A SPITTING IMAGE

How Salivary Cortisol Levels Reflect Stress Levels in High School Adolescents

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THE HORMONE CORTISOL HAS MANY FUNCTIONS, SUCH AS GLUCOSE METABOLISM MAINTAINANCE, BLOOD PRESSURE REGULATION, AND INFLAMMATORY RESPONSE. WHEN CORTISOL LEVELS SPIKE, AS WITH STRESS, THERE CAN BE A NEGATIVE EFFECT ON THE INDIVIDUAL. IT IS REPORTED THAT WHEN UNDER STRESS, ONE TENDS TO TO EITHER EAT MORE OR LESS THAN NORMAL, WHICH CAN POTENTIALLY INFLUENCE THE BODY MASS INDEX (BMI) OF AN INDIVIDUAL. WHILE INCREASED STRESS CAN LEAD TO AN ABNORMAL CORTISOL LEVEL AND BMI IN ADULTS, THE ROLE IN ADOLESCENTS IS NOT ENTIRELY CLEAR. THIS STUDY INVESTIGATED THE RELATIONSHIP BETWEEN STRESS AND BMI IN HIGH-ACHIEVING ADOLESCENTS, AGED 14 TO 18. THE *PERCEIVED STRESS SCALE* SURVEY PAIRED WITH ADDITIONAL QUESTIONS WERE ADMINISTERED TO DETERMINE OVERALL STRESS LEVELS IN EACH SUBJECT. TO DETERMINE CORTISOL LEVELS, A COMPETITIVE ENZYME IMMUNOASSAY WAS USED. THIS STUDY INDICATED THAT THERE WERE NO SIGNIFICANT CORRELATIONS BETWEEN PERCEIVED STRESS LEVELS, SALIVARY CORTISOL LEVELS, AND BMI IN THIS GROUP OF INDIVIDUALS. HOWEVER, A DISTINCT DIFFERENCE IN SELF-ASSESSED STRESS LEVELS WAS APPARENT BETWEEN MALES AND FEMALES. ALSO, A NEGATIVE RELATIONSHIP WAS FOUND BETWEEN BMI AND SALIVARY CORTISOL LEVELS AND PERCEIVED STRESS AND SALIVARY CORTISOL LEVELS.

INTRODUCTION

Stress plays a major role in the lives of numerous adolescents. With the pressure to establish themselves in high school and decide their futures, students are often under a great amount of stress. The stress these teenagers experience oftentimes has a direct influence on their mental health (Turner et al., 1995). Family life, sexuality, relocation, accident and illness, autonomy, high self-expectation, competitive learning environments, and financial concerns are just a few of the factors that impact stress levels (Stilger et al., 2001; Newcomb et al., 1981; Lin et al., 2013). All of these elements, especially parental attachment style and family life (Stenhammar et al., 2010; Parks et al., 2012; Olstad *et al*., 2016) can potentially impact body mass index.

Stress can easily be broken up into two categories: acute and chronic. Acute stress stems from short term demands and pressures. It is much more common on a daily basis. High school students frequently experience acute stressors in the school setting. A few of these stressors are standardized testing, course work, college applications, social bearings and extracurricular activities. In certain situations, acute stress can stimulate the hypothalamic-pituitary-adrenal axis, causing a greater accumulation of abdominal fat (Rodriguez et al., 2015). Chronic stress comes from uncomfortable emotional experiences that occur for long periods of time. It has been shown to increase the consumption of sugary, calorie-dense foods (Tryon et al., 2013). This aligns with the fact that stress often causes people to overeat (Greeno & Wing, 1994). At this point in their lives, adolescents often experience a myriad of emotion. These positive and negative emotions can possibly dictate their eating habits. People who are generally emotional eaters tend to eat more when upset than when happy. This could perhaps be the case for many teenagers as well (Strien et al., 2013).

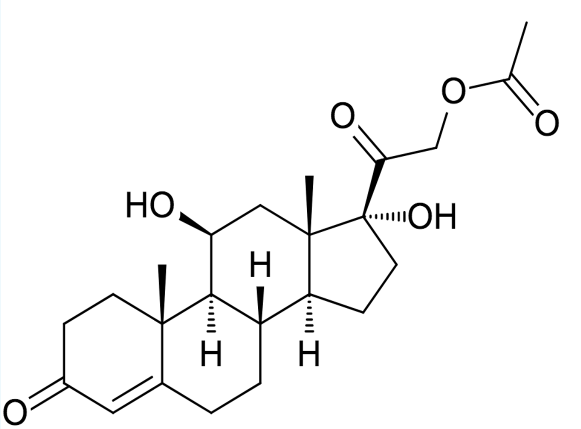

FIGURE 1. CHEMICAL STRUCTURE OF CORTISOL (COURTESY OF *WIKIMEDIA COMMONS*)

