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# Developing a Paradigm for the Measurement of Cortisol

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# Developing a Paradigm for the Measurement of Cortisol

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# Developing a Paradigm for the Measurement of Cortisol

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Stress is a set of physical and mental states accompanied by specific physiological responses. The psychophysiological activation of stress centers around the hypothalamic-pituitary-adrenal axis, a group of structures that help the body cope with stress. Cortisol is the principle glucocorticoid secreted by the adrenal cortex and is known as a stress hormone. Cortisol allows the body to stay on high alert during the stress response and recording changes in cortisol can be useful in a variety of research studies. This thesis focused on the utility and pitfalls of cortisol testing and research design. Cortisol can be testing through blood, hair, and saliva, with saliva being considered among the most viable methods due to its versatility. I learned how to use a salivary cortisol ELISA assay kit and used different technologies such as single-channel and multi-channel pipettes, centrifuge, orbital shaker, and the Epoch 2 Microplate Spectrophotometer. I coordinated with my mentor, Dr. Adam Lawson, and Dr. Bradley Kraemer and Dr. Lindsay Calderon from the biology department. There were several challenges with testing cortisol, including the time of the procedure, the lack of in-depth instructions, and having to coordinate outside of the psychology lab. Testing cortisol is difficult, but not impossible. Cortisol testing could be used in experiments, but due to the challenges and time it takes to learn the procedure from the beginning, it is advisable to be done in a lab that already tests cortisol regularly.

*Keywords and phrases:* Cortisol testing, salivary cortisol, ELISA assay, stress, HPA axis

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# Developing a Paradigm for the Measurement of Cortisol

Cortisol is part of the body's stress response and can be a useful tool in stress research. My thesis focused on the utility and pitfalls of cortisol testing and research design. I assessed the best methods for cortisol testing, how to test cortisol, and the challenges of testing cortisol in a lab without prior hormone testing experience. Testing cortisol is possible, but may not the worth the challenges faced.

Stress is a set of physical and mental states that is driven and accompanied by specific physiological responses. These responses can be measured by changes in stress hormones. A stressor is any real or perceived threat to the stability of "homeostasis", or an organism's balance, and places the body in a stress state (Kaltsas & Chrousos, 2007). Activation of the stress system leads to behavioral and peripheral changes that optimize the ability of the organism to adjust homeostasis and increase its chance of survival. The stress system is one of the most important systems and is an adaptive and helpful response in times of physical danger. Stress is also beneficial when a situation or mental task requires focus. For example, the stress response is beneficial during a job interview or test due to the subsequent physiological changes such as increases in hormones and blood flow.

The psychophysiological activation of stress centers around the hypothalamic-pituitary-adrenal axis, a group of structures that help the body cope with stress. The hypothalamus is a structure in the forebrain beneath the thalamus that is involved in the control of eating, drinking, and emotional behavior (Updegraff & Feist, 2018). The hypothalamus releases corticotrophin-releasing hormone, which stimulates the anterior

pituitary. The pituitary gland is sometimes referred to as the “the master gland” since it produces hormones that affect other glands which then subsequently produce other hormones.

The pituitary gland secretes the adrenocorticotropic hormone (ACTH) (Updegraff & Feist, 2018). ACTH stimulates the adrenal glands, which are endocrine glands located at the top of each kidney. The adrenocortical response occurs when the adrenal cortex, the outer covering of the adrenal glands, is activated to secrete glucocorticoids. The adrenomedullary response occurs when the adrenal medulla, the inner part of the adrenal glands, prompts the secretion of catecholamines.

The activation of the HPA axis results in elevations of glucocorticoids and catecholamines, which are classes of hormones that include epinephrine, norepinephrine, and cortisol (Kaltsas & Chrousos, 2007). The activated receptors of glucocorticoids inhibit the transcription of several genes involved in the function and growth of nonimmune and immune cells. Glucocorticoids also inhibit many functions of leukocytes and immune accessory cells, inhibiting the production of cytokines and other mediators of inflammation.

Along with the hypothalamus, the corticotrophin releasing hormone (CRH) is stimulated by catecholamines and initiates and perpetuates the stress response (Kaltsas & Chrousos, 2007). CRH affects overall arousal and automatic activation. CRH acts in the brain to increase mean arterial pressure and heart rate inhibition. The function of interrelated neuronal pathways is increased, particularly those related to the cerebral cortex, hippocampus, lumbar spine, and locus ceruleus. CRH immunoreactivity has also been detected in lymphocytes, where it plays a role in immune regulation.

The catecholamines epinephrine and norepinephrine increase heart output and liberate glucose from the muscles for additional energy (Kaltsas & Chrousos, 2007). During stress, cardiac output, respiration, and blood flow are enhanced. Blood flow is redirected to the brain, where the brain focuses on the perceived threat. Behavioral changes include enhanced arousal, accelerated motor reflexes, improved attention span and cognitive function, reduced feeding, sexual behavior, and increased ability to withstand pain.

**Psychopathology of Cortisol**

Cortisol is the principle glucocorticoid secreted by the adrenal cortex (Thau & Sharma, 2020). Due to the presence of glucocorticoid receptors in almost every cell of the body, cortisol affects many organ systems including the musculoskeletal, cardiovascular, respiratory, endocrine, and nervous system. Cortisol also has many functions in the human body, such as controlling stress response, blood glucose levels, inflammatory responses, and blood pressure. During the stress response, cortisol allows the body to continue to stay on high alert. Cortisol increases energy levels by increasing glucose, fat availability, and increasing metabolism (Kaltsas & Chrousos, 2007). Cortisol provides a more sustained release of energy than the sympathetic nervous system does, for coping with prolonged stress.

There are several benefits to testing cortisol in saliva. Salivary glands form a highly sophisticated end point in the central nervous system control of local immune defenses (Bosch, 2014). They are capable of responding instantly and with a high level of specificity to potential sources of harm, such as food, stress, and inflammation. Cortisol is an ideal molecule to assess in saliva. Being small, apolar and having a low propensity

to ionize, it readily diffuses through cells and tissues. While saliva seems to be a sufficient measure for cortisol, saliva may not be adequate for other hormones that do not readily diffuse from blood into saliva. Steroid hormones, such as cortisol, enter saliva though passive diffusion. In order to diffuse, the molecules must be able to pass through cell membranes, making lipid solubility important. This lipid solubility allows for a good saliva/plasma ratio (SPR). SPR is necessary for reliably monitoring the contents of plasma, including steroids.

Small molecule size is also conducive to passive diffusion as well as having a low pKa (i.e. the pH at which 50% of the molecules are ionized), which means they are less ionized making them apolar (Bosch, 2014). Ionized molecules are polar and therefore more likely to bind to other molecules. Since the cortisol molecule is apolar, lipid-soluble, very small, and does not ionize at a physiological pH, it diffuses well through cells and tissues making salivary cortisol useful to analyze. In order for salivary hormones to reflect changes in emotional states then salivary gland function must receive inputs from higher neural structures. Another study shows that salivary centers receive direct inputs from various cell groups in the mid-and forebrain, which are parts of the brain implicated in fear, anxiety, and stress. In humans, the salivary glands are capable of producing highly differentiated protein responses. The salivary glands are capable of responding instantly and with a high level of specificity to potential sources of harm such as food, stress, and inflammation. This is why salivary cortisol can be used to gauge emotional states.

Cortisol level analysis can be performed with blood, hair and saliva (Kobayashi & Miyazaki, 2015). The disadvantage of using blood serum is that is costly and may also

lead to falsely elevated cortisol due to the collection alone. Hair cortisol concentration provides an advantage for evaluating chronic stress because hair growth is slow. Conversely, salivary cortisol levels indicate acute response to stress as well as chronic stress. Salivary cortisol is used as a neuroendocrine marker of stress in various fields of science, including physiological anthropology.

