

Stress Response and Salivary Cortisol Levels in Collegiate Wrestlers

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A Research Paper

Submitted in Partial Fulfillment of the

Requirements for the

Master of Science Degree

In

Food and Nutritional Sciences

Approved: Six Semester Credits

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August, 2008

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Title: *Stress Response and Salivary Cortisol Levels In Collegiate Wrestlers*

Graduate Degree/ Major: MS Food and Nutritional Sciences

Research Adviser: Carol Seaborn

Month/Year: August, 2008

Number of Pages: 56

Style Manual Used: American Psychological Association, 5th edition

ABSTRACT

The purpose of this research project was to examine the changes that occur in salivary cortisol in wrestlers during a collegiate wrestling season and to evaluate if this population has higher cortisol levels than the standard for men of the same age. Subjects used consisted of 11 National Collegiate Athletic Association (NCAA) Division III wrestlers. All wrestlers were starters on their team. Salivary cortisol and weight were measured on all subjects leading up to a major competition. The first day of data collection took place one week prior to competition, the second day of data collection took place one day prior to competition, and the third day of data collection took place one month after the season had finished. Salivary cortisol should reflect changes in weight and stress levels leading up to competition.

This study found that mean salivary cortisol was the highest the day before competition when weight reduction and stress were greatest. Subjects had significant increases in off-season cortisol compared to values of standard men of their same age. There was no significant difference in cortisol levels between the one week before competition sampling day and off-season values for the subjects.

In conclusion, weight reduction and stress levels leading to competition in collegiate wrestlers does impact cortisol levels. Increases in weight loss and stress levels resulted in higher morning salivary cortisol levels compared to standard values. More research with a larger sample size needs to be conducted on the topic to examine the impact on health and performance of athletes participating in this sport.

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Acknowledgements

I would like to thank Dr. Carol Seaborn for advising me, not only on my thesis, but during my academic career at the University of Wisconsin-Stout. She has played a critical role in my success and always challenged me to go beyond my own expectations. Dr. Seaborn has been a mentor for me throughout my graduate studies and her commitment to the students is what got me through the tough times. I also want to thank Dr. Charlene Schmidt and Dr. Janice Coker for being members of my committee, for all the great help throughout my research, and for molding my paper into its finished product. They were very patient and always reassured my progress throughout my journey.

I want to thank Dave Malecek for his insight regarding my topic and for helping me gather my subjects during each data collection period and Dr. Hershel Raff and Dr. Douglas Granger for their expert advice in the design of my research paper. I also want to thank the University of Wisconsin-Stout Student Research for funding my research. Without the funding my research would not have been possible.

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Chapter I: Introduction

Wrestling is considered, by many, to be one of the world's oldest sports. It takes strength, power, composure, and self-discipline to become an efficient competitor. These athletes go through rigorous training regimes and weight reduction programs in order to compete at a specific weight class in competition.

Statement of the Problem

Under the stress of rigorous training and weight reduction, it is crucial to maintain strength and power throughout the duration of the lengthy wrestling season. This allows athletes to compete at elite levels. For this reason, hormone testing may prove to be a good marker for performance preparation in situations producing high levels of stress. A hormone is a chemical secreted by a cell or group of cells into the blood for transport to a distant target, where it exerts its effect at very low concentrations (Silverthorn, 2001). More specifically, steroid hormones produced in the adrenal glands effect many different areas of the body. The most important of these hormones applicable to wrestling and the general population are aldosterone, cortisol, and the sex hormones (Gunstream, 2006).

Cortisol is the most important class of steroid hormones called glucocorticoids, according to Gunstream (2006). It is, many times, considered the "stress hormone" because it is increased in the body under high stress situations. When serum cortisol levels are within a normal range, cortisol maintains glucose metabolism, protects the immune system, and has anti-inflammatory action, which is evident in prescription medicine (Bowen, 2006). These issues are of imminent concern with high-level athletes, such as wrestlers. It is important for these athletes to maintain high energy levels and muscle mass. This is why cortisol, which impacts energy and muscle mass, is closely related to performance.

It is a constant challenge to compete at high levels when such drastic weight reduction occurs. A study by Yanovski, Yanovski, Gold, Chrousos, Scavo, Barletta, Buzzetti, and Vagiri, 1997 (cited in Toda et al., 2001), stated that temporary increases in cortisol levels may be considered a normal phenomenon during intense exercise training and competition. During these times of high stress the endocrine system goes through changes to prepare the body to react or retreat. This process is called the “fight or flight” response, which is characterized by increased cortisol secretion from the adrenal glands (“Fight or Flight Response,” 2008). Functions of the cardiovascular system affected by cortisol cause an increase in heart rate and strength, which allows more blood to be pumped to reacting muscles. The respiratory effect causes short, accelerated breathing, which increases oxygen rich air to needed tissues (“What is Anxiety?,” 2002) and the body utilizes glucose for short, concentrated bursts of energy. The issue of concern, however, is not the acute increase in cortisol, but the prolonged increase that may be seen in collegiate wrestlers in association with weight reduction.

Weight reduction to obtain a spot in a lower weight class may occur throughout a wrestling season and is very demanding on the body. There are 10 weight classes, ranging from body weights of 125 lbs. to 285 lbs. Along with the loss of weight, often a loss of power and lean muscle mass follow. It is difficult for many athletes to lose high amounts of weight while maintaining a high level of athleticism. Weight reduction is demanding and stressful on wrestlers; however, weight loss has been part of the sport for many decades. Fluctuations in weight can be seen for months and may bring about prolonged changes in hormone levels. More research is necessary on the relationship between cortisol and weight reduction.

Sampling cortisol is a concern because there are only a few methods from which to choose. Testing, in the past has been done through blood or urine. Blood sampling is a very

invasive process where blood is drawn from a vein, usually on the inside of the elbow or the back of the hand. The puncture site is cleaned and an elastic band is placed around the upper arm to apply pressure and allow blood to flow below it. The needle is then inserted into the vein and blood is collected into a syringe. Urine testing may not be as invasive as blood testing; however, it can be just as uncomfortable for the patient. This test often calls for a 24-hour urine sample. This includes collecting all urine throughout the day into a container; the first urination in the morning discarded. Recent studies have been experimenting with a collection of saliva as an alternative to blood and urine sampling. Reliable determination methods of salivary cortisol levels have now been established (Toda et al., 2001). Saliva samples clearly present an easily manageable, non-invasive and practical assessment method of glucocorticoid production (Poll et al., 2007).

Many people become anxious at the sight of a needle. As already proven, such unwanted stress will elevate cortisol levels in the body and may jeopardize valuable and expensive data collection. In order to perform such sampling on athletes already subjected to great amounts of stress, salivary cortisol testing is an appropriate test to conduct. For clinical use, saliva testing may prove to be an easy test that allows patients to collect their own samples in the comfort of their own homes (Nussey & Whitehead, 2001). Saliva testing is conducted using an Enzyme-linked Immunosorbent Assay (ELISA).

Purpose of the Study

The purpose of this research project was to examine the changes that occurred in salivary cortisol in wrestlers during a collegiate wrestling season and to evaluate if this population had higher cortisol levels than standard men of their same age. Cortisol is a hormone that is increased during stressful situations, that might be affiliated with wrestling training regimens. Past research has shown that prolonged increases in cortisol can have negative effects on elite athletes

including muscle wasting, increased abdominal fat, immunosuppression, impaired glucose metabolism, increased blood pressure, and osteoporosis (Bowen, 2006, & “Addison’s Disease,” 2007). These effects can have detrimental outcomes to performance and health. Wrestlers may have a fluctuation in salivary cortisol due to competitive stress associated with weight reduction, anxiousness, and academic classes that may induce stress and thus, a rise in cortisol. Saliva collection is a non-invasive, stress-free sampling technique that gives representative values of serum cortisol. Currently, little research has been done on wrestlers, although some research has been done on endurance athletes and obese individuals to determine the link between the impact of stress on their health and performance. There are about 342 recognized collegiate wrestling teams (“College Wrestling by Division,” 1996). With over 20 athletes on each roster, this is a topic of concern for many coaches and athletes across the country.

Research Questions

1. Is there a correlation between salivary cortisol concentrations and weight reduction in collegiate wrestlers leading up to competition?
2. Do collegiate wrestlers have different cortisol levels than standard, non-athletic men of the same age?
3. Is there a difference between salivary cortisol levels from one week prior competition to one day prior competition?
4. Is there a difference between salivary cortisol levels from one day prior competition to off-season values?

