faculty interactions. Participants lack of positivity when in concerns faculty does not support what was said by Baker (2013). Building positive relationships with faculty is supposed to help propel the student forward. Based on the data, there does seem to be some type of self-efficacy however, it is hard to tell for sure.

The findings from the survey do show the importance of inclusive excellence. The concept states that all the structures within the institution must work together in order to fully impact retention. It is good that students are forming bonds with each other. However, based on what the literature says, we also have a need for students and faculty to form those same bonds of support. The data collected does make some level of meaningful connection to the pre-existing literature.

This research is limited by the number of participants who were actually surveyed. In order to truly get a good sample of data, this work will have to be revisited again. The survey was meant to reach hundreds of students. When this data was pulled, the number were nowhere near that. Also, even though this is a sample of one particular student group, it would prove beneficial to compare this data to other student groups. This paper aimed to analyze things like self-efficacy and self-esteem. The survey questions really didn't focus in on that too much. It is implied that self- efficacy is in the survey since the students believed in their ability to complete their studies at Wayne State.

Aside from more African American males taking the survey, another means of data collection could have been through focus groups. Focus groups would have allowed for more detailed questions and responses than a survey allows for. It is also more personal, which may lead to people getting more personal in their response. For all of its limitations the survey and the overall paper, contribute to the retention conversation.

Retention issues will not be solved overnight. With that said they can be solved. Using what this paper stated could be a way of starting off new ways of improving retention. Wayne State has actually started utilizing some of the strategic leverage points laid out by Williams (2007) by appointing a chief diversity officer.

Perhaps by doing this and strengthening the student- faculty relationships, we will begin to see positive change in retention.

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HIGH PERFORMANCE COMPUTING USING GRAPHIC PROCESSORS UNITS

By India Owens Major: Computer Science Mentor: Loren Schwiebert, PhD, Department of Computer Science

ABSTRACT

This is a study of High Performance Computing using Graphic Processing Units. The application we are working on uses the Purdue Reactive Molecular Dynamics model, which allows us to simulate DNA molecules, in order to better understand cancer. Before the program ran solely on the CPU, during the course of the study it was partially implemented on the GPUs. In this paper we find that while GPUs can increase the performance of a program, with a lack of optimization and amount of code ported to the GPU the program performance can decrease. In fact we find that the CPU only code is about 2x faster than the partial GPU implementation. This was due to lack of functions implemented on the GPU and code optimization, which lead to overhead latency.

INTRODUCTION

As innovative applications emerge daily, tackling larger and more difficult problems, different ways of processing them have to be explored in order to enjoy the full benefits of them. In the world of computing reliable performance is the key that can make or break the effectiveness of an application. Obtaining peak performance begins with the processor.

The modern Central Processing Unit (CPU), found in every newer computer and performs main tasks for the computer, is a pretty powerful processor. As such most advance applications are going to be executed on them. Applications might get pretty decent performance on the CPU, regardless, performance will never be at its peak. This is because the CPU is a serial processor, when completing a task, it has to do one at a time. Most applications are not just serial they also have parallel qualities, which means there are parts of the program code that have to be done one step at a time, while other parts can be done simultaneously. (Brodtkorb, Hagen and Sætra)

The Graphics Processing Unit (GPU) was developed to be able to quickly handle the mathematical operations needed to produce three-dimension visuals on a two-dimensional screen. The GPU works with pixels, all of the pixels can be processed together in parallel using the same code. The GPU was specifically designed to handle parallel data. Researchers found that pairing the traditional serial CPUs with the specialized accelerators, such as the GPUs produce better results when processing some applications. This is better known as heterogeneous computing. (Brodtkorb, Hagen and Sætra)

The main goal of this combination is to take large problems and solve them in a shorter amount of time. This goal directly aligns with that of High Performance Computing (HPC). (Jin et al.) In this research we converted functions of an application that uses the Purdue Reactive Molecular Dynamics (PuReMD) model to gain a better understanding of cancer. Other PuReMD implementations using GPUs achieved speeds 350x faster than an implementation that solely used the CPU. (Kylasa, Aktulga and Grama). In our study we want to replicate similar results.

CPU AND GPU

The Central Processing Unit (CPU) often referred to as the "Brain of the Computer" and was always the point of interest when it came to advancements of computational speeds. Prior to the year 2000 increasing performance was synonymous with increasing the frequency of the CPU. Frequency describes the rate at which the processor operates. It is measured in Hertz (Hz), clock cycles per second. This method would work for a while and produce desired results, until the CPU hit a power wall. The amount power needed to sustain this trend was inconceivable for the average computer, commercial market, and there was no way to properly cool these higher frequency chips. At the end of this trend the CPU's frequency clocked out a little below 4.0 Gigahertz (GHz). Even so, performance is still a major concern for the advancements of computers. As such other methods had to be developed outside of increasing frequency. (Brodtkorb, Hagen and Sætra)

Around the same time CPUs hit a wall, the GPU performance was steadily being advanced. GPUs are designed for the use of placing three-dimensional image on a two-dimensional display. Due to its capabilities, it is very common in the world of video gaming and can be referred to as gaming cards or video cards. Most modern computers use a GPU because it the component that displays images to the monitors. Prior to the adoption of Graphical User Interfaces, computers were text based. (Alvarez, Romero and Correa-Basurto) As more research developed, studies found that GPUs could be used for non-visual, general purpose computing and are faster than CPUs when computing certain code. (Che et al.)

GPUs experiences a trend where its speeds double approximate every 13 months compare to the CPU which is only about every 22 months. (Alvarez, Romero and Correa-Basurto) This is due to GPUs parallelism, being able to execute the same instruction with different pieces of data at the same time. It was found that many computer applications were "embarrassingly parallel" and GPUs were seen as a suitable way to increase computer performance. Increasing the parallelism of GPUs again produces desired results until the processor encounter serial portions of the application. Serial code is code that has to be executed one by one in a specific order; the CPU as a serial processor best handles these kinds of tasks. Since most advanced application have both parallel and serial qualities, combining the strength of the CPU and GPU leads to impressive results, with computational speeds being faster than with CPU alone. (Brodtkorb, Hagen and Sætra)

GPU ARCHITECTURE

On a GPU there are threads and each thread is responsible for executing a sequence of instructions. Threads are assigned to thread blocks. Thread blocks executing the same kernel, the function that executes the parallel program, are placed in an array called a grid. Within a block, memory is shared between the different threads, which allows for better cooperation between threads. Threads and thread blocks are uniquely identified, and it is the GPU's job to uniquely identify the threads, assign the threads to a block, and a block

to a Streaming Multiprocessor (SM). Each block is assigned to a single SM, but it is possible for two blocks to be assigned to a single SM. This guarantees that all the threads within one block will be processed together. Additionally, multiple SMs will be running at the same time, independently of each other. A SM is made of cores and each core is assigned a thread from the block. When a block is being executed in the SM, it divides the threads into warps of 32 threads, and continues running until every thread in the block has been executed. (NVIDA Whitepaper...Fermi)

This entire process is the Single Instruction Multiple Thread (SIMT) model of computation, which is a combination of the Single Instruction Multiple Data (SIMD), which where each thread works within a block on the same instruction with different data, and Single Program Multiple Data (SPMD), where blocks within the same grid operate independently across SM. This takes two parallelization approaches and combines them to achieve the benefits of both. This architecture is how GPUs are able to process so much data in parallel. (Kylasa, Aktulga and Grama)

In addition to this, the NVIDA Kelper architecture, which is used in the Telsa K40s the GPUs that were used through this experiment, has some important designs. It has 15 SMs, referred to as SMX by NVIDA to distinguish them from their predecessor, with 192 CUDA cores. CUDA means Compute Unified Device Architecture and it is a hardware/software platform developed by NVIDA as an extension program language to enable C language code to work on the GPU. It has quad warp schedulers, which allows up to four warps to be executed concurrently, and two independent instructions can be issued per warp. Other notable features include Dynamic Parallelism, which allows a kernel to launch another kernel. In previous generations the host, CPU, strictly reserved control to launch the kernel. This is important because it deceases transfer of data between the two processors, which takes a lot of time resulting in better performance. The structure of a GPU is important to keep in mind for the optimization of the code. (NVIDA Whitepaper...Kelper)

OPTIMIZATION OF **GPU**

There are multitudes of ways and factors that go into how performance is measured such as performance over price, watt (power), and space. The factors include what kind of CPU and GPU is being used, as this plays a role in how much speedup can be obtained from using a GPU. As well as the application itself, that may have some parallel quality but the latency between copying and sharing the data between the two processor can be so high that it can quash any potential speedup.(Alvarez, Romero and Correa-Basurto) Simply placing functions on the GPU can have the possibility of improving performance, but for the best possible implementation of the code with the CPU and GPU, optimization of the GPU code is important.

Since the warps are done in collections of 32 an important optimization is to have the number of threads per block be divisible by 32. The number of blocks needed depends on any number of factors, but a common approach is to take the size of the data being process and divided it by the number of threads to insure that data is being evenly distributed across blocks. Of course since every number is not divisible by 32 precautions should be taken to insure that some data is not skipped or that the some threads are not going out of bounds. Thread block size and number of threads play a major role in the optimization of the code, but there are other things to be considered, such as memory management, conditional statements, and any other particularity of the code. Memory management is important as data is being shared between devices, the constant copying and updating between the two so that both devices are synchronized can lead to latency. The best way to overcome this is to perform as many calculations on the data while it's on the GPU before returning it to the CPU. As for conditional statement "if..." it best to use a few as possible as they reduce the amount of threads being executed in a warp if only a few meet the conditions. The whole idea behind

achieving high performance with GPUs is to execute the same instruction. If threads within the same warp perform different tasks it decrease overall performance. (Che et al.) Another way is to arrange the data in a way that all the threads in a warp meet the condition to be executed or perform the conditional branch on the CPU, separate the data, make multiple kernels, call the kernel that adheres to the correct condition. (Brodtkorb, Hagen and Sætra) Understanding the code, GPU hardware design, and good CUDA code practices is key in finding ways to optimize the performance.

MATERIALS

The application being used in this experiment is the PuReMD-Cancer code and the GPUs used are the Telsa K40, which uses the Kelper architecture.

METHODS

In order for the code to be able to transition from the CPU to the GPU some preliminary work needed to be done on the code. In the code, Message Passage Interface (MPI) process was used so the code could run on multiple CPUs. (Jin et al.; Kylasa, Aktulga and Grama) To make the code easier to test and modify, the original CPU only code created their own versions of the malloc(); and calloc(); functions that took the MPI group as a parameter, so that everything was properly managed if the system ran out of memory. We mimic the function for our the GPU, creating our own versions of calloc(); and malloc();, calling them GPUmalloc(); and GPUcalloc(); which took the MPI group as a parameter. In these functions instead of calling malloc in the functions we call cudaMallocManaged();, which allowed the host, CPU, and the device, GPU, to share access to the data.