Different procedures for measuring cortisol levels have been established. This includes getting the saliva sample by swabbing the participants' mouth with a cotton or polyester sponge called a Salivette (Bosch, 2014). There is also the method of unstimulated whole saliva collection, where the participants spits into a tube. Current research suggests that researchers should use establish standardized procedures for unstimulated whole saliva collection instead of using a Salivette. Using a Salivette allows for unnecessary confounding variables such as different amounts of saliva and changes in salivary protein composition. Salivary cortisol can be tested using enzyme-linked immunosorbent assay (ELISA).

The time that cortisol is collected is important. Cortisol secretion follows a circadian rhythm, so levels are low or undetectable at midnight, and then begin to increase overnight to peak 30–45 min after awakening (Kobayashi & Miyazaki, 2015). Cortisol then declines slowly throughout the day. This phenomenon is called cortisol awakening response (CAR). Samples collected in the early morning provide the most sensitive measurements of cortisol levels and tend to be highly consistent, even between adult populations of distinct ethnicities.

Cortisol levels have been found to vary depending on stress level and the time the stressor was presented (Miller, Chen, & Zhou, 2007). One meta-analysis found that the

more months that had elapsed since the stress first emerged, the lower a person's morning cortisol, daily volume, ACTH, and postdexamethasone cortisol. These findings are consistent with the hypothesis that when chronic stress first begins, there are elevated concentrations of cortisol. For acute psychological stressors, the peak cortisol response occurs 21–40 min from stressor onset. (Dickerson & Kemeny, 2004). This is when cortisol should be collected if it's used to test a stress response.

As time passes, however, this activity decreases to cortisol levels below normal (Miller, Chen, & Zhou, 2007). Stress posing a threat to the social self was associated with significantly higher cortisol at specific times in the day, including the morning and afternoon or evening. Uncontrollable stress elicited lower morning output of cortisol and higher afternoon or evening secretion. Situations likely to elicit shame were associated with significantly higher afternoon or evening cortisol, whereas those evoking loss were accompanied by lower morning cortisol and higher afternoon or evening cortisol. The impact of chronic stress on hormones is likely to be moderated by the victim's previous exposure to stressful circumstances, ability to call forth effective coping strategies and social support, and need to manage other stressors that compete for their attention and resources.

Adversities during ages 0—5 have not been associated with cortisol outcomes later in life, yet adversities during ages 6—11 are associated with a high cortisol level, even more so in individuals exposed to pre/postnatal adversity (Bosch, Riese, Reijneveld, Bakker, Verhulst, Ormel, & Oldehinkel, 2012). Adversity faced during ages 12-13 and 14-15 are associated with a low cortisol level. Cortisol reactivity depends on the age that the stressor took place. Psychological stressors are capable of activating the HPA axis

just like physical stressors. Studies have reported that laboratory tasks such as public speaking or mental arithmetic can increase cortisol levels (Dickerson & Kemeny, 2014). Some studies have failed to find cortisol changes (Manuck, Cohen, Rabin, & Muldoon, 1991). One review have touted the inconsistent effects of psychological stressors on cortisol activity (Biondi & Picardi, 1999). The differences in the literature suggest that all types of negative situations may not trigger cortisol changes in the same way.

Tasks that included social-evaluative threat provoked larger and more reliable cortisol changes than stressors without this type of threat. (Dickerson & Kemeny, 2014). Social-evaluative threat is characterized by tasks in which others could negatively judge performance. Tasks characterized by social-evaluative threat had an evaluative audience or negative social comparison that was present or the performance was captured on a permanent record. Studies that included multiple social-evaluative components, such has having a videotape and an audience, showed greater cortisol changes than those with only one component. Uncontrollable contexts contained elements that informed participants they were failing or could not avoid negative consequences. This includes manipulated task difficulty, false feedback of poor performance, harassment, or a distraction or other emotionally distressing stimuli when no methods for avoiding the stimuli were possible. Uncontrollable motivated performance tasks performed in the presence of others are the strongest elicitors of cortisol activation.

Cortisol can also be used as a neurobiological marker of empathy. Empathy is the act of perceiving, understanding, experiencing and responding to the emotional state of others (Barker, 2003). Cortisol and other steroid hormones have been used in various types of empathy research, showing a strong connection between cortisol and empathy.

Affective empathy involves the understanding and sharing of others' feelings (Decety & Jackson, 2006). Low affective empathy is related to lower levels of cortisol (Johnson, Caron, Mikolajewski, Shirtcliff, Eckel, & Taylor, 2014). Children with elevated cortisol are also more likely to be able to take another's perspective and elevated cortisol is associated with pro-social behaviors, such as helping another student, and peer acceptance (Oberle, 2018).

Cortisol and empathy are especially intertwined with one another when someone is exposed to emotional stimuli. Viewing another person suffering results in a positive association with empathy and cortisol (Engert, Plessow, Miller, Kirschbaum, & Singer, 2013). These studies show that analyzing cortisol can be used to determine whether emotional stimuli can succeed in producing an empathetic response.

The presentation of stories is useful in evoking different levels of empathy. Exposure to narrative stimuli can have a significant and positive effect on beliefs, attitudes, intentions, and behaviors and can cause stronger persuasive effects than non-stories (Braddock & Dillard, 2016; Appel & Richter, 2010). Viewing stories can also evoke neurological changes as seen through using magnetic resonance imaging (fMRI) (Speer, Reynolds, Swallow, & Zacks, 2009). Neural responses to changes in the stories occurred in the vicinity of regions that increase in activity when people view similar changes or carry out similar activities in the real world.

Research has been done examining empathy and physiological changes from viewing stories. People exhibiting greater empathy, in the form of donations, had increased skin conductance levels and elevated heart rate (Barraza, Beavin, Terris, & Zak, 2015). Viewing an emotional video raised oxytocin significantly compared to those

who watched an emotionally neutral video. There was a positive correlation between the degree of empathy experienced and the change in oxytocin. The increase in experienced empathy was associated with greater generosity. Participants who were more empathically engaged by the video showed greater generosity. Cortisol was elevated in people who reported experiencing empathy while it declined in those reporting distress (Barraza & Zak, 2009). Viewing emotional stories provoked higher empathy and cortisol responses than emotionally neutral stories and the levels of cortisol and empathy were positively correlated. The body of research looking at how stories can provoke empathy is steadily building as researchers find ways to harness the power of empathy in the persuasion of others.

I conducted previous research testing participants' reactions of empathy by giving them a story and then a questionnaire. For my honors thesis, I intended to add on to this study by testing participants salivary cortisol levels as well. This would be more reliable than self-report measures alone, with the intention that cortisol would be a sensitive measurement of stress.

## Previous Research

In my previous experiment, 74 Eastern Kentucky University students completed two questionnaires that included Likert scales about previous bullying history and the Interpersonal Reactivity Index (Davis, 1980). They read one of two different stories about bullying, one group read an emotional story and the other read a non-emotional story, and then completed one final questionnaire, the State Empathy Scale (Shen, 2010). The hypotheses were that individuals with a similar background to the story would have lower levels of empathy than individuals who did not have a similar background to the story

and that individuals who read the emotional story would have higher empathy levels than individuals who read the non-emotional story.