Assumptions and Limitations

This study assumed that the population of wrestlers from this one National Collegiate Athletic Association Division III team was similar to other wrestlers from other National Collegiate Athletic Association teams. It was assumed that each wrestler routinely wakes in the morning of each day. Waking in the morning will set the circadian rhythm in each subject. It is assumed that saliva collection times were an accurate estimate of morning cortisol. It is also assumed that each subject followed the pre-testing instructions. Limitations to the study included all the omitting variables that were not accounted for that may increase stress levels.

Definition of Terms

Adrenalectomy is the surgical removal of one of both of the adrenal glands.

Anabolism is the phase of metabolism in which simple substances are synthesized into the complex materials of living tissue.

Autoimmune disease is a disease in which impaired function and the destruction of tissue are caused by an immune reaction in which abnormal antibodies are produced and attack the body's own cells and tissues.

Catabolism is the metabolic breakdown of complex molecules into simpler ones, often resulting in the release of energy.

Dexamethasone is a synthetic glucocorticoid used primarily in the treatment of inflammatory disorders.

Glucocorticoids are a group of steroid hormones, such as cortisol, that are produced by the adrenal cortex, are involved in carbohydrate, protein, and fat metabolism, and have anti-inflammatory properties.

Gluconeogenesis is a process that forms glucose (energy), by the liver, from noncarbohydrate sources, such as amino acids, lactic acid, and the glycerol portion of fats.

Glucose is a monosaccharide sugar widely occurring in most plant and animal tissue. It is the principal circulating sugar in the blood and the major source of energy of the body.

Homeostasis is the ability or tendency of an organism or cell to maintain internal equilibrium or resist change by adjusting its physiological processes.

Hypercortisolemia is a term used to describe high levels of serum cortisol.

Hypocortisolemia is a term used to describe low levels of serum cortisol.

Lipolysis is the hydrolysis or breakdown of lipids (fat).

Macrophages are large white blood cells that play an immunologic role in humans.

Mineralocorticoids are a group of steroid hormones, such as aldosterone, that are secreted by the adrenal cortex and regulate the balance of water and electrolytes in the body.

Myopathy is a disease of muscle or muscle tissue.

Myostatin is a growth factor that limits the growth of muscle tissue.

Osteoblast is a cell from which bone develops; a bone-forming cell.

Osteoclast is a cell that functions in the breakdown and resorption of bone tissue.

Vasoconstriction means constriction or narrowing of a blood vessel.

Methodology

This study investigated the change in salivary cortisol as it related to weight reduction in NCAA Division III wrestlers from off-season to in-season at University Wisconsin-La Crosse during the months of December, January, and April of 2007/2008. Data was collected one week before competition, one day before competition, and during the off-season. Saliva was analyzed using an Enzyme-linked Immunosorbent Assay.

Saliva samples were collected from collegiate wrestlers from the University of Wisconsin-La Crosse Wrestling Team. Saliva samples were collected two times during the season (one week before competition and one day before competition) prior competition and one

saliva sample was collected out of season. One week before the in season competitive the first set of saliva samples were collected from each subject. On this day two samples were collected from each subject in order to observe a more accurate overall average of cortisol circulating in the body. The second day of sampling was done the day before competition. The levels of cortisol were hypothesized to be directly related to the change in stress levels and weight reduction.

The second set of testing was conducted a month after the season had finished (one sample taken upon awakening and one taken one half hour later). This allowed samples to reflect everyday life without the stress of training and weight reduction. Baseline, off-season cortisol levels for these athletes were compared to standard values for males of the same age and to the athlete's competition values. Immediately after samples were collected they were cooled and placed into a non-frost freezer to ensure the integrity of the samples. Once all samples were collected, they were shipped with dry ice, in order to keep frozen, to Salimetrics. Salimetrics is a diagnostic testing company based out of Pennsylvania that has processed samples for similar studies and has provided instructions on sampling technique and storage. Testing was performed using an Enzyme Linked Immunosorbent Assay (ELISA). This is currently the most accurate and efficient manner to measure salivary cortisol.

Chapter II: Literature Review

This chapter will focus on topics that are pertinent to understanding cortisol and its importance in athletics. The topics will include an overview of the endocrine system, with emphasis on the hypothalamus, pituitary gland, and adrenal glands, an examination of stress and different physiological events that may occur, skills needed to be an effective collegiate wrestler, and the value behind salivary cortisol collection methods.

Stress Response

According to Chrousos and Gold (cited in Hausmann, Vleck, & Farrar, 2007), stress is the sum of biological reactions to intrinsic and extrinsic stimuli that results in a perturbation from homeostasis. It is a physical expression of our “fight or flight” survival mechanism. A situation that threatens life and safety triggers a primal physical response from the body, causing breathlessness, a pounding heart, and racing mind (“Stress System Malfunction,” 2002). This stress response prepares the body to confront or flee possible dangerous situations. This can be a healthy response that allows people to run away from a fire, help somebody who is presented with a life threatening injury (“The Health Effects,” 2008), or compete in a high intensity athletic competition. However, the stress response can also be activated under less physical situations, such as an academic exam, boss conflict, or late bills.

When the brain perceives a threat or danger, messages are sent to a section of nerves called the autonomic nervous system (ANS), which then activates the secretion of certain chemical signals that prepare the body to react (“What is Anxiety?,” 2007). The ANS is a regulatory branch of the central nervous system that helps people adapt to changes in the environment. It adjusts or modifies some functions in response to stress. The ANS helps regulate blood vessel size and pressure, the heart’s ability to contract, and the bronchiole’s diameter in the

lungs (American Heart Association, 2008). It acts through the balance of two components, the sympathetic nervous and the parasympathetic nervous system.

The sympathetic nervous system is the actual system that releases energy and prepares the body for action. This is where the “fight or flight” response is derived. The nerves of the sympathetic system direct more blood flow to major muscles and the brain. During this time heart rate and blood pressure increase. The sympathetic nervous system is catabolic, so this system tears the body down. Energy is created and used for defense, not for nourishment or the elimination of waste. In contrast, the parasympathetic nervous system is associated with nourishing, healing, and regeneration of the body. It is anabolic and is activated by rest, relaxation, and happy mental thoughts (Wilson, 2007). The parasympathetic system restores the body back to normal during ideal conditions.

There are two kinds of stress: acute and chronic. Acute stress is a short-term response that may last for a few minutes or weeks. Hormones released during this type of stress trigger physical responses that increase blood sugar levels, increase the level of red blood cells that carry extra oxygen to muscles and organs, constrict peripheral blood vessels, increase pulse rate, cause blood pressure rises, and stop digestion (“The Health Effects,” 2008). This is a normal response to stress, then the parasympathetic nervous system shuts off the sympathetic nervous system and restores the body back to normal in a relatively short time period. The chronic stress response is when the body is kept on alert continuously, negatively affecting health (“The Health Effects,” 2008). During this type of stress, the body’s ability to turn off the sympathetic system via the parasympathetic system and return itself back to normal does not function properly (“What is Anxiety,” 2007). Collegiate wrestlers may experience many different types of stress throughout the season, causing chronic stress, which may have adverse effects on health and performance.

College life can be very stressful and if involvement in athletics and a job are thrown in the “mix,” everyday routines may be difficult for the body to cope with. A study by Hausmann, Vleck, and Farrar (2007) subjected twelve undergraduate students in the General and Comparative Endocrinology course at Iowa State University to presentation stress, fasting stress, and competition stress to determine the effect on salivary cortisol concentrations. The first experiment was a graded oral presentation that was conducted in front of a panel of professors and peers. The second experiment was a 15-hour fast and the third was an in-class competitive activity where students had to answer questions of varying difficulty. Saliva samples were collected in all three cases and the researchers found that students in each stressful situation had increases in salivary cortisol compared to basal levels. This study is illustrative of how cortisol levels rise due to college academics. Moreover, work related situations have been shown to bring about rises in cortisol. Maina, Palmas, and Filon (2007) investigated the association between work stress measures and salivary cortisol excretion by comparing work days and weekend days. The cortisol excretion on work days was higher than during weekend days. The researchers concluded that the HPA-axis activation was higher during working days than in weekend days.

