

2015-5

# Measuring the Stress Level of Students Preceding an Exam and Post Animal Interaction Through the Use of Salivary Cortisol

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**Measuring the Stress Level of Students Preceding an Exam and Post Animal  
Interaction Through the use of Salivary Cortisol**

Honors Thesis

Presented in Partial Fulfillment of the Requirements  
For the Degree of Bachelor of Science in Biology  
at Salem State University

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Commonwealth Honors Program  
Salem State University  
2015

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## **Abstract**

Stress is a sensation that can be helpful in a dangerous situation but also very harmful in excess. The body naturally begins to fight off the stress response in order to try and diminish the negative effects that it causes in the body. There are many adverse effects to experiencing stress which could include vomiting, panic attacks, changes in breathing, constipation and possibly an increase in drug and alcohol usage (1). College students are known to experience massive amounts of stress mostly revolving around examinations. Animals have been used to reduce stress levels for many years now, and it has been shown that animal owners show significant health benefits due to the reduction of stress. In this experiment we collected salivary cortisol, the hormone responsible for the stress response, from students prior to an exam. Half of the students participated in animal interactions with a friendly dog from the local animal shelter and half of the students did not get contact with the animal. After running the saliva samples through an enzyme-linked immunosorbent assay, no significant difference between the cortisol levels of students with the animal interaction and students without animal interaction were found statistically. Because the sample size was so low, the outliers from each group had a significant effect on the results. If repeated, the sample size will have to increase in order to see the difference between the experiment and the control.

## Introduction

Stress and test anxiety are known to cause short term as well as long-term effects in all animals. It is shown in many studies that students suffer with extreme levels of stress and this can be detrimental to their health. Some effects of stress could include problems with the nervous



system, the musculoskeletal system, the respiratory system, cardiovascular system, endocrine system, gastrointestinal system and the reproductive system (1). The body begins to fight off the threat of stress by sending a response to the nervous system causing a release of cortisol; this makes the heart beat faster and changes the blood stream. At the time same

muscles begin to tense up which can cause muscle pain including headaches. Rapid breathing can occur because stress is associated with a breathing response. This harder breathing can turn into a panic attack. The release of cortisol and epinephrine causes the liver to create more glucose and give the body more unnecessary energy. Stress can also cause vomiting, constipation and increase the use of drugs and alcohol due to the changes in the digestive system (1). Stress is

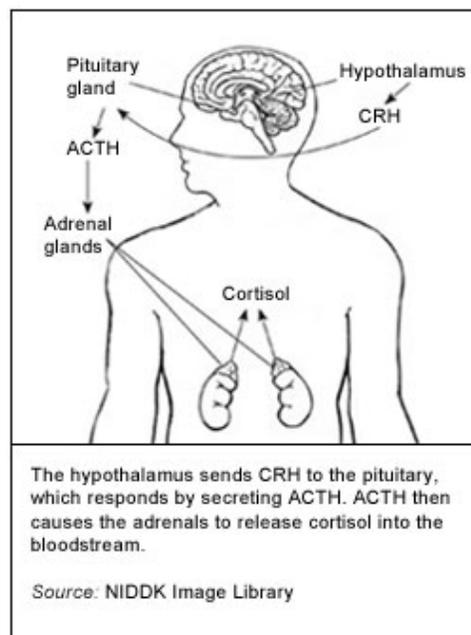
a serious problem that is hard to control but it is understood; there are solutions to managing stress.

College students experience many levels of stress throughout their educational career. The management of stress during this period of their lives is quite difficult. Students tend to resort to substance abuse, poor behavior and eventually an overall lack of care for school. One of the main factors of academic stress results from exams. According to the article, “Emotional intelligence, coping and exam-related stress in Canadian undergraduate students,” written by Elizabeth Austin, et.al stress can cause a decline in academic performance throughout the semester and there is a greater level of stress in pre-exam periods (2).