As the "stress hormone," cortisol (Figure 1) is a viable, accurate way to measure the stress level of an individual (Shirtcliff et al., 2015). Synthesized by the adrenal cortex, cortisol is a paramount glucocorticoid. Cortisol is the most commonly used biomarker because it is linked with a multitude of different physiological processes (Cicchetti et al., 2015). Cortisol production follows a circadian rhythm, peaking in the early morning and dropping at night to its lowest values (Figure 2). Salivary cortisol level is one reliable indicator of stress level. High correlations between salivary and serum cortisol are often reported. Serum cortisol that is unbound goes into the saliva through intracellular mechanisms. Generally, most salivary cortisol is not bound to protein ("Cortisol ELISA Kit (Saliva)"). Salivary flow rate has no effect on salivary cortisol levels.

Body Mass Index (BMI) is often one of the many tools used to determine the general health of an individual. Using height and weight, BMI is calculated through a formula: (Weight (lbs) * 703) / Height (in)^2) (National Institute of Health). A BMI less than 18.5 classifies an individual as

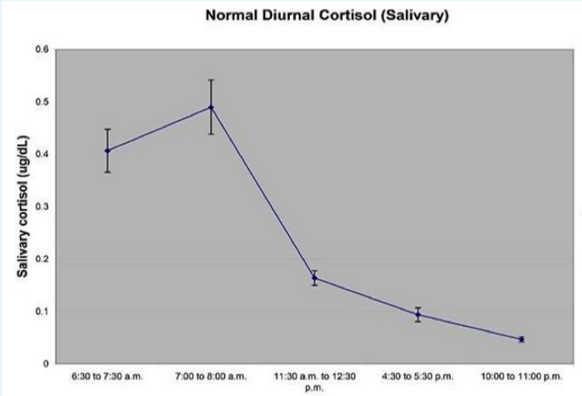

FIGURE 2. SHOWS THE NORMAL DIFFERING SALIVARY CORTISOL LEVELS THROUGHOUT THE DAY, BEGINNING AT 6:30AM-7:30AM TO 10:00PM-11:00PM. AS SEEN IN THE FIGURE, SALIVARY CORTISOL LEVELS ARE NORMALLY THE HIGHEST IN THE MORNING AROUND 7:00AM-8:00AM AND THE LOWEST AT THE END OF THE DAY, AROUND 10:00PM-11:00PM (SALIMETRICS, 2016).

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underweight. Between 18.5 and 24.9 classifies an individual as average weight, and is often referred to as a target range. A BMI between 25-29.9 classifies an individual as overweight, while a BMI of 30 and above is considered obese (National Institute of Health). Additionally, through the measurement of waist circumferences, we are able to better predict the negative health impact of weight.

In recent years, there have been major breakthroughs in the field of nutrition, and this project can further contribute to those findings. The goal of this paper is to add to the existing literature studying the relationship between stress and weight among adolescents. This study hypothesizes that a greater score on the Perceived Stress Scale and a higher salivary cortisol level will be linked to a higher BMI and waist circumference in adolescents. The results of this experiment can potentially foster new strategies for parents, educators, counselors and students themselves, to change the behaviors of teenagers for better health.

METHODS AND MATERIALS

Participants

To determine the relationship between stress, salivary corrtisol levels and body mass index (BMI), a total of 49 subjects participated in this study. Institutional Review Board (IRB) approval and consent from both Long Island University and Sanford H. Calhoun High School were obtained prior to the beginning of this study. Participants were recruited from a pool of high-achieving students at Sanford H. Calhoun High School, mainly from Advanced Placement (AP) classes such as AP Biology, AP Chemistry, Advanced Science Research, and Introduction to Research classes. Subjects ranged in age from 14 years to 18 years, were healthy male and female high school students. Primary recruiting methods included the use of fliers, announcements, and incentives. Science teachers encouraged their students to participate in the study. Teachers could have offered extra credit, but it was ultimately left to the discretion of the teachers to determine how much extra credit, if any, they gave.

Procedure

All paperwork and sample vials were tagged with an identification number that corresponded to an individual participant. No names or identifying information were used in order to protect individuals' privacy and anonymity. For all participants, the procedure took place around 3 pm to minimize inherent salivary cortisol differences throughout different times of the day. Upon arrival at the secure study room, subjects were instructed to sit down and close their eyes as part of a relaxation exercise led by the principal investigator. Subjects listened to five minutes of soothing music, which is considered as an auditory stimulus (Hanser, 1985), to allow the participants to calm down after a busy school day (Rogue et al., 2013). The music was "Spa Music" including *Songs to help relax, Sleep, and Meditate,* and is not associated with normal classroom activities. Musical selections were played through the smartboard speaker (Smart Technologies, Calgary, Alberta) that the room was equipped with. Following the relaxation exercise each subject had their height, weight, and waist circumference measured individually in a private room by two licensed and certified nutrition professionals from Long Island University. A SECA scale (SECA North America, Hanover, MD) Model 700 was used for height, measured in feet and inches and weight, measured in pounds. A flexible tape measure (Tasharina, China) was used for waist circumference measurements in inches.

