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SALIVARY ALPHA-AMYLASE AS AN INDICATOR OF BODY STRESS FOLLOWING AN ACUTE SESSION OF REPETITIVE JUMPING

By

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Bachelor of Science in Biology
Dixie State University
2009

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science - Exercise Physiology

Department of Kinesiology
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ABSTRACT

Salivary Alpha-Amylase as an Indicator of Body Stress Following an Acute Session of Repetitive Jumping

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Determining body stress levels is important when developing training programs for athletes and rehab facilities. Stress levels during exercise are commonly measured using heart rate, oxygen consumption (VO_2), and blood biomarkers. These collection measures involve training on the part of the data collector and the participant, are invasive, and themselves elicit some stress. The purpose of this study is to test whether a 10-min repetitive jumping session will cause a significant increase in salivary alpha-amylase, a potential stress biomarker. This study also looks to determine if a 20-min recovery is enough time for amylase levels to return to baseline. Amylase levels could prove to be a beneficial addition to stress testing as a biomarker of autonomic nervous system activity, or perhaps could be used instead of heart rate and VO_2, thus limiting the need for bulky and costly equipment. Amylase levels could also potentially be used as an assessment of fitness level, and an individual's reaction to training. Salivary amylase may also prove to be a useful biomarker for individuals on medications such as beta-blockers, which affect heart rate during exercise.

Participants included 10 men and women, aged 18-32, without heart, respiratory, or musculoskeletal limitations to exercise. Participants completed a treadmill maximal effort test (GXT), and a jumping session (JP) where saliva was collected, pre, post and 20-min post the jumping session. Data were evaluated using a one-way repeated measures ANOVA. Analysis revealed a significant increase in salivary amylase levels over resting levels ($p<0.001$), and no significant difference between pre levels and 20-min post levels ($p>0.05$). Based on analysis, salivary amylase levels could be used in addition to other stress markers, but further research is needed to consider whether amylase levels could be used in place of heart rate or VO_2 measurements. Further research should also focus on gathering additional amylase during exercise to evaluate a possible dose response of amylase levels.

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CHAPTER 1

INTRODUCTION

The World Health Organization (WHO) called stress the “Health Epidemic of the 21st Century”. They also reported that by 2020, stress-related health conditions will be second only to ischemic heart conditions in the range of disorders experienced (World Health Organization, 1996). It is estimated that $300 billion annually is lost in the United States due to accident, absences, employee turnover, medical, legal and insurance costs due to stress (The American Institute of Stress, 2011). Stress is a state in which an organism’s internal balance, or homeostasis, is disturbed by real or perceived challenges in the external environment (Ali & Pruessner, 2012). The body’s response to restore homeostasis is called the stress response. During the stress response two biological body systems are activated. The Sympathetic Nervous System (SNS), which responds quickly following a stressor through the action of epinephrine and norepinephrine, and the Hypothalamic-Pituitary-Adrenal system (HPA), which responds after a time delay and is mediated through glucocorticords (Chrousos, Kino, & Charmandari, 2009).

Long term response to stress can have numerous negative effects on the body including fatigue, headaches, stomach distress, sleep problems, weight changes, depression, increased heart rate and high blood pressure (Takai et al., 2004). Exercise has been recommended as a means to alleviate stress. The American College of Sports Medicine (ACSM), recommends that adults get at

least 150 minutes of moderate-intensity exercise per week, and train each muscle group two to three days per week (Garber et al., 2011).

In 1979, Gilman et al. found elevated levels of a saliva enzyme called alpha-amylase (sAA) in clinical subjects exposed to 8 days in hyperbaric pressure. The authors concluded that the elevated sAA levels were due not only to the hyperbaric pressure, but the anticipatory stress caused by the procedure. Seventeen years later Chatterton et al, (1996) demonstrated that sAA could be used as a stress marker in both physiological and psychological situations. They collected blood and saliva samples from male medical school students at 15 minute intervals before and after a 20-min outdoor track run, a final examination, or a rest period. There was a significant correlation between sAA and norepinephrine levels from the running session ($r=0.64$, $p<0.001$). Salivary alpha-amylase levels returned to baseline within 30 min after each condition. Chatterton et al, concluded that longer duration activities and higher intensities (walking vs. running) were associated with higher salivary alpha-amylase levels. Norepinephrine is a commonly used measure of SNS activity in the blood. The correlation of norepinephrine and sAA in this study may provide evidence that sAA in the saliva may be used instead of blood measures during stress studies.

Due to the minimally-invasive nature of saliva collection, it is becoming a valuable option for clinicians, coaches, and healthcare providers needing access to biomarkers. Salivary alpha-amylase is currently being utilized in physiological research and clinical facilities for psychological stress testing for populations such as children, those under mental stress, and those with mental or physical

disabilities (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). Sports are often used exercises that are used to teach children discipline and self-control (Capranica et al., 2011). It is unethical to subject children to multiple blood draws for research so other biomarkers should be used to test for stress in children. Caprianica et al. (2011) used salivary alpha-amylase to gauge the amount of stress on twelve 10 year old boys during a taekwondo competition. Salivary alpha-amylase and salivary cortisol were measured 15-min before the competition, 1-min after, 30, 60 and 90-min after the competition. Peak values of sAA at the end of the matches were 169 ± 47.0 $U \cdot ml^{-1}$ (units per milliliter) which is a 208% increase over pre-competition levels. Salivary alpha-amylase levels returned to baseline within 30-min post competition. This study provides evidence that sAA may be a good choice for monitoring sympathetic nervous system activity to stress in specialized participants as well as in the field due to its minimally invasive nature.

Exercise sessions can be done at various durations and intensities. Heart rate and oxygen consumption (VO_2) are common methods used to measure exercise intensity. Exercise studies have proposed sAA be used as a measure of exercise intensity and have conducted studies to evaluate the relationship between sAA, cardiovascular parameters and VO_2. Calvo et al. (2011) recruited 20 military trained, male volunteers to undergo a submaximal treadmill test followed by a maximal treadmill test. They measured blood lactate, salivary alpha-amylase, VO_2 and heart rate in an effort to determine if there was a relationship between the anaerobic threshold measured in blood and saliva.

They found that there was no significant difference in the aerobic thresholds determined by either blood lactate or sAA. There was also a strong correlation between sAA and heart rate ($r=0.95$, $p<0.01$). Similarly Bocanegra et al. (2012) had 12 professional swimmers do an intense, incremental swim session to determine if sAA could be used to measure exercise intensity. All participants did a graded exercise test on a treadmill ergometer 1 week prior to the swim test. The swim test consisted of eight 50-meter swims. This protocol was designed to be finished in 12-min by the swimmers. Blood and saliva was collected at the end of each 50-meter stage, 5 and 10-min into recovery. They found a high correlation between blood lactate and sAA from the swim protocol ($r=0.78$, $p<0.05$). The previous study by Calvo et al. (2011) determined that sAA was not significantly different from lactate and sAA was highly correlated with heart rate. Heart rate was not measured in this study by Bocanegra et al, but the strong correlations of sAA and lactate in each study may demonstrate that had heart rate been measured there also would have been a correlation between sAA and heart rate in this study.

Other intense exercise studies have shown no correlations between sAA and cardiovascular parameters. Gallina et al. (2011) recruited 21 males to participate in a maximal treadmill test. Saliva was collected before, immediately after, 5, 10 and 30-min into the recovery. Heart rate, blood pressure and VO_2 measurements were taken at the same times. Amylase levels increased significantly during the test ($p<0.001$) and decreased throughout the recovery phase. No significant correlations were found between sAA, blood pressure,

RPE or heart rate. Similarly Costa et al. (2012) had 11 competitive endurance runners volunteer 2-hour endurance run at 75% VO_2max on a treadmill. Each participant completed a maximal treadmill test 1 week prior. Saliva was collected pre, post and 30-min post the 2-hour treadmill session. Their results showed a decrease in the flow rate of saliva over the course of the test, which they attributed to dehydration. The change in flow rate did not affect the concentrations of sAA in the saliva. Amylase levels increased significantly throughout the treadmill test. They did not find a significant difference in sAA levels from pre to post test ($p=0.37$). There were also no significant correlations between sAA and any measures of exercise intensity (heart rate, RPE and VO_2). Costa et al. believe the lack of significant relationships between sAA and the cardiovascular measures were due to the chosen intensity of 75% VO_2 max. They believe that a greater intensity exercise for a shorter amount of time would have elicited a significant response in sAA.

Studies have shown that low stress situations and low intensity exercises are not intense enough to elicit a significant response in salivary alpha-amylase levels (Mackinnon & Hooper, 1994; Walsh, 1999). Therefore any stress testing using sAA needs to be done at a great enough intensity to increase the chances of a significant difference in sAA levels from collected samples. Jumping rope is an inexpensive, effective and portable way to exercise. Due to the nature of the exercise, this mode represents a series of recurring jumps with enough height to clear a rope.

There are many activities that also use repetitive jumping such as basketball, volleyball, gymnastics, dancing and boxing. It is estimated that depending on the jumps per minute (jpm), jumping can provide a workload of 8-12 metabolic equivalents (METS), (Getchell & Cleary, 1980). Jump roping at 120 jumps per minute is approximately 12 METs and is considered hard work (Town, Sol, & Sinning, 1980). Exercising at 12 METs is comparable to running at 7.25 miles per hour, playing racquetball, or cross country skiing (Ainsworth et al., 2011).

In an unpublished study, repetitive jumping at 120 jpm for 5-min was approximately 57.1% of the participants VO_2 max and 80.9% of the participants maximal heart rate, when compared with a maximal graded exercise test (Igaune, 2012). This demonstrates the intensity of the exercise and the amount of body stress that repetitive jumping produces. Exercise has been recommended to alleviate stress. However, exercise itself produces varying amounts of stress on and within the body depending on the intensity and duration of the exercise.

It is suggested that sAA can give a more accurate response as to the effect of stress on the body during activities and is faster to respond and recover from stress situations (Ali & Pruessner, 2012) . Measuring the levels of salivary alpha-amylase may show if the repetitive jumping is an intense enough stressor to elicit significant changes in sAA levels.

Purpose of the Study

The purpose of this study is to examine the response of salivary alpha-amylase to a repetitive jumping session, and secondly, to examine the potential relationship between salivary alpha-amylase and heart rate, and salivary alpha-amylase and VO_2.

Research Question

Research Hypothesis : A 10-min repetitive jumping exercise at 120 jpm is intense enough to cause a significant increase in salivary alpha-amylase, which will return to baseline levels within 20 minutes post exercise.

Significance of the Study

The significance of the study is to examine if a 10-min, 120 jpm repetitive jumping session is intense enough to elicit a significant change in salivary alpha-amylase and to examine the relationship between salivary alpha-amylase and heart rate, and alpha-amylase and VO_2. This could potentially benefit individuals looking for biomarkers to monitor the sympathetic nervous system in clinical, research settings, and for generalized population use. Investigating saliva biomarkers could also prove useful for individuals or situations where blood collection is not an option.

Definition of Terms

Alpha-amylase: enzyme in saliva which begins the breakdown of carbohydrates, also inhibits the growth and adherence of bacteria in the oral cavity.

Graded Exercise Test (GXT): a test that evaluates the physiological responses to exercise, usually performed on a treadmill or cycle ergometer, that increases with intensity from one stage to the next.

Metabolic equivalents (METs): the amount of oxygen consumed during activity, (at rest 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$).

Sympathetic Nervous System (SNS): part of the autonomic nervous system which activates the flight-or-flight response.

Sympatho-Adrenomedullary Axis (SAM): responsible for releasing catecholamines (epinephrine and norepinephrine) that regulate short term stress responses such as catabolism prior to a stressor.

Hypothalamic-Pituitary-Adrenal Axis (HPA): regulates long term stress by releasing glucocorticoids (cortisol and cortisone) which promote energy replenishment and cardiovascular efficiency.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Empirical Literature Review

Salivary alpha-amylase has been researched as a stress marker for sympathetic nervous system activity for over 30 years. Salivary alpha-amylase testing is preferable to blood testing due to the minimally invasive nature of collection, the ease at which it can be collected, and because it can be collected repeatedly in the field, clinics, or lab settings. It can be used to test stress from physiological and psychological stresses, and in subjects who may not otherwise be able to communicate properly their stress levels, or individuals who it is unethical to take repeated blood draws from.

Salivary alpha-amylase changes have only been significant if the stimulus is stressful enough to elicit a large response. There is currently conflicting data from studies using sAA and cardiovascular parameters as measurements of stress due to the lack of generalized study participants and the intensities of the stressors in the studies. Repetitive jumping is an intense exercise. Intense exercising puts various amounts of stress on different part of the body depending on rate and duration. Repetitive jumping is an activity that is recommended as a way for adults and children to exercise and train. The following literature review will discuss previous research in salivary alpha-amylase in both physiological and psychological testing. It will also discuss how repetitive jumping can be used as an intense stressor to gauge a stress response in subjects using alpha-amylase levels.

Salivary Alpha-Amylase as a Stress Marker

Salivary biomarkers are being researched extensively for various fields because they are easily accessible, are non-invasive, require little training for researchers, can be measured quickly and repeatedly, and can be collected in the field (Rohleder & Nater, 2009). The use of salivary alpha-amylase (sAA) has been proposed to reflect the activity of the sympathetic nervous system (SNS) during stressful situations. The idea of using sAA as a stress marker was originally proposed in the 1970's by Gilman et al. (1979) and since has been investigated by numerous researchers (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Chatterton, Vogelsong, Lu, & Hudgens, 1997; D. A. Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007; D A Granger et al., 2006; U. M. Nater, La Marca et al., 2006; Rohleder et al., 2004).