One way that has been proven to reduce stress in humans, in both long term and short-term ways, is animal interaction. After many years of study, the knowledge about the importance of the human bond with animals has grown from intuition-based ideas to actual evidence and facts (3). In 1961, a study was conducted showing that children with severe autism learned to socialize and communicate through the use of therapy dogs (3). Veterans that suffer a great deal with posttraumatic stress disorder can usually begin to live a normal life again through the trust and companionship of a dog (3). Also, people with disabilities, which can range from mental to physical disabilities, tend to interact socially with other people, due to having an animal with them. This gives people a topic to discuss and an opportunity to start a conversation with someone they normally would have ignored or avoided. According to the article, “Psychosocial and Psychophysiological Effects of Human-Animal interactions: The possible role of Oxytocin” Andrea Beetz, et, al. reviews 69 different studies that have been completed during the last 10 years regarding these topics (4). In general, these studies showed common effects of human-animal interactions. Many of these studies presented research for an increase in positive social

attention from others for people with companion dogs (4). Two different studies displayed the rise in trust in other people due to having a dog with them. Having a dog, in most of these studies, showed the effects on empathy. Although it is difficult to prove that empathy is caused by having an animal in the house, studies were done to show that students tested higher in empathy towards animals if they grew up with an animal (4). This article continues to talk about the positive effect of having an animal in their life by showing examples of how subjects had a reduction of aggression and depression, lower chances of high blood pressure, and less anxiety (4).

As previously mentioned, cortisol plays an important role in the stress response. Cortisol is synthesized through the adrenal glands. Adrenal glands are two triangular shaped glands that sit on top of the spleen. Cortisol is a hormone that is responsible for proper glucose functions, regulation of blood pressure, insulin release, immune function and inflammatory response (5). Generally, cortisol levels are higher in the early morning and lower throughout the day. When someone experiences sudden energy, low sensitivity to pain, heightened memory, etc. usually coinciding with the common fight or flight sensation it is because of the cortisol hormone (5). When levels of cortisol reach a high level in the blood and in saliva, a negative response could occur. This includes higher blood pressure, impaired cognitive performance, blood sugar imbalances and many more (5). Blood cortisol is usually bound to a protein, the free cortisol is found in the urine and saliva. The hypothalamus (usually for memory) releases a hormone that triggers the



pituitary gland to begin functioning (6). The pituitary gland then releases an adrenocorticotropic hormone that sends a message to the adrenal glands to begin producing cortisol (6). In order for cortisol to be made, all of these steps need to be intact.

Salivary cortisol has been used subsequently in the study of stress levels. In the article, “Salivary cortisol as a biomarker in stress research,” written by D.H Hellhammer, S. Wust and B.M. Kudielka the positive and negative aspects of using cortisol are explained (7). Hellhammer et.al describes there to not be a clear linear relationship between the Adrenocorticotropic hormone (ACTH) which, comes directly to the pituitary gland and is related to biological stress and cortisol in blood and saliva (5). Although salivary cortisol is used frequently in stress research it must be noted that there are dissociations that can cause variability in results. A study completed by Ng, V., Koh, D., Mok, B., Chia, S., and Lim, L., examined “How salivary biomarkers associated with academic assessment stress among dental undergraduates.” (8) Their study tested salivary biomarker before and after an exam, measuring cortisol, IgA (immunoglobulin A) and CgA (chromogranin A). The saliva samples were paired with a survey asking level of stress felt at the time on a one to five scale. After the exam was complete the saliva was tested again. Researchers found no difference in IgA and CgA levels before and after the exam but noticed a correlation between the survey and level of cortisol. Students who had high stress levels according to the survey also had higher cortisol levels, which significantly decreased after completing the exam. This research shows that salivary cortisol is an effective measurement of stress levels. Although the level of stress may have variances, cortisol can be used to test stress levels.

In this study we looked to determine if animal interaction prior to writing an exam, could decrease the stress level of students. Stress level was determined by saliva cortisol levels. The

students that were participating in the animal interaction portion petted and socialized with the dog for up to 15 minutes. There was no statistical significant difference between the salivary cortisol levels in either group, however, preliminary data shows that with increase sample size, change in collection time, and more intimacy with the interaction there may be a decrease in salivary cortisol in students who engage in animal interaction compared to students who did not engage in animal interaction.