Measurements were recorded on an individual form with an identification number corresponding to the subject's cortisol sample vial and survey to ensure confidentiality. Upon completion of these measurements, subjects returned to the secure study room. Here, they were instructed on how to provide saliva samples for cortisol measurements. Saliva was collected via passive collection using SalivaBio oral swabs and a sample vial (Salimetrics, Carlsbad, CA). Dr. Azad Gucwa, Assistant Professor at the Department of Biomedical Sciences at Long Island University (LIU), kindly instructed participants on the collection method as set forth by the *SalivaBio collection insert* (Salimetrics, Carlsbad, CA).

Universal precautions were followed as every subject: researchers wore vinyl gloves during collection and all waste was disposed of as biohazardous waste. Vials for the study were collected in a sealed, iced cooler by Dr. Gucwa and returned to a biomedical lab at LIU to be processed for cortisol levels. Samples were stored in this laboratory at -20°C until tested.

Finally, all subjects answered a questionnaire that was designed to determine their self- perceived stress levels (see Appendix A). This was composed of the widely used, 10-question Perceived Stress Scale (PSS) (Cohen, 1994), as well as seven additional questions to determine possible causes of stress and stress levels. These questions were related to grades, academic class levels, parental education, familial income, physical activity, and eating history from the 60 minutes prior to participating in the study. Participants were given the option to answer “I am unaware of this information” and/or “I prefer not to answer”. These options, however were never chosen, therefore they were not included in the analysis.

Measuring Cortisol Levels in Saliva Utilizing a Competitive Immunoassay

Salivary cortisol measurements were taken using a commercially available enzyme- linked-immunosorbent assay (ELISA) kit obtained from Salimetrics (Figure 3). Once the samples were ready to be measured, they were taken out of the freezer, and the vials containing the swabs were centrifuged through the Beckman Coulter Allegra X-12R Centrifuge to collect saliva at the bottom of the vial. To measure the cortisol, a competitive enzyme immunoassay was used (Cortisol ELISA assays, 1-3002) (Salimetrics, Carlsbad, CA). Samples were processed according to manufacturer recommendations.

FIGURE 3. AN EXAMPLE OF THE ELISA KIT (COURTESY OF *WIKIMEDIA COMMONS*)

Briefly, a 96-well microwell plate coated with monoclonal anti-cortisol antibodies was used. Cortisol from the saliva samples were then added for binding to anti-cortisol antibodies. After washing, horseradish peroxidase enzyme-linked secondary antibodies were added followed by the substrate tetramethylbenzidine (TMB), resulting in a color change detected by a spectrophotometric microtiter plate reader. The processed optical density (OD) value was determined by measuring its absorbance at 450 nm. Next, the concentration of cortisol was determined using a 4-parameter nonlinear regression curve. A standard was used to determine the concentration of our samples. Additionally, low and high cortisol controls were used with every plate and tested within the expected reference range that was provided by the manufacturer. All samples, including standards and controls were tested in duplicate wells to minimize variability.

Determination of Individual Stress Levels

All individual paperwork was kept in a locked vault at S.H. Calhoun High School where it will be kept for a minimum of 5 years, at which point all confidential information will be destroyed. When measurements and stress scale results were organized and analyzed, papers were signed out and returned when completed. The Statistical Package for the Social Sciences (SPSS) was used to organize data. Dr. Bernard Gorman kindly trained the lead investigator in this statistical program.

Statistical Analysis

Regressions were run in SPSS to determine correlations between variables. Subsequently, descriptive tests, frequencies, correlations, and t-tests were run to compare the data. Data was later broken up based on sex and two tables were created to further determine relationships (See Appendix C and D).

RESULTS

Descriptive Statistics

After outliers were removed from the sample, there were a total of 49 students tested. Their ages ranged from 14 to 18, as is expected among high school students. Height and weight between the participants greatly varied. As seen in Table 1, the BMI measurements had a great range from the underweight category under (<18.5) to the overweight category (25.0 – 29.9) ("About Adult BMI") (Table 2). PSS values ranged from 8.00 to 40.00, so there was clearly a distinct difference in stress levels among participants.

As shown in Table 3, the mean values for weight, BMI, and PSS greatly differed between the males and females in this sample. Notably, though self-assessed stress levels were different for males and females, the mean cortisol levels for both sexes were almost the same: females 0.19 ug/dL and males 0.18 ug/dL.

Table 4 (see Appendix B) which is based on the non-PSS questions from the questionnaire, shows much demographic information about the participants. All but one participant had grades in the top tier range from 90%-100%. Additionally, all participants were enrolled in honors and at least one Advanced Placement (AP) course at school. Both factors prove that this group consider themselves high achievers, possibly causing their stress levels to be affected by both their grades and coursework.