The release of sAA is facilitated by the autonomic nervous system. The autonomic nervous system is responsible for the motility of the cardiac muscle, smooth muscle and blood vessels (Gabella, 1987). More sAA can be expected during stressful times when the activation of the autonomic nervous system is high. Early studies using sAA as an indicator of stress involved immersing subjects in cold, waist deep water and administering beta-adrenergic blockers to determine nervous control of sAA release (Speirs, Herring, Cooper, Hardy, & Hind, 1974). These results verified sympathetic nervous control of sAA secretion in humans.

Papcosta et al. (2011) reviewed using saliva as a tool to monitor steroids, peptides and immune markers during sport training. Saliva secretion rate and the

components of saliva are greatly affected by what types of autonomic receptors are being activated. Parasympathetic nerve stimulation appears to stimulate vasodilation, therefore increasing blood flow to the salivary glands. This increase in blood flow is associated with a higher saliva secretion rate (Baum, 1987). Saliva secretion stimulated by parasympathetic innervations is more watery and is low in organic and inorganic compounds. Sympathetic nerve stimulation results in saliva that is low volume and high in organic content, such as sAA, inorganic salts and total protein (Papacosta & Nassis, 2011). Therefore, elevated levels of alpha-amylase can serve as indicators of sympathetic nerve stimulation. Papacosta et al. (2011) concluded that using unstimulated whole saliva is a significant tool for assessing the status of immunological and endocrinological markers associated with exercise and training.

A number of studies have examined the activity of sAA during various laboratory stress tests including the Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993), and the Cold Pressor Task (CPT) (van Stegeren, Wolf, & Kindt, 2008). These tests both found an immediate rise in sAA from baseline levels. They found that sAA levels peak at approximately 10 minutes after the onset of a stressor, and quickly returned to baseline levels after the termination of the stressor (Ali & Pruessner, 2012). According to Ali et al. (2012) the sympathetic nervous system is quick to respond to stress as evidenced by the instantaneous changes in physiological markers such as sAA. The HPA axis is naturally a slower, longer lasting response to stresses, whereas the SAM axis responds quickly to acute stresses.

Chatterton et al. (1996) collected blood and saliva samples from male subjects at four 15 minute intervals before and after a running session, a written examination, or a rest period. Their purpose was to evaluate sAA as a measure of adrenergic activity under different conditions. Thirteen male students completed the written exam, 22 students completed the run and 12 students acted as a control. Participants reported to the lab at 07:30 to have a catheter placed in the antecubital vein. Blood and saliva sampling began at 08:00 and continued at 15-min intervals until 15-min after testing was complete. The written exam consisted of a 3 hour final examination beginning at 09:00 and ending at 12:00. Participants were allowed a 10-min break at 10:30. Participants also completed a study designed to determine the relationship between exercise intensity and sAA concentrations. Saliva and heart rate were taken before and after sequential 10-min sessions of walking, jogging and running. There was a 35-min break in between each exercise. Alpha-amylase and norepinephrine changes were significant for the exercise and the exam protocols. Amylase levels ranged from 150 $U \cdot ml^{-1}$ at baseline to 750 $U \cdot ml^{-1}$ immediately following the running test. Amylase levels did not significantly increase from walking, although heart rate increased 22%. Jogging increased levels of sAA 48% and heart rate 70%. Running increased the mean heart rate by 90% and sAA levels by 158%. There was a correlation between sAA and norepinephrine ($r=0.64$, $p<0.001$). Amylase and norepinephrine returned to normal within 30 minutes after each condition. This study demonstrated the rapid rise in sAA levels from psychological and physiological stressors. It demonstrated that more intense

stressors may elicit a higher sAA response. It also shows a correlation between sAA measured in saliva and norepinephrine measured in blood. Norepinephrine is commonly used to measure stress responses. This correlation of may verify using sAA as a marker of SNS activity.

During further studies by Chatterton et al (1996), the effects of temperature on the relationship of sAA to heart rate and body temperature were investigated. Ten subjects were exposed to both hot (66°C for 40-min), and cold (4°C for 40-min) temperatures. Saliva samples, heart rate and body temperature were taken every 10-min during the different temperature conditions. During heat exposure in a sauna sAA, heart rate and body temperature (36.5 to 38.4°C) all increased. However, during cold exposure, heart rate and body temperature were unchanged, while sAA levels increased rapidly. Chatterton et al. concluded that sAA could be used to predict various plasma catecholamine levels from varied temperature conditions.

These studies evaluated the way sAA is affected by various physiological and psychological events. They show the activation of the stress response and measure sAA along with traditionally measured blood biomarkers such as norepinephrine. This demonstrates that sAA can be measured as a marker of Sympathetic Nervous System activity.

Acute Exercise Effects on Alpha-Amylase

In exercise studies, there are numerous modes of exercise that can be used. Commonly used are swimming (Bocanegra, Diaz, Teixeira, Soares, & Espindola, 2012), cycling, and treadmill exercise (Gallina et al., 2011; Kivlighan &

Granger, 2006). One limitation of exercise is that eventually subjects will tire. This response is partially due to the accumulation of lactate in the muscle. Bocaneegra et al (2012) examined the salivary lactate, alpha-amylase and chromogranin A responses to incremental exercise, and compared them to that individuals blood lactate levels. Because lactate is measure in blood, collecting data is invasive and stressful to subjects. The purpose of this study was to determine if salivary biomarkers could be useful in determining the lactate threshold and exercise intensity of well-trained swimmers.

Twelve, male, well-trained swimmers, ages 21 ± 1.8 yr, were recruited. One week before the test, each subject underwent a VO_2 max test on a treadmill ergometer, with simultaneous measurements of gas exchange. To validate the test, subjects needed to meet two of the following criteria: plateau in VO_2, respiratory exchange ratio> 1.1, and higher than predicted maximal heart rate. On the testing day, subjects completed an incremental test doing the front crawl stroke in a 50 meter pool. The test consisted of eight 100-m swims done 16-24 seconds above the best time of each athlete's 100-m time. Blood and saliva samples were collected before each stage, 5 minutes after each stage and 10 minutes after each stage. Saliva samples were collected by having subjects chew on a sterile cotton swab called a salivette. Blood samples were taken through a fingerstick. After collection, all samples were frozen until processed at the lab.

Samples were analyzed using the Western blotting method. One-way analysis of variance (ANOVA) was used for analysis, followed by Tukey's test for

multiple comparisons. Pearson correlation coefficient was used among variables. There was a high correlation ($p<0.001$, $r= 0.91$) between the concentration of blood and salivary lactate. The concentration of alpha-amylase and salivary chromogranin A showed a similar pattern to that of blood lactate during the incremental test. Calvo et al. (1997) previously explored using sAA as a marker of catecholamines and found a high correlation between sAA and blood lactate ($r=0.93$). Other studies have also reported a correlation between sAA and other markers such as norepinephrine ($r= 0.63$) (Chatterton et al. 1996). This study showed that there is an inflection point during incremental exercise for sAA and salivary chromogranin A, which proved useful in determining the lactate threshold and workload intensity of the exercise.

Similarly Calvo et al. (1997) used salivary alpha-amylase (sAA) to try to determine the anaebolic threshold during both a maximal and a submaximal treadmill test. They recruited 20 healthy, active, young males that were currently a performing military training program that included both aerobic and anaerobic exercises such as jogging, weight training and a military training course. Following an overnight fast subject reported between 9:00-11:00 am. Following a 5-min warm-up on the treadmill, a progressive protocol of 3-min stages for lactate threshold was started. The treadmill was fixed at a 1% incline and running velocity was increased 0.28 $m \cdot s^{-1}$ per stage. The original workload was determined individually for each subject. Tests were terminated when blood lactate levels reached 4 $mmol \cdot L^{-1}$.

After an 80 minute rest period, each subject performed a maximal VO_2 treadmill test. The test was acceptable if two of the following criteria were met: plateau of VO_2 max with increasing heart rate, heart rate exceeding 95% of age-predicted max, respiratory exchange ratio exceeding 1.15 (Jones et al., 1985). Saliva samples were taken 5-min before the submaximal test and during the last 30 seconds of each stage.

They defined the saliva threshold (Tsa) as the point which the first continuous increase in salivary amylase concentrations occurred during exercise (Calvo et al., 1997). The Tsa could not be observed in 4 of the 20 subjects, however the findings of the study showed that the response of sAA to incremental exercise was similar in the majority of the subjects. The results of this study and the previously mentioned one demonstrate the potential relationship of sAA and intense exercise as measured through lactate.

Galinna et al. (2011) examined sAA and chromogranin A (CgA) during high intensity exercise and the possible correlations with cardiovascular and psychological parameters. Twenty-one active men were recruited (age 24±2 years). Before the test, all subjects were familiarized with the Borg 6-20 RPE scale (6-no exertion, 20-maximal exertion). All subjects completed a Bruce protocol test on a treadmill (RAM, Padova, Italy) with a 3-min warm-up walking at 0.765m/s at a 5% grade (Bruce, 1971). Each subject completed at least 5 stages of the Bruce protocol test. After peaking, each subject continued exercising for 1.5 min and then stopped and immediately sat. Heart rate was monitored using a 12-lead electrocardiogram (CARDIOVIT CS-200 Ergo-Spiro; Schiller AG, Baar,

CH). Systolic and diastolic blood pressure was assessed during each stage using the right arm. Saliva was collected at baseline, at the warm-up, at the stopping point, and at 5, 15, 30-min during recovery. Saliva samples were frozen at -20°C until analysis. Saliva samples were centrifuged at 2000 g for 10 min to remove particulates. Salivary CgA and sAA were measure using a Human CgA EIA kit (Yanaihara Institute, Shizuoka, Japan) and Salivary Alpha-Amylase Assay kit (Salimetrics Europe Ltd, Newmarket, UK), according to the manufacturer's directions.

The primary purpose was to evaluate the responses of sCgA and sAA throughout the exercise and recovery. Double product (systolic blood pressure x heart rate) (Kim, Choi, Takahashi, Kurokawa, & Yamasaki, 2003) and RPE was calculated as RPE x duration of the exercise in minutes, and were used as a measure of the intensity of the cardiovascular and psychological responses to the test. The Kolmogorov-Smirnov test was used to test for normality of continuous data distribution. Data was expressed as mean ± SD. Salivary alpha-amylase over time was evaluated by repeated measures ANOVA with Games/Howell post hoc test. Spearman's correlation was used to investigate the relationship between percent changes in DP and salivary values between median percentages of sCgA vs. DP and sAA vs. DP. The RPE was correlated with mean sCgA and sAA throughout the exercise test.

Salivary alpha-amylase increased significantly during exercise, reaching its highest value at the stopping point, then decreased rapidly throughout the recovery stages. The decrease in sAA throughout the 30-min recovery phase

was significant ($p<0.001$) with respect to the stop point. Alpha-amylase is reported in U/ml. (Baseline alpha-amylase: 45.9 ±13.7, warm-up: 67.9 ± 20.2, exercise peak: 110.2 ± 59.9, stop time: 279.3 ± 26.7, recovery time: 5-min 118.3 ±74.7, 15-min 67.2 ± 36.3, 30-min 59.6 ± 23.4). Salivary chromogranin A increased significantly during exercise, reaching its peak at the stop point, then decreasing during the recovery phase. There was no significant difference between the peak and stop stages ($p=0.904$). Recovery of sCgA was no significant until 30-min of recovery ($p=0.034$). No significant correlation was found between sAA baseline-to-stop and DP baseline-to-stop increments. Mean sAA was not correlated with RPE.

This study showed that during a short duration, intense exercise session, sCgA correlates with cardiovascular responses and the subject's perception of exhaustion. No correlation was detected between sAA and the cardiovascular parameters or the RPE-scale. Catecholamines are difficult to measure in saliva, therefore sAA has been proposed as an alternative method of measuring SAM activity (Kennedy, Dillon, Mills, & Ziegler, 2001). During the study sAA levels increased quickly in response to the exercise test, then fell swiftly by the 5-min recovery mark. The researchers believe that this high level of fluctuation may cause some problems in evaluating levels of sAA properly in response to exercise intensity. They also believe that because there was no correlation with sAA, but there was with sCgA, that the two are regulated in different ways. They believe that sAA shows too much interindividual variability and sCgA may be a better indicator of adrenergic response in relation to stress. This study conflicts

the results from Calvo et al. (1997) and Gallina et al. (2011), which did show correlations between sAA and cardiovascular measures. More testing should be done involving intense, acute activity, sAA and cardiovascular measures.

It is important to not only note the differences in sAA in regards to exercise duration and intensity, but also gender, previous experience, and the aspect of competition (Kivlighan & Granger, 2006). Kivlighan and Granger (2006) recruited 21 males and 21 female collegiate rowers, each having at least 3 semesters of experience. The rowing team trained for 5 days per week from 5-7:00 am. For testing, each rower participated in 1 competitive rowing session and one control day where no exercise occurred. They chose rowers because the competitions are brief (6-8 minutes), intense, and provide feedback on the performance and competiveness of the participants.

On testing day, the participants began with a warm-up 45 minutes prior to their competition. Rowing ergometers (Concept II, Morrisville, VT) were lined up in rows facing the same direction. The coaches told the rowers that only the top three finishers would be able to compete at a regional competition. Participants filled out several questionnaires about their competitiveness, bonding, strategic thinking and performance.

Saliva samples were collected before the warm-up, 20- and 40- minutes after the competition. Passive drool saliva samples were collected through a short plastic straw into a 5 ml collection vial (Bateup et al., 2002). Saliva samples were frozen at -80°C until assayed. Samples were thawed, centrifuged at 3000 rpm for 15-min to remove mucins prior to assay. Samples were assayed

for sAA using commercial kits from Salimetrics (Salimetrics, State College, PA). The amount in alpha-amylase in the sample is directly proportional to the increase (over a 2 minute period) in absorbance at 405 nm. All samples were also assayed for cortisol using a highly sensitive enzyme immunoassay used for in vitro diagnostic measure of adrenal function (Salimetrics, State College, PA). Each test used 25 µl of saliva.