An individual measures T-test was conducted in SPSS to find the difference between story one and story two. There was not a significant difference in participants' ratings of empathy between the two stories ($p = .277$). Different bullying levels were separated into groups to conduct a 2-way ANOVA, finding no interaction between the story and previous bullying experience. Correlations were tested between state empathy, trait empathy, and bullying levels. Bullying level was positively correlated with state empathy at a high level of significance (correlation= .489; $p < .0005$) as well as bullying frequency and state empathy (correlation= .334; $p = .003$).

The difference in the stories' emotion did not impact the participants' ratings of empathy. Participants who had a higher level of previous bullying experience had higher levels of empathy in response to the bullying vignette. The lack of differences between the stories may be due to them not being different enough. Individuals may view bullying as emotionally charged regardless of the words used. Participants who experienced higher levels of bullying may have been more empathetic due to their shared history with the protagonist.

In order to expand on this research and to see more substantial differences between the two stories, I planned to augment the stories the participants are given and add a measurement of cortisol. The participants would read either a bullying vignette or a story from the perspective of a boy going to the zoo. Since research suggests that individuals who have undergone chronic stress may have lower levels of cortisol in the

future, I was going to also see if there were below normal cortisol levels in individuals who had suffered the chronic stress of being bullied.

## Honor's Thesis: Investigating the Measurement of Cortisol?

### Hypotheses

After changing my stories and adding a measurement of cortisol my new theses were:

H1: Individuals with a similar background to the bullying story will have higher levels of empathy and cortisol than individuals who do not have a similar background to the bullying story.

H2: Individuals with higher levels of previous bullying experience will have higher empathy and cortisol levels in response to the bullying story than the non-bullying story.

H3: Individuals with higher levels of previous bullying experience will have lower levels of baseline cortisol than individuals with lower levels of previous bullying experience.

### Procedure

This was the original procedure before I changed the focus of the project:

All components of the study will be conducted from the research rooms in the Cammack building on Eastern Kentucky University's campus. Participants will be recruited through the online SONA system. All of the participants will be run individually. Participants will be given a consent form prior to the completion of the questionnaires (Appendix A). Participants will then give a saliva sample by dripping their saliva into a tube. Participants will then be asked to complete two questionnaires and will be verbally asked about their previous bullying history (Appendix B, C). Participants will

then be asked to read one of two different stories, one group will read a bullying story and the other will read a non-bullying story (Appendix D). Participants will complete one final questionnaire on the computer (Appendix E) and give a second saliva sample. Participants will come in on another day and read the other story instead where their cortisol will be tested twice again. They will then be provided a debriefing form that includes additional information about the study and contact information (Appendix F).

**Shifting Gears**

As I completed my preparation for the new experiment, learning how to test salivary cortisol took longer than expected. As I began to focus more on the salivary cortisol design, I realized how complicated and involved the process was. Finding a kit, gathering materials, and figuring out the procedure ended up being a substantial undertaking. Instead of the original experiment idea, my thesis was modified to focus on the utility and pitfalls of cortisol testing and research design from selecting the kit to assessing the efficacy of cortisol research.

**Kit Selection**

To assess the best method for testing salivary cortisol, I looked through different research studies. Using the EBSCO Host database, I combed through research utilizing salivary cortisol, noting the kits used in the analysis. Many studies outsourced the test to outside labs. They would collect the saliva samples from participants and then pay to have them tested at an outside facility. When researchers tested the samples themselves, the company Salimetrics was commonly used. In order to find out the price of the Assay kit from their site, I first had to request a quote through a questionnaire on their site. I

filled out information about the experiment, the University, and the principal investigator. Then, I was sent a PDF of the costs. My mentor, Dr. Adam Lawson, and I ended up ordering a kit from Eagle BioSciences, called, the "Ultrasensitive Cortisol Saliva ELISA Assay Kit." This kit had also been used in a few studies (Fidan, Hakan, Gozeler, Karaaslan, Binay, & Cingi, 2013; Fidan, Hakan, Kalkandelen, & Cingi, 2013; Liu & Vagula, 2015). The cost of the kit is $390.

## Materials

### *Materials Provided*

The kit arrived in a small, cardboard box with glass vials and a foil bag. In the resealable foil bag with desiccant was one 96 well microplate (12x8 breakable strip wells) coated with goat anti-rabbit gamma. The microplate is called a GARGG microplate. The kit can test 78 samples, accounting for two of the wells being filled with controls and seven wells being filled with calibrators. To test more samples, another kit has to be ordered.

There are seven bottles of Salivary Cortisol EIA Calibrators prepared with commercially obtained cortisol. There is 5.0 ml of 0 calibrator and 1.0 ml of 0.1, 0.3, 1, 3, 10, and 30 ng/ml.

There are also two controls. The Salivary Cortisol EIA Control #1 at 1.0 ml and the Salivary EIA Control #2 at 1.0 ml. #1 is the lower concentration of cortisol and #2 is the higher concentration of cortisol.

There is Salivary Cortisol EIA Antibody produced from rabbit, diluted in a phosphate buffer base. The 6 ml bottle of anti-Cortisol contains animal protein and a binding protein blocker. The solution is blue.

There is 1 amber glass bottle containing 0.7 ml of Cortisol-Horse radish peroxidase (HRP) concentrate. The solution is light brown and light sensitive.

There is 6.3 ml of Cortisol-Horse radish peroxidase (HRP) conjugate buffer. The solution is yellow and used for the working reagent preparation.

1 bottle, 50 ml of wash solution is included and to be diluted with deionized water, which is not included.

1 amber plastic bottle, 12 ml of Color Development Reagent EIA #1 (Tetramethylbenzidine plus hydrogen peroxide) is included. It is light sensitive.

There is 1 bottle, 12 ml Stopping Solution EIA #1 (diluted acid solution).

**Materials Not Provided**

***Not Disposable***

A device is needed to dispense 25 µl of saliva accurately. For this, we learned how to use single-channel pipettes (micropipette) from Dr. Bradley Kraemer's lab in the biology department. Multichannel pipettes are also needed. Multichannel pipettes are used to dispense product in multiple wells at once. This makes the process quicker and reduces the chance for error.

A centrifuge is needed to prepare the samples. We used the centrifuge in Dr. Kraemer's lab. The centrifuge has to be balanced, meaning there needs to be equal weight on each side. Each sample always has to have a sample right across from it.

A microplate orbital shaker is used to shake samples in a circular motion. We used the orbital shaker in Dr. Lindsay Calderon's office in the biology department.

A microplate washer is recommended, but not required. We washed the plates by hand by decanting the wells.

If the GARGG microplate well strips are broken, then a holder is needed to keep the wells in place.

A freezer is needed that is -20°C or below to preserve the samples.

To read the final results, a Microplate reader capable of reading 450 nm with 4 parameter data reduction or comparable software is required. We used the EPOCH2 microplate reader in Dr. Kraemer's lab.

### *Disposable*

Saliva collection devices are needed for the samples. They should be plastic, not glass. They also need to be sized to fit the centrifuge. The 1.5 mL MCT graduated Natural from Fisher Scientific worked. The saliva could also be collected in a device specifically for collecting saliva and then transferred over to the plastic tubes.

Pipette tips are used with the pipettes. They have to match the size of the pipettes and be able to dispense 25 µl of saliva.

Disposable gloves are used throughout the sample preparation and procedure. These were available in Dr. Lawson's lab in the psychology department and in the biology department.

The solution will need to be mixed, this could be in a disposable conical or cylinder.

A plate sealer is needed to cover the wells before shaking on the orbital shaker, during incubation, and before the samples are read on the microplate reader. The plate sealer can be Parafilm or tin foil. I used Parafilm. Parafilm is a flexible film that is used to seal and protect containers in the lab.