	N	Min.	Max.	Mean	Std. Deviation
Cortisol	49	0.07	0.93	0.19	0.13
Age	49	14.00	18.00	16.22	1.01
Height (in)	49	59.25	77.00	65.62	3.88
Weight (lbs)	49	88.50	212.10	127.25	22.25
BMI	49	16.20	27.83	20.74	2.84
Waist Circumference (in)	49	24.90	40.10	29.94	3.36
PSS	49	8.00	40.00	21.14	7.34

TABLE 1. THE VARIOUS DESCRIPTIVE STATISTICS FOR THE SAMPLE OF PARTICIPANTS USED IN THE STUDY. OVERALL, 49 PARTICIPANTS WERE USED. THE MINIMUM, MAXIMUM, MEAN, AND STANDARD DEVIATION VALUES ARE SHOWN FOR CORTISOL LEVELS, AGE, HEIGHT, WEIGHT, BMI, WAIST CIRCUMFERENCE, AND PSS LEVEL.

BMI	Weight Status
Below 18.5	Underweight
18.5-24.9	Normal or Healthy Weight
25.0-29.9	Overweight
30.0 and Above	Obese

TABLE 2. BMI CLASSIFICATIONS (*CENTERS FOR DISEASE CONTROL AND PREVENTION*)

The majority of participants perceived their combined familial income to be $100,000 or more. Evidently, this group of students would be considered at least upper middle class, having a high socioeconomic status (SES) (Francis, 2012). 37 mothers of participants earned a bachelor's degree or higher, while only 7 did not finish college or only have a high school diploma. 24 fathers earned a bachelor's degree or higher. 3 fathers received an associate or technical degree and 13 either did not finish college, have only a high school diploma, or did not finish high school at all. The education levels of parents could have influenced the type of students their children came to be, namely if their children were perhaps more motivated to complete school work and advance their education or not, following in the footsteps of their parents.

	Sex	N	Mean	Std. Deviation	Std. Error Mean
Cortisol	Male	17	0.18	0.07	0.02
	Female	32	0.19	0.16	0.03
Age	Male	17	16.12	0.78	0.19
	Female	32	16.28	1.11	0.20
Height	Male	17	65.41	3.92	0.95
	Female	32	65.73	3.91	0.69
Weight	Male	17	121.05	17.85	4.33
	Female	32	130.54	23.88	4.22
BMI	Male	17	19.88	2.24	0.54
	Female	32	21.20	3.05	0.54
Waist Circumference	Male	17	30.65	3.46	0.84
	Female	32	29.48	3.39	0.60
PSS	Male	17	17.11	6.30	1.53
	Female	32	23.28	7.02	1.24

TABLE 3. THE GROUP STATISTICS TABLE, IN WHICH CORTISOL LEVELS, AGE, HEIGHT, WEIGHT, BMI, WAIST CIRCUMFERENCE, AND PSS VALUES ARE REPORTED, BROKEN UP BY GENDER.

"While the relationship was not significant, it appears that both cortisol and BMI were negatively correlated, therefore participants who had higher cortisol levels generally had a lower BMI."

Overall, 42 participants exercised at least twice a week. Notably, this also could have affected their cortisol levels. Overtime, exercise lowers cortisol levels, so this could have been the case for some of these participants (Brogaard, 2015). An additional factor that could have impacted cortisol levels was the participants' eating patterns. 10 of the participants reported that they had eaten in the 60 minutes prior to the testing section. This could have caused their salivary cortisol levels to be higher and not as accurate compared to a fasting sample (Toda et al., 2004).

Overall, the correlation between sex and PSS scores was significant, with a p-value of 0.004. Both height and weight positively correlated with one another. BMI was also positively correlated with weight and waist circumference. Contrary to the hypothesis, there are no significant correlations between cortisol, stress, and BMI (Table 5). While the relationship was not significant, it appears that both cortisol and BMI were negatively correlated, therefore participants who had higher cortisol levels generally had a lower BMI. Notably, BMI and self-assessed stress levels were also negatively correlated. Ergo, participants with a lower BMI generally perceived themselves to have a higher level of stress.

