

Sex Differences in Cortisol Levels

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## Abstract

Any situation that upsets an organism's homeostasis is considered to be a stressor. This study focused on the differences present in salivary cortisol pre- and post-exercise and discusses any differences between males and females. By comparing the cortisol before and after a stressor, one is able to see if males and females handle and respond to stress differently. 10 males and 10 females participated in a 30 minute run at their choice of intensity. Heart rate and saliva samples were taken pre- and post-exercise. An enzyme-immunoassay (EIA) (Salmetrics) was used to analyze the cortisol concentrations within the saliva samples and averages for both the concentrations and heart rates were determined using standard methods. Heart rate post-exercise was significantly higher than pre-exercise heart rate in both males and females. The cortisol data showed a significant difference in males pre-exercise and females pre-exercise where the females had a higher salivary cortisol concentration. This study provided future stepping stones in understanding the differences between male and female stress responses.

*Keywords:* cortisol, exercise, heart rate, stress, sex differences

### **Sex Differences in Cortisol Levels**

Stress is any situation that upsets an organism's homeostasis. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). The result of HPA activation is an increase of cortisol. The activation of the SNS results in an increase in norepinephrine levels. Both the HPA axis and the SNS are attempts the body makes to return to homeostasis. The SNS originally evolved as a survival mechanism, enabling organisms to react to immediate life-threatening danger (Harvard Medical School, 2011). The stress response is an instantaneous, yet complex, series of hormonal changes and physiological responses (Saladin, 2012).

The body reacts to stress in a consistent way known as the general adaptation syndrome (GAS). In 1936, Hans Selye presented the GAS tends to occur in three stages (Saladin, 2012). The first stage is known as the alarm reaction, which is the immediate response to stress that gears the body for fight or flight. The second stage, known as the stage of resistance, is when the body switches to alternate fuels for metabolism when the initial stores are depleted. Once the alternative fuels are gone, the third stage sets in, known as the stage of exhaustion. In this stage the body primarily relies on protein for fuel. If stress lasts too long, the body can break down and weaken during this stage (Saladin, 2012).

The alarm reaction is the initial response to stress and involves activation of the SNS. Activation of the SNS is also commonly referred to as the fight-or-flight response. The SNS prepares the body for physical activity by increasing blood flow to the muscles, alertness, and heart rate. At the same time, the SNS reduces blood flow to the skin and digestive tract (Saladin,

2012). The catecholamines norepinephrine and epinephrine are secreted during a stressful situation and prepare the body to take action (fight) or to escape (flight). Norepinephrine, secreted in smaller doses, is most significant in causing vasoconstriction throughout the body in the skin, viscera, and skeletal muscles, to raise blood pressure. Epinephrine is secreted in larger amounts and increases heart rate, use of fats for energy, cell respiration, decreases peristalsis, and causes vasodilation in skeletal muscles (Scanlon & Sanders, 2007). The SNS also acts as a brake on systems that are not directly involved with stressful situations. Digestive and urinary systems will slow during times of stress to provide necessary energy to other parts of the body (Taylor & Cohen, 2013). The hypothalamic-pituitary-adrenal (HPA) axis is also activated during short-term stress, although it is a much slower response.

Short-term stress, like the stress of an upcoming exam, can be beneficial to the body. Significant, but brief, stressful events have the ability to improve brain performance. Kirby et al. (2013) found stem cells in rats to proliferate into new nerve cells in reaction to short-term stressors. Two weeks later, these cells matured to improve the rats' mental performance, including memory functioning.

If the stressor is not resolved after the glycogen reserves are depleted, the body enters the stage of resistance. The stage of resistance is most common in chronic stress, like ongoing work pressure ("Stress," 2013). Chronic stress also activates the fight-or-flight response while activating the second response to stress: The hypothalamic-pituitary-adrenal (HPA) axis, which is more prevalent during long-term stress. As the brain continues to perceive something as a threat, corticotropin-releasing hormone (CRH) is released. CRH travels to the pituitary gland and triggers the release of adrenocorticotrophic hormone (ACTH). ACTH then travels to the adrenal glands, which prompts a release in cortisol, the stress hormone (Moyes & Schulte, 2008). The

stage of resistance is dominated by cortisol. Cortisol promotes the breakdown of protein and fat, providing the body with an alternate fuel for metabolism (Saladin, 2012).