## Sample Preparation

### *Collection*

Participants avoid food consumption, drinking coffee or alcohol, and smoking or chewing gum one hour prior to sample collection. The mouth is rinsed thoroughly with water 15 minutes prior to collection.

Whole saliva is collected by unstimulated passive drool by allowing saliva to drip off the lower lip into a plastic test tube or by allowing saliva to accumulate in the floor of the mouth and spitting it into a collection device.

Then, the specimen should be timed, dated, and properly labeled. Refrigerate then freeze (-20°C or below) samples until day of assay.

For an experiment held in the psychology department, samples would have been collected there and then stored or testing in the biology department.

### *Storage*

Unopened reagents will retain activity until the expiration date when stored at 2° - 8°C. Reagents should not be used beyond their expiration date, which is on the label, and only reagents supplied with the kit should be used. Opened reagents must be stored at 2° - 8° C and Microtiter wells must be stored at 2° - 8°C. The foil bag holding the GARGG microplate should be tightly resealed if opened. Opened kits retain activity for one month if stored as described above.

### *Assay*

On day of assay, thaw samples to facilitate precipitation of mucins. Then, centrifuge the samples using the centrifuge at 1500xg for ten minutes. The setting “XG” is selected instead of “RPM.” XG stand for relative centrifugal force and RPM stands for

revolutions per minute. The number “1.5” is inputted by hitting the up or down arrow. Multiplying 1.5 by 1000, represents the 1500xg. By using the up or down arrow, the 10 minutes are selected. Bring samples to room temperature and assay. Since the centrifuge used is in the biology department, the testing process must begin there for this step.

### *Reagent Preparation*

Cortisol-Horse radish peroxidase (HRP) working reagent is prepared ahead of time. The amount of working cortisol-HRP needed is determined and diluted 1:10 in conjugate buffer. For example, 0.25 ml of Cortisol-HRP concentrate + 2.25 ml of Cortisol-HRP conjugate is mixed for 50 wells. Unused portions are discarded if not used within 36 hours of mixing. I have worked this out to be the number of wells multiplied by 0.005 for the concentrate and 0.05 for the buffer. The instruction manual only gives the example and says that the dilution is 1:10, however, figuring out the formula had to be done independently.

### **Procedure**

To a 96 well GARGG microplate dispense 25 µl of ready-to-use Salivary Cortisol EIA calibrators. There are seven: 0, 0.1, 0.3, 1.0, 3.0, 10.0 and 30 ng/ml. As well as controls (there are two) and saliva samples. The salivary cortisol EIA calibrators represent a fixed amount of cortisol. They are used to make sure the procedure is working by seeing if the calibrators and controls match the expected mean absorbance, % B/BO, and (ng/ml) value. The substances can be dispensed by using a single-channel pipette (micropipette). The single-channel pipettes in the biology department and I used the ones from Dr. Kraemer’s lab.

Add 50 ul of cortisol-HRP working reagent to all wells. Since the same substance is being added to all of the wells, a multichannel pipette can be used. This is the same for the other steps where the same components are added to every well. Dr. Kramer's lab did not have multichannel pipettes, so I used the one from Dr. Calderon's lab. I met with her for her to show me how to use the pipette. In order to use the single and multichannel pipettes, I had to go to different labs.

Add 50 ul of cortisol EIA antibody, then cover microplate with plastic sealer. I used Parafilm for the plastic sealer that I stretched over the wells.

Incubate the samples by shaking on a microplate orbital shaker set at 500-900 rpm for 60 minutes at room temperature. Above the screen of the orbital shaker was the labels orbital, reciprocal, vibro, and cycle. To the left of the screen was the label "mode", followed by "time." Using the select and arrow buttons at the bottom, the columns on the screen read 50, 60°, 4°, 15, followed by 250, 250, off, and stop on the next row. These input the time, speed at which the machine shakes, and the angle at which it shakes. To insure that the samples shake for one hour, a timer was used. While the shaker is going, someone has to stay with it for the entire duration.

After incubation, decant the contents of the wells. Decanting is the process of separating mixtures. This allows mixtures to settle or separate, pouring off the lighter liquid and leaving the heavier liquid behind. In this case, the antibody to cortisol is already in the wells of the GARGG microplate. The decanting process washes off the product that is not cortisol that has bound to the antibody at the bottom of the well.

For this process, the procedure calls for washing 3 times with 300 µl of diluted wash solution. However, I found that the wells could not take all 300 µl at a time without

spilling over. Instead, I used 200 µl. The diluted wash solution is 10 ml of 10XWash solution EIA #1 diluted with 90 ml of deionized water. To wash the plates, the wash solution is pipetted in and then removed each time. According to Dr. Calderon, after every time product is removed, the plate should be moved in a circular motion. After the 3rd wash, invert the GARGG microplate on an absorbent paper and tap dry.

Pipet 100 ul of Color Development reagent EIA #1 into each well and briefly shake manually. Cover microplate with plastic sealer and incubate for 30 minutes at room temperature. Pipet 100 ul of Stopping Solution EIA #1 into each microtiter well of the GARGG plate. Shake briefly manually and the color changes from blue to yellow.

Read at 450 nm on a microplate reader within 30 minutes. I used the EPOCH2 microplate reader in Dr. Kraemer's lab. The computer required a faculty login, so I had to have Dr. Lawson login each time. The microplate reader had a procedure manual. Figuring out how to use the machine took a while. First, the machine needs to be turned on. Then, a button is pressed on the machine that is hooked up to the computer and a platform comes out where the microplate is placed. Here, it is important to keep track of the orientation of the wells. The EPOCH2 icon is on the desktop. After pressing it, select "task manger." Then select "new", "read," and input for the plate to be read at 450nm. The reader then reads the color of the wells, which is the data that then comes up on the screen.

**Calculation**

1. Compute the average optical density (OD) for the zero (Bo) calibrator.
2. Calculate the percent bound (B/Bo) for each calibrator, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo) x 100.

3. Plot percent bound (B/Bo) versus the calibrator concentrations and draw the best fit for the curve.
4. Plot percent bound (B/Bo) of the controls and unknowns to determine saliva cortisol concentrations.
5. Or determine the concentrations of the controls and unknowns by interpolation using software capable of logistics using a 4-parameter sigmoid minus curve fit.

## Results

There are several steps for the calculation. Dr. Lawson and I used Excel to do these calculations and make a graph (Figure 2). Comparing our results to the "typical" graph (Figure 1) we found that our controls (numbered 2 through 6 on the x-axis of the green graph) were flat, and thus not quite what we expected from the typical curve. This data was one of our first two full runs through the cortisol procedure and we had planned on doing several more full runs right after spring break (Table 1). Unfortunately, the move to online instruction prevented us from doing more runs. Our results do not show a clear change along the controls that we expected. This is likely a result of little minor issues adding up to considerable errors in the process. The process is quite tedious and there are many places where errors can be made. So doing this procedure many times to become efficient and error-free is critical before one could proceed on to using the test in an experiment.

## Perils of Testing Cortisol

There are several challenges when testing cortisol. First, the actual collection process can be challenging. I used a graduated plastic tube to collect the saliva samples

which is generally messy. A swab would be the easiest way to collect saliva, however, this is less accurate than unstimulated whole saliva (Bosch, 2014). A saliva collection aid specifically for passive drool could be used to make the process less messy. However, the saliva will still need to be transferred over to plastic tubes to fit into the centrifuge. Another issue with getting a saliva sample is if the participant feels uncomfortable spitting in the tube or if they struggle because their mouth is dry. If the samples collected are not being used right away, they will need to be stored in a freezer. If the freezer is in a different building, the samples will need to be transported. In my case, participants would give their saliva samples in the Eastern Kentucky University psychology building, Cammack, and then I would have to store the samples the same day or test them across campus in the New Science Building.