The functions that were selected to go on the GPU had to have parallel qualities and be highly used throughout of the code. While reading and learning about the function we could often come some approximate about how often this function is being used. The indicator of parallel qualities would be for loops.

Pointers to structure that were used in the function had to be define on the GPU in order for the GPU to access the data. Additionally structures and any arrays that a structure pointed too needed to be defined on the GPU. Any structures found in the for loop being converted were redefined using the created functions GPUmalloc(); and GPUcalloc();, with regard to how the it was originally define with malloc(); or calloc();. Additional any functions that where call from the inside converted for loop needed to be defined on the GPU and CPU, _____device_____host____was used to do so.

In the two functions I focused on, similar methods where used on both to convert the parallel potions of them. After the selection process, parallel potions of the code were removed from the original function and moved to a kernel definition, saved as .cu file opposed to the original code .c file. Inside of the .cu file, in addition to the kernel definition, was another function that launches the kernel.

All the kernels define the number of thread blocks from the number of iterations in the original for loop, plus the BLOCKSIZE, a defined constant of 128, minus 1, divided by BLOCKSIZE. The number of threads per block was set to BLOCKSIZE. Once the blocks and threads where define, the kernel was called passing any

parameter from the original function that was used in the for loop, followed by cudaDeviceSynchronize(). See Fig. 1.

The kernels were defined with CUDA_GLOBAL, which we used as a defined constant for the declaration specifier __global___. The definition located within the brackets of the for loop was copied and pasted in the kernel body. Any local variables defined in the original function that were used in the for loop were define locally in the kernel definition. The counter variable from the original for loop was redefined from equaling zero to equal blockIdx.x * blockDim.x + threadIdx.x. It was based on the block and thread IDs. An if statement was included stating: if the thread was larger than or equal to the condition statement from the original for loop, return; See Fig. 2.

In the original function, where the section of code was removed, it was replaced with a function call to the function that launches the kernel.

```
int blocks;
blocks = (system->n+BLOCKSIZE-1)/BLOCKSIZE;
Kernel<<<blocks,BLOCKSIZE>>>(some parameters passed);
cudaDeviceSynchronize();
```

Fig. 1 The conditional statement for the original for loop was system->N, total capacity of atoms for a system. In the kernel call blocks where used to define thread blocks and BLOCKSIZE was used to define the number of thread per block.

```
CUDA_GLOBAL void Kernel(some parameters)
{
    int i = blockIdx.x * blockDim.x + threadIdx.x;
    if( i >= condition statement )
        return;
    /*definition of function*/
}
```

Fig 2. This is an example of a Kernel function.

RESULTS

In the CPU implementation of the code it ran 4000 steps for approximately 2.5 hours. In the partial GPU implementation of the code it ran 2500 steps for a little under 4 hours. When dividing the exact times in seconds by steps, the CPU implementation takes about 2.16 per step and the partial GPU implementation takes about 5.15 seconds per step. The CPU implementation is about 2 time faster the partial GPU implementation.

DISCUSSION

The results were not what we wanted but given the limitations of the experiment design, they were understandable. Time was the biggest limitation of the this design, having only 8 weeks there was no wayto fully convert the program over to the GPU and optimize the performance. At the end of the 8 weeks only a small portions of the code was ported over to the GPU. Of that code only one entire function was ported to the GPU while the functions that I worked on was split between the GPU and CPU. The overhead of constantly having to synchronize data between the processor slowed down whatever performance might have been increase by the GPU.

Overhead latency becomes less noticeable with proper optimization, but as mentioned we were not able to perform this task within the time frame. While we made some optimizations to the code it was not enough to make a real difference.

The results highlight the importance of optimization. GPUs have the capabilities to greatly increase performance but without proper implementation, programs will not be able to experience the benefits of them and can actually suffer.

The results should not be seen as a discouragement of the use of GPUs for HPC. As explained by Brodtkorb GPU and CPU programming vastly differ. While getting started with GPU programming it can be relatively simple, but first attempts with GPU computing, and in terms first implementations of the code with GPU, are usually dissatisfactory.

FURTHER DIRECTION

The long term goal is to port most of the PuReMD-Cancer program to the GPU. The future implementation will take advantage of the Dynamic Parallelism featured in the Kelper architecture, where kernels will launch other kernels. This will cut down on the overhead latency, as the CPU will initially send over the data to the GPU and on the GPU all the calculations will be performed before finally going back to the CPU. Work will continue to be done on the program to improve performance until we reach desired results.

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EFFECTIVENESS OF E-INTERVENTIONS: DOES EMPATHY MATTER?

By Damaris E. Pop Major: Psychology Mentor: Emily Grekin, PhD, Department of Psychology

ABSTRACT

Common factors theory suggests that a set of common relationship factors (e.g., positive regard, empathy) is clearly associated with psychotherapy outcome. However, research has not examined whether attention to these factors in the context of a computerized intervention can similarly increase efficacy. The current study examined whether the presence of empathy in a computerized intervention for heavy alcohol use would lead to greater readiness and intention to reduce alcohol use. Participants were 63 university students who met National Institute on Alcohol Abuse and Alcoholism (NIAAA) criteria for heavy drinking. All participants completed an interactive computer-delivered alcohol intervention that was either high or low in empathy. Afterwards, participants completed measures of: 1) readiness to change alcohol use, 2) intention to change alcohol use, and 3) reactions to the computer program. We hypothesized that participants in the high empathy condition would show greater increases in readiness to change and intention to reduce alcohol use than participants in the low empathy condition.

JUSTIFICATION OF NEED

The National Survey on Drug Use and Health estimates that 19.3 million individuals, aged 12 and over, met criteria for an alcohol use disorder in the past year. However, 87% of these individuals neither wanted nor received any treatment in the past 12 months. Barriers to treatment are numerous and include cost, transportation problems, lack of time, and lack of trained providers (National Survey on Drug Use and Health, 2012). In an attempt to address some of these treatment barriers, researchers have begun to develop brief, computer-delivered interventions (CDBIs) that are portable, brief (often single session), replicable, and easy to administer. CDBIs have been proven efficacious in reducing unhealthy drinking (Berholet et al., 2005; Kaner et al., 2007) with effect sizes in meta-analyses ranging from small to moderate (Wilk et al., 1997). Moreover, some data suggest that clients are more likely to disclose sensitive information to a computer program than to a therapist or interviewer (Tourangeau & Yan, 2007; Simoes et al, 2006).

One CDBI component which may increase treatment efficacy is the use of common factors. Common Factors Theory suggests that therapist-delivered interventions vary in efficacy, not just as a function of their theoretical approach, but also with the characteristics of the therapist him or herself. Specifically, a set of common relationship factors (e.g., empathy, therapeutic alliance) has been shown to be associated with positive therapy outcomes (Norcross & Wampold, 2011). However, the degree to which these factors are relevant to interaction with a computer, rather than a person, has not been adequately investigated.

OBJECTIVE/HYPOTHESIS

The current study examined whether one common factor –empathy-increased the efficacy of a CBDI for alcohol use. We compared the effectiveness of two versions of a CDBI; a high empathy version (which uses empathic, reflective statements) and a low empathy version (which presents only factual information about the participant's alcohol use). We hypothesized that the high empathy version of the intervention would produce greater readiness to change and intentions to reduce drinking than the low empathy version.

APPROACH & INTERPRETATION OF RESULTS

Participants were 63 undergraduates who met one of the following criteria for heavy drinking (NIAAA): 1) consumed at least three (women)/four (men) drinks per day, 2) consumed at least seven (women)/14 (men) drinks per week, 3) got drunk at least once per week over the past six months or 4) binge drank at least once per week over the past six months or 4) binge drank at least once per week over the university's campus through advertisements on the university's website and flyers.

Eligible participants came to the laboratory where they provided informed consent. Afterwards, they were randomly assigned to either the high or low empathy version of the intervention, each of which took approximately 20 minutes to complete. The intervention was based on principles of motivational interviewing (MI), a type of therapy that aims to reduce ambivalence about and build motivation for behavior change (Miller & Rollnick, 2002). More specifically, both the high and low empathy versions of the intervention consisted of 3 MI-derived components; 1) decisional balance, in which participants identified what they liked and did not like about alcohol use, 2) normed feedback, in which participants were given information about how their drinking compared to same age, same gender peers, and 3) goal setting, in which participants were offered the option of setting a behavior change goal (e.g., reducing their alcohol use) and were provided with strategies to achieve their goal.

A three dimensional, animated narrator named Peedy guided participants through the intervention. Peedy is capable of performing more than 50 actions, which include talking, waving, and reading messages to the participant. Peedy also has a lifelike personality and is able to interact with participants on a personal level (e.g., he uses their names, reacts to their responses, etc). In the high empathy condition, Peedy provided personalized, empathetic, reflective statements ("I can see that drinking helps you cope with stress"). In the low empathy condition, Peedy provided information about the participant's drinking, but did not use the participant's name or make empathic/reflective statements.

There were two dependent variables in the current study: readiness to change and intentions to reduce drinking. Readiness to change was measured with the 4-item Readiness to Change Scale (Sobel, 1996) a measure that assesses current motivation to reduce alcohol use. Intentions to Reduce Drinking was measured with a 2-item, laboratory-developed scale, that assessed participants' immediate and long-term plans to quit drinking. Both the Readiness to Change Scale and the Intentions to Reduce Drinking Scale were administered both before and after participants completed the intervention. Additionally, after completing the intervention, participants were also asked to respond to 12 questions, which, assessed their reactions to the computer program (e.g. "How much did the computer seem to understand you?").

It was hypothesized that participants in the high empathy condition (n=27) would show greater increases in both readiness to change and intention to reduce alcohol use over the course of the study, than participants in the low empathy condition (n=36). Hypotheses were analyzed with t-tests that compared mean changes in the two dependent variables (readiness and intentions to change) over the course of the study in the high versus the low empathy group.

Contrary to our hypotheses, participants in the low empathy condition showed greater increase in readiness to change than participants in the high empathy condition t(63)=-2.26, $p=.028^*$ (Figure 1). Additionally, there were no between-group, pre-post differences in intentions to reduce alcohol use t(63)=-.266, p=.791 (Figure 2). Finally, there were no significant between group differences in acceptability of the computer program (i.e. high empathy participants did not perceive the computer program to be more empathetic or understanding than did low empathy participants.

Overall, data suggest that participants may not respond to empathy from a computer program in the same way that they do during in-person therapy. It is possible that more sophisticated technology is needed to truly mimic human, empathic interaction. Alternatively, there may be methodological issues that affected results. For example, participants may not have been aware of the different levels of empathy that were embedded in the CDBI, given that they were only exposed to one intervention condition (and therefore could not compare the two). Future research is needed to investigate these possibilities and to explore the effects of additional common factors (i.e. positive regard).

Figure 1: Participants in the low empathy condition (n=36) showed a greater increase in readiness to change than participants in the high empathy condition (n=27).



Figure 2: There were no between group, pre-post differences in intentions to change.