Many teams perform meditation techniques leading up to major competitions in order to relax athletes and relieve stress. This is done because competitive stress can be a significant factor on the performance of the individual. Certain stress is “good” stress and prepares the body for rigorous events; however, persistent stress can hinder performance. A study published in the *British Journal of Sports Medicine* by Kraemer et al. (2008) examined hormonal responses to an ultra-endurance race. This race was a 160-kilometer race across frozen Alaska. They found that cyclists and runners both had significant increases in pre- and post-race cortisol values. This was the body’s way of preparing for an intense race. These research findings were supported in part

by a study titled, “Effects of Dietary Fat and Endurance Exercise on Plasma Cortisol, Prostaglandin E2, Interferon- γ and Lipid Peroxides in Runners,” by Venkatraman, Xiaohong, and Pendergast (2001). They found that, separately from dietary fat intake, that pre, during, and post-exercise plasma cortisol levels were significantly higher in these runners than baseline values. Contrary to the prior studies, a study in the *Journal of Environmental Health and Preventative Medicine* by Toda et al. reported the effect of weight reduction one day before a competition on salivary cortisol levels in judo players. They found that as weight reduction increased cortisol levels had a tendency to decrease. These are interesting findings compared to stress levels of other athletes that were significantly increased leading up to the competition, so the HPA-axis, as compared to studies previously, should have increased in stimulation but actually were blunted.

Past trauma and childhood abuse are difficult situations that have been shown to alter the functioning of the HPA-axis as well. Trauma and childhood abuse provide opposing research on increases in cortisol. Individuals from these groups have been shown to have decreases in cortisol. Preclinical research findings suggest that exposure to stress and concomitant HPA-axis activation during early development can have permanent and potentially harmful physiological effects. Abnormal HPA response to stress challenge has been reported in adult patients with Major Depressive Disorder and Post-Traumatic Stress Disorder (PTSD) (Carpenter et al., 2007). PTSD is a mental health disorder precipitated by a stressful event that produces fear or terror in the individual (King, Mandansky, King, Fletcher, & Brewer, 2001). Carpenter et al. found that adults with severe childhood maltreatment exhibited significantly lower cortisol and ACTH levels after being subjected to various stress tests. The HPA-axis response in these individuals has been blunted, and it seems to be a coping mechanism of the body in response to chronic stress during childhood. This coping strategy of the body can also be seen in the study of sexual

abuse by King et al. (2001). The study was designed to look at the basal functioning of the HPA-axis in response to early sexual abuse in girls aged 5 to 7 years. They found that subjects that had been abused had significantly lower cortisol in comparison to control subjects with no prior history of abuse. This data can possibly be associated with college athletes when looking at subjects who have been competing since early childhood. Similar data has been consistent with findings in male veterans with combat-related PTSD and with survivors of the holocaust. An article by Zarkovic et al. (2003), showed the relationship between cortisol secretion and prolonged psychological stress induced by war. In these individuals prolonged psychological stress was associated with transient suppression of the HPA-axis, manifested by low morning cortisol and reduced cortisol response to ACTH. Fries et al. (2005), hypothesized a trajectory of initial hyper-activation of the HPA system progressing to a state of chronic adrenal stress hyporeactivity as a type of counterregulative adaptation following acute hyper-exposure to ACTH during stressful early development (cited in Carpenter et al., 2007). However, it is not yet completely understood the mechanism behind this HPA-axis suppression.

Endocrine System

The endocrine system consists of endocrine glands, which are ductless, meaning that hormones produced are released directly into the bloodstream and travel elsewhere in the body to target tissues and organs where cellular function is altered (Carter, 2004). This system is interrelated with the nervous system (Gunstream, 2006). The nervous system sends electrical messages to control and coordinate the body. The endocrine system, being very similar, uses chemicals to “communicate” (Carter, 2004). According to King (2006), tissues competent of responding to endocrine hormones have two properties in common: they have a receptor that is attracted to the hormone and the receptor is coupled to a process that regulates metabolism of the target cells. Endocrine glands are dispersed throughout the body from the head to the

reproductive organs. According to Silverthorn (2001), there are three general classes of hormones that are classified by chemical structure: peptide hormones, steroid hormones, and amine hormones.

In order to understand the function and release of cortisol from the adrenal glands, a thorough overview of the hypothalamus and pituitary gland must take place. The pituitary gland is sometimes referred to as the “master gland” but it is under control of the hypothalamus (Carter, 2004). It is a pea-sized organ that lies beneath the hypothalamus in a depression at the base of the skull (Bowen, 2001) behind the nose. The pituitary is made up of two major parts that have different functions: an anterior lobe and a posterior lobe. Being very small, the pituitary gland controls many endocrine functions that are controlled by neurons and chemicals that originate from the hypothalamus (Gunstream, 2006). The pituitary controls hormone functions such as body temperature, thyroid activity, childhood growth, urine production, testosterone production in males and ovulation and estrogen production in females. The pituitary functions to control all other glands of the endocrine system that secrete hormones (University of Pittsburgh, 2008). This gland plays a vital role as to how the human body responds to environmental stress.

The anterior lobe of the pituitary is made up of separate collections of individual cells that are dedicated to produce a specific regulatory hormone messenger or factor that stimulate the release of growth hormones, prolactin, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), or luteinizing hormone (LH). These hormones are needed to respond to the environment. The hormones then travel elsewhere in the blood to its specific target gland. The gland is then stimulated to produce the appropriate type and amount of hormone so the body can react to the environment stress (University of Maryland Medical Center (UMMC), 2008). Environmental stress can encompass numerous

situations. In the case of this research it is most likely stress of collegiate academics, living away from home, competition, weight reduction, or practice. The posterior gland is an extension of the hypothalamus. It is composed largely of the axons of hypothalamic neurons, which extend downward as a large bundle behind the anterior pituitary (Bowen, 2001). This gland controls the conservation of bodily water by antidiuretic hormone (ADH) and oxytocin (UMMC, 2008).

The pituitary gland controls many physiologic functions, but the power behind this “master gland” is the hypothalamus. To appreciate the relationship between the hypothalamus and the pituitary, an understanding of the vascular connections between the two must take place. A branch of the hypophyseal artery runs into a capillary bundle in the lower hypothalamus. Hormones of the anterior pituitary are released into these capillaries via blood, which drains into hypothalamic-hypophyseal portal veins. These veins allow hormones to drain into the anterior pituitary and then are released into veins that release the hormones throughout the system to the specific target gland (Bowen, 2001). This is the technical description of how the hypothalamus releases signals that strictly control the release of hormones from the pituitary. These hypothalamic hormones are referred to as releasing hormones and inhibiting hormones, reflecting their influence on anterior pituitary hormones (Bowen, 1998).

Cortisol and other glucocorticoids are secreted in response to the stimulator ACTH, which is released in the anterior pituitary. However, ACTH secretion from the pituitary is under direct control of the hypothalamic peptide corticotropin-releasing hormone (CRH) secreted by the hypothalamus (Bowen, 2006). As its name implies, ACTH stimulates the adrenal cortex of the adrenal glands. Any type of stress results in an elevation of plasma cortisol levels due to this release of CRH from the hypothalamus, so it is easily seen how the hypothalamus is the “muscle” behind the “motor,” which is the pituitary gland. And vice versa, if there is enough

cortisol to supply the body, cortisol acts on the hypothalamus as feedback inhibition, telling it not to produce anymore cortisol. Another aspect, however, that is intriguing with the relationship of cortisol and collegiate wrestlers is that under severe stress, the body may produce excessive amounts of ACTH, overriding the feedback inhibition (Gunstream, 2006). In many cases, there is a fine line between unwanted stress and overtraining when applied to athletes.

The adrenal glands, acted upon by hypothalamus and pituitary secretions, are small triangular glands located on the top of both kidneys and consist of two separate parts: the outer region called the adrenal cortex and the inner region called the adrenal medulla (Gunstream, 2006). UMMC (2008) reports the inner medulla synthesizes and secretes the hormones epinephrine and norepinephrine. Epinephrine, also called adrenaline, increases heart rate, the force of heart contractions, facilitates blood flow to muscles and brain, and helps with the conversion of glycogen to glucose via the liver. Norepinephrine, also called noradrenaline, has strong vasoconstriction effects, resulting in an increase in blood pressure. These two hormones are classified as catecholamines and have to do with the “fight or flight” response that many people experience when the body is under a great deal of emotional stress (Gunstream, 2006). The inner medulla is not essential to life, but helps people in coping with physical and mental stress (UMMC, 2006).