There are a few times in the procedure where solutions have to be prepared ahead of time. However, the mathematical formula to complete these calculations is not given. While these are not difficult conversions, figuring out what to do at first is confusing. From the perspective of a psychology major, figuring out conversions and the amount of solution needed is not a part of the curriculum, making a clear formula to follow helpful. For the reagent preparation, I was only given an example of the amount used if 50 wells are needed, however, 50 wells are probably not going to be tested at the same time. For the diluted wash solution, 10 ml of Wash solution EIA #1 diluted with 90 ml of deionized water is needed to prepare the diluted wash solution. However, to wash every well three times requires much more than that amount of product.

To complete the procedure, extra equipment is needed. Some of this is obvious, like the orbital shaker, centrifuge, and microplate reader. However, some of the items that

are needed are not listed and I discovered that they were needed throughout trying to complete the procedure. Gloves, a plate sealer, something disposable to mix materials (like a conical or graduated cylinder) are needed. After breaking apart the wells in order to test just a few samples at a time, Dr. Lawson and I discovered that we would need something to hold the wells in place. I also needed a way to prop up our mixing tubes.

When washing the plates, product needs to be put in and out of the wells without anything spilling. However, when I put in the 300 µl of diluted wash solution that the procedure called for, the contents would spill. Dr. Calderon recommended that I instead use 200 µl and she did not understand why the procedure called for such a large quantity of product at once. The instructions were often incomplete or not compatible with typical equipment used. Unfortunately such problems in the procedure would most likely force any new student or researcher to consult with experienced researchers, or to learn by visiting an established laboratory that tests cortisol. Dr. Lawson and I were persistent even in the face of these many obstacles, but recognized that consultation with experienced researchers would be required before we would be comfortable publishing cortisol data.

## Limitations of Cortisol Research

There are several things that can influence cortisol results. Cortisol levels are elevated during the later stages of pregnancy, women on contraceptives, or after long-term use of contraceptives (Eagle Biosciences). Elevated cortisol levels can be found in conditions of sepsis, infection, chronic liver disease, and renal failure. Low cortisol levels result from liver disease, pituitary hyposecretion, hypothyroidism or steroid therapy. The use of topical creams or ointments containing hydrocortisone should be avoided as they

can cause preanalytical contamination of the saliva sample indistinguishable from endogenous cortisol as measured by Immunoassay. Not only that, but the process of having participants spit into a tube may actually produce stress on its own and effect the data.

## Importance of Cortisol Testing

Cortisol exerts a wide range of effects on major organs in the body (Kemeny, 2003). Chronic stress can have severe effects on immune competence. For example, relationship conflict predicts immune system suppression for couples that experience marital conflict (Kiecolt-Glaser & Newton, 2001). Effects of martial conflict may include poorer response to immunization and even slower wound healing (Ebrecht et al., 2004). Alzheimer's caregivers experience poorer psychological and psychical health, longer healing times for wounds, and lowered immune function (Damjanovic et al., 2007; Kiecolt-Glaser, 1999; Kiecolt Glaser, Marucha, Malarky, Mercado, & Glaser, 1995). A meta-analysis of over 30 years of studies showed a relationship between stress and decreased immune function, especially for chronic sources of stress (Segerstrom & Miller, 2004).

Cortisol testing can show physiological differences between different groups of people. Children with a history of bullying have been shown to have higher cortisol than children without a history of bullying (Chen, Kong, Deater-Deckard, & Zhang, 2017). These findings suggest that bullying victimization disrupts HPA axis functioning resulting in chronically higher levels of cortisol. Subtypes of bullying such as physical, verbal, or relational bullying, do not affect the overall pattern of cortisol. These findings suggest that different kinds of bullying elicits the same cortisol reactivity. The findings in

this research indicate a likely association between bullying victimization cortisol levels.

The diathesis-stress model suggests that some individuals are vulnerable to stress-related diseases because they are predisposed to those diseases from either genetic weakness or a biochemical imbalance (Updegraff & Feist, 2018). In this model, a predisposition to disease combined with a stressor causes disease. Examples are mental disorders such as depression, anxiety, and schizophrenic episodes as well as physical conditions such as colds and lung infections, gastritis and ulcerative colitis, headaches, eczema and psoriasis, cardiovascular disease, rheumatoid arthritis, and diabetes mellitus Stress can have severe and long-term consequences and cortisol research can be a valuable asset to this field of study.

## Efficacy in Social Science Research

Learning how to test cortisol has a large learning curve. Learning the different ways it can be tested, choosing a kit, and learning the procedure is a massive undertaking. Learning how to use pipettes, machines, new vocabulary, mathematical equations required, and methods such as decanting takes time. Even if all of these things are mastered, training a new person would then likely also be a long process.

The procedure itself also takes a considerable amount of time, around three hours every time that samples are tested. Not including the added time that it would take when learning the procedure. Added on this is the time that it would take to gather the saliva samples, store them, and then transport them from another building. Every time I went through the procedure, I had to get the materials from the psychology building, go to the biology building, and then take the materials back to the psychology building. Since learning cortisol itself takes a substantial amount of time, this makes it difficult to be used

as an undergraduate project. Cortisol was originally supposed to be incorporated into a larger research project as only one of the dependent variables, which was found to be impossible. Even as a master's level project this would be difficult, and possibly worse, because it could push off someone's graduation date. The amount of time it took to not only learn the procedure but also coordinate with professors in the biology department and wait on materials that were ordered made the project difficult to complete on an expedited timeline.

Testing salivary cortisol was also more expensive than anticipated. While luckily Dr. Lawson and I were able to use the pipettes and machines from another department, things like pipette tips, Parafilm, and saliva test tubes were purchased and would have to be purchased again if they ran out. The GARGG microplate that comes with the kit has 95 wells. A different microplate cannot be used because the microplate that comes with the kit has the cortisol anti-body at the bottom. Therefore, once the 95 wells are used, another kit must be ordered.

Cortisol requires a greater amount of testing consistency than many other measures. Cortisol peaks at different times throughout the day so participants need to be tested at the same time for consistency (Kobayashi & Miyazaki, 2015). There would also be a substantial challenge ensuring that participants did not eat, smoke, or chew gum before the experiment.

The most significant obstacle was not having a lab or an expert that measures cortisol, specifically salivary cortisol using an ELISA Assay, readily available. The biology department was a great asset, but they did not test cortisol, and so their help was greatly appreciated but not directly focused on cortisol testing. While the entire process is

complicated, I believe testing salivary cortisol, even as an undergraduate, is possible within a lab with these procedures already in place. I also believe testing cortisol in the psychology department is possible, however, this would take time to set up before being used in an experiment.

Using salivary cortisol testing in an experiment would take a lot of collaboration. Being able to put the procedure into practice would be impossible without help due to the lack of in-depth instructions and explanations. With my mentor Dr. Adam Lawson, I especially needed help with parsing through the equations and plotting the percent bound on a graph. Even then the procedure used language that was difficult to understand without working with a biology lab. A lot of the challenge would be looking information up and then having to sort through research articles to try and find clear answers. We had to work with the biology department to learn how to not only use their technology, but learn the language of biology research. For this kind of project, collaboration and the time that it takes to collaborate with several different people would need to be accounted for.

This understanding that cortisol testing cannot be easily learned in a span of a year without an experienced lab is a major conclusion of this thesis. However, taking on the difficult talk of learning such a challenging testing process from the very beginning and being able to see the process through till the end has had numerous benefits. The process has improved my confidence as a researcher and my ability to find solutions to situations despite presenting multiple difficulties. I was able to learn more than I ever would have had I not started the project.

