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ABSTRACT

Nutrition education has been shown to improve dietary knowledge and healthy food choices in young children (Pérez-Rodrigo & Aranceta, 2001). This study examined the efficacy of a two-year nutrition education preventive intervention on childhood health in a predominantly low-income sample of preschool children ages 3-5 in Central Pennsylvania. This study assessed the impact of this educational program on body mass index (BMI) and salivary C-Reactive Protein (CRP) levels in 31 children from 5 different preschool classrooms. Parents filled out a survey on their child's health background prior to testing. A single visit was made to each classroom for testing; consented children who were absent due to illness were not included in the sample. During testing, height and weight data were collected for each participant, as well as saliva. Saliva samples were assayed with a high sensitivity Salimetrics C-Reactive Protein ELISA kit. Descriptive statistics were generated to provide sample characteristics. Simple zero-order correlations were conducted to examine associations among variables of interest. Independent t-tests were used to compare participants with non-participating controls. It was hypothesized that participation in the nutrition education program would be associated with decreased BMI as well as levels of CRP, a marker of inflammation that is associated with increased risk for the development of cardiometabolic diseases. The results revealed that there were no significant between-group differences in BMI or CRP. As expected, however, female subjects were found to have higher average BMI than males overall. These results, though largely inconclusive due to a number of limitations, set the foundation for future research on the Healthy Bodies Project participants that employs a longitudinal design and incorporates additional variables of interest.

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Chapter 1

Literature Review

Cardiovascular Disease in the United States

Cardiovascular disease (CVD) places an enormous economic burden on healthcare resources in the United States annually (Trogdon, Finkelstein, Nwaise, Tangka, & Orenstein, 2007). According to the American Health Association, CVD causes 1 in every 3 deaths in the U.S. and represents 17% of all national healthcare expenditures. The direct costs of CVD and loss of productivity amount to $320 billion in annual expenses (Heidenreich et al., 2011). The American Health Association predicts that over half of the U.S. population will be diagnosed with CVD by 2030, which would triple this figure (Mozaffarian et al., 2016).

Although cardiovascular disease is the number one killer of the adult population in Western Societies today, the pathological processes and risk factors associated with its development originate during early childhood (Mozaffarian et al., 2016; Rosamond, Chambless, & Folsom, 1998; Holman, McGill, Strong, & Geer, 1958). Given the major impact that adult cardiovascular disease has in westernized societies, it is crucial to examine the relationships among cardiovascular risk factors early in life (Steinberger & Daniels, 2003).

Childhood Obesity and the Metabolic Syndrome

One of the leading risk factors for CVD is obesity (Hubert, Feinleib, McNamara, & Castelli, 1983). Obesity is an important modulator of the metabolic syndrome, a cluster of biochemical and physiological cardiovascular risk factors such as hypertension, hypertriglyceridaemia, and abnormal glucose metabolism (DeFronzo & Ferrannini,1991). As of 2012, 35% of adults in the United States were obese, while an additional 33.6% of the adult population was classified as overweight (Ogden, Carroll, Kit, & Flegal, 2014). Childhood obesity increases the likelihood of obesity, as well as the risk for the metabolic syndrome, in adulthood. In addition, research suggests that obesity in adulthood that was established during childhood may have more harmful health consequences than obesity that does not arise until adulthood.

Obese children who reduce their weight to become non-obese adults may have a certain degree of protection against metabolic syndrome (Vanhala, Vanhala, Kumpusalo, Halonen, & Takala, 1998). Early intervention to prevent adult obesity, the metabolic syndrome, and cardiovascular risk will decrease risk for preventative chronic inflammatory diseases and curtail the national costs and economic consequences of cardiovascular disease (Ford & Chaoyang, 2008; Varda & Gregoric, 2009).

Circulating plasma levels of markers of vascular inflammFation associated with obesity may help identify individuals at a high risk for future cardiovascular events (Blake & Ridker, 2001). Proinflammatory cytokines and chemokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are upregulated in obesity making them good indicators of excess adiposity (Menzo, 2012). C-reactive protein (CRP), which also reflects low-grade systemic inflammation, is one of the most studied biomarkers in childhood obesity studies (Cook et al., 2000; Ford, 2003; Stoner et al., 2013).

C-Reactive Protein and the Inflammatory Process

C-reactive protein (CRP) is an acute-phase protein produced in the liver that plays an important role in the nonspecific response of the early-defense, or innate immune system, to infection and injury (Gabay & Kushner, 1999). While injury or infection traditionally causes increases in CRP levels within the first 24-72 hours following infection or injury, chronically elevated levels are thought to play an important role in atherogenesis and the development of cardiovascular disease. This role is largely mediated by CRP's facilitation of the uptake of lipids and macrophages to vessel walls (Ridker, 2007).

Researchers have determined several risk factors for elevated CRP levels. In U.S. adults, CRP levels are inversely related to socioeconomic status (Alley et al, 2006). Increased levels of CRP are also associated with higher proportions of body fat (Visser, Bouter, McQuillan, Wener, & Harris, 2001), oxidative stress from smoking or second-hand smoke (Frohlich et al., 2003), food insecurity (Gowda, Hadley, & Aiello, 2012) and chronic infections that elicit a persistent inflammatory response (Eskandari & Sternberg, 2002).

Strong associations between CRP and obesity have been observed in children as young as age 3 (Skinner, Steiner, Henderson, & Perrin, 2010). Epidemiologic studies in children have also shown that elevated body mass index (BMI) is associated with elevated CRP (Singer, Eng, Lumeng, Gebremariam, & Lee, 2014). In addition, because food insecurity impacts all members of a household, many of which may contain children, food insecurity is likely a correlate of elevated CRP in children as well because of the stress imposed on the body by chronic malnutrition (Canpolat et al., 2013; Gowdar, Hadley, & Aiello, 2012). Given the growing evidence for inflammation in the pathophysiology of many chronic diseases, including cardiovascular disease and obesity, elevated levels of CRP during early childhood may increase risk for such disorders later in adulthood (Markus et al., 2006).

National Food Environments and the Rise of Obesity in America

The food environment and children's eating behaviors largely influence obesity and its related morbidities (Benton, 2004). The rise in obesity in the United States over the last forty years has occurred too rapidly to be primarily related to human physiology. For this reason, practitioners and scientists examine changes in the food environment, public policies, and the production system as potential explanations for this national health epidemic (Sallis & Glanz, 2009). The United States food environment and food system has evolved within the last 4 decades to provide an abundant supply of inexpensive, highly palatable, calorie dense foods. These items are conveniently accessible and heavily marketed to consumers (Swinburn et al., 2011). The food environment describes the food options accessible to an individual (fast food chains, convenience stores, school meal initiatives, supermarkets, etc.) as well as the exposure that a consumer has to different items or marketing in said environment (availability of healthy and unhealthy foods, costs, promotions/advertisements, etc.) (Glanz, Sallis, Saelens, & Frank, 2005).

Increased access to supermarkets is related to improvements in fat intake, diet quality, and fruit and vegetable consumption in adolescents and adults, as well as reduced levels of obesity. The opposite holds true for increased access to convenience stores, corner stores, and fast food restaurants, which collectively contribute to increased obesity. Low-income and minority communities have disproportionately higher levels of access to unhealthy food sources (fast food restaurants, etc.), and less access to healthier food outlets (supermarkets, etc.) which may help to explain the disparities in obesity rates among different groups of people (Larson, Story, & Nelson, 2009).

Changes in the food environment impact the eating behaviors of children (Campbell, Crawford, & Ball, 2006). Children's food preferences are influenced by both media (TV) exposure (Taras, Sallis, Nader, & Nelson, 1989) and home food availability, which is largely dictated by the cost and convenience of unhealthy items relative to healthier foods like fruits and vegetables (Gibson, Wardle, & Watts, 1998). Young children exposed to poor food environments have inadequate dietary patterns (Kranz, Findeis, & Shrestha, 2008) and increased preference and consumption of sugar, fat, and salt (Fox, Pac, Devaney, & Jankowski, 2004; Han & Powell, 2013). These findings highlight the need for nutrition education interventions early in childhood.