Timeline

Objectives	Date
Review Literature	Week 1: May 24- May 30
Begin recruitment	Week 2: May 31- June 6
Run participants	Week 3: June 7- June 13
Run participants	Week 4: June 14 - June 20
Continue running participants and begin data analysis	Week 5: June 21- June 27
Begin working on introduction and method section	Week 6: June 28- July 4
Analyze data, review results, and write results/discussion portion of paper	Week 7: July 5 - July 11
Feedback from mentor of rough draft	Week 8: July 12 - July 18
Peer Review	Week 9: July 19 - July 25
	Week 10: July 26- August
Finalizing Paper	18

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HIGH PERFORMANCE COMPUTING USING GRAPHICS PROCESSING UNITS (GPUS)

By Kawthar Shafiekhorassani Major: Computer Science Mentor: Loren Schwiebert, PhD, Department of Computer Science

ABSTRACT

High performance computing has been a topic of high interest recently in the field of Computer Science. Modern Graphics Processing Units (GPUs) have an inherent architecture that can be exploited for high performance computing in addition to their original purpose of rendering graphics. Certain features of the GPU, highly multithreaded and parallel computing, allow for a much more efficient execution of complex computations. This speedup reduces latency and increases the throughput of processes. An operating system is the intermediary between the user of a computer and the computer hardware. GPUs utilize the Operating system to provide a more transparent and effective means of running programs initiated by the user. GPU-accelerated computing utilizes the GPU and the CPU to accelerate applications that otherwise run very slowly on the CPU due to high computation demands.

This paper examines these features of the GPU and delves into the difference between executing a function with complex computation on the GPU in comparison to running it on the Central Processing Unit (CPU). The features of a GPU are examined not only to improve the performance of a code that is running on the CPU but also to continuously optimize GPU performance. The goal of this research is to obtain significant speedup in the running time of a program by exploiting the multithreaded architecture of the GPU.

INTRODUCTION

To accommodate for improvement in speed or the output time of a program, high performance computing can be implemented. Using GPUs alongside the CPU to process certain parts of a program can accelerate computations and improve code efficiency. GPUs are highly multithreaded, process multiple requests simultaneously. This feature allows for more complex computations to be processed in a multithreaded environment thereby allowing processes to run independently of each other.

GPUs are more commonly used for rendering graphics or gaming and can be found in platforms such as a personal computer, embedded systems, robots, mobile phones, and cars. In such a case, the GPU is commonly referred to as a graphics or gaming card. There main purpose in the context of gaming is to render better graphics and to process images on gaming consoles or computers. However, the inherent architecture of the GPU can be directed to completing other tasks as well. The GPU can work in unison with the CPU to process more complex computations that require running in parallel. The multithreaded architecture of the GPU allows for processes to run in parallel. Processing data that deals with complex computations can be done much faster on the GPU than on the CPU due to the parallel structure inherent to the GPU architecture.

The desired outcome for a function implemented on the GPU is to reduce the latency and improve throughput. Latency is related to the amount of time it takes between inputting a process and yielding an output, while throughput refers to the amount of processes being computed at the same time.

GPU vs CPU

Two essential elements that dictate research conducted on GPU performance are: Price/Performance Ratio and Evolution Speed (Fanz, Kaufman, & Yoakum, 2004). Performance ratio refers to exploiting features of the GPU to perform computations at a much faster rate in parallel. Evolution speed reflects on the improvement of GPU performance over time in comparison to the CPU. The gaming industry has had a large influence on the continuous improvement of GPU performance. GPU performance doubles every six months while the CPU performance is improved at a much slower rate (Fanz, Kaufman, & Yoakum, 2004). The evolution of the processor is important for handling code and processes that have also been prone to evolution in coding style. It is crucial to have a processor that can handle such high computations, and therefore the GPU is utilized to do such.

A research conducted on high performance computing using GPUs by the International Journal of Advanced Research in Computer and Technology (IJARCET) found that "GPUs are more performance efficient than CPUs for huge computations involving good scope for achieving parallelism and less data transfers between system memory and the device" (Fanz, Kaufman, & Yoakum, 2004). This research delved into the hardware architecture of the GPU and how that can be utilized to improve performance of a program. The research found that the hardware architecture can be utilized only through an efficient programming methodology (Hemalatha & Kodada, 2013). Two programming methods that were observed in this research were Compute Unified Device Architecture (CUDA) and Open Computing Language (OpenCL). In CUDA, processing data is copied from Main Memory onto the GPU memory. The function call is made in the CPU, which yields parallel process execution in each GPU core. The results obtained from processing these high computations are then copied back onto main memory (Hemalatha & Kodada, 2013). This process emphasizes the difference found in the number of cores on a CPU and GPU. While the CPU has multiple cores, the GPU has thousands. This feature of the GPU is what allows for multiple process to be implemented simultaneously through utilizing each core as a location to execute in parallel.

Another research was done comparing the performance of a program on the CPU and on the GPU. This study resulted in the code running 1.5 times faster than the original. This study maintained the program accuracy and outputted appropriate results. It is difficult to obtain completely accurate results which makes the process of converting functions harder. Memory must be allocated properly and all instances of a variable must be correlated in order to maintain the value that was calculated (Vakulin, Shaw, & Livingston, 2013).

There are different forms of speedup that can be obtained when moving a function onto the GPU. The running time can display a slight change in speed, a significant change in speed, or a fundamentally different time (Kirk 2007). The ultimate goal is to achieve a fundamentally different running time that is over a hundred times faster at computing the required work. However, in many cases this cannot be achieved very easily and requires a lot of debugging and re-structuring of the code.

CUDA

CUDA is a programming model that can be used to express parallel computing on the GPU (Kirk 2007). CUDA is used to implement a code that allows for simultaneously running several threads with unique IDs. A unique feature of CUDA that is essential to maintaining accuracy is synchronization. CUDA can share memory accesses amongst processes, despite the processes being independent of each other (Kirk 2007). Only threads within the same block can share data through their shared memory and synchronize (Kirk 2007). These are all features that make CUDA a unique programming model that works very well with maintaining the accuracy of the results and helping achieve a drastic bandwidth reduction.

METHODS

SOFTWARE

The software used in this research is an open-source software called Purdue ReaxFF Molecular Dynamic Program (PuReMD). The program consists of multiple files that execute functions which interact with each other. To convert functions onto the GPU, the code was analyzed for loops that are implemented multiple times. These loops require high computations that can be performed much more efficiently if they were executed on the GPU. To convert a function onto the GPU, several programming languages and software's were used including Putty for inputting linux commands, and CUDA for implementing GPU versions of the loops.

CONVERSION PROCESS

Several functions in the program were evaluated to determine the amount of computation time required for processing them. This was done either through examination of the loop that was running in the function or through generating a file that showed the time required to output a certain function. This file generated showed the amount of time it took for the function to run on the CPU. The goal of the research was to improve this time by converting specific functions to run on the GPU. Several changes in a code were made in order to conduct such a change and maintain the functionality of that portion of code. Several factors must also be considered during the conversion. A process cannot simultaneously run on the CPU and GPU. The process cannot maintain the value of a variable while running on the GPU that is used on the CPU at the same time. Values must either be referenced by parameter or the function must entirely be run on the GPU and the function call is made in the original loop.

To allocate memory appropriately, any arrays that are referenced in the GPU version of the function must be allocated on the GPU as well. Below is an example of an array allocated on the CPU and the changes made to allocate it on the GPU.

- Allocating an Array (hb top) on the CPU:

hb_top = (int*) calloc(system->local_cap, sizeof(int));

- <u>Allocating an Array (hb_top) on the GPU:</u>

GPUcalloc((void**) &hb_top, system->local_cap, sizeof(int), "hb_top", comm);

These changes were made using CUDA. On the CPU, the term "calloc" is used to allocate memory, and on the GPU, the term "GPUcalloc" is used. Depending on the way GPUcalloc is assigned in the code, there is one format for every allocation of that type. This means that depending on the coding style, the allocation process could look different than the example above but should be consistent throughout the code. Below is an example of a loop on the CPU that is then converted to run on the GPU:

CPU Version of Code:

GPU version of Code:

```
void Calculate_WorkSpace (reax_system* system, int *bond_mark, storage *workspace)
{
    int blocks;
    blocks = (system->N+BLOCKSIZE-1)/BLOCKSIZE;
    CUDA_Calculate_WorkSpace<<<blocks,BLOCKSIZE>>>(system, bond_mark, workspace);
    cudaDeviceSynchronize();
}
```

```
CUDA_GLOBAL void CUDA_Calculate_WorkSpace(reax_system* system, int *bond_mark, storage *workspace)
int i = blockIdx.x * blockDim.x + threadIdx.x;
if( i < system->n )
workspace->bond_mark[i] = 0;
}
```

In the CPU version of the code, an array is assigned depending on the value of i. In the GPU version of the code, two void functions are written, where one calls the GPU version of the code. This is what is used for the function call in Main Memory. Notice that in the GPU version of the code, the value of the integer i is assigned a unique thread ID. This is the feature that allows for multiple processes to run in parallel.

TESTING

After making the appropriate changes to a function, the code is run to test for build errors or inconsistencies in the implementation. This is done through using the Linux operating system. Certain commands are made in linux to run the Makefile for the code, which tests the program for errors. If errors are detected, then the referred lines are changed and corrected depending on what the error is. The code is constantly edited and run until no errors are found. Afterwards, an output command is written in Linux in order to generate a file with the results. This file will take longer to generate depending on how many steps are processed.

RESULTS

The PuReMD source code was run to yield timing results for the code run on the CPU and on the GPU. The function that was converted was called: Init_forces.

Figure 1: Timing Data for Init_Forces Function

% Time	Cumulative	Self Seconds	Calls	Self ms/call	Total	name
	Seconds				ms/call	
9.40	43.21	6.48	101	64.16	85.09	Init_Forces

This table shows the percentage of the total running time of the program used by this function. 9.40 percent of the running time was used by the Init_forces function. The second column, Cumulative seconds, is a running sum of the number of seconds accounted for by the Init_forces function and any functions that were listed above it. The self seconds column is the number of seconds accounted for by the Init_Forces function alone. The fourth column, calls, is the number of times this function was invoked. The self ms/call represents the average number of milliseconds spent in this function per call. The total ms/call is the average number of milliseconds spent in this descendants per call.

The major sort for this listing was the self seconds and the name of the function. The Init_Forces function required 6.48 seconds. This function was amongst five top functions of using up the highest percentage of time to run. While 6.48 seconds may seem like a short amount of time, the code is running for thousands of steps and runs through other process that require longer time as well. The changes made in the code did not yield a change in time when converted onto the GPU. This was due to time constraints, several build errors and the method used to approach this specific function. Therefore, in the long run, if the code is properly debugged, changes made in this function to implement on the GPU can yield significant changes in the running time.