There are obvious drawbacks to the use of cortisol in the lab, but possible benefits from a pedagogical perspective. In fact, one of the labs that used the same kit from Eagle

Biosciences used it as an undergraduate physiological lab module (Liu & Vagula, 2015). The module was developed to enhance student understanding of the functions of the cortisol hormone. They also learned about the circadian rhythm associated with cortisol release, proper ELISA technique, and quantitative data analysis methods. Students made the correct conclusion that cortisol levels are significantly different between morning and evening. The researchers concluded that the lab is suitable for an undergraduate course with a laboratory component. I agree that this test could work for well in a biology lab, and possibly for a psychology lab with a small independent study.

While cortisol is one of the most prominent methods to measure salivary cortisol, there are other physiological methods as well. Heart rate variability (HRV) is sensitive to changes in the autonomic nervous system activity associated with stress (Cheon, Bai, Hwan Lee, & Kool, 2018). HRV variables can change in response to stress induced by various methods. In clinical situations, HRV can be considered a tool that reflects heart activity and overall autonomic health (Kim, Electroencephalography (EEG) is one of the most commonly used neuroimaging modalities to study brain functions (Al-Shargie, Kiguchi, Badruddin, Dass, Hani, & Tang, 2016). EEG measures the fluctuations of electrical fields due to neuronal activity at millisecond resolution. The Alpha and beta frequency bands are linked to negative mood, stress, and depression. There is a negative correlation between EEG alpha power rhythm and stressful events in the prefrontal cortex where alpha rhythm is reduced with stress. Instead of cortisol, or while cortisol is being establish in the lab, for experiments measuring stress, a different physiological measure of stress such as blood pressure, heart rate, galvanic skin response, or respiration rate may be best.

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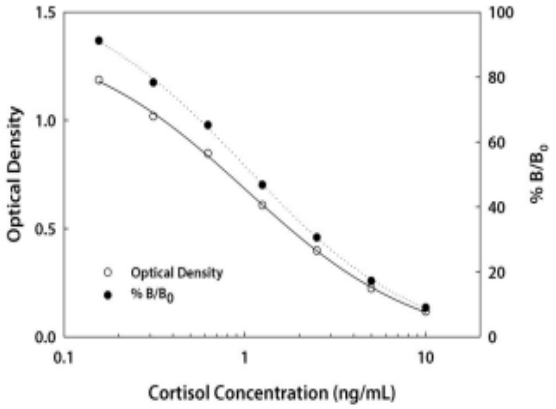
Ultrasensitive Cortisol Saliva ELISA Assay Kit. *Eagle Biosciences,* https://eaglebio.com/product/ultrasensitive-cortisol-saliva-elisa-assay-kit/

Table 1. Cortisol results.

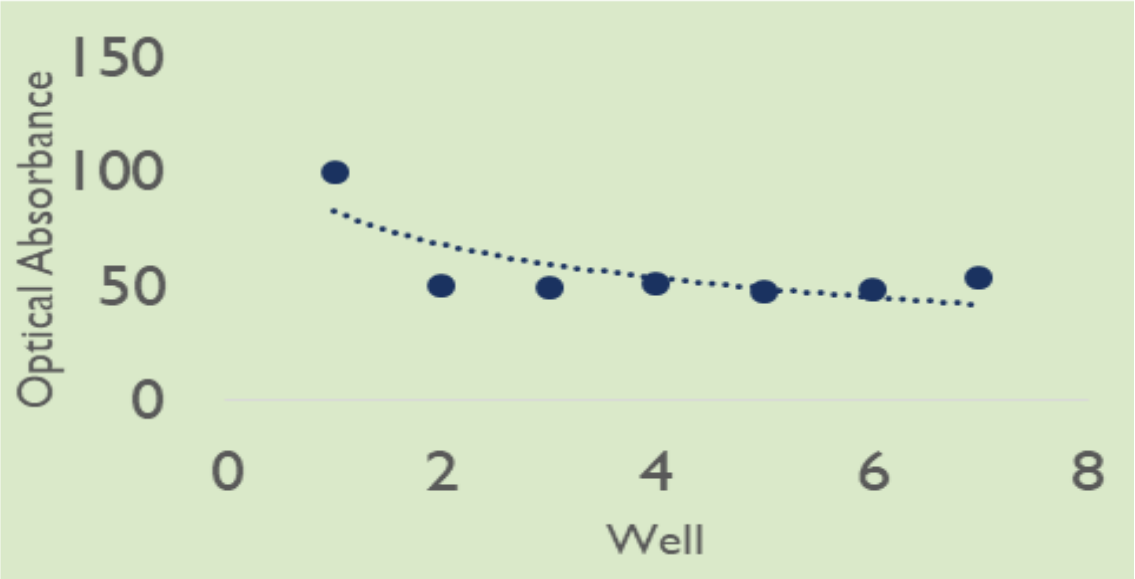
| Saliva Well (ng/ml) | Mean Absorbance (450 nM) | Percent Bound (B/Bo) |
|---|---|---|
| Calibrator 0 | 0.769 | 100 |
| Calibrator 0.1 | 0.483 | 62.80884 |
| Calibrator 0.3 | 1.206 | 156.827 |
| Calibrator 1 | 1.495 | 194.4083 |
| Calibrator 3 | 1.907 | 247.9844 |
| Calibrator 10 | 2.331 | 303.1209 |
| Calibrator 30 | 2.917 | 379.3238 |
| Control 1 | 2.848 | 370.3511 |
| Control 2 | 0.739 | 96.09883 |
| Sample 1 | 1.14 | 148.2445 |
| Sample 2 | 1.006 | 130.8192 |

Figure 1. Typical graph and results.




| (ng/mL) | O.D. | Average | Corrected | % B/$B_0$ |
|---|---|---|---|---|
| NSB | 0.010<br>0.010 | 0.010 | — | — |
| 0 ($B_0$) | 1.298<br>1.302 | 1.300 | 1.290 | — |
| 0.156 | 1.166<br>1.225 | 1.196 | 1.186 | 91.2 |
| 0.313 | 1.008<br>1.047 | 1.028 | 1.018 | 78.3 |
| 0.625 | 0.851<br>0.862 | 0.857 | 0.847 | 65.2 |
| 1.25 | 0.618<br>0.618 | 0.618 | 0.608 | 46.8 |
| 2.5 | 0.403<br>0.413 | 0.408 | 0.398 | 30.6 |
| 5 | 0.231<br>0.234 | 0.233 | 0.223 | 17.2 |
| 10 | 0.124<br>0.129 | 0.127 | 0.117 | 9.0 |

Figure 2. Cortisol results.



Appendix A. Consent Form

# Consent to Participate in a Research Study

# The Impact of Personal Stories on Empathy and Cortisol

## Key Information

You are being invited to participate in a research study. This document includes important information you should know about the study. Before providing your consent to participate, please read this entire document and ask any questions you have.

### Do I have to participate?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study and still keep the benefits and rights you had before volunteering. If you decide to participate, you will be one of about 50 people in the study.

### What is the purpose of the study?

The purpose of the study is to examine how you will respond to different stories. Potential subjects are ineligible to participate if they are under 18 years of age or are pregnant.

### Where is the study going to take place and how long will it last?

The research procedures will be conducted at Eastern Kentucky University. You will need to come to the Cammack building basement during the study. These visits will take about 30 minutes. The total amount of time you will be asked to volunteer for this study is an hour over the next week.