Interventions as a Means of Combatting Childhood Obesity

Children have been the target for a variety of obesity prevention initiatives because many of the risk factors for cardiovascular disease originate in childhood and adolescence (Ford & Chaoyang, 2008). The majority of these interventions have taken place within the school environment because it provides many opportunities to teach children about important health and nutrition behaviors. The influence of schools on student health may be even stronger in low-income communities where children often receive a significant proportion of their daily nutrition requirements via the US Department of Agriculture (USDA) School Breakfast Program and National School Lunch Program (NSLP) (Briefel, Wilson, & Gleason, 2009).

The emergence of many school-based nutrition interventions was motivated by recent policy changes in the past two decades that recognized the importance of nutrition and general wellness on over health in children. The Nutrition and WIC Reauthorization Act of 2004 currently mandates the development of wellness policies at every elementary school that participates in the USDA NSLP (Civic Impulse, 2016). Other federal nutrition-based initiatives emerged out of the 2008 Farm Bill that aimed to increase fresh fruit, vegetable, and whole-grain offerings and opportunities (Food, Conservation and Energy Act of 2008).

A comprehensive review of 139 childhood obesity prevention interventions from the past decade found that the majority (83%) of the programs in their study were school based. These school interventions were employed in both elementary and preschool populations and included a mixture of lessons and activities on healthy eating and physical activity (Hollar et al., 2010; Webber, Osganian, & Felman, 1998; Wang et al., 2015). About half of the interventions analyzed in the literature review by Wang and his colleagues produced statistically significant beneficial effects for some of the adiposity-related measures (Wang et al., 2015). Although promising, the results of many interventions on obesity in the past two decades were still inconclusive (Caballero et al., 2003; Harrell et al., 1998, Sahota et al., 2001).

The unsatisfactory results of several efforts motivated programs to combine school-based approaches with complementary approaches in the community in recent years (Gittelsohn & Kumar, 2007). A 2012 study accomplished this by sending home a daily list of physical activities for children to complete and parents to initial. This same study also worked with popular local restaurants to provide healthier meals with smaller portions for participating families (Crespo et al., 2012). An intervention that targeted middle school girls expanded its outreach by partnering with local YMCA/YWCAs, health clubs, and community recreation clubs to provide an assortment of weekly physical activities that reinforced the health education the children received in the classroom (Webber et al., 2008). Combining school- and community-based efforts into a single initiative increases the effects of interventions by influencing health and nutrition decisions outside of the classroom. Despite some success, however, fewer than half of the combined school-community intervention trials reduced BMI and obesity in children (Wang et al., 2015).

While well intentioned, the limited success of obesity interventions to date can be attributed to a variety of factors. For instance, programs may lack emphasis on the social environment, deliver interventions with limited exposure and intensity, or fail to recognize the large role of policy, which is necessary for long-term stability and stakeholder buy-in (Wang et al., 2013). While current interventions represent valiant efforts towards curtailing the morbidities associated with childhood obesity, new approaches are needed to address the significant aforementioned gaps (Gittelsohn et al., 2014). Such approaches should be evidence-based and may include long-term efforts that involve a combination of active parent/caregiver education and participation, physical activities, nutrition instruction, and corresponding opportunities that challenge families to improve all school-based educational wellness behaviors at home. Success by forthcoming interventions may motivate policymakers to expand accessibility to healthy dietary options within the school and the community, as well as increase opportunities for greater physical activity to fight the national childhood obesity epidemic.

The Healthy Bodies Project

The Healthy Bodies Project is a research effort within the Biobehavioral Health Department at the Pennsylvania State University that is funded by the Food and Nutrition Service (FNS) within the United States Department of Agriculture, and the Pennsylvania Department of Human Services. FNS currently implements Supplemental Nutrition Assistance Program Education (SNAP-Ed), a statewide funded program to deliver nutrition education to SNAP recipients. The Healthy Bodies Project expanded this effort by delivering a food literacy curriculum to over 200 low-income preschoolers in Central Pennsylvania. The program currently serves more than 700 preschoolers and their families.

The selected curriculum, Families Understanding Nutrition (FUN), is a 28-week program during which trained preschool teachers expose children to 26 novel fruits and vegetables. In 30 - 45 minute weekly lessons, children learn how/where the fruit/vegetable grows, and have the opportunity to feel and taste the selected fruit/vegetable. The ultimate goal of the in-classroom curriculum is to increase children's knowledge and enthusiasm about fruits and vegetables, as well as increase their willingness to try new ones.

Parents and caregivers are also participants in the Healthy Bodies Project. Every week they receive weekly newsletters that accompany the FUN curriculum. These newsletters contain information about the foods that children are introduced to in the classroom along with easy, healthy recipes that incorporate the weekly fruit/vegetable. Parents and caregivers are also invited to participate in 13 nutrition education sessions with a focus on food resource management, such as eating healthy on a budget, throughout the academic year. These lessons are delivered in a group format at the child's school or during an individual home visit with the preschool teacher, a standard practice at many of the preschools enrolled in the program. Lastly, parents are invited to participate in a Cooking Matters at the Store Tour where they will receive information on interpreting food labels, comparing unit prices, finding whole grains, and identifying the various ways to purchase produce.

The Healthy Bodies Project aims to achieve four goals. The first goal is to increase food literacy and healthy food acceptance in children and their caregivers through increased nutrition education. The second goal is to provide families with guidance on preparing healthy meals that reduce the risk for obesity, Type 2 diabetes and cardiovascular disease. The third goal is to provide families with guidance on the division of responsibility in feeding, such that families learn how to appropriately include children in decisions about what to eat, when to eat, and how much to eat. The fourth goal is to improve family food resource management in ways that increase the availability of food, particularly healthy items, in the home and reduce hunger.

Study Goals and Hypotheses

The goal of this study was to analyze the impact of the Healthy Bodies Project on children's healthy by addressing the following hypotheses:

1. Participation in the Healthy Bodies Project will be associated with lower levels of BMI.
2. Participation in the Healthy Bodies Project will be associated with lower levels of the inflammatory biomarker, CRP, in children who have participated in the Healthy Bodies Project.
3. Lower levels of BMI will be observed in males regardless of participation in the Healthy Bodies Project. This prediction reflects the natural physiological

differences between boys and girls, with girls having greater total body fat on average than boys (Deurenberg, Westrate, & Seidell, 1991).

4. Lower salivary CRP (pg/mL) values will be observed in males. This is expected because BMI has an established positive association with CRP levels (Rawson et al., 2003) and females in general tend to have higher levels of circulating inflammatory markers (Darnall & Suarez, 2009).

Chapter 2

Methods

Study Design Overview

To test the impact of the Healthy Bodies Project nutrition intervention on childhood immunity, 5 classrooms were selected from the 54 participating schools in Centre and Clearfield County. The intervention group in this study included children who participated in the Healthy Bodies Project during the 2014-2015 academic year, while the control group included children who did not participate during this time frame because they were not enrolled in the classroom. The 31 preschool children involved in this study each provided a small saliva sample (method detailed below) and had their height and weight measured by trained research assistants. Statistical analyses of the BMI data, as well as the salivary levels of the inflammatory cytokine C-Reactive Protein, were performed to compare the two groups.

Subjects

The subjects in this study included 31 preschool children from the Centre County. The children involved in this study were enrolled in 1 of 5 classrooms in Port Matilda, Bellefonte, or State College that participated in the Healthy Bodies Project for the past two years. Within each classroom were children, 3-5 years of age, that had participated in the Healthy Bodies Project previously as well as others whom had not. Out of the 31 children, 14 had participated in the project during the 2014-2015 academic year, and 17 children had not. Consented children who were absent on the day of testing were excluded from the study. Parents/caregivers of the participating children were also included in this research study. They provided responses to a questionnaire concerning their child(ren)'s health; 27 parents provided questionnaire data.