DISCUSSION/CONCLUSION

Due to time constraints in conducting this research, many results were limited and the code was not completely debugged. Certain conversions, while improving the speed of the loop, do not output correct results. This is determined by the allocation of certain parameters on the GPU version of a portion of code which is needed to execute another portion. The code implemented after the function call to the GPU does not use the new value of a variable defined on the GPU, it uses the original initialization of the variable. This ultimately leads to certain aspects of the code not being used in the calculations, or being implemented and then being reinitialized to an incorrect value. As progress was made in converting a function to the GPU, more problems came to light. Proper memory allocation was very important in yielding correct results. Often, the errors in building the code were caused by memory allocation issues. Many factors had to be dealt with including memory allocation, passing appropriate parameters, and converting appropriate functions. A function had to be properly analyzed to determine if it was worth converting onto the GPU. Some functions deal with simple computations that are not worth moving over to the GPU and will not yield a change in throughput. These functions may also have a variable that can lose its value if it is moved onto the GPU.

Time constraints for conducting the research limited the amount of debugging and how much of a function was converted. This is why appropriate running time data for the code after making changes to run on the GPU was not outputted. Several errors that require more time for debugging were not dealt with which led to an inaccurate output of results. The function converted had a large loop that implemented several other loops within it. At first, the smaller loops were individually converted onto the GPU and appropriate function calls were made in the code. However, after noting that individual function conversions yield inaccurate variable values since the values cannot be transferred in the middle of a process running on the GPU onto

the CPU, a different approach was implemented. The entire loop was converted onto the GPU as one function. This required an analysis for appropriate parameter calling and memory allocation. If an array or variable was used in the loop that was not defined directly in that function, the location of the variable definition had to be determined. Once the definition was found, appropriate measures were taken to allocate that variable onto the GPU. This was especially prominent with arrays used. If an array was not allocated on the GPU but was used in the function, many build errors were found and the job was not processed.

Another complication that was met while attempting to convert the entire loop as one function was having a call to a different function within the loop. There was a call to another function implemented in a different file. This function was analyzed to determine whether it would run faster in parallel. This is an important factor in the conversion process because that one function call could be dealing with the majority of the computations. If that is the case, then the original function is not doing much work and the conversion onto the GPU will not yield speedup in running time. Therefore, the function was also converted onto the GPU. However, soon after, it was easy to note that the conversion of the function within the loop was unnecessary. It was a function that did not have loops requiring a multithreaded structure to run. This also led to an observation that certain variable declarations made on the CPU will alter the results generated by the function running on the GPU. This would yield incorrect results, as the variables would be reinitialized inappropriately. Ultimately, the safest approach in such a short time frame was to go back to the original method of converting individual loops within the function separately. For future research on this study, after appropriately debugging the code, the function should run much faster on the GPU and should result in an obvious difference in the time required for executing the function. This only seems possible to achieve if the entire loop is converted together onto the GPU and proper allocation is done. However, this process requires a lot more time and debugging of different files that have interacting functions.

In general, implementing a function that requires complex computations runs faster on the GPU than on the CPU. The difference in running time is also dependent on the coding methodology and efficient programming. It is important to also take into account how the code is processed and written. Implementing changes in a code that was written by other programmers in a different style also makes it difficult to understand the methodologies used and make changes. Certain implementations were incomplete and others were improperly allocated. This led to errors that could cause incorrect results if all factors involved with one function were not appropriately changed.

The highly multithreaded architecture of the GPU and the parallel structure yields a significant change in the speedup of code that is converted from the CPU onto the GPU. This research examined the difference in running time of a function that required a significant amount of time to be implemented on the CPU and how making changes affected this time. Certain parts of the function were converted onto the GPU to yield such results. However, due to time constraints and build errors, the change was not obvious. Future research with this function will examine these build errors and determine what other allocations must be made in order to witness a fundamental change in running time. Experimenting with GPUs is a work in progress and has many aspects that can be utilized to perform different things. This ongoing interest in GPUs allows for research to be very widespread and eventually lead to the development of GPUs with a better or more efficient design.

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DEFINING HOMELESSNESS AND PRECARIOUSLY HOUSED STUDENTS AT MAJOR UNIVERSITIES: IN SEARCH OF INTERVENTIONS AND RESOURCES

By Michael Williams Major: Psychology Mentor: Paul Toro, PhD, Department of Psychology

ABSTRACT

Empirical research has been limited in regards to estimating, homelessness and precariously housed college students. The aim of this paper is to explore the identity of homeless college students, and examine resources, solutions and interventions made available at urban universities. Many college campuses across the United States address a variety of needs for their students in the areas of mental health, physical health, emotional stability and traumatic experience, however, the growing trend of homelessness among college students is unidentified and understudied. According to Carson, C (2015), several analysis indicate that homeless and precariously housed college student differ significantly from their stably housed counterparts in regards to educational outcomes, mental and physical health and stressful events. Stressful conditions may be a contributing factor in the difference between the two groups. What could be considered as a small situation can develop into a major crisis for a homeless student, for instant, an outstanding tuition balance, finding a quiet place to study, receiving a good night sleep, and a secure place to keep course materials and textbooks. Most students do not have these concerns unless they have housing insecurities. This research paper will provide evidence that more attention is needed to address this growing trend of housing insecurities among undergraduates. Optimistically the findings will help contribute to further investigation to assess needs and develop intervention strategies to help students with housing issues obtain their degree in a healthy and safe environment.

INTRODUCTION

For the majority of young students in the United States, college is a requirement to obtain ones occupational and career goals. Many freshmen students are required to make certain decisions that may seem difficult, for example, what major to declare, how to obtain funding, weather to live on campus or commute. For a certain student population the question of where to live is most difficult because they are homeless and lack the immediate solution or resources for current housing. Homeless student are often difficult to locate or identify because, homeless or precariously housed students are hesitant to describe as such due to the stigma attached.

Defining homelessness is not an easy task; most of the public have the notion that a homeless person is just a person without a home. The stereotype brings into one's mind an unkempt, irate, and sleeping on the streets individual. However, if one could imagine for a moment the classmate that heads the study group in your psychology course could very well be homeless. Due to the lack of empirical research and literature about homeless college students, this paper will focus on research questions rather than a hypothesis

HOMELESSNESS DEFINED

How are homeless and precariously housed college students defined? The Department of Housing and Urban Development describes in four ways: literally homeless, imminent risk of homelessness, and fleeing from domestic violence. (U.S. Department of Housing and Urban Development 2014). According to U.S. Department of Health and Human Service defines homelessness as the following in section 330 of the public Health Service Act. The term" homeless individual" means an individual who lacks housing (with regards to weather, that individual is a member of a family), including an individual whose primary residence during the night is a homeless shelter that provides temporary living accommodations and an individual who resides in a transitional housing program. These are definitions most understood by the public, however the less considered larger group is the precariously housed individuals who depend on temporary living arrangements with friends and family the individual who "couch surf" and move quite frequently from place to place. The McKinney-Vento Homeless Assistance Act, which is a part of the no child left behind Act, expands on the definition by including homeless children and youth (National Center of Homeless Education) and adds individual who are sharing housing due to economic struggles, and living in hotels and motels Harber and Toro (2004) defined literal homelessness as often being cyclical and involving episodes of precarious housing, additionally they mention that precarious housing includes individuals who may be one situation or paycheck from becoming homeless. The definitions of homelessness may vary among situation, however, the results are parallel.

REASON FOR HOMELESSNESS

Why are college students homeless? There are many reasons why a person becomes homeless, but homelessness in general is a result of poverty, loss of job or reduction in work availability, lack of affordable housing, etc. For example, a parent may have hardship and funding for the student has been reduced, or stops completely. Most college students are not homeless upon arrival, some student loose financial support from parents, scholarships, as well as poor decision making, etc, that allow the student financial freedom to obtain their college degree. An increase in tuition may also be a result of homelessness student can only afford tuition and have no available funding for basic needs, for example, abusive relationship, living in drug housing or unfit roommates. When supporting a family, consistent full-time employment is vital. Families who are already on a lower income bracket can very quickly find themselves in disastrous situations when losing a job. A 2012 report by the Amherst H. Wilder Foundation reported 20 percent of unemployed homeless parents had lost their jobs within the last six months, nearly three times the percentage reported in 2003. The loss of a job can have a devastating effect on the student and parents.

ACCOMODATIONS FOR HOMELESS STUDENTS

What are some university programs available to assist the homeless and precariously housed student? Due to lack of awareness of homeless and precariously housed college students, it is difficult for universities to determine homelessness among their students. Many universities overlook the immediate services to accommodate the needs of this subpopulation. Many students may come from a working to upper middle class family and may attempt to fix their housing issues themselves, however, most precarious housed students do not identify as homeless. Bates and Toro, (1999), analysis determined a stress buffering association among homeless individuals who had a strong social and family support system, additionally, homeless students with a significant amount of family support had high scores in self-esteem related analysis. The issues of homelessness have forced many universities to develop intervention strategies and

programs to assess the student's needs, for example, Detroit's Wayne States University, Help Individuals Go Higher program (HIGH). The HIGH Program helps financially stressed students at Wayne State University reach their goal to graduate. The program provides resources for students in need, such as housing support, textbooks and other school supplies, clothing, transportation, and child-care assistance. The mission of the program is to ensure that no student abandons their dream of earning a degree solely because of housing or financial challenges. Another great resource for student facing homelessness is The National Association for the Education of Homeless Children and Youth (NAEHCY) is a national membership association dedicated to educational excellence for children and youth experiencing homelessness. Through state and federal policy and technical assistance to our members, students, and the public, help change systems so all children and youth can learn, succeed academically, and achieve their dreams. They provide professional development, resources, and training support for anyone and everyone interested in supporting the academic success of children and youth challenged by homelessness, they also engage in federal policy advocacy to strengthen policies and resources for homeless children, youth, and families. Many universities have become aware of the growing trend of housing and food insecurities, and have adopted similar programs to combat these issues.

BARRIERS FOR UNIVERSITIES

What are some barriers for university programs? Paden, (2012), noted that The biggest barrier for universities were estimating how many of students fall into the category and identify as homeless students in need of assistance. Other barrier included location of contact point, faculty and staff members to run assistance programs. Additionally, funding restraints may also have barriers on how many students can receive direct funding from the university. It is obvious that these barriers may hinder the development of assistance programs at universities. According to Wilson, (2016), some barriers faced by the Wayne State University's HIGH program is funding and housing availability. The program awards 1,500 per student, and would like to get to the point of aiding 50 students per year.