## **Materials and Methods**

### *Study Participants*

Subjects for the study were recruited from a biology course with approximately 50 students enrolled. Ten students engaged in animal interactions. Eight students did not engage in animal interactions. All subjects had given written informed consent to participate. The goals of the study and possible risks were fully explained. This study was reviewed and approved by Salem State University's Institutional Review Board.

### *Animal Interaction*

Subjects were asked to meet a half hour before the exam. The North East Animal Shelter volunteered one of their adoptable dogs for the study. The dog was a black lab and shepherd mix who was about 7 months old. The students participating in the animal interaction met in a microbiology lab that had been prepared for the dog. Subjects sat on the ground and interacted with the dog for a minimum of 10 minutes. The average time was about 15 minutes with the dog. Interaction with the dog included petting the dog, talking at the dog, and socializing with other students about the dog. Students did not get individual time with the dog, the experiment occurred in a group setting.

### *Saliva Collection*

Sterile tubes were labeled by number, students salivated into the tubes. If needed, parafilm was used to aid in saliva production. About 2 ml of saliva was collected from each subject. Students were given a copy of the number that correlated to their saliva and asked to keep the number for further questions. The control group of students salivated in a tube in a separate room and had no contact with the dog. A number was written on the tube and given to the students to keep for further questioning. The saliva samples were frozen post collection.

### *Salivary Cortisol Analysis*

An enzyme-linked immunosorbent assay was used to test the level of cortisol in the saliva samples. The kit is manufactured by Eagle Biosciences, Inc. (Boston, MA). Regents, standards, and samples were prepared as described in the kit's manual. Samples and standards were run in triplets. Samples were incubated for 1 hour at 37°C. The contents from each well were removed and subsequently washed 3 times with wash solution. TMB was used as developing substrate. The plate was read at 450 nm with BioTek HTx plate reader and Gen5 Data Analysis software (Winooski, VT). Graphs were created in Excel.

### **Results**

The results of our experiment were inconclusive. The p value of our data was 0.066138. Further testing needs to be completed in order to get significant results.