Parent Questionnaires

While obtaining consent, parents were asked to fill out a survey concerning their child's health history (see Appendix A). Parents did not always complete every section of the survey in its entirety, resulting in a great deal of missing data.

Data Collection Procedures

A single visit was made to each of the 5 classrooms for testing during the first two weeks of February. Height (inches) was measured in duplicate with a stadiometer and weight (lbs) was measured in duplicate using a research-grade digital scale. Children were asked to remove their shoes before stepping on the scale. Saliva samples were then taken using the Salimetrics SalivaBio Children's Method Swab.

Rationale for Salivary C-Reactive Protein Measurements

Researchers traditionally collect blood or urine samples for measurements of CRP because circulating levels of this protein have been employed clinically as an indicator of both general inflammation and cardiovascular disease risk status (Pearson et al., 2003). Recent technical advances have made the assessment of a wide range of analytes, such as immune markers, in saliva possible. Although blood is still the gold standard, saliva measures of CRP appear to predict reliable information about inflammation and cardiovascular risk, while affording researches several advantages in comparison to other biospecimen collection methods (Slavish, Graham-Engeland, Smyth, & Engeland, 2015). The collection of oral fluids does not require special laboratory equipment or skilled professionals, is generally pain- and stress-free, making it consequently less burdensome for participants, and allows for both home- and self-based collection (Out, Hall, Granger, Page, & Woods, 2012). Because of these advantages, and the young age of the participants, salivary measurements of CRP were collected rather than urine or blood samples.

Salimetrics SalivaBio Children's Method Swab Protocol:

Following Salimetrics' protocol, saliva was collected at least 60 minutes after the children's last meal. To achieve this, classroom visits were made an hour and a half after breakfast or lunch ended, depending on the the classification, AM or PM, of preschool classroom. At least ten minutes before the sample was collected, each child rinsed their mouth thoroughly with water for 10 seconds (Salimetrics, 2015).

To begin testing, a children's swab was removed from its packaging with gloves and inserted into a child's mouth. Children were asked to close their mouths around the swab and chew on it for 60 seconds to stimulate enough saliva for a sample with good volume. Once the 60 seconds ended, the swab was immediately folded and placed into a labeled, sanitary salivette. The salivette was capped and placed in a small ice-packed cooler for temporary storage. Gloves were changed between every participant to maximally limit cross-contamination of other participants (Salimetrics, 2015).

The capped salivettes were brought to the Biobehavioral Health Building within an hour after the first sample was collected at each location. The salivettes were transferred from the small ice cooler to an 80˚C ultra-low temperature freezer in the Pennsylvania State University Biobehavioral Health Building. The samples were stored in this freezer for a maximum of 2 weeks before being assayed.

C-Reactive Protein Assays

Assays of the saliva samples were performed by The Biomarker Core Lab (BCL), a research support and training facility within the department of Biobehavioral Health in the College of Health and Human Development at the Pennsylvania State University. The saliva samples were assayed with a Salimetrics salivary C-reactive protein indirect “sandwich” ELISA kit. This enzyme-linked immunoassay method places pre-coated capture anti-CRP antibodies that bind CRP on two plates opposite one another. The bound CRP antibody complex is bound by an anti-CRP detection antibody linked to horseradish peroxidase. The samples are incubated and then all unbound components are washed away. A Bound CRP Antibody Enzyme Conjugate is measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB), which produces a blue color. The reaction is stopped with an acidic solution, yielding a yellow color. A standard plate reader at 450 nm measures the optical density. The amount of CRP Antibody Enzyme Conjugate detected is directly proportional to the amount of CRP present in the sample (Chard, 1990).

BMI Data

Body mass index (BMI) data was computed using the Centers for Disease Control and Prevention's (CDC's) BMI Tool for Schools, an excel sheet that computes BMI, and corresponding BMI percentiles, using the age, height, weight, and sex of children under the age of 20, as well as the date(s) the measurements were recorded. The BMI Tool for Schools provides a summary of the children's BMIs and identifies the percentages of children who are underweight (<5th percentile), of average weight (5th-85th percentile), overweight or obese (≥ 85th percentile), and strictly obese (≥ 95th percentile) (CDC, 2015).

Statistical Analysis

Data were entered and cleaned in Microsoft Excel and all analyses were run using IBM SPSS 12.0. Descriptive statistics were generated to provide sample characteristics. Simple zero-order correlations were conducted to examine associations among variables of interest. Independent t-tests were conducted to compare means between groups based on gender or participation in the Healthy Bodies Project. Significance levels were set to $\alpha \leq .05$.

Before analyses for salivary CRP (pg/mL) were conducted with SPSS, the data was inspected for skewness. Both the salivary CRP (pg/mL) data with and without outliers were significantly skewed to the right, so a log transformation was applied to correct for the skewness in the CRP variables. Analyses for salivary CRP (pg/mL) were run both with and without the transformations. The transformations did not change the significance of any analysis; for ease of presentation, only the results for the non-transformed variables are presented in this paper.

Chapter 3

Results

Study Sample

There were 17 male children included in the study and 14 females. Of the 31 children, 7 of them were 5 years of age, 15 of them were 4 years of age, and the remaining 9 children were 3 years of age. There were 14 children who had previous exposure to the Healthy Bodies Project, while 17 of the children did not participate during the 2014-2015 academic year. The children in the sample represented 5 different classrooms, Port Matilda (n=7), Bellefonte Elementary's morning (n=7) and afternoon (n=5) classes, and Kindercare's preschool (n=7) and pre-kindergarten (n=5) classes.

BMI Analysis

The BMI data compiled using the CDC's BMI Tool for Schools was separated by group, with one group, participants, representing the children with Healthy Bodies Project participation during the 2014-2015 academic year and the second group, controls, representing the children who did not receive the intervention during that time frame.

Figure 1 shows all of the BMI and weight data by group. As one would expect, there was a positive linear relationship between BMI and weight for both groups. The range for BMI was 7.7 to 23.5 for participants and 13.7 to 17.2 for controls. The range for weight was 30.8 to 80.2 lbs for participants and 26 to 55.6 lbs for controls.

As depicted in Figure 2, the average BMI for participants was 15.45, while the average BMI for controls was 15.65. An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(29) = -.25$; $p = 0.81$) showed no significant difference between groups.

The BMI data was also analyzed by gender and is depicted in Figure 3. The average BMI for male participants, 14.82, was lower than the average BMI for female participants, 16.46. An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(29) = -2.12$, $p = 0.43$) determined that the difference in BMI by gender was significant.

Table 2 displays the BMI data comparison to national percentiles by gender. Of the 31 children in the study, 3% (n=1) were considered obese. This was lower than the 2011-2012 national average (8.4%) for children between the ages of 2 and 5 years old (Centers for Disease Control and Prevention, 2015).

Salivary CRP Analysis

Next, t-tests for independent samples were run to compare salivary CRP levels by participant group. As depicted in Figure 4, the average salivary CRP was 3,741 pg/mL with a standard deviation of 3,879 pg/mL for participants and 12,484 pg/mL with a standard deviation of 27,606 pg/mL for controls. An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(29) = -1.17$; $p = 0.25$) showed no significant group differences.

The data was analyzed for any outliers, values more than 3 standard deviations outside of the mean, which were then removed. Two outliers, one from each group, were identified and removed so that the results of the data could be analyzed with greater accuracy.

After removing CRP outliers, the average salivary CRP value was 2,830 pg/mL for participants and 5,008 pg/mL for controls (see Figure 5). Even with the removal of outliers, the average salivary CRP was still lower for participants than controls. An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(27) = -1.07$; $p = 0.29$) was not significant, although CRP levels were almost twice as high in the control group compared to the intervention group.