A series of repeated measures ANOVAs were used to compute the change in alpha-amylase in response to competition. There were no main effects of gender on sAA in any of the competition phases. Varsity athletes had higher levels of alpha-amylase during each phase of the pre-competition anticipation phase (M=105.42U/ml, SD=60.67). Time-matched baseline and pre-competition samples were compared to determine if alpha-amylase levels were raised in anticipation of the competition and it was found that on average, alpha-amylase levels were lower before competition (M=43.24 U/ml, SD=42.17). Another unexpected effect was that pre-competition sAA levels were lower than baseline levels for women, but not for men ($p<0.001$).

The reactivity phase was classified as the time from pre-competition to 20 min post competition. On average sAA levels increased 156% from pre- to 20-min post competition. There was no significant change in levels on the non-competition day. This indicates that sAA changes in response to, but not in the anticipation of competition. This study was designed to follow the cycle of cortisol and thus missed the peak alpha-amylase response time because they

waited until 20-min after the rowing session to collect saliva. Saliva samples should be taken immediately post exercise to be sure to capture the sAA peak.

These studies demonstrate the reaction of sAA to short, intense exercises. Salivary alpha-amylase levels rise quickly and decrease in as little as 5 to 20 minutes after the stressor has stopped. This shows that it can be a useful tool to gauge the intensity of exercise, especially since it has been shown to be correlated with blood markers such as lactate and the anabolic threshold. Because sAA is only produced in response to a stimulus, there are not high levels of it continuously circulating in the body, which makes it a more accurate measurement tool. When measuring using sAA, it is important to get measurements immediately prior and post exercise to be sure to get crucial basal and peak measurements. There were conflicts between several of the mentioned studies as to whether cardiovascular measurements and sAA levels from exercise are correlated. This relationship should be tested more to properly evaluate if sAA is a good marker of exercise intensity.

Long Duration Exercise Effects on Alpha-Amylase

Aspects of the immune system are temporarily suppressed following high-intensity training exercises (Mackinnon & Hooper, 1994). Components of saliva are in part responsible in maintaining the health of the mouth. Alpha-amylase has been shown to inhibit the ability of bacteria to adhere to oral surfaces (Scannapieco, Solomon, & Wadenya, 1994). According to Walsh et al. (2010), saliva flow rate may be the most influential factor in the protection against oral pathogens. The normal unstimulated flow rate of saliva is 0.05-1.80 $ml \cdot min^{-1}$

(Cole & Eastoe, 1988). Physical exercise causes increased sympathetic nerve stimulation which is expected to decrease the amount of saliva being produced and increase the amounts of alpha-amylase in the saliva. The increased concentration of sAA in the mouth during and following aerobic exercise is thought to be a protective mechanism.

Walsh et al. (2010) completed a study highlighting the effects of high-intensity exercise on salivary alpha-amylase along with salivary IgA and total protein. High intensity exercises are often incorporated into the training routines of athletes. This study recruited eight trained men. Following an overnight fast, the men preformed a 60 minute cycle exercise session comprising of twenty 1-min periods at 100% VO_2 max. These 1-min intervals were separated by a 2 minute recovery at 30% VO_2 max. Saliva samples were collected into pre-weighed tubes using a passive drool technique before exercise, immediately post exercise and at 1, 2.5, 5 and 24 hours post exercise. The final saliva sample was collected following an overnight fast. Saliva samples were stored at -70°C until analysis. To estimate saliva flow rate, it was assumed that saliva density was 1.00 $g \cdot ml^{-1}$ (Cole & Eastoe, 1988). They found that the high-intensity exercise did not affect the flow rate of the unstimulated saliva they collected pre and post exercise bouts. The study emphasized the need for a stress-free control conditions because saliva flow rate can fluctuate due to exercise anticipation. They did find there was an increase in alpha-amylase secretion immediately post exercise. They believe this may improve the protective effects of saliva during exercise.

Using sAA during exercise can provide biomarker results similar to that of cortisol. Salivary amylase-amylase may be a more sensitive marker than cortisol in response to exercise because it is produced locally in the salivary glands instead of needing to be transported through the blood (Rohleder & Nater, 2009). Rohleder et al. also found that the sAA levels were more closely associated with anaerobic threshold than cortisol. Acute increases of sAA were evident in response to acute sessions of exercise as well as to intense exercises of both short and long durations.

Overtraining syndrome (OTS) is the collection of emotional, behavioral and physical symptoms from overtraining without proper recovery. The most common symptoms are fatigue, moodiness, altered sleep patterns, depression, and a loss of the desire to compete (Nederhof, Lemmink, Visscher, Meeusen, & Mulder, 2006). There are few studies comparing OTS in young athletes. Filaire et al. (2012) conducted a study examining the effects of a16 week training on the diurnal patterns of salivary alpha-amylase, cortisol, and the ratio of sAA over cortisol (AOC) in 12 national adolescent female tennis players. Stress and recovery was evaluated using the Recovery-Stress-Questionnaire for Athletes-RESTQ-Sport.

Participants were tested on two periods over 16 weeks between January and April. Each test consisted of a physical examination, determination of biological parameters in saliva and an assessment of recovery-stress state. The first test was conducted after a 2 week rest phase. Participants began with a 30 minute jog, starting slow and increasing up to a high, short sprint. This was

followed by stretching and strength training. The strength training included 40 repetitions of push-ups, pull ups, and sit ups continuously without rest. Participants rated their exertion levels using the CR-10 RPE scale (Borg, 1998). An exercise score was calculated by multiplying the duration and the RPE for the training session. The second test was conducted 16 weeks after the first. During this phase the participants were playing in an average of 30.0 ± 2.5 matches between January and April. Participants were asked to rate each tennis session and each physical training during the sixteenth week. The Recovery-Stress Questionnaire for Athletes (RESTQ-76) (Kellman and Kallus, 2001) was used at each test session. It measures the frequency of activity, mood, focus and recovery profile of each athlete. The scores of the stress-related tests were added and divided by the number of scales to get total stress score. The same was done for recovery questions, resulting in a total recovery score.

Participants were each given a bag containing 5 coded Salivette tubes. Participants were instructed how to complete the passive drool technique for whole saliva collection. They were instructed to take five saliva samples. The first sample was taken immediately after awakening, 30 minutes after waking, 1100 h, 1500 h, 2000 h before dinner. They were instructed to write the time of collection on the tube and to freeze the saliva sample in their home refrigerator until they brought them back to the lab. Saliva volume was estimated by weighing to the nearest milligram, and the saliva density was assumed to be 1.0 $g \cdot ml^{-1}$ (Cole & Eastoe, 1988). The saliva flow rate was determined by dividing the volume of saliva by the collection time. Salivary cortisol was assayed using

kits from Salimetrics (cortisol EIA kit, Salimetrics, Inc., State College, PA, USA). Salivary alpha-amylase was assayed using kits following the manufacturer's protocols (Salimetrics, State college, PA, USA). Results were computed in $U \cdot ml^{-1}$.

Pearson correlation coefficients were used to examine the relationships between cortisol and sAA. The study showed that the participants had a higher sAA output after the 16 weeks of training and tennis matches. Chronic stress may be associated with higher levels of alpha-amylase secretion (Tanaka et al., 2012). The sAA levels at the end of this study are similar to those of young, male ballroom dancers (Strahler, Berndt, Kirschbaum, & Rohleder, 2010), which suggests an imbalance between the Hypothalamic Pituitary Adrenal Axis and Sympathetic Adrenal Medullary Axis. Ali and Pruessner (2012) proposed a model where the SAM has an augmented response to stress and in turn the HPA response is blunted. They also reported that a ratio of sAA over cortisol may be a more sensitive stress marker than either cortisol of sAA alone.

Rosa et al. (2013) conducted a study to determine the effects of moderate exercise on saliva parameters of the immune system such as cytokines, IgA, alpha-amylase and total protein over a 24 hour period. Ten male volunteers took part in the study. The volunteers were all trained, having performed regular activity at least three days per week. On the first day of testing, each volunteer completed a progressive exercise test on a treadmill (LifeFitness, 9700HR, Schiller Park, IL, USA) to determine VO_2 max and confirm that all of the volunteers were trained. The initial velocity of the test was 6.0 km/h and the

speed was increased by 1.0 km/h every minute until voluntary exhaustion was achieved. The expired gases were collected at the end of each stage. Respiratory and metabolic measurements were analyzed using the expired respiratory gases with a metabolic system (COSMED PFT4, Rome, Italy).

One week later, each of the ten, male volunteers completed 60 minutes of exercise at 70% VO_2 max. Saliva samples were taken before, immediately after, 12 hours and 24 hours after the second test session using a salivette that was placed in their mouth for 2-min. The cotton roll was centrifuged at 10 000 g for 20-min to extract the saliva, the centrifuged at 10 000 for an additional 15-min to remove any debris. Samples were then frozen at -70°C until analysis. Alpha-amylase levels were assessed by enzymatic kinetic kits from Bioblin (San Paulo, Brazil). To eliminate interassay variance, all samples from one individual were analyzed in the same run.

Results are presented as means ± standard error. Significant differences were determined using one-way ANOVA and Tukey's post hoc tests. Significance was set at $p<0.05$. The alpha-amylase levels increased at 12 and 24-h after exercise (529 ± 18.39 and 524.36 ± 14.78 U/μg) relative to immediately post exercise. These are increases of 25.14 and 23.91% in the 12 and 24-h times after exercise, respectfully. These amounts are not significant when compared to resting levels. A previous alpha-amylase study by Costa et al. (2012), found no differences in alpha-amylase levels after exercise at 75% of VO_2 max. These studies demonstrate that acute exercise lower than 75% of VO_2 max is not enough of a stressor to elicit a significant sAA response.

These studies do not measure sAA levels often post exercise and therefore may not have measured to fast acting decline in sAA levels. Previous studies have indicated that sAA levels can return to baseline levels in less than 20 minutes and have recommended that studies involving sAA should take measurements every 10-min during recovery to accurately gauge the recovery response (Rohleder & Nater, 2009). Long duration exercise studies tend to take samples to gauge the response of slow response, long acting biomarkers such as cortisol and therefore miss the significant response times of fast acting biomarkers such as sAA. These longer duration stressors may also lack the intensity to stimulate enough of a stress response to facilitate significant sAA production.

Alpha-Amylase and Psychological Stress Testing

Anticipatory coping responses may occur before major stressors such as surgeries, major life changes, traumatic events, and when control is out of someone's hands. Chatterton et al. (1997) looked to examine the relationship between hormonal and psychological responses of young men preparing to skydive. Subjects were male college student who had never before been skydiving. On the jump day, each subject had a indwelling catheter with a heparin lock inserted into their antecubital vein at 7:45 am at a medical center. They then had a 45-min car ride to the skydiving location. During the drive, 4 samples of blood and saliva were taken, one sample every 15-min. Saliva was taken by having the subject chew sugarless gum and saliva was collected into a beaker and stored on ice. Additional samples were collected after a 1-hr

classroom instruction session at the skydiving school and just before entering the airplane. A control group was left at the skydiving school to watch TV while the test subjects boarded an airplane with instructors. After a 30-min flight, each of the subjects jumped with an instructor harnessed to them. Immediately after landing, blood and saliva samples were taken, and continued to be collected at 15-min intervals for 45-min.

Saliva samples were prepared using commercially available kits from Sigma Chemical (St. Louis, MO). Saliva samples were tested for alpha-amylase, cortisol, and testosterone. Blood serum was tested for cortisol, testosterone, luteinizing hormone, and growth hormone. An ANOVA with repeated measures was used to analyze the results. Plasma and salivary cortisol levels were lower in the skydivers then the control group at 8:45 am. Cortisol levels did not increase until after the subjects had landed. Salivary alpha-amylase concentrations were significantly higher on the morning of the jump than the control group ($p<0.001$). Growth hormone levels were significantly higher in the morning in the skydivers ($p<0.013$) across all measured points and remained elevated longer then the alpha-amylase levels post jump. Plasma and salivary testosterone levels were slightly higher throughout the day, but were not significant ($p<0.001$). Plasma luteinizing hormone levels were higher in the skydivers than the control group ($p<0.05$). None of the hormone levels increased significantly in the morning leading up to the skydive. Alpha-amylase levels, however, were significantly elevated at the time of the first collection when compared with the control group. Those levels remained elevated, with the

highest levels being immediately after landing. This shows the quick response of sAA when compared to cortisol from the skydive stress.

Salivary alpha-amylase has been speculated to be a valid measurement to reflect catecholaminergic changes due to an increase in sympathetic-adrenal-medullary (SAM) response (U. M. Nater, La Marca et al., 2006). A study by Nater et al. (2006), aimed to evaluate the possible relationship between salivary alpha-amylase, cortisol, norepinephrine, epinephrine and cardiovascular activity. They used an intra-individual repeated measures design and had 30 healthy, men (age 24.8 ± 2.4 years) undergo the Trier Social Stress Test (TSST), which consists of mental arithmetic and a speech in front of an audience. The TSST has been shown to induce an endocrine and cardiovascular response in 70-80% of tested subjects (Kirschbaum et al., 1993).

Prior to testing subjects were told to not undergo physical activity for 48-h, not to intake ethanol or caffeine for 18-h, and not chew gum for 24-h. Subjects were told not to brush their teeth within 60-min of testing. Fifty minutes prior to the TSST, a catheter was inserted into the antecubital vein. Basal salivary and blood samples were taken and subjects were slowly and quietly led into another room and given 10-min to prepare for their speech. Following their speech, each subject was taken back to another room where they were exposed to a simulated job interview for 5-min and some mental arithmetic for another 5-min in front of an audience. Sample of saliva were taken by having the subject chew on a cotton swab, and blood samples were taken immediately before, 5-min into, immediately after, and 10-min after the TSST. An additional saliva sample was

taken 20-min after the TSST. All testing was done between 1400 and1800-h. A control group was also tested between 1400 and 1800-h. Control subjects were taken to a room and told to sit quietly. Collection times were the same as the test group.

All samples were kept frozen until analysis. Upon thawing, samples were centrifuged at 3000 rpm for 5-min for saliva, or 10-min for blood. All samples from one subject were run in the same assay. Alpha-amylase activity was determined using the automatic analyzer Cobas Mira and assay kits obtained from Roche. Autonomic changes were assessed using heart rate variability (HRV). A Polar System (S810, Polar Finland) was placed on the lower part of the subjects sternum. Perceived chronic stress was measured using the Trier Inventory for the Assessment of Chronic Stress (TICS). Subjects were required to indicate how often they described stressful situations they had experienced within the past 3 months. The TICS is comprised of 10 subscales and a 36-item questionnaire.

Analysis of variance (ANOVA) with repeated measures was computed to reveal possible time and condition effects. Correlations between physiological measures were computed as Pearson correlations. The TSST was evaluated by the subjects as significantly more stressful than the control condition ($p<0.001$). Interaction of time and condition was significant ($F=5.68$; $p=0.02$). The TSST resulted in a significant increase in sAA (10.49U/ml; $p<0.001$). Changes over time in the control condition were also significant (3.12 U/ml; $p=0.043$). Salivary cortisol increased significantly (7.4 nmol/l; $p=0.002$), but there was no significant

effect in the control condition (2.42 nmol/l; p=0.12). There was a significant increase in plasma norepinephrine in both the TSST and the control condition (39.18 pg/ml; p<0.001 and 39.18 pg/ml; p<0.001). There was also a significant increase in plasma epinephrine from the TSST (14.82 pg/nmol; p<0.001), but not in the control condition (1.52 pg/ nmol; p=0.22).

Nater et al. (2006) determined that sAA is a valid and reliable stress marker, although they found no correlations between sAA and the measured catecholamines. This contradicts the findings of Chatterton et al. (1996), who did find significant correlations between sAA and norepinephrine during a 20-min running test.

Prior to salivary testing, measurements of the sympathoadrenal medullary system were restricted to electro-physiological measurements (heart rate), and plasma measurements (epinephrine and norepinephrine) (Rohleder et al., 2004). Rohleder et al. (2004) investigated the association between sAA and peripheral markers of SAM activity. In their first study 12 healthy subjects, 7 women (aged 41.56 y ± 2.53) and 5 men (aged 39.25 y ± 9.23) had a catheter inserted. Following a 30 minute resting phase, all subjects underwent the Trier Social Stress Test (TSST). Blood and saliva was taken immediately before, immediately after, 10 minutes and 20 minutes after the test. Salivary amylase and serum norepinephrine were analyzed. It was found that both parameters showed a similar response to the stress test, but were not correlated with each other at any of the four measurement points. In a second study, Rohleder et al. looked for a circadian rhythm in sAA. Twelve women (aged 23.18 y ± 177) and 5

men (aged 20.51 y ± 0.88) were instructed to collect saliva samples throughout a normal day. Sample times were immediately after waking, 30-min and 60-min after waking as well as 11:00, 15:00 and 20:00 hours. Salivary amylase levels were shown to decrease sharply after waking and increase throughout the afternoon and evening. These two preliminary studies demonstrate that sAA levels appear to be in a circadian rhythm and may be used as an indicator of sympathetic activity in healthy individuals. This was one of the first reports to analyze sAA and the response to waking. The results of the TSST are in line with previous studies demonstrating the acute effects of sAA to psychological stress.

In later testing, Rohleder et al. (2006) again used the TSST to examine alpha-amylase. With this study they investigated if saliva flow rate is affected by psychosocial stress. Secretion of saliva is activated by stimulation in the mouth and also by olfactory and visual stimuli (Ehlert, Erni, Hebisch, & Nater, 2006). Rohleder et al. evaluated if sampling method influences flow rate of saliva and if different saliva collection methods influence the amount of alpha-amylase. They also measured heart rate and salivary cortisol.

Twenty-six, Caucasian men were recruited. Participants were randomly assigned one of the sampling conditions (salivette or passive drooling). Participants in the salivette condition had a mean age of 23.2 y ± 2.5 years and a means BMI of 22.3 kg/m² ±1.7. Participants in the passive drooling condition had a mean age of 24.8 y ± 4.1 and a mean BMI of 22.6 kg/m² ± 1.8. All testing was completed between 14:00 and 17:00 h.

During the first laboratory session, a written explanation of the study was given out and an informed consent was signed. The first testing day was used as a control day. This fixed timing was chosen to eliminate carryover effects or any anticipatory responses. On both days a Polar S810 heart rate monitor (Polar Electro Oy, Kempele, Finland) was attached to the participants immediately after arrival. After a 30-min rest period the first saliva sample was taken. Depending on the group, saliva was taken by either passive drooling or salivette. On the control day, the participants were then taken to the testing room. On the control day there was no audience or video equipment set up. On testing day after sitting for 5-min, the participant was asked to stand up and read aloud for 10-min. After the TSST or control condition, participants were taken back to the waiting area and additional saliva samples were taken at 1, 10 and 20-min following the TSST or control condition. These times were chosen based on Nater et al. (2005) results showing that sAA levels rise quickly following a stressor and return to baseline levels following 10-20 min of rest. Heart rate was measured using the software “Heart Rate Variability Analysis” (Niskanen Tarvainen, Ranto-Aho, & Karjalainen, 2004) using R-R intervals.

Saliva samples were frozen immediately after collection at -20°C until analysis. Upon thawing, salivette samples were centrifuged at 3000 rpm for 5-min. Passive drooling samples were centrifuged at 20,000 rpm for 5-min to move any mucous compounds to the bottom of the tube. Alpha-amylase levels were measured in a Genesis RSP8/150 liquid handling system (Tecan, Crailsheim, Germany).

ANOVAs with repeated measures for the between subject factor (salivette vs. passive drooling) and the within subject factor (control vs. stress) and time (-1 min, +1 min, +10 min, +20 min) were calculated to test for stress induced sAA changes, sAA output, and to test for differences with sampling method. Similar analysis was run for heart rate. Pearson correlations were calculated to test for associations between increases in heart rate and salivary parameters. The ANOVAs show that saliva flow increased in response to the TSST, but not the control condition when the passive drool technique was used (Stress effect: $F[1,11]=5.11$, $p=0.045$) (Time effect: $F[3,33]=3.50$, $p=0.026$). Flow rate did not respond in the salivette condition. Amylase concentrations were also lower in the samples collected by passive drooling when compared with the salivette method (main effect condition: $F[1, 24]=10.44$, $p=0.004$). Heart rate increased in response to both the control and TSST condition, but failed to reach significance. Pearson correlations found that there were no associations between increases in saliva flow rate, amylase concentrations, and amylase output with any of the heart rate parameters. The results show that there were slight increases in saliva flow during passive drooling and increased alpha-amylase concentrations in the saliva, but not significant amounts. There were increased amounts of sAA in response to the TSST which corresponds to previous studies (Bosch, Ring, de Geus, Veerman, & Amerongen, 2002; Chatterton et al., 1996; U. M. Nater, La Marca et al., 2006; Rohleder et al., 2004).

These studies demonstrate that the passive drool method of collection is the better choice when trying to get whole unstimulated saliva flow, but saliva

flow rate should be taken into account. These studies also showed no correlations between sAA levels and cardiovascular measurements. Cardiovascular parameters and sAA levels were correlated in some exercise studies, but not others. Psychological stress testing may not be an intense enough stressor to elicit a higher enough stress response to elevate sAA levels significantly. These studies and their conflicting results verify the need for intense stress or exercise protocols when trying to evaluate the relationship between sAA and cardiovascular measurements.

Amylase Testing in Special Populations

Due to the easier nature of collecting saliva samples for testing, it is quickly becoming the method of choice for children, handicapped individuals, and those individuals who are unable to give blood for testing. Basal sAA levels do not change significantly over the adult life span (Salvolini et al., 1999). Salivary alpha-amylase is a valuable tool for accessing biomarkers for studies involving theses special populations. Capranica et al. (2011) wanted to evaluate the effects of a taekwondo competition on children using salivary alpha-amylase. The competition consisted of three 1-min rounds with a 1-min recovery in between. Saliva samples were collected 15 minutes before the match, 1-min after, 30, 60 and 90 min after using cotton swabs which the children chewed on 20 times. Heart rate was also measured to evaluate the intensity of the competition by placing a heart rate belt on the athlete's chest under their body armor before their match. They found that the child athletes spent 78% of the match working at HR>90% of their max heart rate. Saliva samples were

analyzed for cortisol and amylase using commercial kits (Salimetrics LLC, State College, PA, USA). A repeated measures analysis of variance (ANOVA) was applied to the salivary amylase and cortisol findings. The finding showed that the matches were highly stressful to the young athletes. The sAA showed a fast response with peak values at the end of the fighting and recovering to baseline within 30 minutes of recovery.

Chia et al. (2013) conducted a comparison of the oxygen use and physiological responses to exercise in children with Developmental Coordination Disorder (DCD). Developmental Coordination Disorder (DCD) is a condition in children where movement skills are below average in fine and/or gross motor skills, specifically the ability to run proficiently (Chia, Reid, Licari, & Guelfi, 2013). The researchers hypothesized that due to their difficulty moving, children with DCD would have higher oxygen consumption rates than control children, which were matched for age, height and body mass. They also believed that there would be a higher metabolic cost in children with DCD. To assess this they measured RER, heart rate, blood lactate, salivary alpha-amylase, and pain threshold.

Thirty boys ages 7-10 participated in the study. Fourteen of the boys had a DCD diagnosis and 16 boys were matched controls. Each subject reported to the exercise physiology laboratory on 2 separate occasions, which were separated by 1 week. During the first visit, each boy had his height, body mass, body composition, motor proficiency and VO_2 max measured. Body composition was measured using a dual energy X-ray absorptiometry scan (DEXA; encore

2004, Lunar Prodigy, GE Medical Systems, Madison, WI, USA). Images were analyzed for fat free mass, fat mass and body fat percentage. The Movement Assessment Batter for Children-2 (MABC-2) was administered for motor proficiency. Peak aerobic capacity was carried out on a treadmill. Each subject began walking on the treadmill at 2.4 km/h. The speed was gradually increased to 4.8 km/h and was maintained for 5-min. Each subject then had their fastest walking speed (FWS) determined by increasing the speed 0.2 km/h every 20-sec until the subject had to transition from walking to running. Next, VO_2 was determined by having each subject walk at a constant speed 1 km/h below their FWS. The treadmill gradient was raised 2% every 20-min until volitional exhaustion was achieved. During the test respiratory gases were collected and analyzed using gas analyzers (Ametek Applied Electrochemistry S-3A/1 and CD-3A, AEI Technologies, Pittsburg).

On the second visit, participants arrived at the lab in the morning following an overnight fast. First oxygen consumption was measure by having each subject stand quietly for 5-min while breathing through a mouthpiece connected to the gas analyzers. They then completed a warm-up on the treadmill by running at 6.8 km/h for 4-min. They then ran on the treadmill at 7.2, 8.0, and 8.8 km/h for 4-min while oxygen consumption was measured. The average VO_2 from the last minute of each speed was recorded as the steady state VO_2. The subject's heart rate was also measured during the final 10-sec of each running speed (Polar Electro Oy, Professorintie 5, FI-90440 Kempele, Finland). Between each running speed subjects rested for 8-min, or until heart rate returned to

baseline. Immediately after each speed, rating or perceived exertion was recorded and a blood sample was taken through a capillary tube from a finger tip. In addition a salivette was placed in each subjects mouth to collect saliva for 2-min. Lastly, pressure was applied using a pressure threshold algometer (Wagner Instruments, Greenwich CT) applied to the tibia and the mid-belly of the upper trapezius, middle deltoid and rectus femoris. Each subject was seated while increasing pressure was applied until each subject felt discomfort and verbally said 'stop'.

One-way between group analysis of variance (ANOVA) was used to compare subject characteristics and aerobic fitness between the DCD and control groups. Two-way between groups repeated measures ANOVA were used to compare oxygen use, blood lactate, RER, rating of perceived exertion, sAA, and pressure threshold. Post hoc analyses using one-way ANOVA were used to determine where any differences were. Analysis were done using SPSS version 19.0 for Windows (SPSS, Inc, Chicago, IL). Total body composition and fat free mass were similar in both groups, however, body fat percentage was higher in the DCD group ($F=5.6$, $p<0.05$). The DCD group had a lower aerobic capacity than the control boys ($F=22.1$, $p<0.001$). Four boys from the DCD group and five boys from the control group achieved a RER≥1.00 and a heart rate of ≥95% of their age predicted maximum. All 14 boys from the DCD group and 16 boys from the control group were able to complete the running speeds of 7.2 and 8.0 km/h. Only 11 boys from the DCD group and 15 boys from the control group were able to complete the fastest speed of 8.8 km/h. When oxygen

cost was expressed as a percentage of peak aerobic capacity, it showed that the DCD boys were working at a higher percentage of their capacity than the control boys at all speeds. RER was also higher in the DCD group.

Heart rate was higher in the DCD group as well. The main effect for blood lactate approached significance ($F=3.7$, $p=0.088$) with the higher levels being in the DCD group. There were no pre-exercise differences in salivary alpha-amylase between the groups ($F=2.5$, $p=0.124$). The main effect of group for the alpha-amylase response to running approached significance ($F=3.9$, $p=0.072$). Post hoc analysis revealed the DCD boys had higher levers of sAA following the two faster running speeds when compared with the control group (8.0 km/h, $F=5.9$, $p=0.023$, 8.8km/h, $F=6.3$, $p=0.020$). The boys with DCD had significantly lower pain thresholds over all four sites then the controls ($p<0.05$). The DCD boys were shown to be working at a energy cost which was seen by their higher heart rates and blood lactate levels at each running speed. They also had higher salivary alpha-amylase levels at each speed and at rest. This may be attributed to high levels of stress from anticipation of the test to come. It demonstrates the need to have relaxed conditions prior to taking basal measurements.

These studies demonstrate the ease of accessing salivary alpha-amylase and the various populations that can benefit from its measurements. Alpha-amylase can be used as an indirect marker of the SNS activity in children and adults. They also demonstrate that sAA can be measured in the field for subjects who are unable to travel or during competitive matches.

Not only may there be differences in the mechanisms of coping with stress, but there may also be different coping mechanisms between males and females. Takai et al. (2007) designed a study to determine whether there are gender differences in the HPA and SAM in response to an acute psychological stressor. To evaluate the HPA, the group used salivary cortisol, and salivary alpha-amylase was used as an indicator of SAM activity. A total of 83 volunteers were recruited ages 20 to 27 years. Volunteers were screened for physical or mental illness, pregnancy, and oral contraceptives. Each volunteer had their anxiety level assessed using the Spielberger's State-Trait Anxiety Inventory (STAI). This is a 40 questions quiz where results are based on a 4 point Likert scale. High test results (STAI score ≥ 55) indicate high anxiety, while low scores (STAI score ≤ 45) indicate low anxiety. The high anxiety group consisted of 18 females and 14 males while the low anxiety group consisted of 8 males and 4 females.

At the time of testing, each subject rinsed their mouth with water, then sat comfortably in a chair opposite a 19-incg TV monitor that was placed 100 cm away at eye level. A video recording or a corneal transplant surgery, which included images of injections into the eye and incisions into the cornea, served as the stressor for 15-min. While watching the video, subjects were instructed to tilt their heads slightly forward while keeping their eyes on the screen. This allowed saliva to accumulate in the floor of their mouth. A pre-stress saliva sample was taken 5-min prior to the start of the video and a post-stress sample was collected immediately after viewing. Saliva samples were centrifuged and

the supernatant was collected and stored at -20°C until analyzed. Salivary cortisol was analyzed using ELISA kits (Correlate-EIA kit, Assay Designs Inc. USA). Alpha-amylase was assayed using a blue starch kit as the substrate (Neo-Amylase test, Daiichi Pure Chemicals, Japan).

There was a wide range of basal levels of cortisol and amylase between individuals. No significant differences in alpha-amylase levels between male or females were observed in either the low or high anxiety group (male, low anxiety: 121.5 ± 57.9 U/ml; female, low anxiety: 132.1 ± 51.0; male, high anxiety: 141.1 ± 36.7 U/ml; female, high anxiety: 119.1 ± 48.2). Post stress levels were reported as percentages of the resting pre-stress levels (male, low anxiety: 160% above resting; female, low anxiety: 170% above resting; male, high anxiety: 200% above resting; female, high anxiety: 190% above resting). These amylase responses showed no significant gender differences in either the high or the low anxiety groups (Takai et al., 2007).

Another recent study to investigate the response of alpha-amylase to a stressor between genders is van Stegeren et al. (2008). They presented a group of male and female participants with two mildly stressful tasks to compare the responses of sAA and cortisol between the sexes. They also wanted to determine if sAA and cortisol responses were related during two consecutive stress tasks.

Eighty subjects (21 male and 59 female, mean age=20.7 ± 3.2 years) participated in the study. Testing was completed between noon and 1800 h. The first stress task consisted of the subjects watching a series of 144 pictures

derived from the International Affective Picture System (IAPS). The pictures were divided into 4 categories and depicted neutral items or tools (CAT1) to extremely negative emotional (CAT4) images depicting mutilation or serious injuries. Categories from 1 to 4 were related to IAPS norms ranging from neutral (5.0) to extremely negative pictures (2.0), and in low arousal (3.2) to high arousal (6.2). No positive pictures were shown. After each picture was shown on a screen a pop-up appeared with the prompt “how emotional did you feel the picture to be?”. Participants were then prompted to press one out of four buttons to indicate their emotional response (0=not emotional at all, 3=extremely emotionally intense). The picture task lasted between 10-15 min.

The second stress task was the cold pressor stress task (CPS) and evoked a physical stress response. Subjects were told to place their left arm including their elbow in an ice water tank at a temperature or 3°C. A container with warm water at 35-40°C served as a control situation. Subjects were randomly assigned to either the CPS of the control situation. Subjects were told to keep their arm in the water for 3-min or as long as they could. Immediately after the CPS or control procedure, subjects were asked how stressful he procedure was for them. They could score between 0 (no stress) and 10 (most stressful experience ever).

Saliva samples were collected using salivettes five times over the course of the two stressors. The first sample was taken just before the picture presentation started (t1= -30 min), the second sample was taken immediately after the picture presentation ended (t2=0). The following three samples took

place at +10 (t3), +20 (t4), and +60 (t5) after the start of the CPS. Salivary alpha-amylase was assayed using a quantitative enzyme kinetic method. Analysis included the evaluation of sex group differences in demographic characteristics with t-tests. An ANOVA was used to compare the stress ratings using Stress Task and Sex as independent variables. All measurements were log-transformed using Shapire-Wilk due to non-normality. ANOVA was also used to analyze baseline cortisol and sAA levels by Stress Task and Sex. There were no differences in the emotional rating of Task 1 (Picture Task) for subjects belonging to the control group or the CPS group. The mean stress rating of Task 1 did not differ between men and women, and were not correlated with sAA or cortisol baseline levels. Participants rated the CPS procedure as more stressful than the control task ($F1,75)=125$, $p<0.001$). There was no main effect of sex or interaction effect of sex by stress task. There were differences in baseline sAA levels by sex ($F(1,62)=5.141$, $p<0.05$), with men having higher baseline sAA levels then women at rest. Salivary alpha-amylase levels rose significantly between -30 and 0 ($F(1,51)=9.16$; $p<0.01$), just after the picture task. The sAA response to the CPS task revealed a just significant interaction effect of sAA x task ($F(1,51)=3.93$, $p=0.05$), where the CPS group displayed higher sAA elevations than the control group, who showed a drop in sAA levels. There was a main effect of Sex of sAA levels during the experiment with men having higher sAA levels throughout the experiment ($F(1,49)=5.191$, $p<0.05$), with no interaction of sex by stress task or time. The study found sex differences in basal sAA levels as well as sAA levels throughout the experiment, with men

having higher levels than women. They concluded that the sAA response pattern between men and women may be comparable, but baseline sAA levels should be considered when using mixed male and female populations in studies.

These studies demonstrate that baseline levels of sAA may differ in men and women, but there was a comparable amount of reactivity in both males and females to both physical and psychological stress tasks. These show that studies may choose to include both sexes in their studies, or if a study if looking at something very specific, may want to exclude specific populations for testing.

Jumping Exercise

Cycling, running, swimming and walking are commonly used exercises to meet the American College of Sports Medicines (ACSM) guideline recommendations for exercise in adults (Lyons, Navalta, & Callahan, 2010). Repetitive jumping is another option that is becoming more common. Rope jumping is one of the activities that can best improve cardio respiratory fitness (Haskell et al., 2007). It is important to know what pace is best for the desired goal when undergoing repetitive jumping exercises. The ACSM recommends 30 minutes or aerobic exercise at 64-76% of one's maximal heart rate to maintain fitness. Individuals may be jumping to burn calories, improve speed or endurance, tone muscle, warm up or cool down.

A study by Town et al. (1980) examined the energy expenditure in 19 males and 11 females using a 5-min rope skipping session using rates of 125 jpm, 135 jpm, and 145 jpm. Their results showed that females have higher heart rates and lower VO_2 max compared to males, but there were no differences in

metabolic values between the sexes. This study also showed the MET values of the subjects to be between 11.7 and 12.5 which demonstrated the intensity of the exercise.

Lyons et al. (2010) conducted a study evaluating the metabolic stresses associated with repetitive jumping at different cadences. Twenty-eight subjects, males and females, ages 18-25 were recruited to complete two jumping cycles, one at 120 jumps per minute (jpm) and one at 100 jpm. Each jumping session lasted up to15 minutes or until volitional exhaustion of the subject. Jumping sessions were separated by a minimum of 48 hours. Participants were instructed to abstain from caffeine, nicotine and alcohol for 24 hours prior to each test. Prior to the first session subjects were assessed for height, weight and body fat percentage. Body fat was calculated by using the sum of three Skinfold sites (males: chest, abdomen and thigh; females: tricep, suprailiac and thigh) using Lange Skinfold calipers. This study was conducted on an electrical jumping machine called a Digi-Jump, which has a beam of light for participants to jump over while the machine plays a set cadence. Subjects completed one exercise trial either at 120 or 100 jpm for 15-min or until volitional exhaustion. The second trial at the other speed was conducted a minimum of 48-h later. During each jumping session, metabolic measurements were obtained using a two-way low-resistance breathing value and mask. Expired gases were analyzed using a Vacumed Vista Mini-CPX (Vacumed, Ventura, CA). A heart rate monitor was worn by each subject during testing (Polar Vantage XL, Port Washington, NY).

Rating of perceived exertion (RPE) was determined using Borg's 15-point scale (Borg, 1970).

All data is reported as mean ± standard deviation. Analysis of variance (ANOVA) was used to test for differences among the subject's responses from the two different jump speeds. Lyons et al. (2010) found the peak metabolic values were: VO_2, (120 jpm: 40.88 ml·kg^{-1}·min^{-1} ± 4.74, 100 jpm 41 ± 6.16, p=0.904), heart rates (120 jpm: 174 ± 15.95 bts·min^{-1}, 100 jpm: 175.33 ± 16.46, p=0.57), RER (120 jpm: 1.08 ± 0.087, 100 jpm: 1.17 ± 0.1, p<0.001) and RPE (120 jpm: 15.89 ± 3.44, 100 jpm: 16.11 ± 3.17, p=0.602).

Lyons et al. (2010) found that the 100 jpm cadence was more anaerobic than the 120 jpm cadence. The subjects were able to maintain the repetitive jumping rate for a longer period during the 120 jpm trial. The subjects reported more upper leg fatigue during the 100 jpm session, while more lower leg fatigue was reported during the 120 jpm session. Blood lactate was not measure during this study, but a study from (Quirk & Sinning, 1982) revealed higher blood lactate levels from slower cadences. This is believed to be due to the deceleration required between jumps to maintain the slower cadence. Subjects reported preferring the 120 jpm cadence over that of the lower 100 jpm speed. Subjects were able to maintain the 120 jpm pace for 12.4-min ± 3.42 and the 100 jpm pace 9.68-min ± 4.31. Because salivary alpha-amylase levels are believed to peak at around 10-min of exercise, the 120 jpm pace would work better for an alpha-amylase based study.

For an unpublished study, Laura Igaune (2012) used the Digi-Jump machine to determine the steady state metabolic cost of repetitive jumping, and to determine if the Digi-Jump is a more or less strenuous exercise than a jump rope. Twenty-seven subjects, 12 males and 15 females, (aged 18 to 44 years) who participated in at least 30-min of moderate intensity physical activity most days of the week volunteered for the study. A maximal graded exercise test (GXT) was performed on a treadmill, and a Digi-Jump machine was used for the jumping trial. During both tests a two-way low resistance breathing valve was used to take metabolic measurements. Expired gases were analyzed using metabolic analysis equipment (ParvoMedics TrueOne 2400, Sandy, Utah). A heart rate monitor was worn at all times during each test (Polar Vantage XL, Port Washington, NY). Ratings of perceived exertion were taken the last 15 seconds of each stage of the maximal exertion test and the last minute during the jumping test, using Borg's 6-20 scale. Percent body fat was taken using Lange Skinfold calipers.

Each subject completed two laboratory sessions. The first session consisted of descriptive data measurements and the GXT. Descriptive data included age, height, weight, hip circumference and percent body fat. The Bruce protocol was used for the GXT test. Each subject will run at a pre-determined speed and grade until 2 criteria were met: 1. Subject's heart rate reaches within 10 beats of age-predicted max; 2. Respiratory Exchange Ratio is ≥1.15; 3. A plateau is observed in the subject's VO_2; 4. Rating of perceived exertion (RPE)

is greater than 17 on the Borg scale (6-20). The GXT should take approximately 8-12 minutes.

For the second session, the subjects engaged in a repetitive jumping session lasting for 5-min at a height of 0.5 inches at a cadence of 120 jpm on the Digi-Jump machine. Subjects were instructed not to eat for 4 hours prior to each testing session. They were also instructed to abstain from heavy exercise 48 hours prior and to avoid alcohol.

Statistical Package for the Social Sciences (SPSS) software was used to perform the analysis. Data is reported as mean ± standard deviation. Paired t-tests and one-way ANOVA and Tukey's Post-Hoc tests were used to test differences between the subject's responses to the differences testing protocols. Pearson correlations were used to determine the relationship between variables measured on the GXT and the Digi-Jump machine.

The one-way ANOVA and Tukey's Post-Hoc test indicated that steady state VO_2 during the 5-min jumping session occurred at minute 3 ($p<0.05$). VO_2 steady state at that point averaged 31.1 ± 5.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (8.9 METs). This study consisted of undergraduate and graduate college students, some of whom were collegiate athletes. When separated into groups (male, female, athletes, recreationally active) the time to reach steady state VO_2 did not change significantly.

The same analyses were used to determine the average time to reach steady state heart rate was 2-min 30-sec during the 5-min jump time. Mean heart rate was 149.2 ± 20.1 beats per minute (bpm), and the median was 152.4

bpm. Separating the groups showed the time to reach steady state heart rate did not change significantly for any group. Since steady state VO_2 occurred at 3-min, that time point was used to determine the rest of the tested variables.

Ventilatory threshold occurred during the GXT at 9 ± 2.2 minutes (median 8.3-min) at an average of 77.8 ±11.8 % of the subjects VO_2 max. Females reached their ventilator threshold at 8.5 ± 1.7 minutes (80.4% VO_2 max). The males reached their mean ventilator threshold at 9.8 ± 2.6 minutes (74.5 ± 14.4 % VO_2 max). The competitive athletes achieved median ventilator threshold at 10.2 ± 2.5 minutes (76.8 ± 13.1% VO_2 max). The recreational athletes reached ventilator threshold at 7.9 ± 0.6 minutes (77.8% VO_2 max). Jumping steady state for all subjects was 57.1% of VO_2 max, but ventilator threshold occurred at 77.8% of VO_2 max. This mathematically suggests there is a 20.7% difference between steady state and ventilator threshold. These findings are slightly lower than pervious jumping studies. This could be attributed to the short jumping duration, the calculating means at minute 3, when steady state was achieved, or that the subjects were not using a rope and thus did not have the added strain on shoulder and arm muscles.

Average heart rate for all subjects was during jumping was 149 ± 20.1 bpm with a steady state during the GXT of 184.7 ± 9.9 (80.9% max). There was no significant difference between male and female groups, showing that while females had a lower VO_2 max, they were still physically fit. Average Respiratory Exchange Ratio (RER) was 0.99 ± 0.6 with a max RER of 1.15 ± 0.07 (86%

max). Each of the groups showed very close results. These percentages suggest that all the subjects were performing close to their maximal performance, verifying that jumping is a strenuous activity. Ratings of perceived exertion using the Borg (6-20) scale averaged 13.5 ±1.5, with a max of 17.9 ± 1 (75.2% max) which shows the elevated intensity the subjects were feeling during the jumping session.

Chen et al. (2010) investigated the impact of rope jumping on visually impaired students. They recruited 16 visually impaired students, ages 15-17 from the National Taichung School for the Blind. The study included a 10 week continuous jump rope training with students doing three 50-min sessions per week. Participants warmed up for 10 minutes. They then completed 8 cycles of rope jumping. Each cycle consisted of 2-min of jumping, followed by 2-min of rest. This lasted for 30-min before a 10-min cool down. The exercise intensity was maintained using the Borg 6-20 scale. The students were randomly assigned to the experimental group, or the control group. The experimental group was provided with 10 weeks of rope jumping exercise training. RPE values from 11-15 were the set points for the exercise intensities. Level 11 was set as fairly light and level 15 was set as being difficult. After each of the eight 2-minute jumping session, each participant was asked about their RPE experience. Fitness levels of the experimental group were evaluated pre and post the 10 week rope jumping instruction.

The results of the study showed that rope jumping is a good alternative to improve the aerobic capacity of visually impaired students. The rope jumping

exercises can help the visually impaired to overcome movement restrictions and learning limitations. The study found that it was simple for the visually impaired students to learn the mechanisms of rope.

These studies demonstrate that repetitive jumping is a strenuous exercise that has a high metabolic demand. They also show the high MET and VO_2 values from repetitive jumping sessions. Their results show the intensity of jumping at quick cadences and the fatigue that individuals feel after an acute, repetitive jumping session. They also show the very little prep work needed to begin a repetitive jumping session. Very little instruction is needed. Individuals of various ages and fitness levels are able to participate and benefit. The activity can be lessened for a less strenuous exercise for children or those with disabilities, or can be increased for competitive athletes and those wishing to gain cardiovascular benefits from the exercise. The heart rate, RPE and VO_2 measurements of jumping found in these studies may show that repetitive jumping at 120 jpm may be intense enough to elicit a significant sAA response.

Diurnal Cycle of sAA

The autonomic nervous system is one of the main stress sensing systems in humans, and it has a distinct pattern. Sympathetic activity increases during the day and decreases at night, while parasympathetic activity decreases during the day and increases at night (Yamasaki et al., 1996). A distinct diurnal rhythm has been shown for salivary α-amylase in young adults and children (Rohleder et al., 2004; Rohleder, Wolf, Maldonado, & Kirschbaum, 2006).

Strahler et al, (2010), conducted a study to assess the diurnal profile of sAA and salivary cortisol in ballroom dancers. There were two groups of dancers recruited, younger dancers aged 15-30 and older ballroom dancers aged 49-75. All the dancers with the exception of one couple were lifelong dance competitors. Each participant had 5 saliva samples collected, the first being immediately after waking, 30 minutes after waking, 11 am, 3 pm, and 8 pm. Samples were collected using cotton swabs. After collection samples were frozen until analysis. Samples were thawed and centrifuged at 3000 rpm for 3 minutes. The concentration of sAA was analyzed using a quantitative enzyme kinetic method. The data was tested for normal distribution and homogeneity. Significant deviations in cortisol and α-amylase were found. Univariate ANOVAs were computed for a comparison between the groups. There were significant differences in groups with respect to BMI and awakening time. After controlling for BMI and awakening time, it was found that the older ballroom dancers had elevated outputs of sAA when compared with the younger population. Female older dancers had the lowest cortisol outputs.

The results concerning sAA show its usefulness as a sympathetic activity marker due to the elevated levels in older adults which they contribute to a so-called sympathetic drive that increases with age. They were also able to see a rise in sAA throughout the day in all of the ballroom dancers showing that levels of sAA increase throughout the day and in response to stressful conditions.

Rohleder et al. (2004) completed a study using seventeen healthy, young subjects, 12 women (aged 23.18 ± 1.77 years) and 5 men (aged 20.51 ± 0.88

years). The subjects were instructed to use a rolled up piece of cotton called a salivette to collect saliva samples throughout a normal day. Subjects placed the salivette in their mouth for several minutes to absorb saliva, then placed the salivette into a plastic bag and into the freezer. Collection times were immediately after awakening, 30 and 60-min after awakening, 11:00, 15:00 and 20:00 hours. They measured sAA and cortisol from the saliva samples. They found that sAA levels were lowest in response to awakening increased throughout the day and into the evening.

There had been several studies evaluating the flow rate and changes in sAA due to stressors, but no studies looking at the various factors that could influence a biological rhythm of a substance (U. M. Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). These factors include age, sex, emotions, stress and lifestyle. Nater et al. (2007) recruited 76 volunteers to take part in a study to establish if there was a diurnal cycle for sAA. They excluded those with psychiatric and somatic diseases, dental problems, abuse or drug use, smokers and those who worked night-shifts or transmeridian flights. Volunteers were instructed on the use of the collection instruments and procedures so collection could be done at home during a normal day of the subject. All subjects were university students so daily activities were comparable.

On the day of testing, a total of 15 saliva samples were collected by chewing on cotton rolls for 1-min (Salivettes, Sarstedt, Numbrecht, Germany). Samples of unstimulated saliva were collected immediately after awakening, 30, 60 minutes later, at 0900 h and each hour after until2000 h. Each subject was

instructed to wake up before 0900 h. The first sample was taken while the subject was still lying in bed. Time compliance for each subject was monitored by an electronic device that recorded when the box containing the cotton rolls was opened (MEMS 6 TrackCap Monitor, Aardex, Ltd., Switzerland). After chewing on the cotton swab, the salivette was stored in a plastic tube in a bag in the subject's refrigerator until all samples were collected the next day and frozen at -20°C until analysis. Both salivary cortisol and alpha-amylase were analyzed from the same saliva sample.

Prior to analysis, each sample was thawed and centrifuged at 3000 rpm for 5-min. All samples from one subject were run in the same assay. Alpha-amylase was measured by quantitative enzyme kinetic method. Due to positively skewed distributions, salivary alpha-amylase levels were log-transformed to approximate normal distributions. There were no differences between men and women in their average sAA levels. There were also no difference in the diurnal profile of men or women, nor did it differ by body mass, activity level, food and drink. Participants who woke up with an alarm clock showed higher sAA levels in the first hour after awakening ($t=2.29$, $p=0.025$). Overall the levels of sAA were lowest at the beginning of the day, within the first hour of waking and increased in the afternoon. They also found that sex, momentary stress, moderate activity, ingestion of food and drinks and body mass did not have a significant effect on the diurnal rhythm of sAA.

These studies evaluated the diurnal cycle of sAA and demonstrate that there is a distinct rhythm in sAA levels. Salivary alpha-amylase levels are lowest

within the hour following waking and rise throughout the afternoon and evening. Studies involving sAA should be done at a standardized time to account for the cycle of sAA.

Saliva Collection and Analysis Methods

There is a wide variety of methods available to those wishing to collect saliva. The most commonly used methods are absorbent materials, passive collection, and suction devices (Rohleder & Nater, 2009). The salivette is the most commonly used absorbent material used in saliva collection. A salivette is a cotton roll that is inside a plastic container. To use the subject will remove the plastic container and place the cotton roll in their mouth for 1to 5-min. In some protocols, subjects are instructed to chew on the cotton roll to stimulate saliva flow. This stimulated saliva is different in flow rate and composition than unstimulated saliva (Rohleder & Nater, 2009). A disadvantage is depending on where the salivette is located within the mouth, different saliva composition from the different producing glands can be gathered (Harmon, Hibel, Rumyantseva, & Granger, 2007). The use of salivettes in research may be beneficial to those wishing to collect samples from young children, or individuals unable to comply with another method. Suction devices have been developed to harvest saliva directly from specific glands in the oral cavity. These devices allow specific and precise sampling and are usually only employed by those with specific questions in the field of oral biology (U M Nater & Rohleder, 2009)

Another method of saliva collection is through passive drooling or spitting. This is the preferred method for collecting unstimulated whole saliva (Navazesh,

1993). For this passive draining method, subjects are instructed to empty their mouths by swallowing all saliva in their mouths. The subsequently secreted saliva is collected by allowing it to drain out of the mouth, down a straw into a collection device for 2 to 5-min. The major advantage of this method is that it gathers saliva from all of the glands that produce it. The disadvantages are that it is somewhat more difficult for the subject and it sometimes yields unfiltered samples, where there may be particulates or mucous present.

For any method of saliva collection, participants should refrain from eating or drinking anything but water at least 1 hour before collection. This will help to eliminate the risk of contamination of the samples. Subjects should also rinse their mouths with water immediately prior to the first collection to remove debris. Saliva samples can be stored for up to three weeks at room temperature without a significant loss in alpha-amylase activity (DeCaro, 2008). For long term storage, samples should be frozen below -20°C. Amylase has been shown to not be affected by repeated thaw and freeze cycles (D A Granger et al., 2006).

Measuring salivary alpha-amylase can be done by enzymatic measurement techniques. Assays for enzyme kinematic measurements are commonly done using 96-well microtitre plates and absorbance readers as described by Bosch et al. (1996, 2003). This process works by alpha-amylase cleaving the substrate into smaller intermediates, which are then broken down by another enzyme into p-nitrophenol (PNP). PNP absorbs light at a wavelength of 405nm. The more alpha-amylase present in the sample, the higher the optical

density that is measured in the reader at the 405nm (Salimetrics, State College, USA).

Amylase levels are typically reported in enzyme units per milliliter (U/ml). An enzyme unit is the amount of enzyme that catalyzes the conversion of 1 µmol of substrate per minute (Rohleder & Nater, 2009). Alpha-amylase levels can also be reported as amylase activity relative to saliva flow rate. This is obtained by multiplying the amylase activity measured (U/ml) with flow rate (ml/min). Saliva flow rate is determined by collecting saliva for a specified amount of time (usually 2 to 5-min) and weighing the collecting vial before and after, assuming the density of saliva is 1.0 g/ml (Cole & Eastoe, 1988).

CHAPTER 3

METHODOLOGY

Subject Characteristics

Institutional Review Board (IRB) approval for testing with human participants was obtained from the Protection of Human Subjects Committee at the University of Nevada, Las Vegas prior to any testing (Approval #1303-4393). Using the expected pre to post change in salivary alpha-amylase observed by Gallina et al. (2011) an effect size of 0.80 was calculated. Utilizing an α of 0.05 and a ß of 0.95, power analysis revealed a total sample size of 8 subjects would be sufficient to observe a significant difference if one was present.

In order to take a conservative approach, ten, apparently healthy, male and female volunteers, ages 18-44, were recruited for this study (men N=4, women N=6). Physical characteristics of the participants can be seen in Table 1.

Table 1: Participant Descriptives

Variable	Mean ± SD
Age (y)	25.1 ± 4.25
Height (cm)	173.48 ± 8.81
Weight (kg)	73.96 ± 16.74
% Body Fat	20.48 ± 7.36

Each participant was informed of the particulars of the study and asked to sign an informed consent form. Each participant was given a copy of the consent form for their records. Each participant completed a Par-Q questionnaire to ensure they had no contraindications which would prevent them from participating in the study. Participant inclusion criteria included the requirements of being apparently healthy as determined by the American College of Sports Medicine health and fitness facility pre-participation screening questionnaire and be free of any cardiovascular, metabolic, and/or pulmonary diseases (American College of Sports Medicine, 2006).

Collection of the Data

All testing was completed at a standardized time between 10 am and 2 pm due to the diurnal nature of salivary alpha-amylase. Testing consisted of two laboratory sessions. On the first day of testing, participants arrived at the University of Nevada, Las Vegas and proceeded to the Exercise Physiology Laboratory, MPE 312. The participant's height and weight were obtained using a balance scale with an attached height measuring device. Barometric pressure, relative humidity and air temperature of the lab was also recorded. Chronic smokers, women who were pregnant, those not classified as ACSM 'low-risk' and those with oral diseases were excluded from the study.

On the first visit, each subject participated in a graded exercise test (GXT) performed on a treadmill (Precor C954/C956, Precor Incorporated, Los Angelas, CA). Prior to the test, each subject was instructed on the use of Borg's 6-20

Rating of Perceived Exertion scale (RPE). Participants were fitted with a heart rate monitor placed on the lower portion of their sternum under their clothing (Polar Electro Inc., Lake Success, NY). Participants put on a nose clip and a head gear was placed on them to monitor oxygen uptake using a Moxus Metabolic System (AEI Technologies, Pittsburg, PA). Participants started with a 2-min warm-up (2.8 ± 0.4 mph) with no incline, followed by two minutes of jogging at a self-selected speed (6.2 ± 1.4 mph). The incline of the treadmill was then increased by a 3% grade every 2-min while the jogging speed remained constant until volitional exhaustion. RPE was taken at the end of each 2-min stage. Participant's VO_2 max was determined by averaging the two highest, consecutive values in a 30 second rolling average of VO_2. Average time for GXT completion was 10.3 ± 1.7 minutes.

The second day of testing took place 48 hours after the first. Participants again reported to the exercise physiology laboratory MPE 312. To avoid contamination or interference with saliva analysis, each subject was asked to do the following: Avoid alcohol for 12 hours prior to participation, refrain from eating within 60 minutes of collection, avoid caffeine, nicotine and over-the-counter medications for 1 hour prior to collection, participants were told not to brush their teeth within 45 minutes of collection, refrain from exercise for 48 hours, and not to have dental work 48 hours prior to collection.

Participants were each given a small glass of sterile water. The participant put the water in their mouth and rinsed to remove any debris or residue that may have been present. Participants waited a minimum of 10

minutes after mouth rinsing before giving the first saliva sample. During the wait time, Resting Metabolic Rate (RMR) for each participant was measured using the MOXUS Metabolic analysis system. A heart rate monitor was applied to the participant's lower sternum under their clothing (Polar Electro Inc., Lake Success, NY, USA). The participant then lay down on a table. A heart rate sensor was placed in proximity of the heart rate monitor to receive the signal from the monitor worn by the participant. Resting metabolic rate was measured for 15 minutes. The last 5 minutes of the 15 minute session were averaged to calculate each participant's RMR. The baseline RMR and heart rate measurements were used during analysis as a measure of how hard each participant was working during the repetitive jumping session and to compare with the GXT measurements previously taken.

Following RMR, each participant had their body fat calculated using an ELG III Tetrapolar Bioelectrical Impedance Analysis system (Electrolipograph System, ELG, Bioanalogics, Portland, OR). Participants remained lying down. A sterile alcohol wipe was used to clean a place on each participant's right wrist and right ankle. An electrode was placed on the participant's right wrist and right foot. BIA measurements were taken three times and averaged to get a final number. Each participant then used the passive drool technique to give the first saliva sample (Salimetrics, State College, PA). Saliva samples were collected using the protocol outlined by Salimetrics, a commercial saliva analysis company. Each participant sat up on the table and was given a 2 ml sterile cryovial and a sterile 2 inch plastic straw. The participant was instructed to put

one end of the straw into the cryovial and the other end in their mouth. They then tilted their head down and allowed saliva to run down the straw into the vial. Approximately 1 ml of saliva was collected for analysis.

After the first saliva collection, participants were outfitted with headgear and a mouthpiece for the MOXUS so the participant could begin the jumping protocol. The jumping protocol consisted of a 10 minute jump session at 120 jumps per minute (jpm). Participants were monitored during the session and participants were instructed that they could stop the session at any time if an issue should arise. Participants were monitored throughout the session for safety. During the session, heart rate and VO_2 were monitored. A metronome (JM-1000, J & H Technology Co., China) was used to assist the participant in maintaining the 120 jpm cadence. Each participant jumped for 10 minutes. After completing the jumping session, the mouthpiece and headgear were removed so a second saliva sample could be obtained. Participants were given a new sterile straw and cryovial and the passive drool technique was again used. No rinsing of the mouth was required before the second sample was taken. Participants were asked to sit quietly for a 20 minute rest period following the jump session, after which a third saliva sample was collected. Collection vials were weighed pre and post collection. Saliva samples were frozen at -80°C in BHS 119 until samples from all participants were collected. Analysis of saliva samples was done using Salimetrics saliva assay kits (Salimetrics Inc., State College, PA). Samples were run in duplicate with pre- and post- saliva with the two samples. Analysis was done using instructions provided from Salimetrics.

Data Analysis Methods

Samples and reagents were brought to room temperature prior to analysis. Plate layout was determined prior to analysis. The Salimetrics high and low salivary alpha-amylase controls were run with each assay. The microplate reader (Epoch, BioTek Instruments Inc, Winooski, VT) was set up to read in center measurement kinetic mode initially at 1 minute, then again 2 minutes later. The 405 nm filter was used with no reference filter. A 1:10 dilution of saliva was prepared by combining 10 µL saliva with 90 µL of alpha-amylase diluent. The mixture was diluted further by pipetting 10 µL of the 1:10 dilution with 190 µL of additional alpha-amylase diluents. This gave a final concentration of 1:200. The alpha-amylase solution was heated in a heater block bath incubator (Thermo Fisher Scientific, Livonia, MA) to 37°C in the provided warming trough. Eight microliters of prediluted controls and/or unknowns was added to individual wells. Three hundred and twenty microliters of preheated amylase-amylase substrate was then added to each well simultaneously using a multichannel pipette (Thermo Fisher Scientific Inc., Finland). The assay tray was then placed in the plate reader and read at exactly 1 minute and 3 minutes. The 1 minute readings were subtracted from the 3 minute reading and multiplied by the standard conversion factor. Results of sAA levels are expressed in U/mL.

Alpha-Amylase Calculations

$$\frac{\Delta Abs/min \times TV \times DF}{MMA \times SV \times LP} = \text{U/mL of α-amylase activity in sample}$$

Where: ΔAbs./min = Absorbance difference per minute

TV = Total assay volume (0.328 mL)

DF = Dilution factor (200)

MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)

SV = Sample volume (0.008 mL)

LP = Light path = 0.97(specific to plate received with kit)

Statistics

A one-way repeated measures analysis of variance (ANOVA) was used to analyze the data from the 3 amylase collection times of the 10 participants. Statistical Package for the Social Sciences (SPSS version 20, IBM Corporation, Armonk, NY) software was used. Results are reported as Mean ± Standard Deviation. Heart rate during the jumping protocol was compared to heart rate during the maximum treadmill test and reported as a percentage to compare the intensity of the two exercise sessions. Oxygen uptake (VO_2) during the jumping protocol and max test is also reported in this manner.

CHAPTER 4

RESULTS

Findings

Analysis revealed amylase pre jump levels to be 29 ± 20. 87 $U \cdot ml^{-1}$ (M ± SD), amylase post 123.73 ± 104.09, and amylase 20-min post 25.28 ± 16.32 (Figure 1). Pairwise analysis using Sidak adjustment for multiple comparisons revealed that a 10-min repetitive jumping session significantly elevated salivary alpha-amylase concentrations compared to baseline levels ($p=0.032$). Additionally, sampling at 20-min post exercise revealed that amylase concentrations had returned to baseline levels ($p=0.779$). Heart rate and VO_2 during RMR, JP, and GXT can be seen in Tables 2 and 3 respectively. Workload intensity comparison of GXT and JP can be seen in Table 4. Ratings of perceived exertion (RPE) can be seen in Figure 2.

Table 2: Heart Rates During Testing. Heart rates (bpm) obtained following a 15-min resting metabolic rate (RMR) session, a 10-min repetitive jumping session (JP), and an exhaustive treadmill test (GXT).

Variable	Mean ± SD
RMR	69.5 ± 10.11
JP	176.9 ± 18.38
GXT	191.6 ± 7.01

Table 3: VO_2 During Testing. VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) obtained following a 15-min resting metabolic rate (RMR) session, a 10-min repetitive jumping session (JP), and an exhaustive treadmill test (GXT).

Variable	Mean ± SD
RMR	4.22 ± 1.03
JP	41.25 ± 6.47
GXT	53.11 ± 10.67

Table 4: Jumping Protocol and Maximum Effort Test Comparison. Workload percentage Comparison of Heart rate and VO_2 during jumping protocol compared with GXT

Variable	Mean ± SD
HR	92.33 ± 7.79
VO_2	77.67 ± 9.70

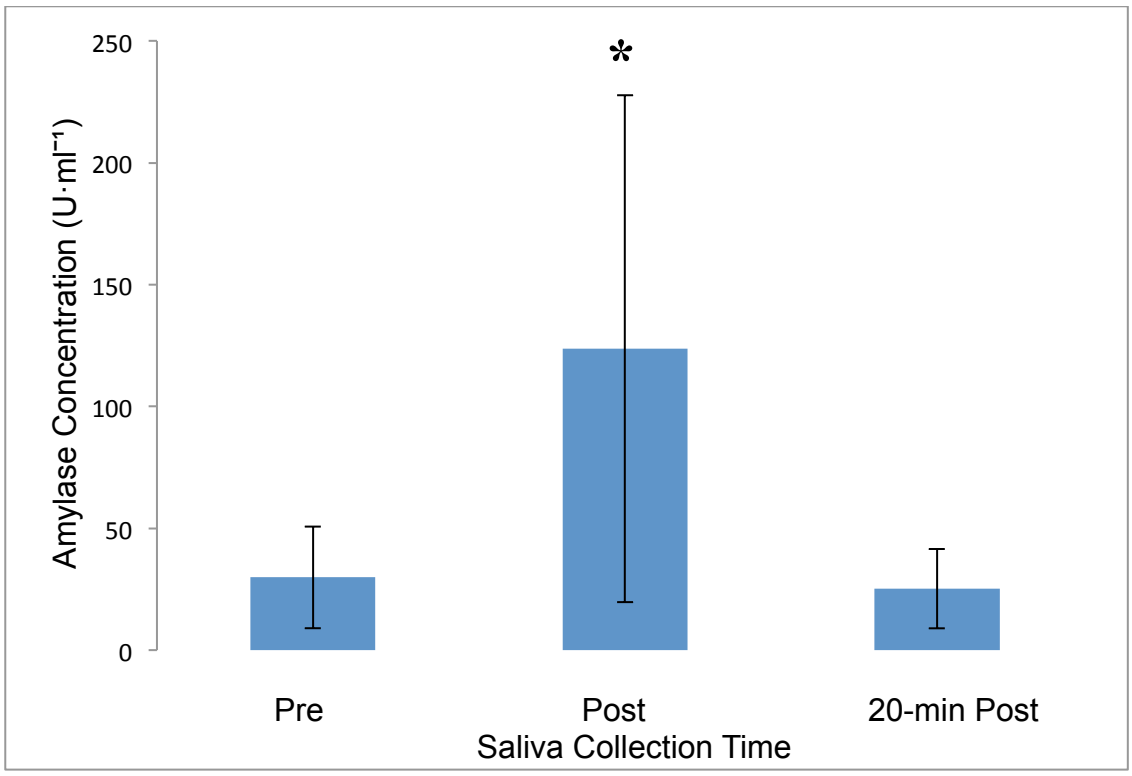

Figure 1: Salivary Alpha-Amylase Levels During Testing. Salivary alpha-amylase concentration before (Pre), immediately after (Post), and 20-min post repetitive jumping. * indicates significant difference compared to pre and 20-min post measures ($p<0.05$)

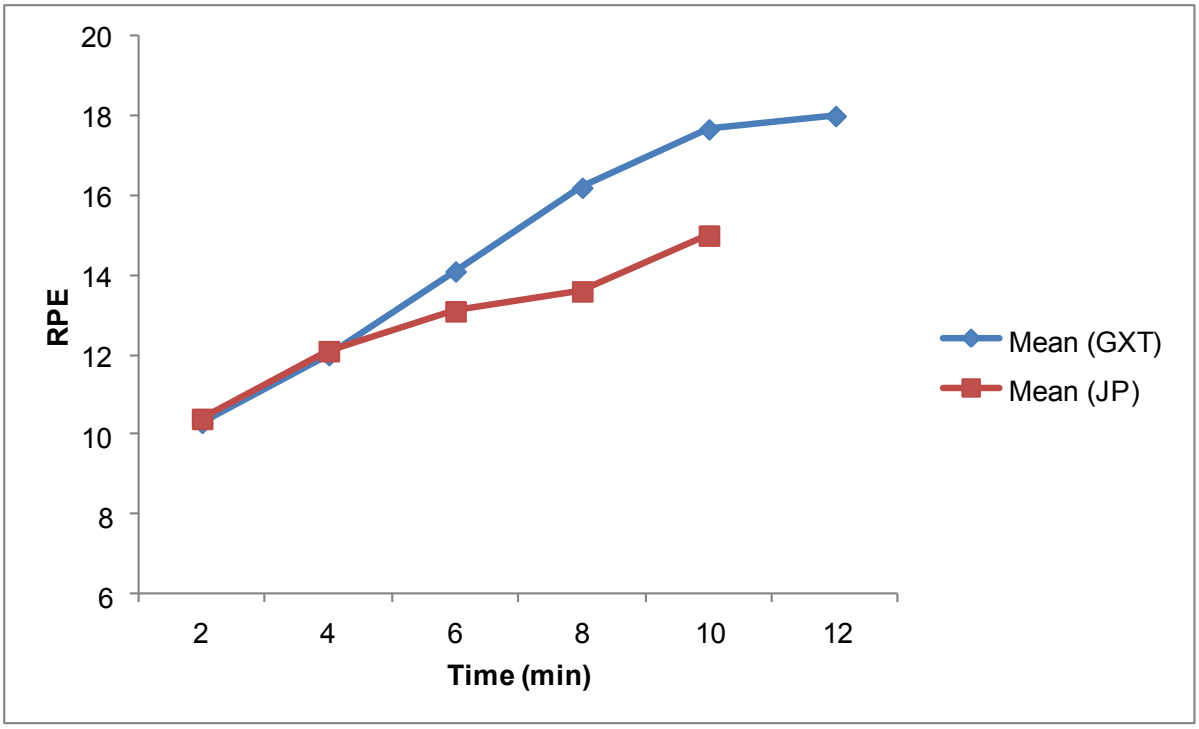

Figure 2: RPE Means During Testing. RPE Means taken every 2-min during JP and GXT

CHAPTER 5

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Discussion of Results

The most important observation in this study was that amylase levels did increase significantly from resting levels in response to the 10-min repetitive jumping session, and decreased significantly within the 20-min rest period. There was no significant difference between baseline amylase levels and concentrations following the 20-min recovery period. This provides evidence to support the hypothesis that a 10-min repetitive jumping session at 120 jpm is of sufficient intensity to elicit a response in salivary amylase, and a 20-min recovery is enough time for amylase levels to return to baseline levels. This demonstrates the possible use of amylase as a stress indicator during short, intense exercise stress studies. These results are similar to previous investigations utilizing moderate to intense activity such as track running (Chatterton et al.), and treadmill running (Gallina et al., Rosa et al.) which also stimulated an increase in salivary amylase levels, and provide evidence that these findings can be extended to repetitive jumping as a mode of exercise. The amylase levels found in the present study was at similar levels to those from previous amylase studies (Chatterton et al: peak; 169 ± 44 $U \cdot ml^{-1}$, Gallina et al: baseline; 45.9 ± 13.7 $U \cdot ml^{-1}$, peak; 110.2 ± 59.9 $U \cdot ml^{-}$).

Salivary alpha-amylase may prove to be a valuable stress marker due to the saliva glands releasing enzymes upon stimulation of the sympathetic nervous system during exercise (Proctor & Carpenter, 2007). Amylase levels have been

correlated with other markers of sympathetic nervous system activity, such as norepinephrine and epinephrine (Chatterton et al., 1996), cortisol (Papacosta & Nassis, 2011), and blood lactate (Gilman, Thornton, Miller, & Biersner, 1979). The increased amylase concentrations immediately post jumping session may be part of the protective benefits of saliva, since amylase has been shown to inhibit bacterial attachment to oral surfaces (Scannapieco et al., 1994). Amylase also plays a role in the breakdown of starch into glucose and maltose. Nater and Rohleder (2009) discussed how short-term increases in amylase, and thus starch breakdown may be useful in the preparation for the flight-or-flight response, by increasing the amount of available glucose for energy. A short, intense exercise session such as a GXT or repetitive jumping may have elicited this same physiological response. Dehydration and hyperventilation from the exercises may have contributed to the increased levels of amylase post exercise due to some evaporation of the liquid portion of saliva. Salivary alpha-amylase levels may have recovered quickly during the 20-min rest phase as the participant's bodies returned to a homeostatic state following the 10-min repetitive jumping session.

Research by Costa et al. (2012) demonstrated that activity needs to be more intense than 75% of a participants' maximum to elicit a significant amylase response. The jumping protocol used in this study was 92.33% ± 11.01 of participants' heart rate max and 77.67% ± 17.78 VO_2 max as calculated by comparing heart rate and VO_2 taken during each participants' GXT and jumping test. This demonstrates the intensity of the 10-min jumping session used for this

study and gives further evidence of repetitive jumping as a relatively intense workout. Reported RPE values taken every 2-min during the JP were an average of 93% to those taken every 2-min during the GXT. Some participants claimed that the JP was more muscle fatiguing than the GXT because repetitive jumping was a movement they were unaccustomed to.

Many studies have been done to try to correlate salivary amylase with various markers of nervous system activity such as heart rate (Gallina et al., 2011), cortisol levels (Papacosta & Nassis, 2011), blood lactate (Bocanegra et al., 2012; Calvo et al., 1997), and norepinephrine (Kang, 2011; U. M. Nater, La Marca et al., 2006). These studies believe that amylase gives a more accurate reflection of body stresses during situations because of its fast acting and easily collectable nature. A practical application of using the faster acting amylase could be stress testing populations on medications such as beta-blockers. These medications affect heart rate during exercise and amylase may provide a better indicator of stress in these individuals. Studies have shown that amylase levels peak after 10-min of activity making it ideal for use with short, intense stressors such as repetitive jumping (U. M. Nater, Abbruzzese, Krebs, & Ehlert, 2006; U. M. Nater, La Marca et al., 2006; Rohleder et al., 2006).

Conclusions and Recommendations for Further Study

The focus of this study was using amylase as an intensity and body stress indicator during a short, intense jumping session. The amylase levels elevated significantly above baseline levels and returned to resting levels within the 20-

min rest period as hypothesized. There was a range of ages (18-32 y), fitness levels (VO_2 max of 35.5-70.6 $ml \cdot kg^{-1} \cdot min^{-1}$), current activity levels, gender, and ethnicity between participants. Previous studies have often used specific samples of participants to study amylase responses. This study used a convenience sample of college students and had less specific guidelines for participation. Future studies may wish to use more specific sample populations, and other short term exercises to examine salivary amylase. Amylase may also be examined in trained populations as a potential way to assess training status, stress levels from different training protocols, and as a way to examine how players cope with competition and training.

The purpose of the current study was to determine if a 10-min repetitive jumping session at 120 jpm would cause a significant increase in salivary alpha-amylase levels, and whether the amylase concentrations would return back to baseline within a 20-min recovery period. Amylase levels did increase significantly above baseline levels ($p=0.032$) and decrease significantly back to baseline levels within the 20-min recovery time ($p=0.022$). The significant increase in amylase concentration post exercise is believed to be due to the activation of the flight-or-flight response through the sympathetic nervous system. According to previous studies this may increase glucose levels short term as the body attempts to return to homeostasis.

Salivary alpha-amylase through further research may prove to be a useful biomarker in stress research due to the ease of collection, the minimally invasive nature of collection, and the ease of analysis. Amylase requires minimal time to

collect, can be collected repeatedly in many populations which may prove useful when other biomarker collection methods are unethical and difficult, such as children, those with special needs, and water sports. It can also be collected in the field and requires minimal training. Future studies could potentially use other exercise activities to examine if amylase levels increase in a similar fashion as those in the present study. Studies may also wish to include the effect of different intensities to examine a possible dose response of amylase to exercise conditions. This possible dose effect could be examined in many populations, during other short-term or longer duration exercises. Salivary alpha-amylase may also be potentially used a way to asses training status as opposed to traditional measures such as heart rate, VO_2, and body fat percentage. These factors as well as the swift nature of amylase may prove useful to future stress studies.

APPENDIX A

INFORMED CONSENT

Department of Kinesiology and Nutrition Sciences

TITLE OF STUDY: Salivary Alpha-Amylase as an Indicator of Body Stress Following an Acute Session of Repetitive Jumping

INVESTIGATOR(S): James Navalta, Ph.D

For questions or concerns about the study, you may contact Dr. Navalta at **(702)895-0996.**

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted, contact **the UNLV Office of Research Integrity – Human Subjects at 702-895-2794, toll free at 877-895-2794 or via email at IRB@unlv.edu.**

Purpose of the Study

The purpose of this study is to determine the intensity of a repetitive jumping exercise using salivary alpha-amylase as an indicator of body stress.

Participants

You are being asked to participate in the study because you fit this criteria: are between the ages of 18-44, have no cardiovascular, respiratory, or orthopedic limitations, have no oral diseases, are not pregnant.

Procedures

If you volunteer to participate in this study, you will be asked to do the following:

- Attend two testing sessions that will last less than 1 hour each in MPE 312 at the UNLV campus in Las Vegas, NV.
- On your first testing session you will undergo a maximum effort graded exercise test on a treadmill. You will be fitted with a heart rate monitor and a headgear. You will then walk at a pace of your choice for 2-min on a treadmill. You will then choose a running speed which will remain

constant throughout the test. The incline of the treadmill will be raised 3% every 2-min until you stop running.

- The second session will take place a minimum of 48 hours after the first. This session will begin with a 15-min Resting Metabolic Rate (RMR) session. You will put on the heart rate monitor and headgear mask and lie on a table for 15-min. After the RMR, you will have electrodes attached to your right wrist and ankle to have your body fat measured. You will then be asked to give a saliva sample. After giving a small saliva sample, you will begin jumping in place at 120 jumps per minute (jpm) for 10-min or until you are too tired to continue. After 10-min, or you choose to stop jumping, you will give a second saliva sample. You will then sit quietly for 20-min and then give a final saliva sample. Initials:_____1 of 2

Benefits of Participation

There may not be direct benefits to you as a participant in this study. You will be given information on your maximum heart rate, oxygen consumption and body fat, if you choose.

Risks of Participation

There are risks involved in all research studies. This study may include only minimal risks. Because you will be participating in intense activities, there is a risk of soreness.

Cost /Compensation

There will not be financial cost to you to participate in this study. The study will take less than two hours total of your time, less than 1 hour at each of the two testing sessions.

Confidentiality

All information gathered in this study will be kept as confidential as possible. No reference will be made in written or oral materials that could link you to this study. After the storage time the information gathered will be destroyed.

Voluntary Participation

Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with UNLV. You are encouraged to ask questions about this study at the beginning or any time during the research study.

Participant Consent:

I have read the above information and agree to participate in this study. I have been able to ask questions about the research study. I am at least 18 years of age. A copy of this form has been given to me.

Signature of Participant _____ Date _____

Participant Name (Please Print) _____

APPENDIX B

TESTS OF NORMALITY

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Amylase_Pre	.258	10	.059	.829	10	.032
Amylase_Post	.223	10	.174	.795	10	.013
Amylase_20min_Post	.301	10	.011	.850	10	.058
HR_JP	.250	10	.076	.876	10	.116
VO2_JP	.188	10	.200*	.889	10	.163
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

APPENDIX C

REPEATED MEASURES ANOVA DATA

Descriptive Statistics

	Mean	Std. Deviation	N
Amylase_Pre	29.8718	20.872416	10
Amylase_Post	123.72680	104.086768	10
Amylase_20min_Post	25.281	16.3169	10

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time	61738.040	2	30869.020	10.815	.001	.546
Error(Time)	51376.144	18	2854.230			

Pairwise Comparisons for pre, post, and 20-min post testing conditions

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]
1	2	-93.855	29.382	.032

	3	4.591	5.139	.779
2	3	98.446	28.684	.022

b. Adjustment for multiple comparisons: Sidak.

APPENDIX D

INDIVIDUAL DATA

Individual heart rate data (bpm)

Subject	HR (RMR)	HR (JP)	HR (GXT)
1	56	148	182
2	76	184	192
3	74	197	191
4	85	195	191
5	66	154	186
6	62	184	201
7	68	186	192
8	66	171	197
9	86	194	202
10	56	156	182
Mean ± SD	69.5 ± 10.11	176.9 ± 18.38	191.6 ± 7.01

Individual VO_2 data ($ml \cdot kg^{-1} \cdot min^{-1}$)

Subject	VO2 (RMR)	VO2 (JP)	VO2 (GXT)
1	2.4	43.8	70.6
2	4.2	48.2	50.5
3	3.4	30.2	35.5
4	6.6	48.3	58.2
5	4.1	47.3	67.2
6	4.1	38.6	50.2
7	4.9	46.7	54.2
8	3.7	32.5	46.5
9	4.4	34.8	42.3
10	4.4	42.1	55.9
Mean ± SD	4.22 ± 1.03	41.25 ± 6.47	53.11 ±10.67

Individual Amylase levels Pre, Post and 20-min post repetitive jumping

Subject	Amylase Pre ($U \cdot ml^{-1}$)	Amylase Post	Amylase 20-min post
1	59.07	93.64	25.26
2	8.53	78.23	21.81
3	26.73	176.96	20.17
4	55.43	143.99	22.63
5	11.15	21.98	12.63
6	10.50	21.65	3.44
7	41.82	125.30	45.76
8	14.92	92.09	20.50
9	55.27	386.06	60.56
10	15.29	97.39	20.05
Mean ± SD	29.87 ± 20.87	123.73 ± 104.09	25.28 ± 16.32

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Dixie Ophthalmic Specialists, St. George, UT
Ophthalmic Technician

LDS Hospital, Salt Lake City, UT
Pharmacy IV Technician

Dixie Regional Hospital, St. George, UT

Pharmacy Technician

Professional Presentations
Physically Interactive Video Games Increase VO_2 Above Resting Metabolic Rates.
Annual Meeting of the American College of Sports Medicine, Indianapolis, IN, 2013.

Certifications and Skills
Professional Rescuer CPR/AED
Fitness Assessments (body composition, strength and cardiovascular field tests)

Thesis Title: Salivary Alpha-Amylase as an Indicator of Body Stress Following an Acute Session of Repetitive Jumping

Thesis Examination Committee:
Chair, Dr. James Navalta, PhD
Committee Member, Dr. Richard Tandy, PhD
Committee Member, Dr. John Young, PhD
Graduate College Representative, Dr. Daniel Young, DPT