SOLUTION TO BARRIERS

What are some possible solutions to barriers at university programs? Paden, (2012), noted that, conducting research on the prevalence of housing and food insecurities at university could help determine which students are in need of assistance. Other solutions consisted of a point of contact on campus, this location should be established early in the program, and easy to find. Staffing should consist of a full time program coordinator, counselor and several student interns rotated throughout certain department on campus. Students can also volunteer. Each year a fundraising event held to generate necessary funding. Additionally, collaborating with other local service organization and food pantries to assure that student has available support year around. According to Wilson, (2016). Programs like the HELP program "will need short-and long-term investments to stabilize their revenue and create an endowment of \$5 million". Five million would help secure the wellbeing of students facing housing and food insecurities. Also suggested by, Wilson, (2016). "Universities should embrace the challenge and explore ways to help students in need on their campuses. They must establish solid relationships with their local shelters, food banks, and clothing closets near their location". These partnerships help students year round, even when semester has ended. Wayne State University is building a new student-housing complex in the near future and will reserve 10 units for HIGH programs students who have been homeless without intervention. Once a university has made a decision to address homelessness and precariously housed students on campus an effective communication system is important for creating awareness of the needs of students by the university and awareness of the student of the assistance offered. Determining a contact point on urban campuses should be considered early, some suggestions are that the location be anonymous and free from embarrassing images of homelessness. Testimonials from former homeless and precarious housed student would help persuade students in need of assistance to seek help.



Figure 1: Communication Flows - Informing and Persuading Homeless Students

CONCLUSION

Above all, the finding confirms that: unstable housing places a significant burden on the lives of college students in many different areas. Several analysis in this study indicates that student with housing insecurities are at a greater risk of not obtaining their college degree as compared to stably housed students, due to stressful occurrences. The hypothesis questions posed in this study focused on the identity of the new face of homelessness, the solutions, barriers, early intervention, communication strategies, funding, collaborations and partnerships, suggested by urban universities that take on the challenges to assist students on their campuses. This provides evidence that more attention needs to be address toward undergraduates college students who have been homeless without intervention. It is important for universities to spread awareness of the need to help students on campus, in their community, and nationwide, so to combat this understudied and unidentified trend. Now there is a small platform of empirical information on precariously housed students. The current study under way will inform us about the size of the population at Wayne State University. Duplication on future studies will examine and estimate the prevalence of homeless and precariously housed students among rural and community colleges, this would be informative to evaluate the impact of intervention strategies.

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EXPLORING ENZYME-LINKED IMMUNOSORBENT ASSAY INACCURACIES DURING CORTICORTISONE STUDY

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ABSTRACT

Corticosterone (CORT) is a stress hormone, and its level change in response to stress. The enzyme-linked immunosorbent assay (ELISA) is a standard wet laboratory technique that can be used to measure CORT concentration in serum. Due to assay results outside of the normal range, our lab halted the original study and explored plausible causes for the varied results. The areas explored included: the assay, the plate reader, human error, and environmental factors. Researchers wanted to optimism ELISA performance. After ruling out multiple confounders, environmental factors in the lab ultimately contributed to poor assay performance.

Key words: enzyme-linked immunosorbent assay (ELISA), corticosterone (CORT), restraint stress, diagnostic test validation, basal level

INTRODUCTION

Corticosterone (CORT) is the main stress steroid produced by non-human mammals. CORT is regulated though the hypothalamic-pituitary-adrenal (HPA) axis. CORT can be measured through serum. CORT levels increase in response to stressors. Thus, one way to measure an animal's stress responsivity is exposing it to acute stressors and measuring CORT levels before, during, and after the experiment. For instance, animals should be the least stressed before the experiment and the most stressed after the initial stressor.

Our study used a restraint stress test as the behavioral model to stimulate a physiological response in young adult rats. The restraint stress test involved placing the rat into a Plas-Labs broome-style plastic tube that limited the rat's mobility. The confinement in the tube acted as the stressor. In a stressful situation, basal levels change in response to a new unconditioned stimulus (Kalil, Leite, Carvalho-Lima, & Anselmo-Franci, 2013). CORT concentrations were based on serum obtained from blood collected from the saphenous vein of the rat at four points during the experiment: pre-stressor (T0), 30 minutes after stressor (T30), one hour post-stressor (T1), and two hours post-stressor (T2). The CORT concentrations of the blood should be within a normal unstressed range at T0, which is the basal level, spike at T30, and slowly return to a basal level or go under the initial T0 CORT concentration. Basal stress levels for male rats should be around 50 ng/mL, and basal stress levels for females should be around 100-150 pg/mL (Kalil et al., 2013). Stressed levels range from 250-1000 pg/mL for both sexes (Kalil et al., 2013).

The primary way to measure CORT concentrations in rats is through enzyme-linked immunosorbent assays (ELISAs). ELISAs are useful tools to determine the concentration of a given antigen or antibody in a substance. ELISAs are often used in hospital and laboratory settings, and samples can be tested via serum, plasma, urine, extracted dried fecal samples, or tissue culture media samples. Along a series of steps, a given substance is quantified.

Diluted samples are pipetted into a clear microtiter plate coated with an antibody. A corticosteroneperoxidase conjugate is added to the wells of the microtiter plate. The binding reaction is initiated by the addition of a polyclonal antibody to corticosterone. After one hour incubation, the plate is washed and substrate is added. The substrate reacts with the bound corticosterone-peroxidase conjugate. After a short incubation, the reaction is read at an optical density. The optical density, for a given wavelength, is an expression of the transmittance of an optical element. Optical density results reflect the substance and are determined by the color reaction between the substrate and stop solution. This color change is essential.

There are different methods of ELISA. This study utilized the sandwich method, which is used to detect and quantify a given antigen. The presence of an antigen results in a color change, and the amount of the antigen present in the substance results in the range of color upon reaction. The deeper the color upon reaction indicates the greater amount of an analyte present in the sample (Berg, Tymoczko, & Stryer, 2002). For sandwich ELISAs, an antibody is bound to a plate well, and the analyte sits between the bound antibody and detecting antibodies. The sandwich ELISA is more sensitive and specific than the direct and indirect ELISA methods. For direct ELISA, an antigen is absorbed from an antibody conjugate. For indirect ELISAs, a secondary antibody binds to a primary antibody. The binding of multiple antibodies makes this method more sensitive than a direct ELISA. The most important aspect of the assay is the creation of standard curve.

The CORT concentrations are based off the standards, which produce a curve for the sample results to be analyzed. Standard curves are used to determine the concentration of substances. If the standard curve for the ELISA is off, the data from the trial is useless. Without the standard curve, the sample results do not have a basis for comparison (Natarajan, & Remick, 2008). While a standard curve can be assumed based on the concentration of CORT stock for each standard, it is not ethnical to alter the standard curve numbers for a publishable piece of work. Standards must be prepared and tested with each assay plate that is run.

A sample's CORT concentration is aligned on the standard curve in comparison to optical density readings of the standards and the CORT concentrations. To determine the antibody concentration in each sample well, the optical density readings of the wells are compared to the optical density readings of the standard curve. The standards have a known concentration. These standards provide a reference to determine unknown concentrations. The standards' concentration range need to span the range of concentrations expected to be found in the "unknown" sample concentrations. The typical range is 0-10,000 pg/mL (Arbor Assays, 2016).

Samples are always run in duplicates and with inter-assay samples to ensure comparability between different runs/plates. Inter-assay samples (IAS) are samples collected from a previous study to serve as a consistent between different plates. The IAS values should not change between trials. There are generally two IAS used, a high concentration sample and a low concentration sample. Samples are run in duplicate to assess intra-assay validity. Intra-assay validity tests whether the sample same yields the same results. If the paired wells of the same sample are not similar, this lets the researcher know there was an error during the ELISA. Pipetting errors are the most likely cause of intra-assay validity variances (source).

While trying to implement the intended study, the lab could not get consistent results. Based on the results from an ELISA CORT assay, our lab believed an error occurred at some step during the testing process. This paper goes through the different stages of the study, including the animal environment, the restraint stress procedure, blood collection, pipetting, ELISA protocol, and plate reader, to determine which step(s) of the process, if any, likely caused the data errors. We predicted that any one or a combination of the stages could be the issue. The aims of the present investigation were to identify variations in animal CORT concentrations and then determine if this was the cause of error during the experiment or if the results

reflect the animals' true stress levels. By identifying the cause in result variability, we hope to optimism ELISA performance.

The data from the assays were inconclusive. The concentrations of the samples were either unable to be analyzed because of a faulty standard curve or the observed concentration levels were higher than previous studies suggest. This paper addresses different aspects of the process to determine what steps could have been affected. It was important to not simply start with the ELISA itself, but to review if any errors could have been affected prior to running the assay. The subsections related to an error occurring outside of the ELISA are: environmental changes and the restraint stress test/blood collection. The subsections related to errors that occurred during or involved with the ELISA are: pipetting techniques/assay procedure and plate reader alignment.

METHODS

SUBJECTS

98 young adult male and female Sprague-Dawley rats (bred in-house) were used for this study. Animals lived in a reverse-light cycle setting (12:12 light-dark cycle (light from 7 p.m. to 7. a.m. and dark for 7 a.m. to 7 p.m.) and had ad libitum access to food and water.

All procedures were approved by the Wayne State University Institutional Animal Care and Use Committee, and all animal care and use were in accordance with all applicable portions of the Animal Welfare Act and the current NIH "Guide for the Care and Use of Laboratory Animals". The University maintains Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited animal facilities under the Division of Laboratory Animal Resources.

RESTRAINT STRESS TEST PROCEDURE

The animal was taken from its cage and poked with a 21 gauge hypodermic needle in the saphenous vein of either foot for the basal sample (T0). The researcher collected a minimum of 200 microliters (μ L) of blood in a 500 μ L collection tube within three minutes for the T0 testing point. Blood was not collected after the three minute point because CORT levels in the blood would have increased due to the stress of being poked with the needle. The needle poke served as a stressor as well. Once the sample was collected, the sample was immediately placed on ice to preserve accurate CORT levels. The puncture site was briefly gauged, and the animal crawled into the plastic tube (Plas-Labs; broome-style; Animal size range: 250 to 500 g; L x Dia.: 8.5 x 2.5 in. (21.6 x 6.4cm), which served as the stressor, and was secured by a plastic ring. The ring prevented the animal from moving forward, backward or rotating in the tube. The animal remained in the tube until the next collection time (T30). The researcher watched for distress signs from the animal such as an inability to breathe.

After 30 minutes of the animal being in the tube, the ring of the tube was removed and the animal was encouraged to back out of the tube into the researchers' hands. This was done by holding the tube at a 90 degree angle. Blood was collected from the saphenous vein within five minutes. The time frame for blood collection was longer because the animal was already stressed and basal levels were not being accessed. The sample was then iced. The animal's puncture site was briefly gauged before the animal was placed back in its cage and returned to the holding room. One hour post-stressor, the animal was removed from the holding room and the blood collection procedure stated earlier was performed. This step was repeated for

the last collection two hours post-stressor. Noise was limited during the test to avoid disturbing the animal being tested and other animals that were waiting to be tested.

BLOOD COLLECTION

Blood from the procedure was iced immediately after collection and then refrigerated (4°C). This was important because blood loses it property once removed from its host. Samples should be centrifuged within 24 hours. Centrifuge samples at 8,000 to 10,000 g for 10 minutes speed or until serum and whole blood have separated.