The CRP (pg/mL) data was also separated and analyzed by gender. The average salivary CRP value was 5,765 pg/mL for males and 11,901 pg/mL for females (see Figure 6). An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(29) = 1.25$; $p = 0.27$) showed no significant difference by gender.

The data was analyzed for any outliers and values more than 3 standard deviations outside of the mean were removed. Two outliers, one from each group, were identified and removed so that the results of the data could be analyzed with greater accuracy. After removing outliers, the average salivary CRP value was 3,127 pg/mL for males and 4,352 pg/mL for females (see Figure 7). Even with the removal of outliers, the average salivary CRP was still lower for males than females. An independent samples t-test was utilized to determine whether the difference between these two values was significant. The results of the t-test ($t(27) = -0.98$; $p = 0.35$) were not significant, although mean salivary CRP values were almost twice as high in females compared to males.

Salivary CRP and BMI Correlations

As depicted in Figures 8, 9, and 10, Salivary CRP (pg/mL) was plotted against BMI for all children and then separately for participants and controls. There was a negative correlation between salivary CRP (pg/mL) and BMI for the entire study sample, but this association was not significant ($r = -0.4$, $p = 0.084$). Figure 9 showed that children with higher levels of BMI exhibited higher levels of CRP, but statistical analysis revealed that this relationship was not significant as well ($r = 0.15$, $p = 0.62$). Lastly, as shown in Figure 10, the plot of salivary CRP (pg/mL) vs. BMI for controls showed a negative correlation, which was not significant ($r = -0.22$, $p = 0.40$).

Chapter 4

Discussion

BMI and Salivary CRP Results

This cross-sectional study analyzed the effectiveness of a nutrition education intervention by analyzing differences in measures of body composition and inflammation between participants with and without intervention exposure and participation. The overall hypothesis was that children who participated in the intervention would have lower levels of BMI and salivary CRP, an indicator of inflammation. The results of this study did not provide support for this hypothesis; however, several findings are worth discussing.

One goal of the present research was to investigate the effect of the intervention on the most commonly used measure of body composition is the body mass index (BMI) (Daniels, Khoury, & Morrison, 1997). BMI is both a non-invasive, low-cost measure of adiposity for use in the clinical and research setting because of its reliability and the convenience of its collection in a variety of settings (Himes & Dietz, 1994). Given the potential implications of nutrition education on body composition, as well as the young age of the participants in the study, BMI was an appropriate, noninvasive measure (Must & Anderson, 2006). The average BMI for participants was slightly lower than the average BMI for controls. An independent samples t-test confirmed that these results were not significant. Aside from body composition, this study also sought to analyze the potential impact of the nutrition intervention on levels of a known inflammatory marker. Circulating levels of CRP, an acute phase protein, have been utilized clinically as indicators of general inflammation and markers of cardiovascular disease (Pearson et al., 2003). C-reactive protein can also be detected and reliably assessed in oral fluids (Pfaffe, Cooper-White, Beyerlein, Kostner, & Punyadeera, 2011). Research shows that salivary CRP levels accurately reflect systemic levels and, consequently, inflammation (Out, Hall, Granger, Page, & Woods, 2012). Given the reliable correlates between these different measures, saliva collection was ultimately selected because it was a minimally invasive measure.

When compared to national obesity prevalence in 2011-2012 (8.4%), the sample in the current study had a smaller proportion of obese children (3%). The prevalence of obese children in the U.S. population between the ages of 2-5 decreased from 13.9% in 2003-2004 to 8.4% in 2011-2012 (Centers for Disease Control and Prevention, 2015). Although recent declines in national obesity rates are modest, researchers postulate that recent progress in obesity prevention among young children will concurrently decrease the prevalence of obesity related morbidities in later childhood and adulthood (Pan, Blanck, Sherry, Dalenius, & Grummer-Strawn, 2012).

An effect of the intervention on neither BMI nor CRP was detected in the present research. The average BMI for participants was equivalent to the controls. The average salivary CRP value for participants was not significantly lower than the average for controls. Given the large difference in these values, the data was analyzed for any outliers, values more than 3 standard deviations above the mean, and those numbers were removed. Even with two outliers removed, the average salivary CRP value for participants was not significantly different from controls.

As expected, an independent samples t-test determined that the difference in average BMI between males and females was significant. These results are consistent with the differences in physiology between boys and girls, with girls having greater total body fat than boys on average (Deurenberg, Westrate, & Seidell, 1991).

BMI is positively associated with CRP (Chu, Chang, & Shieh, 2003; Ford et al., 2001; Brasil, Norton, Rossetti, Leão, & Mendes, 2007). Given that females were heavier on average than males in the present research, it would be expected that females would have larger salivary CRP values. However, the difference in average salivary CRP between males and females was not significant. When outliers were removed, independent samples t-test still showed that the difference in average salivary CRP between males and females remained not significant.

Plots of salivary CRP (pg/mL) vs. BMI for each group showed a positive correlation for participants, but a negative correlation for controls, and a negative correlation for the entire study sample. These results contradict one another. CRP is the prototypic biomarker of inflammation and has a strong positive association with BMI (Hak, Stehouwer, & Bots, 1999). While the data for participants reflects this known relationship, the data for controls and the entire sample does not.

The findings for BMI and salivary CRP (pg/mL) were insignificant and inconclusive. These indecisive results and the discrepancies between the plots of salivary CRP (pg/mL) vs. BMI, were likely due to several limitations of the study, which are discussed below.

Research Limitations

The sample size (n = 31) was much lower than the initially intended sample size (n = 50+), making it difficult to extrapolate information from the data and draw conclusions. The small sample size was largely due to the lengthy institutional review board (IRB) approval process, which limited the amount of time available to recruit participants.

The IRB for this study was approved on January 14, 2016, leaving approximately a month and a half for participant recruitment and data collection. Due to the young age of the children, parents needed to provide consent for preschoolers' participation in the study. The only times to meet with parents in-person with such short notice were during parent pick-up and drop-off at the preschools. These times varied amongst the 5 classrooms, all of which were located in different areas of Centre County.

Consent was collected for as many children as possible who were attending school on the days that the classrooms were visited for recruitment. The cold weather, and resulting illnesses, impacted attendance. Some parents also refused to provide consent because they were in a rush. It would have been beneficial to receive consent during family events or home visits with the preschool teachers to avoid some of these issues, but the time constraints and scope of this study made those options impossible.

The small sample size was also composed of a mix of genders and ages, from different classrooms which makes it difficult to generalize findings, even with significant results. Children between the ages of 3-5 are at very different stages of their development, which can contribute to differences in eating behaviors, physical activity, body composition and a variety of other factors (Birch & Fisher, 1998; Kohl & Hobbs, 1998). These differences are also evident between males and females (Cooke & Wardle, 2005; Deurenberg, Weststrate, & Seidell, 1991). With a larger sample size, BMI data and salivary CRP could be isolated and analyzed with respect to just gender or age, consequently eliminating any confounding effects of the other variable. It would also be useful to compare effects within different classrooms and administer a teacher questionnaire to analyze whether or not certain teaching tactics and attitudes produce better results.

The salivary CRP (pg/mL) measurements were also a source of contention. There were two statistical outliers, as well as other values, that were much more elevated compared to the other data. This, coupled with the small sample size, likely distorted the relationship between salivary CRP (pg/mL) and BMI for controls.

Unlike serum CRP (mg/L) values, which have statistically confirmed reference ranges, salivary CRP (pg/mL) reference ranges and their correlation with serum concentrations have yet to be investigated in detail (Pfaffe, Cooper-White, Beyerlein, Kostner, & Punyadeera, 2011). This gap in the research largely limited interpretation of the salivary CRP (pg/mL) measurements. In addition, while research demonstrates that salivary CRP (pg/mL) has systemic origins, poor oral hygiene and local inflammatory processes may contribute to elevated levels and distort data analysis (Megson, Fitzsimmons, Dharmapatni, & Barthold, 2010; Noack et al., 2001). The present research, however, was conducted with young children who have relatively low rates of gum disease, perhaps limiting the degree to which this issue could have affected results.