		Sex	Cortisol	Age	BMI	PSS
Sex	Pearson Correlation	1.00	0.02	0.08	0.22	0.40**
	Sig (2-tailed)		0.90	0.59	0.12	0.00
	N	49	49	49	49	49
Cortisol	Pearson Correlation	0.02	1.00	-0.09	-0.07	-0.12
	Sig (2-tailed)	0.90		0.52	0.62	0.41
	N	49	49	49	49	49
Age	Pearson Correlation	0.08	-0.20	1.00	0.08	-0.07
	Sig (2-tailed)	0.59	0.52		0.59	0.64
	N	49	49	49	49	49
BMI	Pearson Correlation	0.22	-0.07	0.08	1.00	0.14
	Sig (2-tailed)	0.12	0.62	0.59		0.35
	N	49	49	49	49	49
PSS	Pearson Correlation	0.40**	-0.12	-0.07	0.12	1.00
	Sig (2-tailed)	0.00	0.41	0.64	0.35	
	N	49	49	49	49	49

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

TABLE 5. OVERALL STATISTICAL CORRELATIONS

DISCUSSION

Studies relating BMI to stress among adolescents have been very inconsistent. Some find that the higher the cortisol level, the higher the BMI (Kiess et al., 1995; Abraham et al., 2012; Melbin & Vuille, 1989). Others find that the lower the cortisol level, the greater the BMI (Odeniyi et al., 2015). Others do not find much of a correlation at all. The group of participants used in this study proved to be very valuable. Oftentimes, there are a multitude of factors that influence stress in adolescents, such as income level, parental education, and academic workload. Because the subjects of this study were high achieving adolescents, their stress levels could have been inherently higher (Hopkins, 2012). Additionally, the majority of students also all came from households with a familial income of over $100,000. Thus the subjects' stress levels could have been lower due to their high socioeconomic status (Chen et al., 2004). Generally, social disadvantage relating to low socioeconomic status and minority race/ethnicity causes greater stress levels (Goodman et al., 2004; Champaneri et al., 2013). This relatively homogenous group of subjects was able to control for several of these factors that influence stress, because numerous participants had the same background. Consequently, we were able to better understand the relationship between stress and BMI.

The participants in this study have a great sense of will power. With 42 of them exercising at least 2 times a week, it is evident that they are conscious of their health. This exercise directly impacts their BMI, and it helps to prove why the BMI range in this group is predominantly in the healthy range. Oftentimes, physical activity is associated with healthier dietary patterns (Miles, 2007), which serve as another reason for their healthy BMIs. It is often observed that over time, chronic stress contributes to higher

BMIs (Tryon et al., 2013). On the other hand, instances of acute stress can often contribute to a lower BMI (Kivimäki et al., 2006). In these adolescents' lives, they experience acute stressors often, from college applications, to standardized testing, to coursework. These acute stressors could potentially explain why there seems to be a negative relationship between perceived stress and BMI.

As found in previous studies, males and females differed when it came to perceived stress (Yamamoto et al., 2010; Jaarsveld et al., 2009). Females appeared to have a higher self-assessed stress level. Overall, more females are depressed than males (Kessler, 2003). Consequently, they would perceive their stress levels to be higher than men do. However, there was not much of a difference between the sexes in salivary cortisol levels, which aligns with past studies (Kiess et al., 1995; Kjolhede et al., 2013). Because the procedure was conducted around 3 pm, cortisol levels were inherently affected. With the peak of cortisol occurring in the morning and the trough in the evening, the cortisol levels obtained during this experiment were naturally lower in samples due to the time of day. Overall, though not significant, relationships between self-assessed stress, BMI, and salivary cortisol were found. Consequently, all of these factors impact one another

LIMITATIONS

The sample used in this study serves as a convenience sample. These high school students were an easily obtained group of adolescents. However, the small sample size of this study proved to be a limitation. With only 17 males and 32 females, we were not able to gain results that accurately represent all adolescents. Self-selection was prevalent in this study because of the sample of participants. Because subjects self-reported whether they ate, there could have been error, which would have in turn affected their salivary cortisol levels. Medications can also possibly affect cortisol levels (Granger et al., 2009), and this was not taken into account. Additionally, cortisol levels are drastically higher in depressed people, and this variable was not measured in this study (Booij et al., 2015). This variable could have potentially changed the results if it was accounted for, so it would be valuable to look into in the future.

FUTURE WORK

There is still a multitude of research to be done regarding this topic. It would be beneficial if the sample size of adolescents in a future study was much larger than 49 participants to improve the accuracy of the results. Race/ethnicity have proven to have an impact on stress levels (Goodman et al., 2004), which although not considered during this experiment, would be beneficial to take into account when pursuing further studies regarding this topic. Additionally, the use of a heterogeneous sample with people hailing from different ethnic and SES backgrounds could be beneficial to examine if these factors impact stress, BMI, and/or cortisol levels.

Adolescents may possibly be one of the most stressed age groups. With this, many of them suffer from sleep deprivation. This lack of sleep could potentially contribute to weight gain as well (Bose et al., 2009). Therefore, it would be valuable to determine the hours of sleep each participant gets per night to see if it has an impact on his or her stress levels and BMI.

As mentioned, it would also be valuable to determine if participants are currently taking any medication that could impact cortisol levels.

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REFERENCES

"About Adult BMI." *CDC*, www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/.

Abraham, S. B., et al. "Cortisol, obesity, and the metabolic syndrome: A cross sectional study of obese subjects and review of the literature." *Obesity*21.1 (2013): E105-E117.