### What will I be asked to do?

You will be asked to come in and answer a couple questionnaires and give a saliva sample by spitting into a tube. Then, you will read a story, answer another questionnaire, and give another saliva sample. On a different day you will read a different story and give two saliva samples again.

There is minimal risk involved in this study. All data collected and responses will be confidential, and no identifying information will link you to any of the data collected.

### Are there reasons why I should not take part in this study?

You should not participate in this study if you are under the age of 18, or pregnant.

### What are the possible risks and discomforts?

To the best of our knowledge, the things you will be doing have no more risk of harm or discomfort than you would experience in everyday life.

### What are the benefits of taking part in this study?
You are not likely to get any personal benefit from taking part in this study. Your participation is expected to provide benefits to others by increasing our understanding of factors that influence empathy.

### If I don't take part in this study, are there other choices?

If you do not want to take part in the study, there are other choices, there are alternative opportunities to gain outside activity credits for your class by a) participating in other psychological research, b) attend presentations that impact psychology, and d) completing writing assignments of a psychological nature.

Now that you have some key information about the study, please continue reading if you are interested in participating. Other important details about the study are provided below.

## Other Important Details

### Who is doing the study?
The person in charge of this study is Megan Hurley at Eastern Kentucky University. She is being guided in this research by Dr. Adam Lawson. There may be other people on the research team assisting at different times during the study.

### What will it cost me to participate?
There are no costs associated with taking part in this study.

### Will I receive any payment or rewards for taking part in the study?
You will receive 3 SONA credits for taking part in this study. If you should have to quit before the study is finished, the credit you will receive will be based on the amount of time you were in the study.

### Who will see the information I give?
Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about this combined information. You will not be identified in these written materials.

This study is anonymous. That means that no one, not even members of the research team, will know that the information you give came from you.

The information or saliva you provide as part of the research will not be used or distributed for future research studies even if identifiers are removed.

### Can my taking part in the study end early?
If you decide to take part in the study, you still have the right to decide at any time that you no longer want to participate. You will not be treated differently if you decide to stop taking part in the study.

The individuals conducting the study may need to end your participation in the study. They may do this if you are not able to follow the directions they give you, if they find that your being in the study is more risk than benefit to you, or if the University or agency funding the study decides to stop the study early for a variety of reasons.

### What happens if I get hurt or sick during the study?

If you believe you are hurt or get sick because of something that is done during the study, you should call Megan Hurley at 502-316-8555 immediately. It is important for you to understand that Eastern Kentucky University will not pay for the cost of any care or treatment that might be necessary because you get hurt or sick while taking part in this study. Also, Eastern Kentucky University will not pay for any wages you may lose if you are harmed by this study. These costs will be your responsibility.

Usually, medical costs that result from research-related harm cannot be included as regular medical costs. Therefore, the costs related to your care and treatment because of something that is done during the study will be your responsibility. You should ask your insurer if you have any questions about your insurer's willingness to pay under these circumstances.

### What do I need to know about the use of the biospecimens I provide?

The saliva you provide will not be used for commercial profit.

Clinically-relevant research results, including individual results, not be disclosed to you.

This study will not include whole genome sequencing (i.e., sequencing of a human germline or somatic specimen with the intent to generate the genome or exome sequence of that specimen).

### What else do I need to know?

You will be told if any new information is learned which may affect your condition or influence your willingness to continue taking part in this study.

We will give you a copy of this consent form to take with you.

## Consent

Before you decide whether to accept this invitation to take part in the study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact the investigator, Megan Hurley at megan_hurley15@mymail.eku.edu. If you have any questions about your rights as a research volunteer, you can contact the staff in the Division of Sponsored Programs at Eastern Kentucky University at 859-622-3636.

If you would like to participate, please read the statement below, sign, and print your name.

*I am at least 18 years of age, have thoroughly read this document, understand its contents, have been given an opportunity to have my questions answered, and voluntarily agree to participate in this research study.*

_____

Signature of person agreeing to take part in the study Date

_____

Printed name of person taking part in the study

_____

Name of person providing information to subject

# Appendix B. Materials

Interpersonal Reactivity Index

The following statements inquire about your thoughts and feelings in a variety of situations. For each item, indicate how well it describes you by choosing the appropriate letter on the scale at the top of the page: A, B, C, D, or E. When you have decided on your answer, fill in the letter next to the item number. READ EACH ITEM CAREFULLY BEFORE RESPONDING. Answer as honestly as you can. Thank you.

A: DOES NOT DESCRIBE ME WELL

B

C

D

E: DESCIBES VERY WELL

1. ___ I daydream and fantasize, with some regularity, about things that might happen to me.

(FS)

2. ___ I often have tender, concerned feelings for people less fortunate than me. (EC)

3. ___ I sometimes find it difficult to see things from the "other guy's" point of view. (PT)

(-)

4. ___ Sometimes I don't feel very sorry for other people when they are having problems. (EC)

(-)

5. ___ I really get involved with the feelings of the characters in a novel. (FS)

6. ___ In emergency situations, I feel apprehensive and ill-at-ease. (PD)

7. ___ I am usually objective when I watch a movie or play, and I don't often get completely caught up in it. (FS) (-)

8. ___ I try to look at everybody's side of a disagreement before I make a decision. (PT)

9. ___ When I see someone being taken advantage of, I feel kind of protective towards them.

(EC)

10. ___ I sometimes feel helpless when I am in the middle of a very emotional situation. (PD)

11. ___ I sometimes try to understand my friends better by imagining how things look from their perspective. (PT)

12. ___ Becoming extremely involved in a good book or movie is somewhat rare for me. (FS) (-)

13. ___ When I see someone get hurt, I tend to remain calm. (PD) (-)

14. ___ Other people's misfortunes do not usually disturb me a great deal. (EC) (-)

15. ___ If I'm sure I'm right about something, I don't waste much time listening to other People's arguments. (PT) (-)

16. ___ After seeing a play or movie, I have felt as though I were one of the characters. (FS)

17. ___ Being in a tense emotional situation scares me. (PD)

18. ___ When I see someone being treated unfairly, I sometimes don't feel very much pity for

them. (EC) (-)

19. ___ I am usually pretty effective in dealing with emergencies. (PD) (-)

20. ___ I am often quite touched by things that I see happen. (EC)

21. ___ I believe that there are two sides to every question and try to look at them both. (PT)

22. ___ I would describe myself as a pretty soft-hearted person. (EC)

23. ___ When I watch a good movie, I can very easily put myself in the place of a leading character. (FS)

24. ___ I tend to lose control during emergencies. (PD)

25. ___ When I'm upset at someone, I usually try to "put myself in his shoes" for a while. (PT)

26. ___ When I am reading an interesting story or novel, I imagine how I would feel if the events in the story were happening to me. (FS)

27. ___ When I see someone who badly needs help in an emergency, I go to pieces. (PD)

28. ___ Before criticizing somebody, I try to imagine how I would feel if I were in their place.

(PT)

# Appendix C. Materials

## Demographics

What is your age? ____

Gender? _____

Year in school? (Freshman, sophomore, etc.) _____

## Previous Bullying Scale

On a scale from 1 (I wasn't bullied at all) to 5 (experienced extremely serious bullying) rate your level of previous bullying experience:

On a scale from 1 (never bullied) to 7 (constantly bullied) rate the frequency of your previous bullying experience:

# Appendix D. Materials

Story One:

**Lions and rodents and birds, OH MY!**

**But no penguins.**

I can still remember the first time I went to the zoo. I grew up in a rural area and had only been to the city to shop at the mall. I had just started high school and had joined the Future Farmers of America (FFA) club. At the second FFA meeting of the year, we discussed differences between domesticated and wild animals. Our teacher and club advisor, jumped up and said we should have a field trip at the zoo. I got really excited because I had always wanted to go to a zoo, and this was my chance.