Aside from BMI and salivary CRP (pg/mL) data, it would have been useful to analyze the results in respect to background information on the children's parents, health history, and household and neighborhood environments. This was the intention of the parent questionnaire, which had a 68% (n = 21) completion rate. Many parents chose not to fill out the survey because of their own personal time constraints. Without a higher proportion of completed surveys, the information garnered from them would not be indicative of much.

Considerations for Future Research

Future research in this field would benefit from the incorporation of variables that were not measured in this study. Ideally, researchers implementing a nutrition intervention should assess food literacy, BMI, and inflammatory and/or immune biomarkers at both the beginning and the end of the intervention for comparison. Time constraints largely limited this study to a cross-sectional analysis. A longitudinal study would provide more information about changes in BMI and salivary CRP (pg/mL) over time.

It would also behoove researchers to collect data on a variety of known inflammatory or immune biomarkers, so as to create a more comprehensive profile of children's cardiovascular disease risk. For instance, elevated circulating levels of inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha have also been associated with cardiometabolic diseases in overweight pediatric populations (Weiss, Dziura, & Burgert, 2004; Gupta, Ten, & Anhalt, 2005; Reinehr, Stoffel-Wagner, & Roth, 2005). Due to funding limitations, only salivary CRP (pg/mL) could be collected and assayed in this study.

If possible, researchers should also avoid biomarker collection during the cold/flu season. Influenza virus is associated with elevated CRP levels as well as elevated activities of other cytokines such as IL-6, tumor necrosis factor-alpha and interferons. (Jaye & Waites, 1997; Conn et al., 1995). Although data was not collected on present or recent sicknesses for participants, it is hard to tell if any of the salivary CRP (pg/mL) measurements from this study were elevated due to influenza virus or other seasonal illnesses around the time of data collection.

Conclusions

Statistical analysis of data from the Healthy Bodies Project did not support the hypotheses that participants would have lower BMI and CRP than controls, nor the hypothesis that salivary CRP values would be higher for females than males. The only significant finding was that of higher female BMI, compared to males. These results, though inconclusive, set the foundation for further research of this project. To date, nutrition interventions have not produced reliably significant decreases in BMI and obesity (Wang et al, 2013). Critics of traditional school based nutrition interventions believe that these initiatives alone cannot combat obesity, but provide a great foundation (Gittelsohn & Kumar, 2007). Statistically successful interventions often incorporate components outside of the classroom to reinforce nutrition and general wellness behaviors. The Healthy Bodies Project currently employs both a school based and family based initiative. A longitudinal study with a larger sample size that compares BMI and salivary CRP (pg/mL) at two points in time may validate the utility of this particular nutrition intervention as well as general nutrition education within this target group. The data will also help identify program strengths and weaknesses, so that alternations can be assessed and, perhaps, eventually implemented in future programs.

Appendix A

Supporting Documents

Parent Questionnaire

PARENT SURVEY
NUTRITION EDUCATION AND CHILDHOOD IMMUNITY THESIS

Instructions: Parents, please answer the following questions as truthfully as possible. Circle your answers and respond to all questions that apply to you and your child.

1. What is your child's sex? MALE / FEMALE

2. What is your child's age? ____________ YEARS OLD

3. Did your child participate in the Healthy Bodies Project in their preschool last year (2014-2015)? YES/NO

4. In general, how would you describe your child's health?
POOR / FAIR / GOOD / EXCELLENT

5. Does your child currently need or use medicine prescribed by a doctor, other than vitamins?
YES / NO

    a. If YES, what medication(s)?

        i. ______________________________
        ii. ______________________________
        iii. ______________________________
        iv. ______________________________
        v. ______________________________

6. Does your child need or get special therapy, such as physical, occupational, or speech therapy? YES / NO

a. Is this because of a medical, behavioral, or other health condition?

MEDICAL / BEHAVIORAL / OTHER

i. What condition? ___________________________________

b. How long has your child's condition affected him/her?

______________ YEARS ______________ MONTHS _____________ DAYS

7. Does your child have any kind of health care coverage, including health insurance, prepaid plans such as HMOs, or government plans such as Medicaid. YES / NO

a. If YES, is the child insured by Medicaid or the Children's Health Insurance Program, CHIP?

MEDICAID / CHIP

8. During the past 12 months, did your child see a doctor, nurse, or other health care professional for any kind of medical care including sick-child care, well-child checkups, physical exams, and hospitalizations? YES / NO

a. If YES, how many times did you child have one of these visits?

__________ TIMES

9. During the past week, how many days did all the family members who live in the household eat a meal together?

__________ DAYS

10. During the past 12 months, did your child receive Food Stamps or Supplemental Nutrition Assistance Program benefits?

YES / NO

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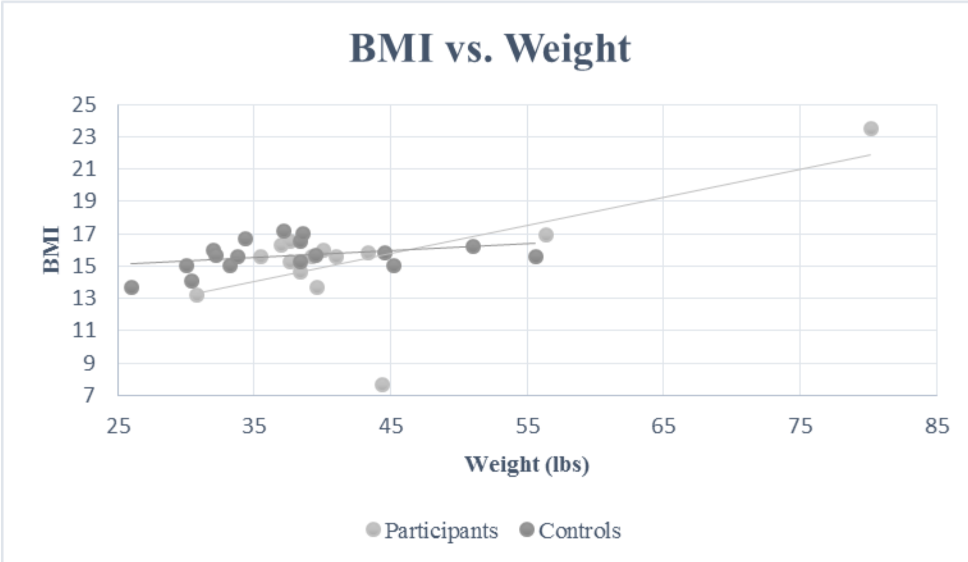