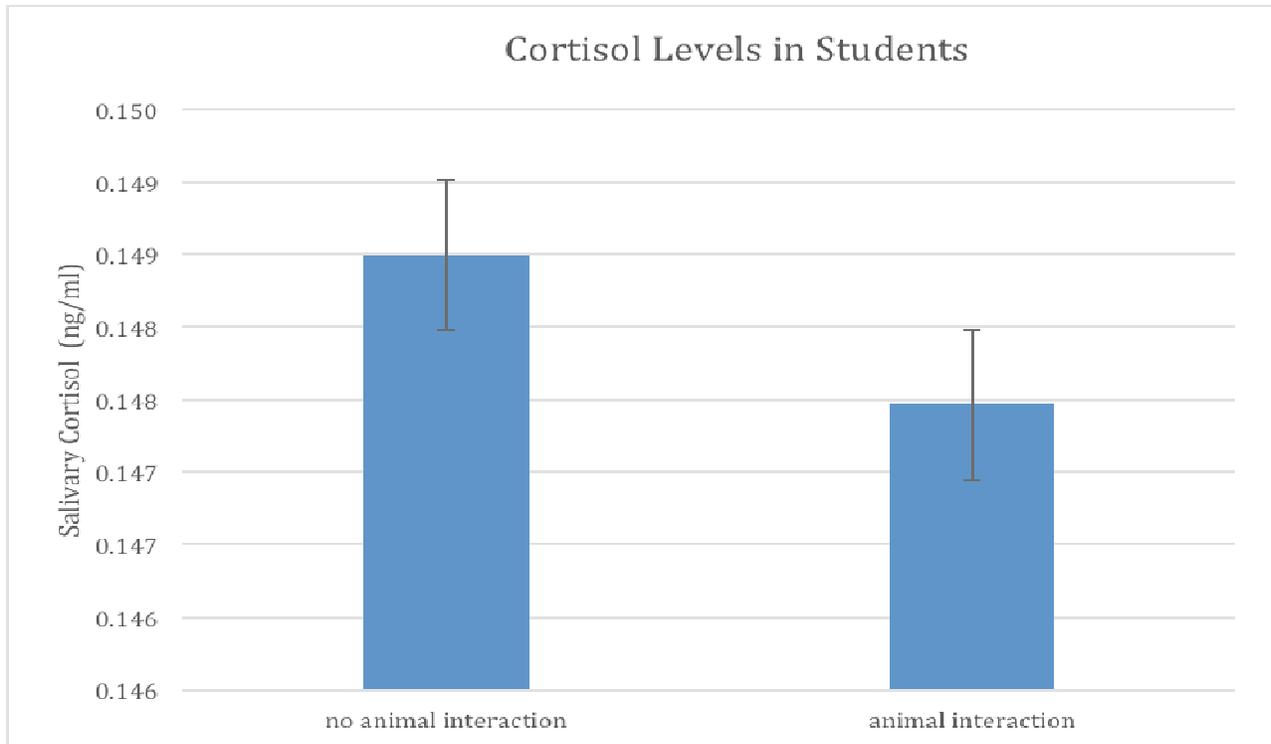


Figure 1: This graph shows the Cortisol Levels in Students comparing animal interaction and no animal interaction. The standard deviation lines slightly overlap showing that there is not a significant enough difference between students cortisol levels.