The outer portion of the adrenal gland, adrenal cortex, secretes several classes of steroid hormones: glucocorticoids and mineralocorticoids. Aldosterone is a mineralocorticoid, meaning it regulates the concentration of electrolytes, such as sodium and potassium. It increases blood levels of sodium and water, and it decreases blood levels of potassium (Gunstream, 2006). Mineralocorticoids play a crucial role in regulating concentrations of minerals, mainly sodium and potassium. Without aldosterone and other mineralocorticoids the concentration of potassium

in extracellular fluid becomes dramatically elevated, urinary excretion of sodium increases and the concentration of sodium in extracellular fluid decreases, volume of extracellular fluid and blood decreases, and the heart will function poorly. Because of this action to conserve body sodium by stimulating its absorption, aldosterone has effects on sweat glands, salivary glands, and the colon (Bowen, 1999).

Glucocorticoids are steroids, and as the name implies, affect mainly the metabolism of fats, proteins, and carbohydrates, decrease inflammation, and increase resistance to stress (St. Edwards University Chemistry and Biochemistry Department, 2007). The majority of glucocorticoid activity in humans is from cortisol, also known as hydrocortisone. This activation of the hypothalamus and the pituitary gland all the way down to the release of cortisol from the adrenal cortex is called the hypothalamic pituitary adrenocortical axis (HPA-axis). Cortisol is synthesized from cholesterol and is the precursor for all steroid hormones. The cholesterol precursor comes from cholesterol synthesized within the cell from acetate, from intracellular cholesterol esters, or from uptake of cholesterol-containing low density lipoproteins (LDL) (Bowen, 2001). In the adrenal cortex, about 80% of cholesterol required for steroid synthesis is captured by receptors which bind LDL. Recent evidence also suggests that high density lipoproteins (HDL) may also be taken up by adrenal cells. The first step in the synthesis of adrenal steroids is the hydrolysis of cholesterol esters in the mitochondria of cells, converting it to pregnenolone. Pregnenolone is then shuttled from the mitochondria to the smooth endoplasmic reticulum where it is converted to androgens and cortisol through subsequent hydroxylations.

In the fasting state, cortisol stimulates several processes that increase and maintain normal concentrations of glucose in blood (Bowen, 2006). This is important for athletes that

restrict calorie intake to reduce weight. When a reduction in energy is present, this secreted cortisol will make glucose for uptake by cells from protein and fats. This process is known as gluconeogenesis and it is the main function of glucocorticoids. Other processes include inhibiting glucose uptake in muscle and adipose tissue, a mechanism to conserve glucose and stimulate fat breakdown in adipose tissue. The fatty acids released by lipolysis are used for the production of energy in tissues of muscles, and glycerol is shuttled to the liver to make glucose, which is also a purpose of gluconeogenesis (Bowen, 2006).

Proteolysis

When collegiate wrestlers are not getting enough glucose from their diet for their body to utilize during the intense season, cortisol will promote the breakdown of muscle proteins and fats for energy. This can have implications on performance. Simmons, Miles, Gerich, and Haymond (1984) studied the effect of increased cortisol on proteolysis in seven healthy subjects. After cortisol infusion the researchers found there was an increase in the rate and percentage of leucine nitrogen converting to alanine. This increase in alanine illustrates proteolysis as a result of cortisol infusion. These amino acids are the products of lean muscle mass destruction. These increases in cortisol may be evident in wrestlers as a result of their increase in food restriction and physical and mental stress, which would result in a loss of lean muscle mass.

In correlation, Long, Barrett, Wei, and Lui (2003) tested the amount of protein synthesis after a reduction of cortisol from an adrenalectomy in rats. They found that an adrenalectomy increased insulin sensitivity, which significantly increased protein synthesis. Upon reintroduction of cortisol there was a decrease in insulin sensitivity and protein synthesis. The researchers concluded that a deficiency in glucocorticoid secretion enhances insulin sensitivity and protein synthesis in rats.

These catabolic actions on protein synthesis and muscle mass may result from changes in the production of two growth factors which control muscle mass, IGF-I and myostatin. IGF-I is anabolic and myostatin is catabolic toward skeletal muscle. The decreased production of IGF-I as well as the increased production of myostatin have both been demonstrated to contribute to the muscle atrophy caused by glucocorticoids (Schakman, Gilson, & Thissen, 2008). Increased cortisol, due to an increase in stress and weight reduction, possibly from malnutrition, may cause problems for wrestlers. This recurrent malnutrition will add to alterations in protein metabolism, as seen in a study by Lewis et al. (2006). In this research study male rats were tested for the amount of change in diaphragm smooth muscle as a result of nutritional deprivation (ND). They concluded that ND altered the synthesis of key proteins of several pathways involved in protein turnover. Many factors of the daily routine are going to affect the maintenance of lean muscle mass in collegiate wrestlers and this may be seen in alterations in performance.

Immune Response

Along with proteolysis, changes in immune response and inflammatory action will be seen with alterations in cortisol levels. Glucocorticoids have potent anti-inflammatory and immunosuppressive properties. This is normally evident when glucocorticoids are administered in pharmacologic doses, but also important in normal immune responses. Glucocorticoids are widely used in the clinical setting as drugs to treat inflammatory conditions, such as arthritis or dermatitis, and are also used in the treatment of various autoimmune diseases (Bowen, 2006). Many studies conducted have illustrated the physiologic affects of administration of dexamethasone on the immune system, which mimics the action of cortisol in large doses. Hench et al. (1949) discovered the inflammatory action of glucocorticoid hormones over 50 years ago. These researchers found that small doses of cortisone dramatically improved the symptoms of patients with rheumatoid arthritis. From this point, powerful synthetic glucocorticoids were then

developed, that became a mainstay of anti-inflammatory and immunosuppressive therapy (cited in Saklatvala, 2002). The side effects of glucocorticoids that severely limited their use were osteoporosis, diabetes, hypertension, cataracts, thinning of the skin, and the appearance of Cushing's syndrome. Glucocorticoids also inhibit expression of many genes involved in inflammatory and immune responses (Saklatvala, 2002).

The body can become ill due to disease producing viruses and bacteria, called pathogens. In response to the presence of pathogens the immune system induces the inflammatory response. This is the result of the body trying to offset the actions of these pathogens (Lim, Muller, Herold, Brandt, & Reichardt, 2007). Prolonged increases in cortisol will suppress this inflammatory response and will make it more difficult for the body to rid itself of disease. Humans, athletes or not, cannot efficiently perform athletic tasks or daily activities if the body's immune system is constantly suppressed due to increased cortisol. Lim et al. (2007), found that glucocorticoids exerted an opposing effect on macrophage function. Macrophages play a key role in stimulating the immune system when under attack from infectious material. Lim et al. also found that glucocorticoids are involved in the modulation of macrophage function and thereby control the host's immune responses to pathogens. The conclusion was that high concentrations of corticosterone strongly repressed macrophage function.

Elevated blood pressure and induction of osteoporosis can also be linked to increases in cortisol. Cortisol decreases osteoclast function and decreases new bone formation. Osteoclast numbers increase and measures of osteoclast activity also increase. Increased cortisol also decreases gut calcium absorption and decreases renal calcium reabsorption, which adversely affects bone formation, thus causing osteoporosis. In the cardiovascular system, cortisol is required for sustaining normal blood pressure by maintaining normal myocardial function and

the responsiveness of arterioles to catecholamines and angiotensin II (Nussey and Whitehead, 2001). Cortisol has vasoconstrictive action on blood vessels, which makes it difficult for the heart to pump an ample amount of blood to the rest of the body. The heart must work harder to accelerate and increase the force of contractions. If this acceleration is constantly occurring, a cardiac event could take place.

Disease States and Cortisol

There are a couple of disease states that result from a flaw in the HPA-axis that may cause a chronic state of hypo or hypercortisolemia. Hypocortisolemia is encountered in Addison's Disease and hypercortisolemia is encountered in Cushing's Syndrome. Addison's disease is an uncommon disease caused by partial or complete failure of the adrenal cortex. For this reason, a deficiency of cortisol and aldosterone occurs in these patients. Failure to produce adequate amounts of cortisol can occur for different reasons. The problem may lie in the insufficiency of the adrenal glands themselves, termed primary adrenal insufficiency, or in the inadequate secretion of ACTH by the pituitary gland, termed secondary adrenal insufficiency. Addison's Disease is most often caused by an autoimmune destruction of the adrenal glands with symptoms of worsening chronic fatigue, muscle weakness, loss of appetite, and weight loss. These symptoms are accompanied by nausea, vomiting, diarrhea, decreased blood pressure upon standing, hyperpigmentation, depression, and hypoglycemia ("Addison's Disease," 2007).