Figure 1: BMI vs. Weight by Participant Group

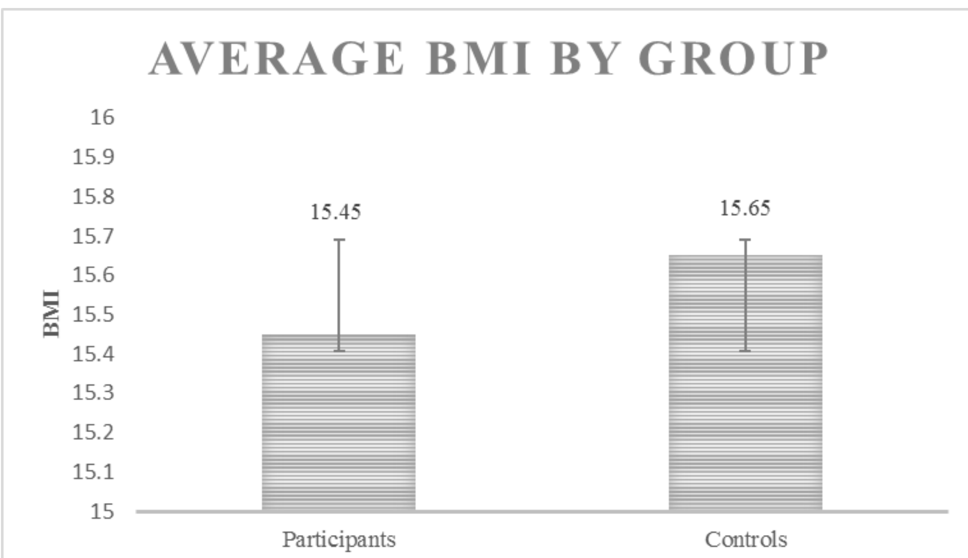

Figure 2: Average BMI by Participant Group

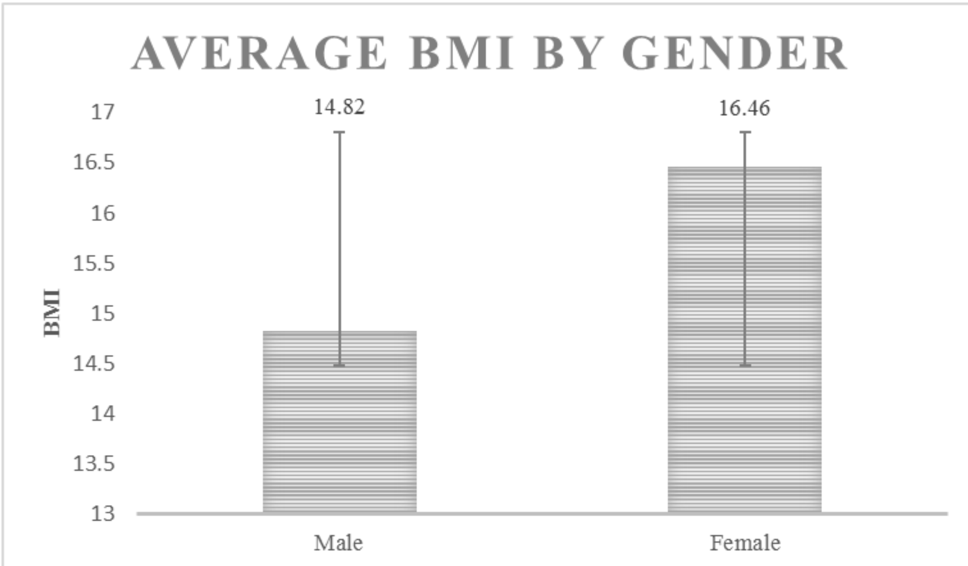

Figure 3: Average BMI by Gender

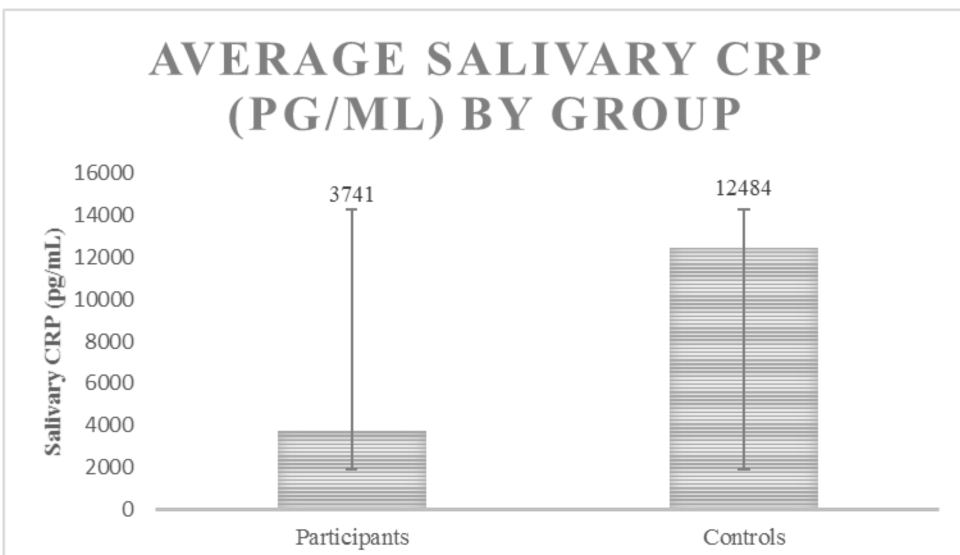

Figure 4: Average Salivary CRP (pg/mL) by Participant Group

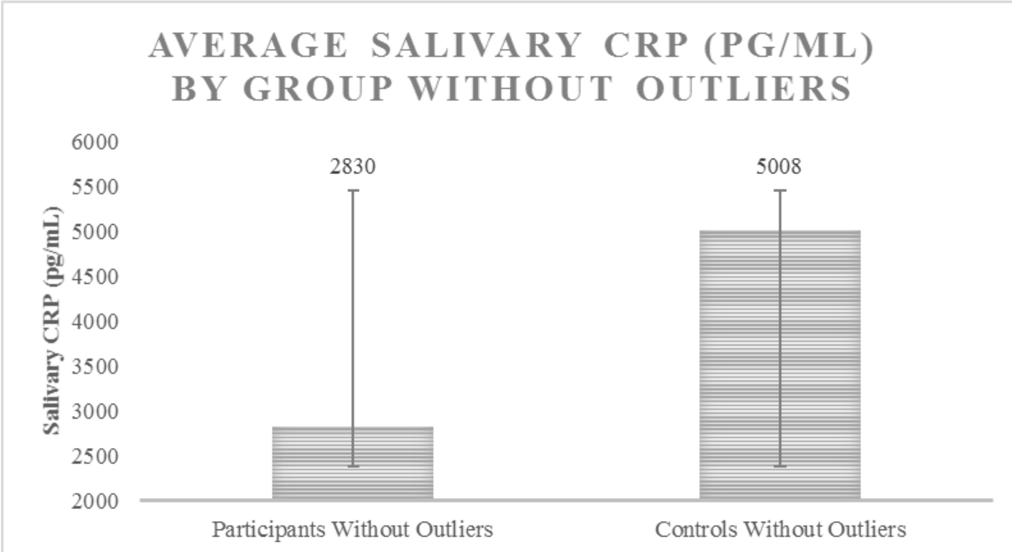

Figure 5: Average Salivary CRP (pg/mL) by Participant Group Without Outliers

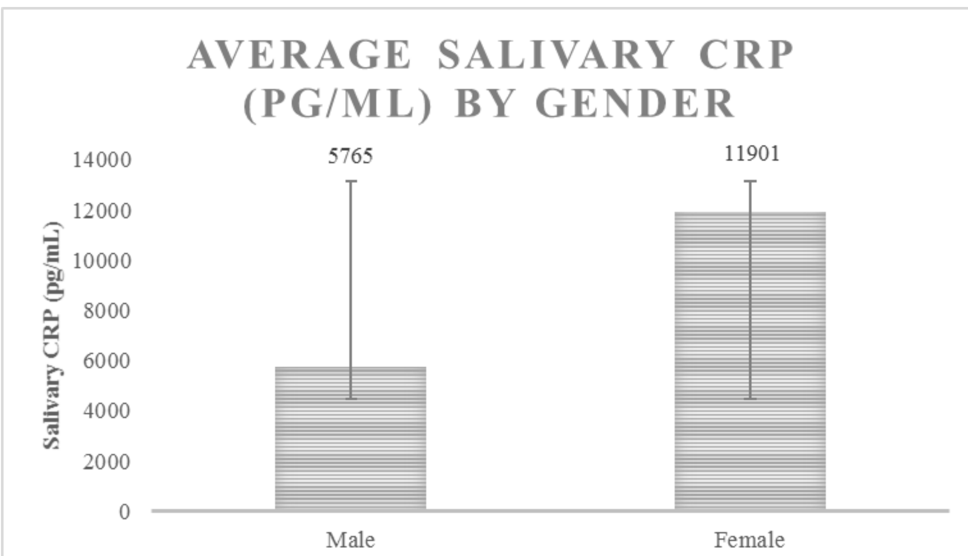

Figure 6: Average Salivary CRP (pg/mL) by Gender