On the day of the field trip, most of my club met in front of the school at 7 am. We were all very tired, as it was a Saturday and we would usually be sleeping in. We slowly climbed onto the bus, and I sat next to my friend Derek near the middle. As we drove along the country roads, we started to wake up and talk about the day to come.

Our bus ride took two hours, with a restroom break in the middle at a gas station. When the bus finally stopped at the zoo, we rushed off, and I was energetic from the soda and candy I purchased at the gas station. Before we could see any animals, our teacher had to register us up at the front. I was surprised by how packed the zoo entrance already was, and with my sugar rush, I was pacing in front of the ticket booth. Finally, after ten minutes we entered the gates.

The first exhibit was a large cage of birds, with many of them staying tucked in the corners of the cage. The ones that I could view up close seemed pretty quiet. The group was led between two bird cages, where most of my classmates quickly pushed past to see some of the larger animals.

“This zoo is boring” Derek said.

At this point, our teacher had already given up on keeping the group together. Derek and I ended up falling farther towards the end of the pack. I did not see any reason to rush, as we would be at the zoo all day. On my right, I could see a smaller cage. The sign read “capybara”. After about a minute of searching, I could see a large rodent sitting under a tree next to a small pail of water. This trip is not quite as exciting as I thought it would be.

Next to the capybara cage, I saw a sign promoting their penguin exhibit. Now that’s more like it. Before I could find the building for the penguin exhibit, I saw an enclosure with two zebras. I had never seen a zebra in-person before. I got really close to the enclosure, but could barely make out the zebras hidden behind the brush. I started to get frustrated by all of the animals not doing anything, so I began shaking the fence. A zookeeper ran out and told me to stop.

Finally, I got to the penguin exhibit and felt devastated—the exhibit was currently closed! My parents would never take me anywhere I wanted to go as a child, and now I wouldn't even get to have fun on a school fieldtrip.

I slogged from the penguin exhibit, not really paying much attention to the inhabitants of the smaller cages when I stopped in my tracks. Up ahead I could see several children all huddled in front of a much larger cage. In my excitement, I pushed past a couple of kids in order to get to the front of the cage. Walking from side to side was a strong, majestic lion. I had never seen anything like it before. If it wasn't for the foul manure smell, it would have made my day.

In the end, both Derek and I agreed that the next zoo field trip needs to end with an hour at the mall.

Story Two:

**Sticks and stones may break my bones**

**But words will never hurt me.**

I can still remember sinking into my chair when my middle school teacher wrote that ridiculous rhyme on the blackboard. Every day I was living the reality of being hurt by words. My well-meaning teacher gave me yet another reason to feel small and stupid, as if I needed one.

I was bullied throughout most of middle school, mostly by one girl. It was that insidious type of bullying that can go unnoticed by teachers for years. My stress at the time was so high that I do not remember all of the details. But, I do remember often lying in bed and trying to stay awake as long as I could on school nights. I wanted to delay having to wake up and face another day of humiliation. There were weeks when this girl and I would have an odd kind of pseudo-friendship, but it never lasted. The wind would change and I would again become the target of her bullying. Because she was quite a popular girl, some of my classmates would join her in tormenting me.

Kind and well-intentioned adults who had probably never been bullied said things like "Don't rise to it", "Ignore her and she'll get bored", and "Ask your teacher to sort it out." I trusted adults so I did what they told me to do. But it didn't work, it just added 'tattle-tale' to the list of verbal sticks and stones that were flung at me when teachers were out of earshot.

There were also negative consequences for my family. It is often said that a school bully is likely to be someone with a difficult home life, but the humiliations at school is why I had problems at home. No one thinks about the child who, after a day of diligently ignoring bullies and keeping their rage inside, goes home and takes it all out on family members. Because I was so powerless at school, I became a complete nightmare at home. Anger does not disappear when it is suppressed, it simply looks for a different outlet. Throughout high school I had a destructive relationship with my family, constantly yelling at my parents and wanting nothing to do with my brother.

When I began high school, I was relieved that another girl was the class punching bag. Making fun of her was a team sport, and I desperately wanted to belong. I had been on the margins throughout most of middle school and I finally had a chance to free myself from that.

One day I made a cruel remark to her. She turned around with hurt and anger in her eyes and snapped back at me. I don't remember what she said – but I do remember how disgusted I was with myself at that moment. Her reaction reminded me of exactly how it felt to be bullied.

I cried much of that night, and I resolved to tell her that I was sorry the next day. I walked up to her before classes and apologized. I told her that I had been badly bullied in

middle school and was mad at myself for being cruel. She asked me if I wanted to talk after school, and I said okay. After school I told her my story and she told me some of hers. She told me about cutting herself on the arm, and how the physical pain eased her hurt from the bullying. We left agreeing to be friends, and that we should talk to each other when things went wrong at school.

We met after school a couple days later when a few girls stuffed a hateful message in her locker. They called her a whore. The following weeks we briefly talked a couple more times, but then she transferred schools.

It was years later that I saw her on Facebook. I sent her a message and we spoke on the phone. She said that she switched schools to get away from the bullying. Her parents had also saw the cutting on her arms and took her to counseling. I thought that my brief time as a friend did not do much, but she said that it made a huge difference. She was on the brink of suicide, and the friendship motivated her to get help.

# Appendix E. Materials

State Empathy Scale

The following questions relate to your feelings after reading the story. Rate the following statements on a scale of 0 to 4 with 0 meaning you agree "not at all" and 4 meaning you agree completely. Write you answer next to each statement.

1. The character's emotions are genuine.

2. I experienced the same emotions as the character when reading this story.

3. I was in a similar emotional state as the character when reading this story.

4. I can feel the character's emotions.

5. I can see the character's point of view.

6. I recognize the character's situation.

7. I can understand what the character was going through in this story.

8. The character's reactions to the situation are understandable.

9. When reading this story, I was fully absorbed.

10. I can relate to what the character was going through in this story.

11. I can identify with the situation described in this story.

12. I can identify with the characters in the story.

# Appendix F. Debriefing Form

## "The Impact of Personal Stories on Empathy and Cortisol"

Thank you for participating in this experiment! The purpose of this study was to examine story content and participant background on empathy and cortisol. The independent variables in this study were participant background and the different stories and the dependent variables were empathy and cortisol. Empathy was measured through the Interpersonal Reactivity Index and the State Empathy Scale, and cortisol was measured from a saliva sample.

This study tests the hypothesis that all participants will have higher empathy and cortisol levels to the bullying story than the non-emotional story. Also we expect that individuals who have experienced bullying will have higher levels of empathy and cortisol for the bullying story than individuals who were not bullied. Last, we expect that prior bullying experience will be associated with lower levels of baseline cortisol.

With this information, we hope to learn more about the different factors that influence empathy.

If you have any questions, please contact us. Megan Hurley, the Principal Investigator responsible for this project, can be reached at megan_hurley15@mymail.eku.edu. If you would like to learn more about the concepts in this study, including bullying or how cortisol can measure emotions and empathy, then read the articles listed below:

Barraza, J. A. & Zak, P. J. (2009). Empathy toward strangers triggers oxytocin release and subsequent generosity. *Annals of the New York Academy of Social Sciences, 1167,* 182-189.

Chen, G., Kong, Y., Deater-Deckard, K., & Zhang, W. (2017). Bullying victimization heightens cortisol response to psychosocial stress in chinese children. *Physiology & Behavior, 46,* 1051–1059.