The third stage of stress is known as the stage of exhaustion. Exhaustion sets in when stress has been present for an extended period of time (Saladin, 2013). At high or chronic levels, stress will make a person more susceptible to illness, infection, and some forms of cancer (Salleh, 2008). Stress can become harmful when there is a long-term increase of cortisol. Too much cortisol decreases one's immune response, which leaves the body susceptible to disease and infection (Scanlon & Sanders, 2007). Chronic stress also suppresses the ability to secrete sex hormones causing disturbances in fertility and sexual function. After prolonged stimulation, it becomes difficult for the HPA axis to maintain glucose homeostasis. When the body's fat reserves are depleted, the body primarily relies on protein breakdown to meet energy needs, which results in a breakdown of muscles and the weakening of the body. Heart failure, kidney failure, or infections can result in death at this stage (Saladin, 2013).

Exercise is considered a voluntary stressor because it activates the SNS as well as the HPA axis, but it is controllable. There is an increased demand for energy during exercise. The response levels of the SNS and the HPA axis may vary between different kinds of exercise, depending on the rigor and intensity of the exercise. The more strenuous the exercise is, the more it will promote the secretion of cortisol and be considered a stressor. Tanner, Nielsen, and Allgrove (2014) confirmed that salivary cortisol post-exercise was higher than pre-exercise in interval and tempo runs with endurance-trained men. The researchers collected saliva and blood samples pre- and 0, 15, 30, and 60 minutes post-exercise. Their trials included interval training, tempo runs, and bodyweight-only circuit training.

Obmiński (2008) confirmed that pre-competition female athletes had higher cortisol levels as compared to their male counterparts. He also found that menstrual cycles did not have an impact on the cortisol levels. Obmiński placed both males and females under similar conditions within different sports including volleyball, judo, and taekwondo. The researchers measured blood cortisol before a competition and again during a non-exhaustive period. This study supports the hypothesis of sex differences in cortisol secretion when the body is placed under other voluntary stressors.

Both bound and free cortisol can be measured in blood, while only free cortisol appears in saliva. Salivary tests provide a measurement of the biologically active fraction of cortisol (Kirschbaum & Hellhammer, 2000). Blood samples are the preferred method of measuring cortisol levels, but the tests can be invasive and painful. The assessment of salivary measures of free cortisol has become an increasingly important tool in stress research (Kirschbaum & Hellhammer, 2000). The fact that saliva samples can be taken in the field while being non-invasive and without raising ethical issues associated with venipuncture leads salivary testing to be a convenient alternative for researchers. The levels of unbound cortisol in the saliva and the blood have been found to be correlated. Due to the similar concentrations of cortisol in blood and saliva, researchers are able to use salivary samples to measure stress as they would with blood samples.

There are numerous studies done comparing salivary cortisol levels pre- and post-exercise, as well as differences in men and women. There has not been a study done combining both aspects. This study focused on the differences present in salivary cortisol pre- and post-exercise and discusses any differences between males and females. It was hypothesized that there will be an increase in cortisol levels post-exercise and men will have a

higher overall cortisol levels throughout the trials. Participants provided saliva samples before and after a 30-minute run. Data was analyzed to show differences, if any, between the samples.

### **Materials and Methods**

Funding for this research was applied for and granted through the Department of Natural Sciences at the University of Wisconsin-Superior. Materials and methods were submitted to the Institutional Review Board (IRB) and deemed acceptable for testing. Recruitment began after approval was granted by the IRB. Volunteers were sought out to collect samples before and after exercise.

Twenty participants (10 males and 10 females) were included in this study. The average age of the male group was 27 years. The mean age of the female group was 21 years. The males weighed an average of 198 pounds and the females weighed an average of 153 pounds.

The subjects completed a written form of consent and a questionnaire about their general characteristics including age, height, and weight. They then performed a workout, which consisted of 30 minutes of running at their own intensity and comfort level. Saliva was collected twice, once before exercise and once after exercise, in order to measure cortisol. Heart rate was measured twice (pre- and post-exercise) using a finger heart rate monitor.