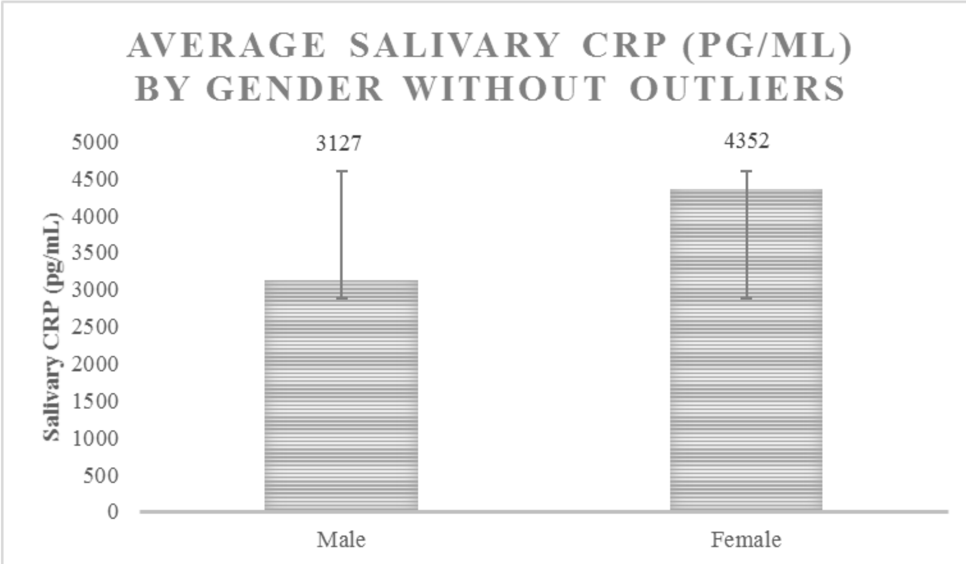

Figure 7: Average Salivary CRP (pg/mL) by Gender Without Outliers

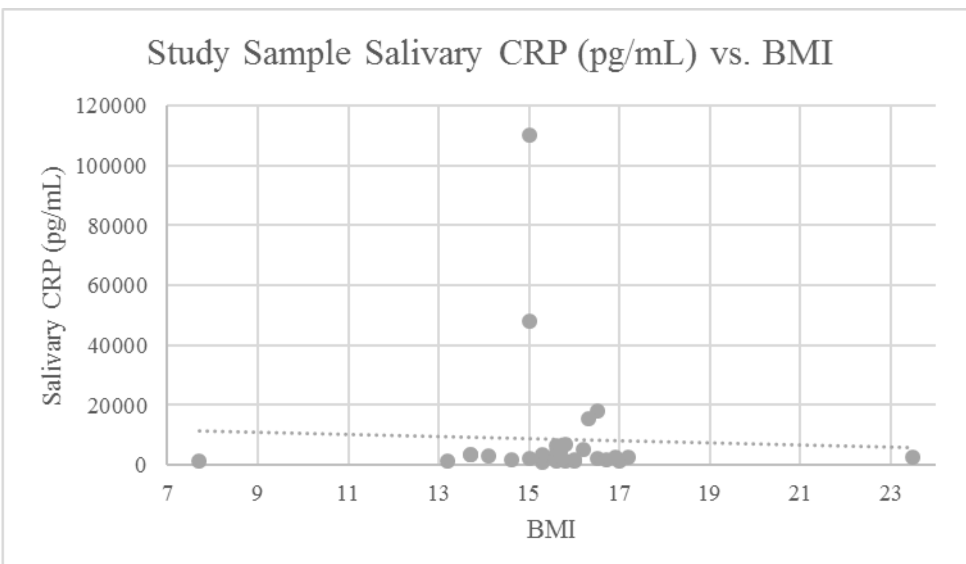

Figure 8: Study Sample Salivary CRP (pg/mL) vs. BMI

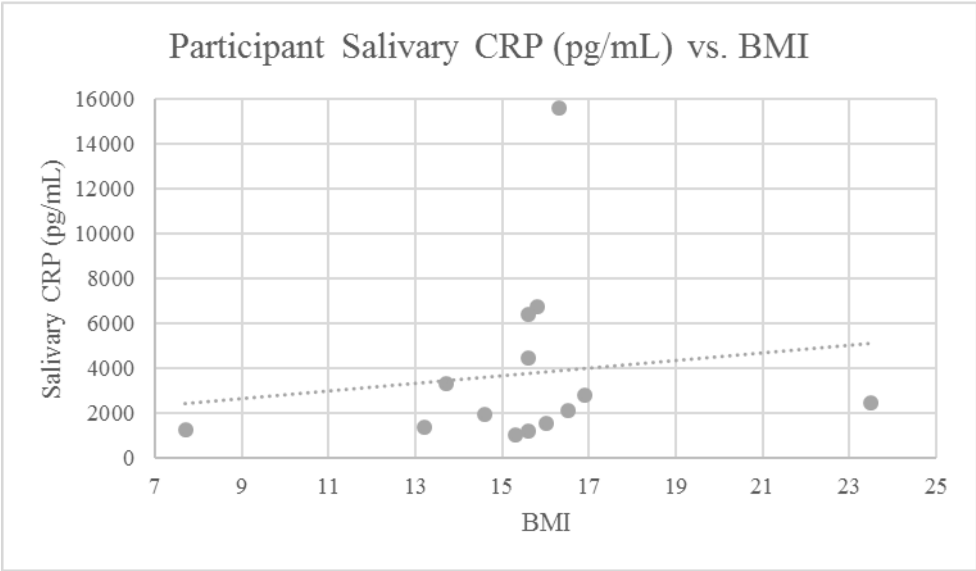

Figure 9: Participant Salivary CRP (pg/mL) vs. BMI

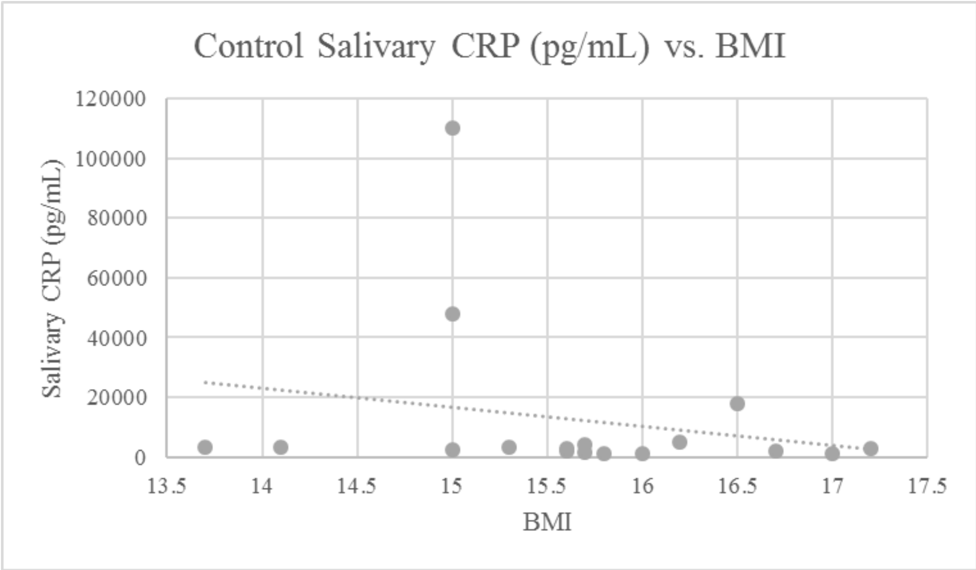

Figure 10: Control Salivary CRP (pg/mL) vs. BMI

Table 1: BMI Statistics by Participant Group

Group	N	Mean	Standard Deviation	Standard Error of the Mean
Participants	14	15.45	3.26	0.87
Controls	17	15.65	0.94	0.23