Using a 10 μ L pipette tip, the pipette was angled below the surface of the serum in the tube. Forward pipetting was used to transfer serum into smaller tube centrifuge tubes (1.5 μ L). In forward pipetting, the tip is placed into the substance. The release button is pressed to the first stop and the sample goes into the pipette tip. Once the release button is pressed again, the sample will be dispensed into the given well or tube. The researcher collected as much serum as possible, 10 μ L or above was preferred. Blood was and serum were placed in a freezer (-20°C) after collection.

ELISA PROCEDURE

The experiment used the DetectX Corticosterone Immunoassay kit from Arbor Assays. The present study used standards that ranged from 78.125 to 5,000 pg/mL. The assay can be used for standards up to 10,000 pg/mL. Sensitivity was determined as 18.6 pg/mL. Limit of detection was determined as 16.9 pg/mL.

Using reverse pipetting, two μ L of the sample was deposited into its respective new vial. In reverse pipetting, a greater amount of the substance in taken into the pipette tip and will be left in the tip, unless willingly removed by pressing the release button an addition. The dissociation reagent container was vortexed and two μ L was added to each sample vial. The dissociation reagent helped to isolate and breakdown the CORT in the substance. Pipette tips were changed for every sample. The tips were pre-rinsed in order to ensure all of the substance made contact with the pipette to prevent capillary action or sticking. Assay buffer was added to each new vial using the 1:400 dilution (796 μ L). The samples were returned to the freezer.

For making standards, 475 μ L assay buffer was added into vial one. 250 μ L assay buffer was pipetted into vials two through seven. 25 μ L corticosterone stock solution was added to vial one, and the solution was thoroughly vortexed. 250 μ L of the corticosterone stock solution from vial one was added to vial two and the procedure continued to vial seven. The finished solution was vortexed. The tips were rinsed several times because the CORT solution was very viscous.

The time was noted and a timer was set. 50 μ L of standards and samples vortexed and then pipetted into each well. Samples were run in duplicate, side-by-side. Each duplicate of the sample was treated before moving on to the next sample. Pipette tips were pre-rinsed tips and change tips between samples.

50 μ L of assay buffer was pipetted into B0 wells. 75 μ L of assay buffer was pipetted into NSB wells. The time was noted. 25 μ L of corticosterone conjugate was pipetted into each well using the repeater pipette and appropriate labeled tip. 25 μ L of corticosterone antibody was pipetted into each well except the NSB well using the repeater pipette and appropriate tip. The sides of the plate were gently tapped to ensure adequate mixing of the reagents. The plate was covered with a plastic adhesive film and place on the shaker. After 60 minutes, the contents of the well were poured into a discard trough. Using multichannel pipette, 300 μ L of wash buffer was added to each well and then discarded. The process was repeated three more times. 100 μ L of TMB substrate was added to each well using a repeater pipette and appropriate tip. The well was

incubated for 30 minutes. After 30 minutes, 50 μ L of stop solution was added to each well using a repeater pipette and appropriate tip. The well was placed in the plate reader for Gen5 analysis

GEN5

The Gen5 program was cued to read at 450 nanometers (nm). The program analyzes well plate results. The user programs the software to read the appropriate number of rows, either six or seven, and layout protocol mapped on a 96- well plate. Background absorbance of the microplate is removed by subtraction of a blank well.

At this point, the researchers identified inconclusive results from the assay. The results did not make sense, so the lab shipped some samples to the assay manufacturer. The manufacturer ran several tests for potential confounders as described below.

SHIPMENT PROCEDURE

Serum samples were shipped to Arbor Assays, the manufacturer of the ELISA kit used for this study, for CORT analysis. The serum samples were classified as "non-regulated biological" samples because they proposed no hazardous risks to human health or the environment.

Two μ L of the serum from each of the samples were placed in a .5 mL centrifuge tube. The tubes were placed small plastic storage bag and then placed into in a small Styrofoam container with ice packs. The package was shipped to the manufacturer the same day.

PLATE READER ALIGNMENT

While waiting for samples to be analyzed at Arbor Assays, the lab ordered an absorbance test plate to check the alignment of the plate reader. An absorbance test plate can be used to test for plate reader errors. The plate is an aluminum block manufactured to specific tolerances. Holes in the plate hold neutral density glass disks. The alignment feature tests if the absorbance of the plate reads between -0.015-0.015. The accuracy feature tests pre-determined numbers. A data sheet provides a range of numbers to evaluate the accuracy of the reader at specified wavelengths.

ARBOR ASSAYS VISIT

Exhausting all other plausible causes of error, the lab visited the assay manufacturer to perform some mock assays. The results from the mock runs were successful. This confirmed that researchers were skilled in ELISA techniques. The lab discussed other possible errors including water contamination and temperature variances.

QUALITY CONTROL

The lab has certain equipment to aid in everyday lab tasks. Two of these materials are a water distiller and a thermometer. The lab ensured the water distiller was working probably. The lab even purchased distilled water to use for assays. The lab was not temperature controlled. The temperature for the lab was generally around 21°C. The assays were run near large windows, which could interfere with the temperature. At Arbor Assays, the average temperature was around 25°C. The lab began running assays on a heating pad and heating a windowless room with a space heater to maintain temperature results around 25°C. Since implementing the new heating procedure, the lab has experienced successful assay runs.

RESULTS

Assay/CORT Measures

There was no unity between the testing points, in reference to the expected alignment along the standard curve (see Figure 3). Serum CORT concentrations increased after restraint and then decreased for each subsequent testing point (see Table 1). Even though Arbor Assays results followed this descending CORT trend, Arbor Assay results were at least twice as high for all samples over all testing points (see Table 1). Female samples had higher CORT serum concentrations than males during the same testing point (i.e., T0) for our lab and Arbor Assays) (see Figure 1; Table 1).

PLATE READER ALIGNMENT

All results were within normal range. The analysis for the plate reader included alignment, accuracy, and repeatability results (see Tables 2.1-2.3).

TIMING

No relationship could be found between the circadian rhythm and CORT levels (see Table 3).

QUALITY CONTROL

There was a difference in results for the IAS samples with and without the use of a heating pad (see Table 4).

DISCUSSION

Determining the cause of scientific error is a process. Examining past data and procedures to determine which steps are the likely causes of the error and determining if there is a way to resolve the issue is tedious. Assays are extremely sensitive and the researcher should be aware of errors that can skew the results of the assay. By understanding the different aspects that go into the project, the researcher is better able to safeguard against potential errors. Results comparing data from different labs of the same specimens and results from performance tests on equipment yielded the likely cause of error in this experiment.

ANIMAL ENVIRONMENT

The animal environment did not play a role in the assay performance. The key components of the animal environment were circadian rhythm and human contact. In addition to stress levels, corticosterone plays an important role in the circadian rhythm. Circadian rhythms are the physical, mental, and behavioral changes that occur during a 24-hour cycle and are cued by daytime (light) and nighttime (darkness) for most living things (National Institute of General Medical Sciences, 2012). Further analysis is needed on the correlation between stress and immediate removal from a reversed light-cycle environment. Peak CORT levels should occur toward the end of the inactive period (Kalsbeek, van Heerikhuize, Wortel, & Buijs, 1996).

Rats are nocturnal and their active period occurs during the night. In the reversed light-cycle setting, the rats experience their active period when lights are off during daytime hours. The CORT peak should occur toward the end of the inactive cycle as corticosterone rises and stimulates carbohydrate and fat intake

(Kalsbeek, 1996). The CORT peak should diminish within one hour because of the effect of the vasopressin V1 receptor antagonist (Lemmens-Gruber, & Kamyar, 2006). Vasopressin stimulates contraction of the muscular tissues of the capillaries and arterioles, raising the blood pressure (Lemmens-Gruber, 2006). The role of the antagonist is to oppose the effects of the receptor. The antagonist effect of vasopressin is increased blood flow.

Change in scenery from dark to light may produce a stress response in rats (Prager, Bergstrom, Grunberg, & Johnson, 2011). This change in scenery cued a response. To reduce the effect of scenery change from having an effect on animals during testing, it is best to allow the subject to adjust to the present environment. It is up to researchers to determine how much time to allow the subject to acclimate prior to experimentation because the National Institute of Health does not have set standards (Prager, 2011). The researcher should take into account the method results will be tabulated (i.e., blood, fecal material, tissue, etc). Acclimation allows the animal time to adjust to the new environment. Prager (2011) suggests a minimum of 30 minutes for acclimation. Prager's study was based on serum results.

The circadian rhythm did not seem to be a factor in high basal levels. The active cycle started for the animals at 7 a.m. and animals were not tested minimally until at least an hour into the active cycle. In Table 3, TO samples are analyzed according to time frame. The selected samples were tested between 8:14-9:29 a.m. This data suggests that the time the animals were tested for the TO testing phase did not impact stress responses, which reflects CORT concentrations.

During the restraint stress test, it is important to limit the external stressors near the animals. Despite trying to prevent animals from being stressed, there is only so much control that researchers have over an environment. There is not a definite way to do this. Wayne State University Division of Laboratory Animal Resources (DLAR) is responsible for operating the university's laboratory animal care and use program. The animals could have been stressed from things happening in their housing. DLAR staff makes sure that the animals are feed and changes cages. If there are any health concerns, DLAR provides assistance. Since animal care was eliminated as the cause for poor assay performance, the next likely cause was examined—pipetting.

PIPETTING

It is important for the researcher to have adequate pipette training. This is the most crucial aspect of the experiment. The experiment utilized both forward and reverse pipetting. Forward pipetting is used for large amounts (greater than two microliters). Reverse pipetting is used for smaller amounts (two microliters or less). Reverse pipetting is used to reduce the risk of measurement displacement. Displacement is often the result of bubbles. Forward pipetting can be used for substances of limited quantity.

Calibrating pipetters is important for proper substance dispensement. Pipetters that are not calibrated could dispense more or less than the desired substance. The conductor of the experiment must also ensure that the tips used for the experiment fit properly and are autoclaved. The pipetters used in the experiment were calibrated, and the lab ruled out the pipetters and pipetting techniques as the cause of poor assay performance. The next step was to review the assay procedure.

Assay Procedure

Contamination of products could cause errors in results. It is important to make sure that all instruments used for the experiment are sterile. Pipette tips should be autoclaved and placed into appropriate container

before use for the experiment. Glassware should be autoclaved as well. The glassware is used to store the assay buffer and wash buffer. Contaminated containers could alter the chemistry of the substances causing varied results. Assays should be examined prior to beginning the experiment. Notations should be made for any scratches or dust. Scratches inside or outside the well could interfere with the optical density readings.

Intra-assay validity compares mean absorbencies for the samples in plates. If the duplicates of the samples are showing vast differences there is a problem. Inter-assay variation, or assay to assay reproducibility, can be evaluated by comparing reruns of samples or the results of the same samples performed by different researchers.