Cushing's Syndrome has the opposite impact on the body as Addison's Disease; excessive increases in cortisol are observed. These increased levels can be caused by excessive endogenous production of cortisol, which can result from a primary adrenal defect (ACTH-independent) or from excessive secretion of ACTH (ACTH-dependent) from the pituitary gland. It may also be seen in administration of synthetic glucocorticoid drugs for therapeutic purposes. This is the most prevalent disorder involving glucocorticoids, showing symptoms of

hypertension, apparent obesity, muscle wasting, thin skin, and metabolic abnormalities such as diabetes mellitus (Bowen, 2006).

Many of the symptoms seen in Cushing's Syndrome may be seen in collegiate athletes, especially wrestlers who go through drastic alterations in weight and body composition throughout the season. It is especially important for wrestlers to lose weight in the most efficient manner possible. This means losing the weight, keeping it off, and obtaining all or most of their lean muscle mass, which is so important to success. Increased cortisol has been shown to cause weight gain. Individuals may find it difficult to maintain healthy eating habits and may eat more in attempts to fulfill emotional needs during stressful situations. These are all possible explanations of weight gain due to cortisol increases. It is true that prolonged exposure to high levels of cortisol, such as seen in Cushing's patients, can cause weight gain (Mayo Clinic, 2007). Prolonged exposure is the key. Are the months of increased stress from weight reduction, competitive stress, and classes long enough to be considered "prolonged" and to see changes in body composition? Currently there is not enough evidence to show that the amount of cortisol produced by a healthy individual under stress is enough to cause weight gain (Mayo Clinic, 2007). However, collegiate athletes teeter on the edge of drastically unhealthy nutritional practices in hopes to achieve elite performance.

Weight Reduction

Weight reduction has long been a part of the sport of wrestling. The issue many times lies in the topic of controlling caloric intake or starvation. Johnstone et al. (2004) examined the effect of short-term dietary weight loss on obese men and found that starvation produces acute stress and further activation of cortisol secretion. Although, this research was conducted with obese men, it could bring insight about the relationship between cortisol and weight reduction in wrestlers. Moreover, research has been studied on the relationship between cortisol excretion rate

and a range of important cardiovascular risk factors in 439 subjects. Increased cortisol was correlated with increases in body mass index (BMI), increases in hip measurements, and decreases in HDL cholesterol (Fraser et al., 1999). This research shows that glucocorticoids regulate components of cardiovascular disease. While this research may not affect in-season athletes, it may affect the rate and difficulty of wrestlers reducing weight in the off-season leading into the season. The more difficult the weight reduction, the more drastic measures will be taken to lose weight to meet certain criteria of different weight classes. This will have a result of decreasing energy, muscle mass, and thus, performance. Previous research shows that results of cortisol increases may have detrimental health effects later in life for athletes subjected to chronically high amounts of stress. Studies on cortisol excretion and athletics is a topic that has had little investigation. And the area of weight reduction and wrestling should be investigated further in order to offset health and performance difficulties.

Chapter III: Methodology

The purpose of this research project was to examine the changes that occurred in salivary cortisol in wrestlers during a collegiate wrestling season and to evaluate if this population had higher cortisol levels than standard men of their same age. This chapter first describes the subjects that were chosen and the sample selection for the experimental data. Next, is a description of the instrumentation used and reliability and validity of the instrumentation. Then the chapter provides information on the data collection and data analysis procedures. Finally, the chapter concludes with a brief overview of the limitations that originated from the method, sample, and procedures.

Subjects

The subjects used for this study were 14 men from the National Collegiate Athletic Association (NCAA) Division III university wrestling team. This group of wrestlers was serving as the subset for the entire population of collegiate wrestlers. Hopefully, through the results of the data inferences can be made from the subset regarding the entire population of collegiate wrestlers. The subjects were all from the same team, ranged from the lowest weight class of 125 pounds through the highest weight class of 285 pounds, and were of 18 to 23 years of age. The subjects were chosen based on their level of success and commitment to the sport, their willingness to participate in the study, and the fact that they were a good representation of the entire population of collegiate wrestlers. Approval for the study was obtained through the University of Wisconsin-Stout Institutional Human Subjects Review Board. All subjects completed a consent form, which explained the study and provided risk and benefit information (Appendix A). Subject's names were randomly coded to maintain confidentiality.

Instrumentation and Collection Procedure

An Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure cortisol. An article by Haussmann et al. (2007), describes the ELISA as a modern laboratory tool utilized to determine the amount of endogenous antigens in plasma or saliva. In the same study researchers found increased cortisol levels in saliva when testing increased stress levels, dealing with educational situations, such as oral presentation, and competitive class activities. Also, a study by Shimada, Takahashi, Ohkawa, Segawa, and Higurashi (1995), (cited in Toda et al., 2001) found that salivary cortisol can be accurately determined using ELISA. Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes. Studies consistently report high correlations between serum and saliva cortisol, which indicate that salivary cortisol levels reliably estimate serum cortisol levels (Salivary Cortisol Enzyme Immunoassay Kit, 2006). Many studies have validated the ELISA protocol in measuring salivary cortisol. According to Putignano et al., in the *European Journal of Endocrinology* (2001), salivary cortisol measurements are a valuable and convenient alternative to plasma cortisol measurement. With the establishment of more specific and widely acceptable cut-off values for dynamic testing, measurement of salivary cortisol could largely replace plasma cortisol measurement.

All samples were assayed for salivary cortisol in duplicate using a highly sensitive enzyme immunoassay. The test used 25 μL of saliva per determination. The test has a lower limit of sensitivity of 0.003 $\mu\text{g/dL}$, standard curve range from 0.012 to 3.0 $\mu\text{g/dL}$, and intra- and inter-assay coefficients of variation 3.5% and 5.1%, respectively. Method of accuracy, determined by spike, recovery and linearity, was determined by serial dilutions of 100.8% and 91.7%. Values from matched serum and saliva samples show the expected strong linear

relationship, $r(63) = 0.89$, $p < 0.0001$ (M. Curran of Salimetrics, personal communication, June 6, 2008).

Collection of saliva samples took place in La Crosse, Wisconsin. Saliva samples and weight (Appendix B) were collected two times during the season (one week before competition and one day before competition, prior competition) and one time out of season. One week before the in season competition the first set of saliva samples were collected from each subject. On this day two samples were collected from each subject in order to observe a more accurate overall average of cortisol circulating in the body. All subjects completely saturated salivary cotton swabs and inserted them directly from the mouth into salivettes shortly after waking and approximately 30 minutes later, as instructed by consultants. The preferred saliva collection method is to collect saliva by passive drool, allowing the saliva to pass through a short straw into a polypropylene vial (Salivary Cortisol Enzyme Immunoassay Kit, 2006). Due to weight reduction and lack of saliva stores, these athletes do not have enough drool for this type of test. That is why salivary cotton swabs were the collection method of choice.

Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and drop at night according to *Salimetrics*, a diagnostic testing company located at State College, Pennsylvania. Studies consistently report high correlations between serum and saliva cortisol indicating that salivary cortisol levels reliably estimate serum cortisol levels according to Francis et al., Hiramstu, and Vining (1987, 1981, & 1983) (cited in Salivary Cortisol Enzyme Immunosassay Kit, 2006). An article by Kudielka, Bellingrath, and Hellhammer (2003) corroborated the idea of higher cortisol levels in the morning relative to evening types in a sample of healthy middle-aged male and female adults. To obtain accurate and reliable measures throughout the experiment, testing was done the same way and at the same

time during each day of sampling. The second day of sampling was done the day before competition, again upon awakening and 30 minutes later. The levels of cortisol were hypothesized to be directly related to the change in stress levels and weight reduction. It was expected that increased weight reduction leading to competition would correlate with an increase in salivary cortisol levels.

The second set of testing was conducted a month after the season had finished (one sample taken upon awakening and one taken one half hour later). This allowed samples to reflect everyday life without the stress of training and weight reduction. Baseline, off-season cortisol levels for these athletes was compared to standard values for males of the same age and to the athlete's competition values.

Subjects were instructed not to consume a major meal 60 minutes before sampling and not to consume alcohol within 12 hours of sampling. Subjects were also warned against applying any topical cream containing steroids of any kind. If the directions were neglected false readings would appear (Salivary Cortisol Enzyme Immunoassay Kit, 2006). Immediately after samples were collected they were cooled and placed into a non-frost freezer (at or below -20 degrees C) to ensure the integrity of the samples. Once all samples had been collected, they were shipped with dry ice, in order to keep frozen, to *Salimetrics*. As *Salimetrics* has processed samples for similar studies, instructions were provided on sampling technique and storage.