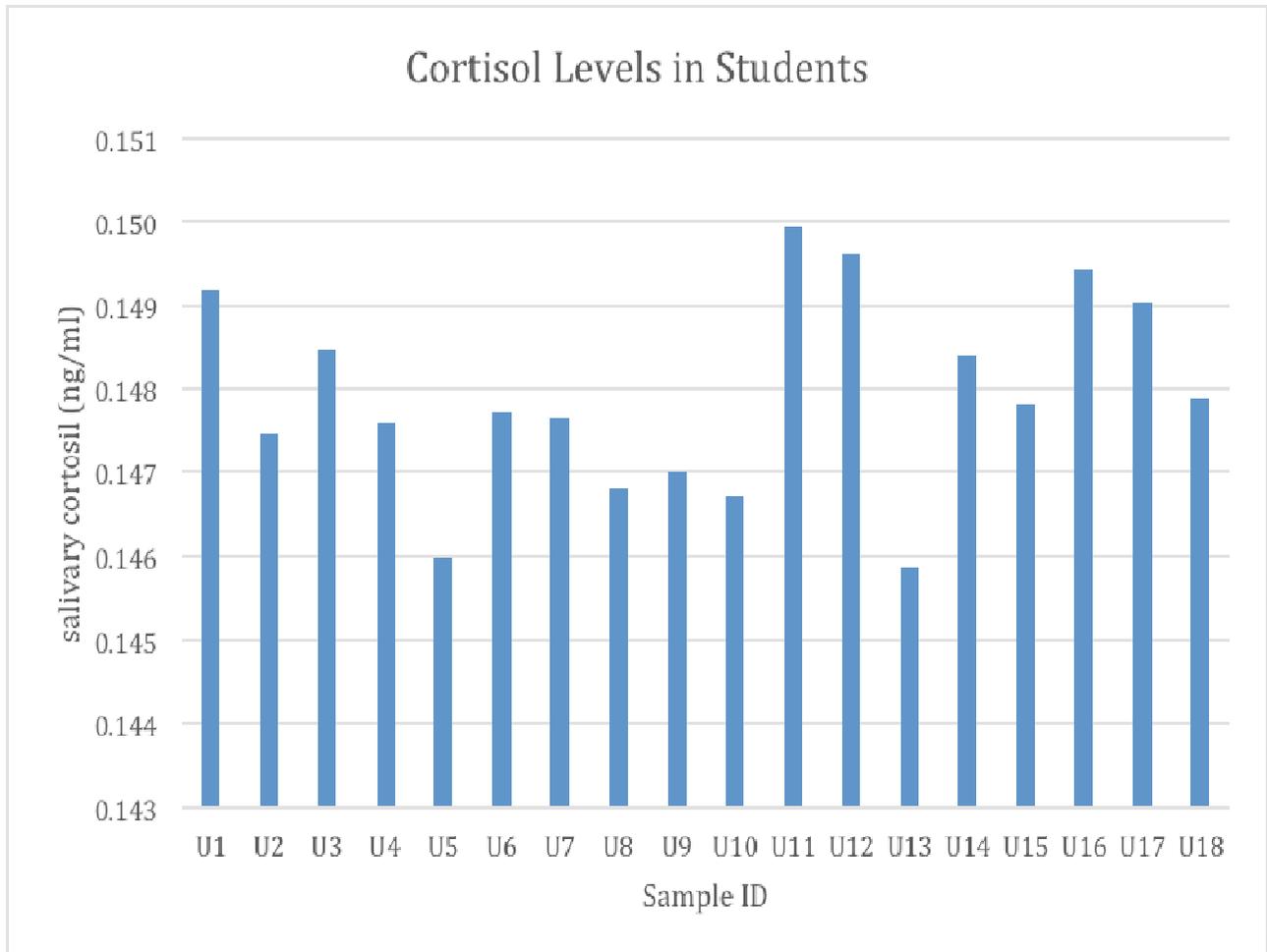


Figure 2. This graph shows the Cortisol Levels in individual students. U1 through U10 correlates with the students that participated in animal interaction, U11 through U18 were the students who had no animal interaction.

## Discussion

After completing the experiment it is evident that the experiment does not have a large enough sample size to support a statistical significant difference between the two groups-no animal interaction vs. animal interaction. In the future, the experiment should be repeated with a larger sample size. The results of the experiment had a p value of 0.06. Additionally, collecting surveys from subjects to determine if they were taking multiple different exams, interacted with

animal prior to arriving on campus, and even foods ate earlier in the day could help reduce error as this factors could effect the results of the salivary cortisol levels. There could be other factors causing stress in the students so having a larger sample size would be ideal.

In the future, beginning the experiment with a written survey to measure the level of stress from the subject's opinion may yield additional insight. It would also serve as gaining more information about the students, such as, whether they studied for the test or if they were even nervous for the exam. If this information was available more connections could be made to the positive benefits of reducing stress with animal interaction. This would require more of a time commitment for the subjects and the researchers but it will add a new level to the experiment that could be valuable.

As with any experiment with animal subjects, it was impossible to control what the students ate and drank before the collection. In order to get the most accurate levels of cortisol in saliva, eating and drinking should be limited as it changes the natural hormone levels. Including a pre-animal interaction survey which asks if they ate anything prior would allow us to determine if a subject's sample should be used.

Another important aspect of salivary cortisol collection is the time of day when the samples were collected. Through research, it is known that the highest and most accurate levels of cortisol appear in the morning. This experiment was completed during the middle of the day due to the time of the exam and convenience for the students and animal shelter. In future experiments, courses in the morning should be utilized. Naturally, everyone has different levels of cortisol. If samples were collected on a different day at the same time calculations, the change in cortisol levels could be measured due to heightened stress the examination is causing (6).

The animal interaction took place in a room near the exam location. This room is where many of these students have spent time for certain classes, which could bring back feelings of stress and discomfort. The environment would ideally be a calm and serene place that brings about relaxation in the students. Also, the students who interacted with the dog did so in a group setting. Ideally, the animal interaction should be limit to one on one or smaller group size. If students got to interact with an animal privately or in a small group, the levels of stress may be more likely to decrease and level of relaxation to increase.

The results of this experiment are inconclusive, but it leaves an opening for more testing. This experiment should be completed again with the changes mentioned.

### **Acknowledgments**

We would like to thank Salem State University's Honors program, the Institute Research Board, Dr. Nelson Scottgale and the Northeast Animal Shelter for their contributions

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