During the process of this experiment, the manufacturer modified the amount of serum needed for each sample from two microliters to five microliters. The researchers were unable to conclude if the increase in serum required to perform the assay impacted earlier poor assay performances. The researchers were unable to conclude if the increase in volume was due to other labs having issues with the kits as well. When performed properly, a standard curve (see Figure 2) should provide a scale for measurements to be judged. The standard curve creates the basis for the assay. When the standard curve is inaccurate, the results from the assay must be ruled null and void. While a suggested standard curve could be used for estimates of how the results align, a standard curve performed outside of the assay in question cannot be used because the samples were not run with the other samples. Inter-assay validity is a major aspect of assays. Since the lab was not getting consistent results, it was important to see if the manufacturer would get similar results.

INTER-ASSAY VALIDITY

The results suggest that the animals were stressed, but do not explain why the lab results were not near Arbor Assays level. The samples from Arbor Assays displayed overall higher numbers than the results from our lab for all the samples in the TO-T2 testing points. The pattern of change from TO-T2 was consistent with previous data. As shown in Table 1, our predictions were supported that T30 had the highest CORT concentrations. The results also provided that the inter-assay validity was outside of the 18.9 ng/mL sensitivity of the ELISA. The results suggest that the animals were stressed prior to the beginning of the study. This might be the result of handling the animal prior to the experiment, thus causing stress. If this was the case, true basal levels cannot be determined. Further research needs to be done to figure out why the CORT concentration of the same animals differed between the labs. To ensure that the results were accurate, the machine that analyzed the results were tested.

PLATE READER ALIGNMENT

The results from the plate reader manufacturer concluded that the plate reader was reading at accurate levels for 450 nm wavelength (see Tables 2.1-2.3). This invalidates the argument that an issue with optical density readings was the cause for high basal readings. For the alignment results, the four wells (B2, B12, G1 and G11) all passed the tolerance level for 450 nm. If the readings did not read pass this test, the machine would need to be recalibrated.

It is best to perform an absorbance test prior to preforming assays. This will ensure that the reader is analyzing the results properly. The absorbance test plate has predetermined results that determine if there is an issue with the reader. If the results are off, the reader should be recalibrated or may need a replacement item such as a bulb. It is important to limit movement of the reader because the bulb inside the machine can be easily damaged.

Hemolysis, or serum that contains a lot of red blood cells, is another aspect of the project that could interfere with the plate reader. The lab excluded samples that appeared extremely hemolyzed; however, the exact

amount of hemolysis was not scientifically tested. Samples with moderate to severe hemolysis should not be used with the ELISA kit. The hemolysis could have affected results. The samples used were a biegish color, which suggested the samples contained 40 mg or less of hemoglobin. 40 mg was an acceptable level to use with this kit. The prediction that hemolysis was cause for error regarding optical density was disproved. Specimens were kept at the proper temperatures by being placed on ice immediately. After centrifuging the specimen, the serum was inspected for a pink or reddish tint, which is the result of hemolysis. The sensitivity of the assay kit to hemolysis determines to what extent the data will be affected. The error that results from hemolysis has to do with the color of the product.

It is important to find the optimal temperature for the enzyme-based assays to work. Proteins disintegrate at too high temperatures, but enzymes have lower activity in low temperatures. Lower temperatures in the lab led to suboptimal performance of the assay and low recovery of CORT, thus producing unreliable results. These results were shown with the IAS samples and the standard curves. Maximum binding recovery was lower with low temperatures.

CONCLUSION

In conclusion, researchers must be aware that quality control issues can impact assay performance. Even though the lab followed the protocol and ensured the substances were brought to room temperature, the actual temperature in the lab could not be controlled throughout. In addition, the location where the assays were performed was not ideal, especially during colder months.

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Table 1

Comparison of Our Lab and Arbor Assays Sample Concentrations

Samples	Arbor Assays Conc. (ng/μL)	Lab Results Conc. (ng/µL)
3 F1 T0	301.168	123.062
15 M1 T0	258.019	120.667
15 M1 T30	789.179	223.212
25 M2 T1	96.855	38.212
3 F1 T2	54.459	11.685
33M1 T2	47.764	9.522

Tables 2.1-2.3 refer to absorbance test plate results.

Table 2.1

Alignment Results

Wells	B2	B12	G1	G11
Read	0	0	0	0
Tolerance	0.015	0.015	0.015	0.015
Result	PASS	PASS	PASS	PASS

Note. Wavelength = 450 nm

Table 2.2

Accuracy Results

Wells	C1	E2	G3	H6	F5	D4
Reference	0.128	0.579	1.121	1.619	2.059	2.55
Min Limit	0.105	0.547	1.079	1.567	1.957	#N/A
Max Limit	0.151	0.611	1.163	1.671	2.161	#N/A
Read 1	0.131	0.582	1.133	1.621	2.053	2.525
Result	PASS	PASS	PASS	PASS	PASS	#N/A

Table 2.3

Repeatability Results

Wells	C1	E2	G3	H6	F5	D4
Read 1	0.131	0.582	1.133	1.621	2.053	2.525
Min Limit	0.124	0.571	1.117	1.599	1.987	#N/A
Max Limit	0.137	0.592	1.15	1.642	2.12	#N/A
Read 2	0.131	0.582	1.134	1.621	2.053	2.524
Result	PASS	PASS	PASS	PASS	PASS	#N/A

Table 3

T0 Collection Times and CORT Levels

Sample ID	T0 Time	CORT Average Concentration			
		N	11	Ν	12
7	9:15 a.m.	1116.477	953.161	634.293	457.368
8	8:23 a.m.	829.523	730.822	781.818	781.818
9	9:29 a.m.	2201.746	2348.695	1611.968	931.213
10	8:35 a.m.	2123.546	919.596	861.216	1133.896
11	8:14 a.m.	705.959	1438.074	1393.33	1494.228
12	8:32 a.m.	2584.805	2685.72	685.758	665.501
14	8:40 a.m.	1145.574	555.051	231.915	695.191
19	9:23 a.m.	543.832	820.776	679.687	796.585



Table 4

The Effect of Heat on CORT Sample Levels

Samples	With Heat	Without Heat
IAS 2013	580.43075	916.31635
IAS AD 3	103.2505	86.38575
IAS AD 1/2	101.99585	110.32325

Note. All samples are in ng/ μ L.



Figure 1. Comparison of our lab and Arbor Assays sample concentrations. Arbor Assay results were higher for all samples over all time periods.



Figure 2. A typical standard curve. Source: Arbor Assays, 2016.



Figure 3. A faulty standard curve. This is the result of standards not being properly prepared.

THE HOMERIC CONCEPTION OF THE SOUL. EXPLORING THE PHYSICAL NATURE OF THE SOUL IN THE ILIAD.

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ABSTRACT

The objective of my research was to determine whether or not Homer understood the soul in physical terms. It was common among ancient Greek philosophers to define the soul in physical terms: Thales (625 - 555 BCE) thought that the soul was made of water, Anaximenes (570 - 505 BCE) thought that the soul was made of air, Democritus (470 - 400 BCE) thought that the soul was made of fire, etc. Since there is no explicit, systematic psychology or psychophysiology apparent in Homeric literature, this research is based on the etymology of the most prevalent term used in Homeric literature for the denotation of the soul – θ uµoç (*thumos*) – which is often translated into English as 'spirit', 'soul', 'passion', 'emotion', 'courage' or 'anger'. The etymology of *thumos* led me to think that Homer might have conceived of the soul as a type of hotwind or smoke, if he did conceive of it in physical terms, as the root meaning of *thumos* pertains to thinks like 'smoke', 'burnt offering', and 'movement'.

To test whether this theory is true, I used the *lliad* to find where *thumos* occurs in proximity of words related to fire, heat, wind, and smoke (or simply fire- and wind-related words); and I then used those occurrences to see how often *thumos* is modified by or used significantly with said words. The results were taken to reflect how Homer might have wanted to depict the soul in physical terms. To support my findings further, I did the same search and analysis for other words in the *lliad* that are used for the denotation of the soul. Throughout this essay, I refer to these words as 'soul-terms': they are $\phi p \eta v$ (*phren*, meaning, mind), vooç (*noos*, meaning, mind), and $\psi u \chi \eta$ (*psyche*, meaning, soul). The results show that Homer used fire- and wind-related words to depict physical and behavioral characteristics of the soul. But the number of occurrences found in the *lliad* are so few that it is difficult to say whether or not these results actually reflect how Homer conceived of the soul.

INTRODUCTION/ BACKGROUND

Traces of influence of Homeric literature, which was written as early as the eight century B.C.E., can be found in the works of many different philosophers who wrote many centuries later, e.g., like Plato, Aristotle, the Atomists and the Stoics. And we know of specific psychological concepts that were introduced in Homeric literature that had an influenced on the writings of later philosophers. In particular, Homeric literature was influential in respect to the behavior and personality associated with a warrior, or simply the area of warriorpsychology. In both the *lliad* and the *Odyssey* – two of Homer's most famous works – Homer uses ' $\theta u \mu o \varsigma'$ (*thumos*) as the choice Greek word to denote the soul and a broad range of different psychological phenomena typical of a warrior, like anger, passion, fear, bravery etc. His use *thumos* is so unique that when we see other ancient authors who wrote later than him make use of the term '*thumos*' to denote the soul or similar psychological phenomena, we often believe that it is the result of Homeric influence and look for more similarities in their usage of *thumos* to support that claim. My goal here is to study the physical nature of the soul in one of the Homeric works. The study of the physical nature of the soul is an area in psychology called 'psychophysiology'. This study will be interesting because defining physical characteristics of the soul was not uncommon for ancient authors who come later than Homer, even the same authors whose psychology was arguably influenced by Homer. Discovering this aspect of Homeric psychology could provide us with another way besides or in addition to the use of *thumos* to connect his works with that of later thinkers, whose extant works are not always complete or easy to understand.

It is unclear, however, whether there was an exact physical nature of the soul in the time of Homer. It is very possible that Homer did not conceive of the soul in physical terms at all. A.W.H Adkins, noting the abundant use of *thumos* to denote the soul in the Homeric writings, says that *thumos* was used to describe human beings' internal life before much was known about human physiology. For this reason, others like John Lynch think that we should 'be careful not to demand a physiologically exact reference for *thumos'*. Lynch does well to begin his study in search of a physical nature of $\partial u\mu o\zeta$ by studying the etymology, which allowed him to connect *thumos* is a sort of inner wind that 'stirs up and rouses the person to action'. We might conclude from their studies that *thumos* was physically something like wind or smoke or something similar to air. If this is true, then it suggests that we should take the physical nature of the soul as *thumos* to be something along those lines.