Booij, Sanne H., et al. "Cortisol and a-amylase secretion patterns between and within depressed and non-depressed individuals." *PloS one* 10.7 (2015): e0131002.

Bose, Mousumi, Blanca Oliván, and Blandine Laferrère. "Stress and obesity: the role of the hypothalamic–pituitary–adrenal axis in metabolic disease."*Current opinion in endocrinology, diabetes, and obesity* 16.5 (2009): 340.

Bouma, Esther MC, et al. “Adolescents’ cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study.” *Psychoneuroendocrinology* 34.6 (2009): 884-893.

Brogaard, Berit. “What Causes Adrenaline Rushes?” *Livestrong,* 17 June 2015, www.livestrong.com/article/196498-what-causes-adrenaline-rushes/.

Champaneri, Shivam, et al. “Diurnal salivary cortisol is associated with body mass index and waist circumference: the Multiethnic Study of Atherosclerosis.” *Obesity* 21.1 (2013): E56-E63.

Chen, Edith, et al. “Socioeconomic Status and Health in Adolescents: The Role of Stress Interpretations.” *Child Development*, vol. 75, no. 4, July-Aug. 2004, pp. 1039-52, sites.northwestern.edu/foundationsofhealth/files/2013/03/04-ChDev-SES-and-Health-In- Adol-Role-of-Stress-Interpret.pdf.

Cicchetti, Dante, et al. “Beyond the Stress Concept: Allostatic Load—A Developmental Biological and Cognitive Perspective.” *Developmental Psychology*, vol. 1, 6 Sept. 2015, onlinelibrary.wiley.com/doi/10.1002/9780470939390.ch14/summary.

“Cortisol ELISA Kit (Saliva) - Salimetrics Assays, 1-3002.” *Salimetrics,* www.salimetrics.com/assay-kit/cortisol-salivary-elisa-eia-kit.

Francis, David. “Where Do You Fall in the American Economic Class System?” *US News,* 13 Sept. 2012, money.usnews.com/money/personal- finance/articles/2012/09/13/where-do-you-fall-in-the-american-economic-class-system.

Goodman, Elizabeth, et al. “Social disadvantage and adolescent stress.”*Journal of Adolescent Health* 37.6 (2005): 484-492.

Granger, Douglas A., et al. “Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science.” *ISPNE,* vol. 34, no. 10, Nov. 2009, www.psyneuen-journal.com/article/S0306-4530(09)00205-4/abstract.

Greeno, Catherine G., and Rena R. Wing. “Stress-Induced Eating.” *Psychological Bulletin*, vol. 115, May 1994, pp. 444-64, psycnet.apa.org/psycinfo/1994-32685-001.

Hanser, Suzanne B. “Music therapy and stress reduction research.” *Journal of Music Therapy* 22.4 (1985): 193-206.

Hopkins, Kate. “Weigh the Benefits, Stress of AP Courses for Your Student.” *US News,* 10 May 2012, www.usnews.com/education/high-schools/articles/2012/05/10/weigh-the- benefits-stress-of-ap-courses-for-your-student.

“How Valid Is BMI as a Measure of Health and Obesity?” *Examine*, examine.com/nutrition/how-valid-is-bmi-as-a-measure-of-health-and-obesity/.

Jaarsveld, Cornelia HM, et al. “Perceived stress and weight gain in adolescence: a longitudinal analysis.” *Obesity* 17.12 (2009): 2155-2161.

Kessler, Ronald C. “Epidemiology of Women and Depression.” *Journal of Affective Disorders,* vol. 74, no. 1, Mar. 2003, pp. 5-13. *ISAD*, www.jad- journal.com/article/S0165-0327(02)00426-3/fulltext.

Kiess, W., et al. “Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight.” *Pediatric Research* 37.4 (1995): 502-506.

Kivimaki, M., et al. “Work stress, weight gain and weight loss: evidence for bidirectional effects of job strain on body mass index in the Whitehall II study.” *International Journal of Obesity*, vol. 30, no. 6, June 2006, pp. 982-87, www.ncbi.nlm.nih.gov/pubmed/16418750.

Kjölhede, E. Allansson, et al. “Overweight and obese children have lower cortisol levels than normal weight children.” *Acta paediatrica* 103.3 (2014): 295-299.

Lin, Hau Jett, and Muhamad Saiful Bahri Yusoff. “Psychological distress, sources of stress and coping strategy in high school students.” *International Medical Journal* 20.6 (2013): 672-676.

Melbin, T., and J. C. Vuille. “Rapidly developing overweight in school children as an indicator of psychosocial stress." *Acta Paediatr Scand*, vol. 78, no. 4, July 1989, www.ncbi.nlm.nih.gov/pubmed/2782072.

Miles, Lisa. "Physical Activity and Health." *British Nutrition Foundation*, 15 Nov. 2007, onlinelibrary.wiley.com/doi/10.1111/j.1467-3010.2007.00668.x/full.