An enzyme immunoassay (EIA) was used to analyze the cortisol in the saliva samples. The samples were brought to room temperature and centrifuged for 10 minutes each. The reagents were brought to room temperature and mixed briefly. A wash buffer was prepared by diluting the wash buffer concentrate with deionized water in a 1:10 ratio. The plate was then prepared by pipetting 25 uL each of the standards, controls, unknown, and assay diluent into the appropriate wells. Next 15 uL of the conjugate was added to 24 mL of the assay diluent, and

then 200 uL of the mixture was immediately pipetted into each well using a multichannel pipette. The plate was mixed for 5 minutes then incubated at room temperature for an hour. The plate was then washed four times using the wash buffer, and 200 uL of the TMB solution was added to each well. The plate was mixed again for 5 minutes and incubated in the dark for 25 minutes. Finally, 50 uL of the stop solution was added to each well then mixed for 3 minutes. The plate was read by a plate reader at 450 nm.

### **Statistical analysis.**

Averages for heart rate and cortisol concentrations were determined using standard methods. Relationships between the cortisol concentrations were assessed using T Tests. The data was examined as a pooled group, by gender and time period.

### **Results**

There was no statistical difference between heart rate in males versus females pre-exercise and post-exercise. Heart rate post-exercise was significantly higher than pre-exercise heart rate in both males and females (See Appendix A). The cortisol data showed a significant difference in males pre-exercise and females pre-exercise where the females had a higher salivary cortisol concentration. There were no significant differences between males pre- and post-exercise or females pre- and post-exercise. There was a non-significant trend present between males and females post-exercise.

### **Discussion**

This study focused on whether or not there was a significant difference between cortisol levels in males and females pre- and post-exercise. It was hypothesized that females would have



a higher cortisol level post-exercise than the male participants. Previous studies considering the heart rates, salivary cortisol concentrations, and differences between males and females pre- and post- a 30-minute run do not exist. This study is the first to consider these trends.

Similar studies have discovered a difference between male and female cortisol concentrations post-exercise. Obminski noticed that females have a much higher cortisol level post-athletic competition than males after the same competition (2008). Obminski (2008) concluded that females may have a higher susceptibility to psychological stress, resulting in higher cortisol levels after exercise. This is concurrent with the results found in this study. The average cortisol concentration of females post-exercise was higher than that of males post-competition (See Appendix B).

A significant difference in cortisol concentrations between males or females pre- and post-exercise was not found, as previous studies have done. VanBruggen et al. (2011) reported a higher cortisol concentration after high-intensity exercise in aerobically trained males. The researchers had the subject perform at 80% of their maximal oxygen uptake and provided a saliva sample before and after exercise. Sperlich et al. (2012) also reported a higher cortisol concentration after a qualifying heat in elite male downhill racers. Participants took a saliva sample before and after the qualifying heat for a championship race. The absence of a difference within our study could be due to the short amount of time spent exercising. Participants may not have put enough stress on their bodies during the run to see a difference. An increase in time exercising may increase the cortisol concentrations within the saliva samples post-exercise, creating a larger difference within the results.

Another variable that may have altered the data was intensity of the exercise. Controlling the intensity of a run for 30 minutes poses multiple challenges. Some participants may have trouble running a full 30 minutes while others may do so with ease. This lack of control over the exercise method may have affected the observed results. By having more control over the intensity of the exercise, there would be a higher chance of a significant trend to be present within the samples.

This study also found an increase in heart rate post-exercise in both the male and female participants. This is concurrent with other studies requiring participants to measure their heart rate. In research conducted by Sperlich et al. (2012) participants had a significant increase in heart rate after an elite downhill mountain bike race. This trend is relevant in almost all individuals post-exercise, since the body needs more oxygen in the muscles to be efficient.

Heart rate was also a factor in the intensity and overall fitness of the individuals. Being able to control the fitness level of the participants and grouping them accordingly could have shown us a trend in the data. A higher fitness level may mean an increase in the ability for an individual to handle stress adequately. Having volunteers from a sports team that undergoes the same conditioning may have eliminated outliers within the data sets. These athletes may have been able to control their heart rate throughout the run, as well. Having a consistent heart rate throughout the participants may also eliminate some of the outliers.

This study was a stepping-stone for future research to be conducted. The trends found within this study allow for more questions to be asked. Eliminating any outliers and putting more control over the variables may lead to more compelling answers to the questions left unanswered.

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## APPENDIX A

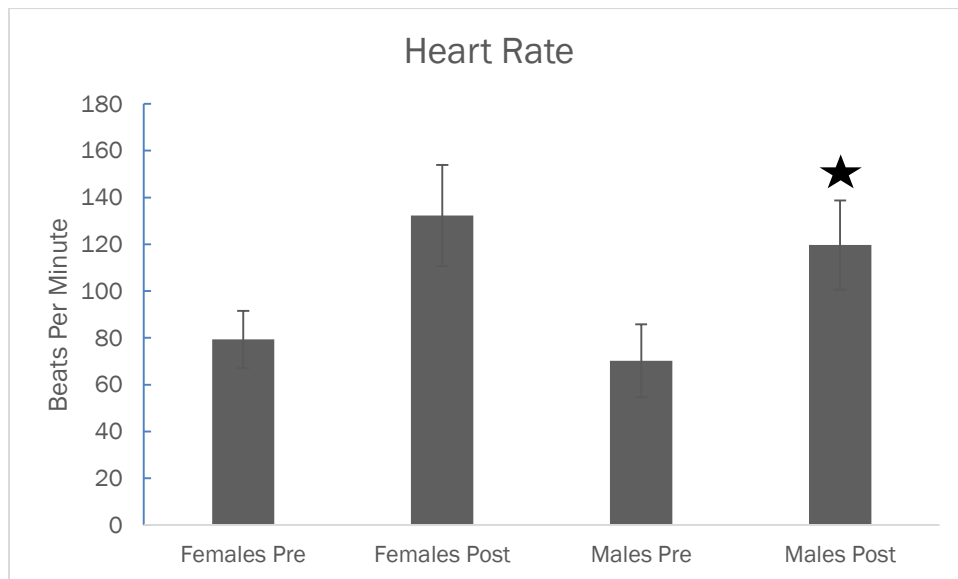


Figure A1: Heart rate in males and females before and after exercise. Heart rate post-exercise was significantly different from pre-exercise in both males and females. ★ =  $p < 0.05$  in each cohort versus pre-exercise.

## APPENDIX B

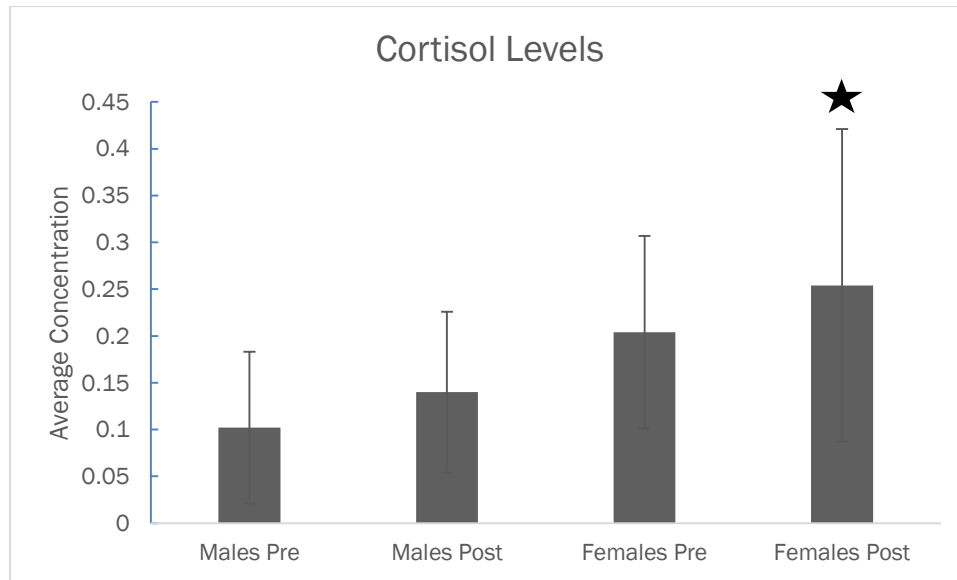


Figure B1: Cortisol concentrations for males and females before and after exercise. Cortisol concentrations in females post-exercise were significantly different than males post-exercise.

★ =  $p < 0.05$  versus males post-exercise.