Data Analysis

The Statistical Program for Social Sciences (SPSS), version 16.0, computer software program, was used to analyze the changes in salivary cortisol levels as they relate to stress and weight reduction leading to competition. Descriptive statistics including mean, median, and standard deviation were conducted on cortisol levels of each day. Linear regression and Pearson correlation were used to test the relationship between pre-competition weight and pre-

competition cortisol levels. A paired sample t-test was used to compare the differences in cortisol from one week pre-competition to one day pre-competition and from one day pre-competition to off-season values. Next, a one-sample t-test was calculated to compare cortisol levels of standard men of the same age and off-season values of the subjects. Finally, a linear regression and Pearson correlation was used to test each individuals change in weight versus the individual change in cortisol from one day pre-competition to off-season values. Because there are many factors that exist to explain fluctuations in cortisol levels, this test will only take into account weight change as the explanatory variable when trying to explain the change in cortisol levels.

Limitations

A major limitation of study was the sample size ($n=11$). The study started with 14 subjects, however, at each testing date a different person was absent. For most of the statistics run this brought the total subjects to 11 wrestlers. Another limitation was that each value of cortisol was based upon each subject's honesty and their ability to follow pre-testing instructions. Each subject is unique and may handle life stressors in different ways. Thus, there may be many omitted variables that were not accounted for during the study that may have changed cortisol levels between each subject and at collection times. These variables include, but are not limited to academic difficulties, family issues, significant other factors, and nagging injuries. The time of cortisol peak will vary in individuals relative to their normal wake-up time. Also, only two samples were taken to give an overall estimation of morning cortisol values. It would have been ideal to have taken additional samples. Another limitation was if the time of data collection was not part of the subject's normal routine then values may be misrepresented. If saliva samples were contaminated with blood, false readings could occur (Salivary Cortisol Enzyme Immunoassay Kit, 2006). Finally, money and time were limitations to the study. It is very expensive to run multiple ELISA assays, so the number of samples collected in the morning were

reduced to fit within the budget allowed. Moreover, there are many competitions throughout a wrestling season; however, there are only a few major competitions that are ideal to test before in order to detect high stress levels. The season becomes very limited when working around certain competitions. Not only time for the researcher, but the time that the team is able to meet for the collection of data is a limitation. On occasion, planes get canceled and buses get delayed, so many time plans needed to be changed in order to accommodate unexpected delays in the schedule of the team.

Chapter IV: Results

The purpose of this research project was to examine the changes that occurred in salivary cortisol in wrestlers during a collegiate wrestling season and to evaluate if this population has higher cortisol levels than the standard for men of the same age. Prolonged alterations in cortisol levels can have negative effects on health and performance. Salivary cortisol was measured and cortisol testing used an Enzyme-Linked Immunosorbent Assay (ELISA).

Summary Descriptives

Table 1 illustrates the mean values of cortisol at all three collection stages. The day before the competition, when weight reduction was at its greatest, the mean cortisol was highest at 0.801 $\mu\text{g/dL}$ ($SD = 0.422$). There was not much difference in mean values during off-season and 1 week prior competition, 0.610 and 0.582 $\mu\text{g/dL}$, respectively

Table 1

Summary of Descriptive Statistics for Cortisol Across Three Collection Times

Collection Time	Minimum ($\mu\text{g/dL}$)	Maximum ($\mu\text{g/dL}$)	Mean ($\mu\text{g/dL}$)	Std. Dev. ($\mu\text{g/dL}$)
1 Week Before	0.258	0.857	0.582	0.165
1 Day Before	0.346	1.798	0.801	0.422
Off-Season	0.220	0.852	0.610	0.216

Cortisol Comparison between Off-Season and Standard Values

A one sample t-test was run in order to see if the subjects, in general, had higher salivary cortisol levels off-season than standard values from men of the same age. A normal morning cortisol range for men of the same age is 0.112 - 0.743 $\mu\text{g/dL}$ (Salivary Cortisol Enzyme

Immunoassay Kit, 2006). Expected ranges for 8 to 18 years of age were reported from an unpublished manuscript, from Pennsylvania State University's Behavioral Endocrinology Laboratory. Adult ranges were obtained from published literature. For this reason, the value being tested against was 0.4275 $\mu\text{g/dL}$. This is the average of the low normal and the high normal values. There was a significant difference in salivary cortisol levels, $t(10) = 2.804$, $p < 0.05$, with off-season values being higher in wrestlers compared to standard men of their same age.

Table 2

One Sample t-Test Comparing Wrestler's Off-Season Salivary Cortisol to Standard Values of Men of the Same Age

	Test Value = 0.4275					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Cortisol ($\mu\text{g/dL}$)	2.804	10	.019	.1828182	.037528	.328108

Linear Regression Comparing Cortisol and Weight Reduction

The linear regression showed a tendency for a negative relationship between average mean cortisol and average mean weight reduction (Table 3). When weight decreased just before competition, average mean cortisol levels increased. A third (33%) of the variability in cortisol could be explained from weight. A weak correlation was found, $r(33) = -0.326$, $p > 0.05$.

However, the correlation was not statistically significant at $\alpha = .05$. The quadratic regression

in Figure 1 displays that cortisol levels were the highest in the middle-weights where weight reduction was highest.

Table 3

Comparison of all Cortisol Levels and all Weights Recorded at Every Collection Time

Correlations

		Weight (lbs.)	cortisol ($\mu\text{g/dL}$)
Weight (lbs.)	Pearson Correlation	1.000	-.326
	Sig. (2-tailed)		.064
	N	33.000	33
cortisol ($\mu\text{g/dL}$)	Pearson Correlation	-.326	1.000
	Sig. (2-tailed)	.064	
	N	33	33.000

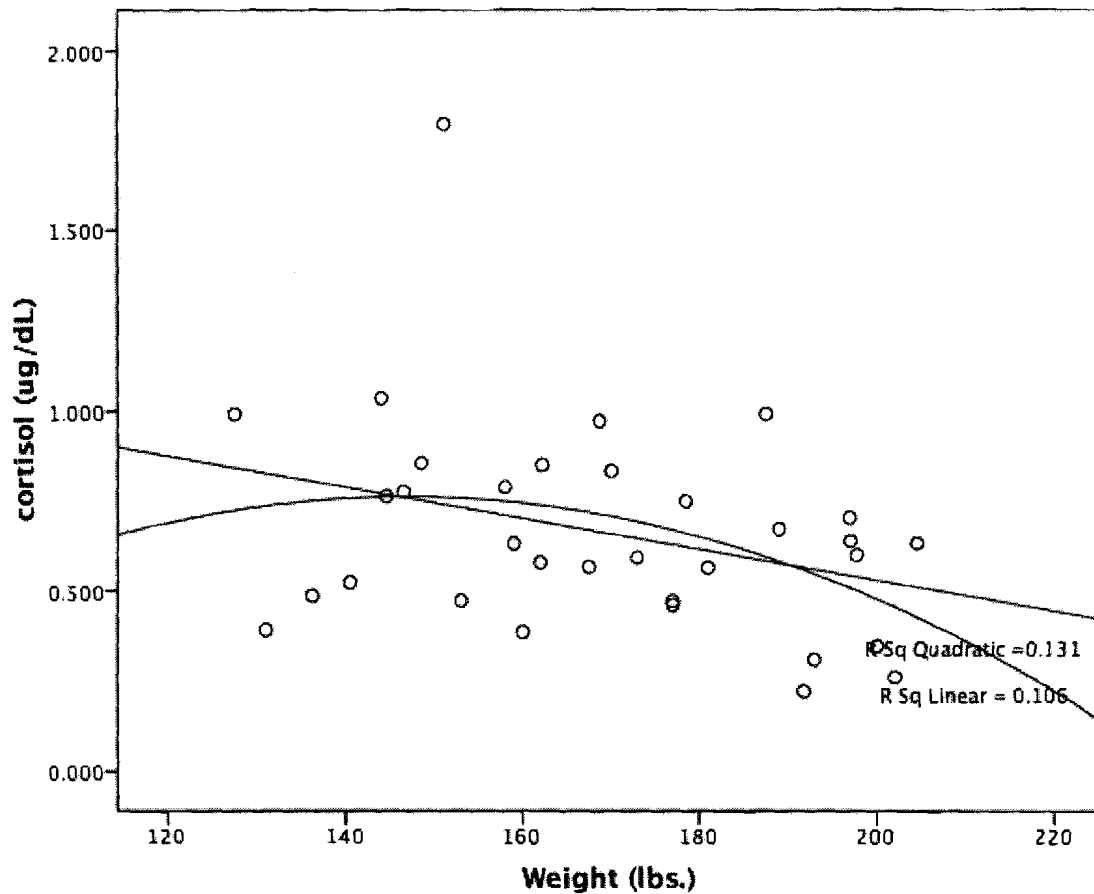


Figure 1: Linear and quadratic regression comparing all cortisol levels and all weight recordings

Regression Comparing the Individual Change in Cortisol Between Individual Change in Weight Reduction

The individual changes in cortisol levels from one day before competition and off-season cortisol levels were compared to the individual changes in weight from one day before competition and off-season for all subjects. Results did not significantly support the relationship due to the lack of linear relationship between the two variables, $\beta = -.014$, $t(33) = -1.212$, $p > .05$.