Newcomb, Michael D., George J. Huba, and Peter M. Bentler. "A multidimensional assessment of stressful life events among adolescents: Derivation and correlates." *Journal of health and social behavior* (1981): 400-415.

Nicolson, Nancy, et al. "Salivary cortisol levels and stress reactivity in human aging." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 52.2 (1997): M68-M75.

Odeniyi, I. A., et al. "Body mass index and its effect on serum cortisol level."*Nigerian journal of clinical practice* 18.2 (2015): 194-197.

Olstad, Dana Lee, et al. "Hair cortisol levels, perceived stress and body mass index in women and children living in socioeconomically disadvantaged neighborhoods: the READI study." *Stress* 19.2 (2016): 158-167.

Parks, Elizabeth P. "Influence of Stress in Parents on Child Obesity and Related Behaviors." *Pediatrics*, vol. 130, Nov. 2012, pp. 1096-104, www.ncbi.nlm.nih.gov/pmc/articles/PMC3483892/.

Rodriguez, Angela C. Incollingo, et al. "Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: a systematic review."*Psychoneuroendocrinology* 62 (2015): 301-318.

Roque, Adriano L., et al. "The effects of auditory stimulation with music on heart rate variability in healthy women." *Clinics* 68.7 (2013): 960-967.

Shirtcliff, Elizabeth A., et al. "Salivary cortisol results obtainable within minutes of sample collection correspond with traditional immunoassays."*Clinical therapeutics* 37.3 (2015): 505-514.

Stenhammar, Christina, et al. "Family stress and BMI in young children." *Acta paediatrica* 99.8 (2010): 1205-1212.

Stilger, Vincent G., Edward F. Etzel, and Christopher D. Lantz. "Life-stress sources and symptoms of collegiate student athletic trainers over the course of an academic year." *Journal of athletic training* 36.4 (2001): 401.

Toda, Masahiro, et al. "Effect of snack eating on sensitive salivary stress markers cortisol and chromogranin A." *Environ Health Preventive Medicine*, vol. 9, no. 1, Jan. 2004, pp.27-29,www.ncbi.nlm.nih.gov/pmc/articles/PMC2723385/.

Tryon, M. S., Rashel DeCant, and K. D. Laugero. "Having your cake and eating it too: a habit of comfort food may link chronic social stress exposure and acute stress-induced cortisol hyporesponsiveness." *Physiology & behavior* 114 (2013): 32-37.

Turner, R. Jay, Blair Wheaton, and Donald A. Lloyd. "The epidemiology of social stress." *American Sociological Review* (1995): 104-125.

Van Strien, T., et al. "Emotional eating and food intake after sadness and joy."*Appetite* 66 (2013): 20-25.

Wolf, Oliver T., et al. "The relationship between stress induced cortisol levels and memory differs between men and women." *Psychoneuroendocrinology*26.7 (2001): 711-720.

Yamamoto, Kazuhiko, Ai Okazaki, and Susumu Ohmori. "The relationship between psychosocial stress, age, BMI, CRP, lifestyle, and the metabolic syndrome in apparently healthy subjects." *Journal of physiological anthropology* 30.1 (2011): 15-22.

Questionnaire for Advanced Science Research

Age_____years **Sex** (Circle) **M** **F** **Other** _______________

All individual data will remain confidential throughout the study. No names will be paired with any answers to this questionnaire; the ID number will be the ONLY thing associated with the answers. Once the data is released, it will be done in an aggregated form and no ID will be released. Everything will be confidential and protected.

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?

0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life?

0 1 2 3 4

3. In the last month, how often have you felt nervous and "stressed"?

0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

0 1 2 3 4

5. In the last month, how often have you felt that things were going your way?

0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life?

0 1 2 3 4

8. In the last month, how often have you felt that you were on top of things?

0 1 2 3 4

9. In the last month, how often have you been angered because of things that were outside of your control?

0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

0 1 2 3 4

For the following questions, please circle the answer choice that pertains the most to you. If you do not feel comfortable answering any of the following questions, there is an answer choice labeled "I prefer not to answer."

11. Please choose the range of grades that is most similar to your overall average in school.
a. 90%-100%+
b. 80%-89%
c. 70%-79%
d. 60%-69%
e. 0%-59%
f. I prefer not to answer.

12. Are you enrolled in honors courses?
a. Yes
b. No
c. I prefer not to answer.

13. Are you enrolled in 1 or more Advanced Placement (AP) courses this year?
a. Yes, I am enrolled in one.
b. Yes, I am enrolled in more than one.
c. No, I am not enrolled in any Advanced Placement courses.
d. I prefer not to answer.

14. Which category do you perceive your household income (yearly earnings of your parent(s)/guardian(s) to be?
a. Less than $24,999
b. $25,000 to $49,999
c. $50,000 to $99,999
d. $100,000 or more
e. I am unaware of this information.
f. I prefer not to answer.

15. What is the highest degree or level of education your mother/female guardian has completed?
a. 12th grade or less (no diploma)
b. High school diploma
c. Some college, no degree
d. Associate or Technical degree
e. Bachelor's degree
f. Graduate degree/Professional
g. I am unaware of this information.
h. I prefer not to answer.

16. What is the highest degree or level of education your father/male guardian has completed?

a. 12th grade or less (no diploma)
b. High school diploma
c. Some college, no degree
d. Associate or Technical degree
e. Bachelor's degree
f. Graduate degree/Professional
g. I am unaware of this information.
h. I prefer not to answer.

17. Have you eaten within the past 60 minutes?

a. Yes
b. No
c. I prefer not to answer.

18. How often do you exercise each week?

a. Everyday
b. 6 days a week
c. 5 days a week
d. 4 days a week
e. 3 days a week
f. 2 days a week
g. 1 day a week
h. I typically do not exercise.
i. I prefer not to answer.

APPENDIX B. NON-PSS QUATIONS (TABLE 4)

Table 4. Questions

Q11: Please choose the range of grades that is most similar to your overall average in school.
Q12: Are you enrolled in honors courses?
Q13: Are you enrolled in 1 or more Advanced Placement (AP) course this year?
Q14: Which category do you perceive your household income (yearly earnings of your parents(s)/Guardian(s) to be?
Q15: What is the highest degree or level of education your mother/female guardian has completed?
Q16: What is the highest degree or level of education your father/male guardian has completed?
Q17: Have you eaten within the past 60 minutes?
Q18: How often do you exercise each week?

Results

		Frequency	Valid Percent
Q11	90%-100%+	48.00	98.00
	80%-89%	0.00	0.00
	70%-79%	0.00	0.00
	60%-69%	0.00	0.00
	0%-59%	1.00	2.00
Q12	Yes	49.00	100.00
	No	0.00	0.00
Q13	Yes, I am enrolled in one.	12.00	25.00
	Yes, I am enrolled in more than one.	31.00	64.60
	No, I am not enrolled in any Advanced Placement courses.	5.00	10.40
Q14	Less than $24,999	2.00	7.40
	$25,000 to $49,999	1.00	3.70
	$50,000 to $99,999	5.00	18.50
	$100,000 or more	19.00	70.40
Q15	12th grade or less (no diploma)	0.00	0.00
	High school diploma	6.00	13.30
	Some college, no degree	2.00	4.40
	Associate or Technical degree	0.00	0.00
	Bachelor's degree	15.00	33.30
	Graduate degree/Professional	22.00	48.90
Q16	12th grade or less (no diploma)	3.00	7.50
	High school diploma	5.00	12.50
	Some college, no degree	5.00	12.50
	Associate or Technical degree	3.00	7.50
	Bachelor's degree	13.00	32.50
	Graduate degree/Professional	11.00	27.50
Q17	Yes	10.00	22.20
	No	35.00	77.80
Q18	Everyday	8.00	17.40
	6 days a week	11.00	23.90
	5 days a week	4.00	8.70
	4 days a week	5.00	10.90
	3 days a week	6.00	13.00
	2 days a week	5.00	10.90
	1 day a week	0.00	0.00
	I typically do not exercise.	7.00	15.20

APPENDIX C. MALE AND FEMALE CORRELATIONS (TABLE 5 AND 6)

Table 6: Male Correlations

		Cortisol	Age	BMI	PSS
Cortisol	Pearson Correlation	1.00	-0.20	0.44	0.35
	Sig (2-tailed)		0.44	0.08	0.17
	N	17	17	17	17
Age	Pearson Correlation	-0.20	1.00	0.33	-0.03
	Sig (2-tailed)	0.44		0.19	0.91
	N	17	17	17	17
BMI	Pearson Correlation	0.44	0.33	1.00	0.12
	Sig (2-tailed)	0.08	0.19		0.68
	N	17	17	17	17
PSS	Pearson Correlation	0.35	-0.03	0.12	1.00
	Sig (2-tailed)	0.17	0.91	0.68	
	N	17	17	17	17

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 7: Female Correlations

		Cortisol	Age	BMI	PSS
Cortisol	Pearson Correlation	1.00	-0.08	-0.17	-0.24
	Sig (2-tailed)		0.65	0.37	0.18
	N	17	17	17	17
Age	Pearson Correlation	-0.08	1.00	-0.02	-0.14
	Sig (2-tailed)	0.65		0.97	0.45
	N	17	17	17	17
BMI	Pearson Correlation	-0.17	-0.02	1.00	0.03
	Sig (2-tailed)	0.37	0.97		0.85
	N	17	17	17	17
PSS	Pearson Correlation	-0.24	-0.14	0.03	1.00
	Sig (2-tailed)	0.18	0.45	0.85	
	N	17	17	17	17

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).