Table 2: CDC's BMI Tool for Schools Summary of Children's BMI Percentiles by Gender

Group	N	Underweight	Normal BMI	Overweight or Obese	Obese
Boys	17	12%	76%	12%	0%
Girls	14	7%	79%	14%	7%
Total	31	10%	77%	13%	3%

a. Underweight: (<5^{th} percentile)
b. Normal BMI: (5^{th} – 85^{th} percentile)
c. Overweight or obese (≥85^{th} percentile)
d. Obese (≥95^{th} percentile)

Table 3: BMI Statistics by Gender

Gender	N	Mean	Standard Deviation	Standard Error of the Mean
Male	17	14.82	2.06	0.50
Female	14	16.46	2.22	0.59

Table 4: Salivary CRP (pg/mL) Statistics by Participant Group

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Standard Error of the Mean (pg/mL)
Participants	14	3,741	3,879	1,037
Controls	17	12,484	27,606	6,696

Table 5: Salivary CRP (pg/mL) Statistics by Participant Group Without Outliers

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Standard Error of the Mean (pg/mL)
Participants	14	2,830	1,927	534
Controls	17	5,008	11,779	2,945

Table 6: Salivary CRP (pg/mL) Statistics by Gender

Gender	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Standard Error of the Mean (pg/mL)
Male	17	5,765	11,571	2,806
Female	14	11,901	28,481	7,612

Table 7: Salivary CRP (pg/mL) Statistics by Gender Without Outliers

Gender	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Standard Error of the Mean (pg/mL)
Male	17	3,127	4,074	1,019
Female	14	4,352	3,789	1,051

ACADEMIC VITA

CORINNE RENNER

Education

Schreyer Honors College, Pennsylvania State University
Bachelor of Science, Biology (Neuroscience)
Honors Distinction: Biobehavioral Health
Minor: Spanish
Dean's List

Research Experience

Department of Psychology, Bilingualism and Language Development Lab, Professor Van Hell – Pennsylvania State University, State College, PA

Research Assistant 08/2013- 05/2014

- Explored the phenomenon of code-switching with English-Spanish bilinguals
- Contacted, scheduled, and conducted experiments with participants using MATLab software and electroencephalography
- Coded data for later analysis
- Attended weekly lab meetings

Department of Biobehavioral Health, family and Child Health Project, Professor Francis – Pennsylvania State University, State College, PA

Research Assistant 11/2013-05/2016

- Organize and facilitate nutrition education programs for preschool children and parents/caregivers in Central Pennsylvania
- Design educational materials to incorporate into classroom lessons and family events
- Accumulate and analyze nutrition literacy data

Additional Experience

Schreyer Honors College Orientation – Pennsylvania State University, State College, PA

Orientation Leader, SHO-Time 2013 02/2013-08/2013
Orientation Leader, SHO-Time 2014 02/2014-08/2014
Orientation Leader, SHO-Time 2015 02/2015-08/2015

- Organize and facilitate move-in and arrival events for parents and students.
- Mentor new students during a three-day introduction to the Honors College.

Department of Sociology – Pennsylvania State University, State College, PA

Teaching Assistant 08/2014-12/2014

- Monitored the class blogs, served as a student resource, led in-class discussion and assisted in grading assignments.

Department of Sociology – Pennsylvania State University, State College, PA

Learning Assistant 01/2015-05/2016

- Facilitated active learning exercises in weekly lectures
- Hosted weekly tutoring sessions for students.

Memberships

Member, Honor Society of Phi Kappa Phi 2015-Present

Honors and Achievements

Academic Excellence Scholarship 2012-2016

- A $4,000 Academic Excellence Scholarship is available for all Schreyer Scholars entering directly from high school. This is a merit-based scholarship that was renewable for a total of eight semesters.

President's Freshman Award 2013

- This award is presented annually to undergraduate degree candidates and provisional students who have earned a 4.00 (A) GPA based on at least 12 graded Penn State credits completed by the end of the fall semester of the academic year the award is given.

Schreyer Ambassador Travel Grant 2013-2015

- The Schreyer Honors College awarded $1,200 for medical/public health service trips to Panama and Nicaragua with Global Medical Brigades.

President Sparks Award 2014

- This award is presented annually to undergraduate degree candidates who have earned a 4.00 (A) GPA based on at least 36 graded Penn State credits completed by the end of the fall semester of the academic year the award is given.

Edward C. Hammond Jr. Memorial Scholarship 2014

- This scholarship is awarded to a student who demonstrates excellence in Biology. This award provided $2,500 in additional academic funding for the 2014-2015 academic year.

Wheeler P. Davey Memorial Scholarship 2015

- This scholarship is awarded to students demonstrating academic excellence at the graduate or undergraduate level in the Eberly College of Science. This award provided $2,100 in additional academic funding for the 2015-2016 academic year.

Co-curricular Activities

Global Medical Brigades – Pennsylvania State University, State College, PA

Member/Brigader 08/2012-05/2015

- Participated in weeklong brigades in Panama (2013) and Nicaragua (2014 and 2015).
- Triaged patients, shadowed physicians during consultations, distributed medications and presented educational information about preventive medicine during 3-day medical clinics on brigades.
- Constructed sustainable public health projects such as latrines, septic tanks, showering units, and concrete floors in local communities on in-country brigades.
- Promoted student awareness on campus for sustainable development and healthcare in under-resourced communities.

Medical Coordinator 04/2014-03/2015

- Ordered medications and solicited donations for the 4 medical clinics in Panama, Nicaragua, and Honduras during the 2014-2015 academic year.
- Recruited medical professionals for in-country brigades.

Vice President/Education Coordinator 04/2015-03/2016

- Spearheaded the committee that develops all of the educational materials for in-country brigades.
- Designed and delivered meeting spotlights on health disparities, policy, and common diseases in the communities we serve abroad.
- Served on the Global Brigades Penn State campus council as a liaison for the medical chapter.

IFC/PanHellenic Dance Marathon (THON) – Pennsylvania State University, State College, PA

THON Committee Member 09/2012-03/2015

- Maintain and build relationships with THON's individual, small business, and corporate donors
- Organize, support, and facilitate year-long fundraising efforts

THON Donor Alumni Relations Captain 09/2015-03/2016

- Build and uphold THON's relationship with thousands of Penn State Alumni across the country
- Play an active role in promoting and facilitating all THON family events and fundraisers throughout the year, including the 46-hour dance marathon in February

Work Experience

Casa Toro Mexican Grill, Coopersburg, PA

Hostess/Waitress 08/2009-08/2013

Camelot or Children, Inc., Allentown, PA

Summer Camp Coordinator and Counselor 05/2014-08/2015

- Designed, organized, and orchestrated a 7-week camp (2014 and 2015) for children with chronic illnesses and/or disabilities.
- Established educational activities that allowed children to improve their social skills while further developing their physical, mental, and emotional capabilities.

PSU Know How Private Instruction, State College, PA

Private Instructor 02/2016-05/2016

- Provided individualized or group instruction in organic chemistry and biochemistry.

Service Experience

Camelot for Children, Allentown, PA

Summer Camp Volunteer 06/2013-08/2013

- Participated in summer camp with the children and encouraged their participation in all activities

Fundraising/Events Volunteer 09/2008-05/2016

- Attended, supported, and ran yearlong fundraising efforts for the non-profit organization that fosters a developmentally enriching environment or children with a variety of disabilities and/or chronic illnesses.
- Participated and helped facilitate monthly events with the children and their families.

Adult Basic Education Center, State College, PA

Literacy Corps Tutor 08/2015-12/2015

- Worked three hours weekly with an English as a Second Language (ESL) learner on improving her conversational skills, expanding her vocabulary, advancing her grammatical understanding, and developing the skills necessary to communicate professionally and effectively via email.

Skills

Proficient in spoken and written Spanish, Red Cross childcare certification, CPR/AED certification.