This may be partially explained by the small sample size. If significance had been determined, it could be explained that for every pound lost there was a tendency for cortisol to increase by .014 $\mu\text{g/dL}$. Figure 2 depicts the regression comparing individual changes in cortisol to individual changes in weight reduction from one day before competition to off-season.

Table 4

Comparison of Individual Change in Cortisol Levels to Individual Change in Weight:
Coefficients Analysis^{ab}

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	deltaweight	-.014	.012	-.358	-1.212	.253

^a Dependent Variable: deltacort

^b Linear Regression through the Origin

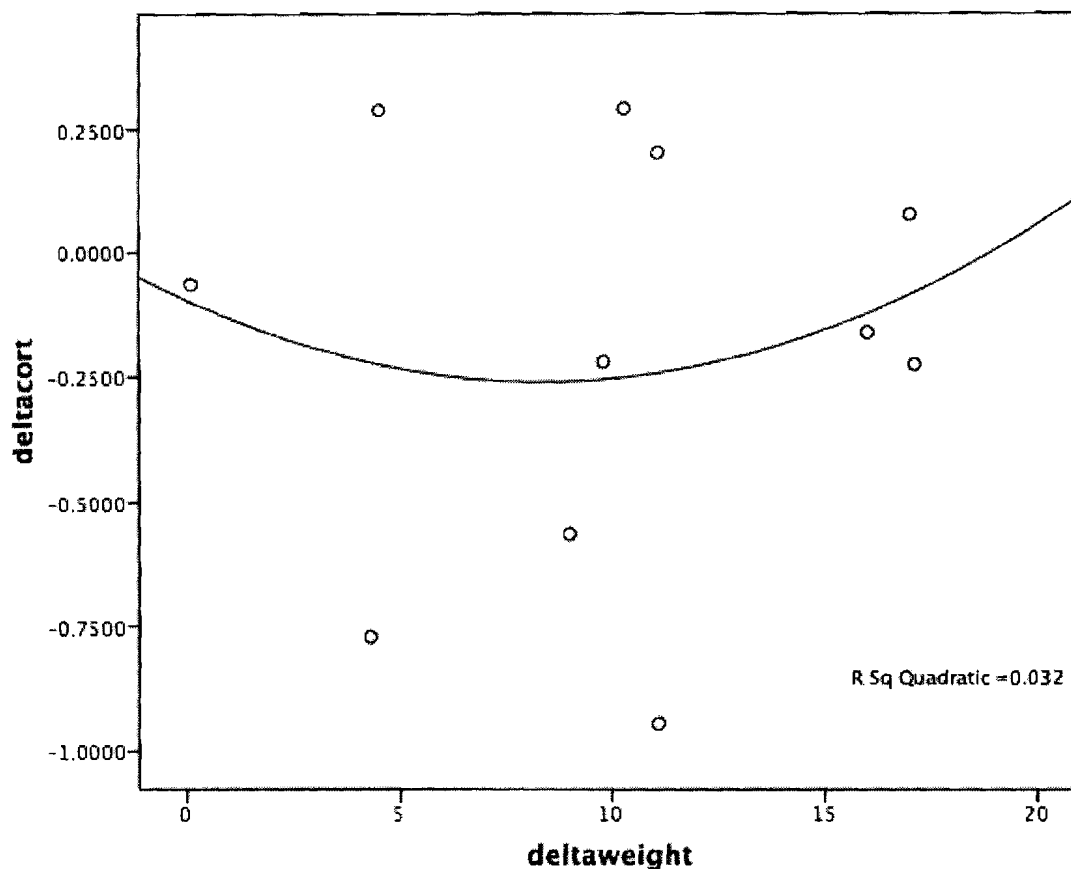


Figure 2: Regression comparing individual change in cortisol to individual change in weight reduction

Paired Sample t-test

A paired sample t-test was used to compare one week before competition cortisol values to one day before competition cortisol values and one day before competition cortisol values to off-season cortisol values. The mean cortisol value for one week before competition was 0.582 $\mu\text{g/dL}$ (SD = 0.165) and the mean cortisol value for one day before competition was 0.801 $\mu\text{g/dL}$ (SD = 0.422). The mean cortisol value for off-season was 0.610 $\mu\text{g/dL}$ (SD = 0.216). There was not a significant difference in cortisol, $t(10) = -2.11$, $p > 0.05$, between one week before competition and one day before competition. There was not a significant difference in cortisol,

$t(10) = -1.51, p > 0.05$, between one day before competition and off-season values. The comparison between one week before competition to one day before competition was not significant at $\alpha = 0.05$, however it would be significant at $\alpha = 0.10$.

Chapter V: Discussion

The purpose of this research project was to examine the changes that occur in salivary cortisol in wrestlers during a collegiate wrestling season and to evaluate if this population has higher cortisol levels than the standard for men of the same age. Prolonged increases in cortisol, related to weight reduction and competitive stress, can have detrimental effects on performance and health. Each athlete submitted a saliva sample and was weighed at different times leading up to a major competition within the season. With almost 10,000 competing collegiate wrestlers, this study has implications for many athletes across the country.

Limitations

A major limitation to the study was the sample size ($n=11$). The study started with 14 subjects, however, at each testing date a different person was absent. For most of the statistics run this brought the total subjects to 11 wrestlers. Another limitation was that each value of cortisol was based upon each subjects honesty and their ability to follow pre-testing instructions. Each subject is unique and handle life stressors in different ways. Thus, there were many omitted variables that were not accounted for during the study that may have changed cortisol levels between each subject. These variables include, academic difficulties, family issues, significant other factors, and nagging injuries. Time of cortisol peak will vary in individual patients relative to their normal wake-up time. Also, only two samples were taken to give an overall estimation of morning cortisol values. It would have been ideal to have taken a few more samples. Another limitation was if the time of data collection was not part of the subject's normal routine then values may be misrepresented. Any saliva samples contaminated with blood would give false readings and the samples would have to be screened for blood using a reliable screening tool (Salivary Cortisol Enzyme Immunoassay Kit, 2006). Finally, money and time were limitations to

the study. It is very expensive to run multiple ELISA assays, so the number of samples collected throughout the morning were reduced to fit within the budget allowed. Moreover, there are many competitions throughout a wrestling season; however, there are only a few major competitions that are ideal for testing in order to notice high stress levels. The season becomes very limited when working around certain competitions. Not only coordinating time for the researcher, but the time that the team is able to meet for the collection of data was a limitation.

Discussion

Subjects in the study were found to have higher mean salivary cortisol levels one day before competition ($M = 0.801$, $SD = 0.422$) than one week prior competition ($M = 0.582$, $SD = 0.165$) and baseline, off-season levels ($M = 0.610$, $SD = 0.216$). At the one day prior competition collection period, wrestlers experienced the most weight reduction and the highest stress levels, shown with the negative slope of linear regression. This illustrates the implications that weight reduction may alter cortisol, which may affect performance and health of these athletes. This type of athlete is consistently competing and making weight starting in November and possibly extending through March. Weight reduction appeared to be a factor related to increased cortisol levels. This is an interesting finding because, as cited earlier, the study by Toda et al. (2001), found that Judo players experiencing weight reduction had decreases in salivary cortisol levels. However, they informed readers that saliva collection was taken at different times throughout the day at certain points within the study which may not have taken into account the peak levels of cortisol on awakening. Cortisol levels vary greatly during the day, with the highest levels being early in the morning then dropping considerably throughout the day and into the night (Salivary Cortisol Enzyme Immunosassay Kit, 2006). Also, Kudielka et al. (2003), corroborated the idea of higher daytime cortisol levels in the morning relative to evening levels. This may be reasoning behind the contradicting conclusions. Also, collegiate wrestlers have different stress factors than

Judo players. A question of concern is which most affected the rise in cortisol weight reduction, competitive stress, or other omitting variables?