Caswell says that it was typical for early Greeks to model their understanding of the soul on the natural elements in this way. Some argue that thumos was connected to the element of wind or air because it was thought of as the breath within an individual. Onians, e.g., holds that the organ of mind in Homer is the lungs, which was considered identical with breath. Purriance, similar to Onians, says that thumos was localized in the thoracic cavity, which is where the motions of the diaphragm and the lungs occur. She explains further that thumos was probably placed there because of the close connection between breathing and different 'psychic conditions': the ancient Greeks noticed that one's breathing pattern is often indicative of and affected by one's psychological state. By a similar line of thought, thumos was not only linked with breath, but also with warmth: since different psychological states, like anger or rage, cause one's body temperature to rise, *thumos* was taken to be something responsible for warmth or heat as well, like fire. (For more on the relation between soul and the body in Homer, see Crivellato and Ribatti, (2007)). These studies that connect *thumos* to breath and warmth within the individual are consistent with the thinking of some of the philosophers after Homer, who conceived of the soul as air or fire. Although the term 'thumos' is not always used by these philosophers, their conception of the physical nature of the soul could still be inspired by the psychophysics associated with thumos, especially if they were inspired by Homeric psychology and if Homer conceived of and portrayed thumos in his works along similar lines. Establishing, therefore, if and how Homer conceived of the soul physically would help us to link his works with those later philosophers who do not use the term 'thumos', yet who, among other things, based their psychophysical concepts of the soul on the *thumos* in Homeric literature.

To see whether Homer conceived of the soul in physical terms, I will explore his use of the term *thumos* in the *lliad*, which is one of the most famous and influential of the Homeric works. We have so far seen how a few studies of *thumos* suggest that its physical nature may be something like wind, smoke, air or fire. My theory is that Homer could have chosen *thumos* as the primary term to refer to the soul because he, and perhaps his contemporaries, conceived of the soul as something like wind or fire. This claim I hope to explore by examining how often *thumos* is called or compared to air, smoke, fire, heat, etc., in the *lliad*. And since others words are used to denote the soul in the *lliad* – not just *thumos* – I will perform the same analysis for those words as well. These words are $\phi p \dot{\gamma} v$ (mind), vooc (mind), and $\psi v \chi \eta$ (soul). For convenience, I shall refer to them as 'soul-terms'.

METHOD AND MATERIALS

The present study involves a close look at several passages from the *lliad*, in particular, any passage in which one of the Greek words, or a cognate of the Greek words, $\theta u \mu o \zeta$ (*thumos*), $\phi p \eta v$ (*phren*), $v o o \zeta$ (*noos*), or $\psi u \chi \eta$ (*psyche*), can be found near a word whose meaning is related either to fire or to wind. By 'near', I mean within twenty-five words. The Greek words that pertain to fire (fire related words) are:

- 1. πῦρ (fire)
- 2. θερμός (heat)
- 3. κατακαίω (to burn completely)
- 4. φλόξ (flame)
- 5. ὑποθερμαίνω (to warm)
- 6. λιαρός (warm)
- 7. αἶθοψ (fiery-looking)
- 8. ἰαίνω (to heat)
- 9. θεσπιδαής (kindled by god)

The Greek words that pertain to wind (wind-related words) are:

- 1. ἄνεμος (wind)
- 2. οὖρος (wind)
- 3. καπνός (smoke)
- 4. ἄελλα (stormy wind)
- 5. πετάννυμι (to fly)
- 6. ἐμπνέω (to blow)
- 7. ἀίσθω (to breathe out)
- 8. κεκαφηώς (breathing forth)
- 9. ἀσπαίρω (paint or gasp)
- 10. ἄημι (breathe)
- 11. ἀποπνέω (breathe forth).

This study seeks to determine how strongly *thumos, phren, noos,* and *psyche* are connected to the element fire or wind by answering the following two questions: 'How often in the *lliad* do we find an occurrence of *thumos, phren, noos,* and *psyche* in close proximity with a fire- and wind- related word?' and 'How often do these occurrences contain a modification or strong comparison of *thumos, phren, noos,* and *psyche* and a fire- or wind- related word?' I shall consider an occurrence relevant if the fire- or wind-related term is modifying or used significantly with one of the soul terms; and I shall consider an occurrence irrelevant if the fire- or wind-related term is used in the proximity of a soul term, but is neither modifying nor used significantly with a soul term.

To define any Greek words for this research, I used *Liddell and Scott's Greek-english Lexicon*, At the Clarendon Press, 13th ed., Cambridge, 1915. To collect from the *Iliad* the relevant passages, I used TLG (Thesaurus Linguae Graecae), which is a digital library of Greek texts. To analyze the passages from the *Iliad*, I used a translation by A.T. Murray, Cambridge, MA., Harvard University Press; London, William Heinemann, Ltd. 1924. This translation can be found at Perseus, which a digital library of Latin and Greek texts.

RESULTS

Table 1 – A ratio between the relevant (R) and the irrelevant (I) uses of soul-terms as wind and fire:

Elements		The	Different	Soul-Terms		
		θυμος	φρήν	νοος	ψυχη	
Wind	R	11	0	1	2	
	Ι	38	14	6	2	
Fire	R	6	1	0	0	
	Ι	40	8	2	5	

Table 2 – References for the relevant uses of $\theta \upsilon \mu o \varsigma$ and $\phi \rho \eta \varepsilon \varsigma$ as wind and fire:

θυ	μος	ф	φρήν				
WIND	FIRE	WIND	FIRE				
	Book Reference						
21.386	9.587		1.103				
1.593	18.1						
18.110	24.321						
16.468	23.597						
20.403	23.600						
5.698	24.321						
3.293							
10.492							
21.386							
4.524							
13.654							

Table 3 – References for the relevant uses of vooc and $\psi u \chi \eta$ as wind and fire:

vo	ος	ψι	νχη			
WIND	FIRE	WIND	FIRE			
	Book References					
11.813	813 23.100					
		5.696				

Two examples of relevant uses:

Example 1

>>>Hom. Iliad, 23.597: 'So spake the son of great-souled Nestor, and led up the mare, and gave her into the hands of Menelaus. And his heart ($\theta u \mu \delta \varsigma$) was warmed with cheer ($i \dot{\alpha} v \theta \eta$) even as the corn when with the dew upon the ears it waxeth ripe, what time the fields are bristling.'

Ή ῥα καὶ ἴππον ἄγων μεγαθύμου Νέστορος υἰὸς ἐν χείρεσσι τίθει Μενελάου· τοῖο δὲ θυμὸς ἰάνθη ὡς εἴ τε περὶ σταχύεσσιν ἐέρση ληΐου ἀλδήσκοντος, ὅτε φρίσσουσιν ἄρουραι·

Example 2

>>>Hom. *Iliad*, **23.100**: 'So saying he reached forth with his hands, yet clasped him not; but the spirit $(\psi \nu \chi \dot{\eta})$ like a vapour (καπνός) was gone beneath the earth, gibbering faintly.'

Ώς ἄρα φωνήσας ώρέξατο χερσὶ φίλῃσιν οὐδ' ἔλαβε· ψυχὴ δὲ κατὰ χθονὸς ήΰτε καπνὸς ὤχετο τετριγυῖα·

Two examples of irrelevant uses:

Example 1

>>>Hom. Iliad, 7.2: '... in their hearts ($\theta \cup \mu \tilde{\omega}$) were both eager for war and battle. And as a god giveth to longing seamen a fair wind ($\tilde{\upsilon \upsilon \rho ov}$) when they have grown weary of beating the sea with polished oars of fir, and with weariness are their limbs fordone.'

... ἐν δ' ἄρα θυμῷ ἀμφότεροι μέμασαν πολεμίζειν ἠδὲ μάχεσθαι. ὡς δὲ θεὸς ναὑτῃσιν ἐελδομένοισιν ἔδωκεν οὖρον, ἐπεί κε κάμωσιν ἐϋξέστῃς ἐλάτῃσι πόντον ἐλαύνοντες, καμάτῳ δ' ὑπὸ γυῖα λέλυνται

Example 2

>>>Hom. Iliad, 18.15: 'While he pondered thus in mind ($\phi p \epsilon v \alpha$) and heart ($\theta u \mu \delta v$), there drew nigh unto him the son of lordly Nestor, shedding hot ($\theta \epsilon p \mu \dot{\alpha}$) tears, and spake the grievous tidings . . .'

ἂψ ἐπὶ νῆας ἴμεν, μηδ' Ἔκτορι ἶφι μάχεσθαι. Εἶος ὃ ταῦθ' ὥρμαινε κατὰ φρένα καὶ κατὰ θυμόν, τόφρά οἱ ἐγγύθεν ἦλθεν ἀγαυοῦ Νέστορος υἱὸς δάκρυα θερμὰ χέων, φάτο δ' ἀγγελίην ἀλεγεινήν·

DISCUSSION

I was able to find a total of 136 occurrences of the different soul-terms in proximity to some fire- or windrelated term. After taking a closer look at these occurrences, to see how many were relevant and how many were irrelevant, it became very clear that the results show little evidence that Homer took the soul to physically resemble either fire or wind in the *lliad*. There are very few relevant occurrences of soul-terms in the proximity of fire- or wind- related terms – only 21 of the 136 occurrences were significant in the way that I hoped. As mentioned in **Methods and Materials**, I considered an occurrence relevant or significant only if a soul-term was modified by or used significantly with a fire- or wind-related term (See **Results** section for two examples of relevant occurrences and two examples of irrelevant occurrences).

Table 1 shows a ratio between what I considered relevant and irrelevant occurrences for each soul-term. It also shows that *thumos* yielded most of the results for the relevant occurrences: it provided 17 of the 21 relevant occurrences. 6 of these 17 occurrences were where *thumos* occurred near some fire-related term and 11 were where *thumos* occurred near some wind-related term. See **Table 2** for the exact references to these occurrences.

How we ought to interpret the 21 occurrences is debatable, though: on the one hand, the 21 is insignificant compared to the overall number of 136. On the other hand, the 21 is significant in that it does show that Homer did want to depict the soul as either fire or wind a total of 21 times. It is nevertheless unclear how this information should be interpreted for now, since the numbers are so small. In the future, it might be more helpful to search for fire- or wind- related terms separate from soul-terms. This would be more helpful for two reasons. First, that search would provide us with more data than the present research, as fire- and wind-related terms occur both near and distant from soul-terms in the Iliad. Second, if the soul is comparable to fire or wind at all, it is possible that a soul-term is substituted for a fire- or wind-related term, and thus does not occur in the passage at all. The present research does not accommodate such occurrences.

CONCLUSION

To be sure, even if the results of this research should be considered insignificant, we do not have to take these results to mean that the soul lacked a physical nature in the time of Homer; the results could mean that the physical nature of the soul resembles something other than fire or wind, or simply that this area of psychology, namely, psychophysiology, is not strongly represented in the *lliad*. More work must be done in order to establish a strong connection between soul-terms and a physical nature of some kind or in order to prove that the soul lacked a physical nature all together.

There are several further steps that could help us get a better idea of the psychophysiology in Homeric literature: we could begin exploring other Homeric works to see if there is a connection between soul-terms and fire- or wind- related terms; we could try some alternative terms for fire or wind; and we could even try different terms all together just in case the physical nature of the soul is not consistent with the etymology of *thumos* on which this research is based. Be that as it may, establishing the physical nature of the soul could be a key to better understanding later philosophers whose psychology is influenced by Homeric literature and to better understand the psychology in other works that were written during or near time of Homer.

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