It was found that these athletes had significant increases in baseline cortisol levels ($M = 0.610$, $SD = 0.216$), compared to standard values of men of the same age. The value tested against was an average of a normal standard range ($0.4275 \mu\text{g/dL}$). Many of these athletes have been competing their entire lives and have experienced similar stress influences. Prolonged increases in cortisol can cause muscle wasting, increased abdominal fat, immunosuppression, impaired glucose metabolism, increased blood pressure, and osteoporosis (Bowen, 2006 & "Addison's Disease," 2007). Athletes experiencing these effects are not going to efficiently compete at elite levels due to proteolysis, immunosuppression, and impaired glucose metabolism. These are crucial areas that will affect performance.

When comparing all three mean cortisol levels with paired sample t-tests, there was not a significant difference between them; however, it can be observed that the mean of the one day prior was well above the other two collection days. Even though it is not statistically significant, it may have more significance clinically. Though off-season data was similar to the one week prior competition data, both values support the idea of these individuals experiencing baseline increases of cortisol when compared to standard values of normal men. This may help illustrate the idea of prolonged increases in this type of athlete.

This research was conducted in hopes that follow-up studies will be conducted on the same type of athlete in order to see if something can be done to off-set difficulties with performance and health. Follow-up research may need to implement a nutritional supplement or some other type of nutritional intervention. Relaxation techniques and coping strategies for student athletes may prove to be beneficial in reducing in-season and off-season cortisol levels.

Much research is needed in the future, not only to help collegiate athletes perform better, but to help maintain a healthy athletic career and future.

Conclusion

In conclusion, leading up to competition there is a negative relationship between weight and cortisol levels. As weight decreased, there was a tendency of cortisol to increase. The data analysis can explain 33% of the variability in cortisol from weight changes. These athletes have higher baseline cortisol values than men of similar age, which will have implications on health and performance. More research with a larger subset of similar athletes must be done in order to prove cortisol and weight reduction have a strong correlation with each other, although this data on this population suggests such a relationship exists. These wrestlers do exhibit significantly higher salivary cortisol than men of the same age, which suggests both their health and performance may be at risk.

Recommendations for Future Research

- Include a larger sample size of NCAA wrestlers from all divisions of multiple programs.
- Collect saliva samples from athletes multiple times throughout the day to obtain a more accurate representation of cortisol values.
- Conduct a retrospective study to determine health risks of elevated cortisol levels to those who were athletes.
- Take different omitting variables into account when establishing stress levels, such as childhood abuse, PTSD, winning percentage, class, and work schedules.
- An interesting side study would be cortisol levels of normal weight populations involved in weight reduction.

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Appendix A: Consent to Participate

This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.

Consent to Participate in UW-Stout Approved Research

Title: Stress Response and Salivary Cortisol in Collegiate Wrestlers

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Description:

Cortisol is a hormone in the body that is released during high stress situations. Prolonged increases in cortisol have many side effects that may be damaging to the performance of collegiate wrestlers. These side effects include, muscle wasting, increased abdominal fat, impaired glucose metabolism, immunosuppression, and increased blood pressure. Cortisol has not been determined in wrestlers, but has been evaluated in endurance athletes. Therefore, these findings would provide data of significant value to the existing research on athletes.

Saliva samples will be collected from collegiate wrestlers from the University of Wisconsin La Crosse Wrestling Team. Six saliva samples will be collected on three different days during the season just prior competition and two samples will be collected out of season. One week before the in season competition, the first two samples will be collected from each subject in order to observe a more accurate overall average of cortisol circulating in the body. All subjects will submit saliva into salivettes shortly after waking and around 30 minutes after that. This takes into account the circadian rhythm of cortisol in the body. Levels are highest in the morning with a spike occurring 30 minutes after waking. To get accurate measures throughout the experiment, testing will be done the same way and at the same time during each day of sampling. The second day of sampling will be done the day before competition (one sample taken upon awakening and another taken 30 minutes later). The levels of cortisol should be directly related to the change in stress level and weight reduction. In order to document the correlation between weight loss and salivary cortisol levels, weight will be recorded for each subject on a weight sheet during each testing time. The third sampling day for the competition will take place 2 days after competition. Cortisol levels should be related to the amount of weight that is regained and the level of stress that may be relieved. The correlation in weight gain and salivary cortisol levels will be documented on the weight sheet.

The second set of testing will be conducted a month after the season has finished. This will allow samples to reflect everyday life without the stress of training and weight reduction. Baseline, off-season cortisol levels for these athletes will be compared to standard values for males of the same age and to the athlete's competition values. Immediately after samples are collected they will be cooled and placed into a non-frost freezer to ensure the integrity of the samples. Once all samples have been collected, they will be shipped with dry ice, in order to

keep frozen, to Salimetrics, a diagnostic testing company based out of Pennsylvania that has processed samples for similar studies and has given me instruction on sampling technique and storage. Testing will be performed on an Enzyme Linked Immunosorbent Assay (ELISA). This is currently the most accurate and efficient manner to measure salivary cortisol.

Risks and Benefits:

The testing that is being done on the athletes may be outside their realm of comfort in preparing for the competitive event. In order to make sure their pre-game strategy is not compromised, testing is going to be done on site where minimal effort is going to have to be provided by the athlete. This should allow them to relax as they would if they were not being tested. There is also a fear factor that comes into play during diagnostic testing, however, saliva collection is non-invasive and with subject education, should be less intimidating.

Prolonged increases in cortisol can have negative effects on elite athletes including, muscle wasting, increased abdominal fat, immunosuppression, impaired glucose metabolism, increased blood pressure etc. These effects can have detrimental outcomes to performance and health. Subjects may have a fluctuation in salivary cortisol due to competitive stress, associated with weight reduction, anxiousness, classes, etc. If this increase in salivary cortisol is observed, then follow up research can be done to try and reduce cortisol in this population. Something can then be done to try and lower the level of cortisol, which would be very beneficial to the health and performance of collegiate athletes. Saliva collection is a non-invasive, stress free sampling technique that gives representative values of serum cortisol.

Time Commitment and Payment:

Each subject will have two saliva samples collected from them 1 week before a competition, 1 day before a competition, and 2 days following the competition. Two different samples will be collected from each wrestler at each testing time, one upon awakening and one 30 minutes later. One month after the wrestling season has ended, the athletes who were tested will have baseline saliva samples collected, one upon waking and one half hour later. During each testing time, the weight of each subject will be recorded on a weight sheet. Collection of salivary samples should take no more than 3 – 5 minutes and the recording of weight will take less than a minute each. Levels will be compared to standard salivary cortisol values for males of the same age and will be compared using paired t - test with competition values. Basically, each subject will have to submit 8 saliva samples into a "test-tube like" container. For the importance of collecting a saliva sample upon waking, it is anticipated that all subjects will stay together. There is no payment for being a research subject; however, individual cortisol values will be shared with that individual only.

Confidentiality:

A saliva sample will be collected into a collection salivette container. Each sample container will be labeled with a 3 digit numerical code. The code will only be associated with the day, time of saliva sample, and person. However, using codes will ensure confidentiality. Your name will not be included on any documents. I do not believe that you can be identified from any of this information. For publication, all subjects will be labeled as wrestlers from a NCAA Division III program in the United States.

Right to Withdraw:

Your participation in this research study is completely voluntary. You may withdraw at any time with no consequences. If you agree to participate and later feel that it is not in your best interests, you may withdraw immediately without any punishment.

IRB Approval:

This study has been reviewed and approved by The University of Wisconsin-Stout's Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University policies. If you have questions or concerns regarding this study please contact the Investigator or Advisor. If you have any questions, concerns, or reports regarding your rights as a research subject, please contact the IRB Administrator.

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Statement of Consent:

Signature:..... Date:

Appendix B: Weight Collection Form

<p>This research has been approved by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46.</p>
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Names/Code Number	Weight (lbs.)		
	1 week before	1 day before	Off- season
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

Comments: