8-2019

The Salivary Alpha-Amylase Response to Resistance Training

Asher Flynn
East Tennessee State University

The Salivary Alpha-Amylase Response to Resistance Training

A dissertation

presented to

the faculty of the Department of Sport, Exercise, Recreation, and Kinesiology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy in Sport Physiology and Performance

---

by

Asher L. Flynn

August 2019

Michael H. Stone, PhD, Chair

Caleb Bazyler, PhD

Brad H. DeWeese, PhD

Jeremy Gentles, PhD

Nick Fiolo, PhD

Keywords: Athlete Monitoring, Performance Biomarker, Vertical Jump

ABSTRACT

The Salivary Alpha-Amylase Response to Resistance Training

by

Asher L. Flynn

Purposes of this dissertation were to investigate the response of salivary alpha-amylase to a single resistance training session and to a week-long resistance training over-reaching protocol. The major findings of this dissertation are as follows:

Study 1 – A single resistance training session consisting of 5 sets of 10 repetitions of squat and bench press at 95 percent of repetition maximum creates a statistically significant increase in salivary alpha-amylase concentrations.

Study 2 – Two resistance training sessions consisting of 5 sets of 10 repetitions of squat and bench press at 95 percent of repetition maximum within 5 days does not create a statistically significant change in resting baseline salivary alpha-amylase concentrations. These results are corroborated by not causing statistically significant change in perceived stress, as measured by Total Mood Disturbance, calculated from the Profile Of Mood States questionnaire, nor causing a change in perceived stress calculated from the Daily Analysis of Life Demands for Athletes survey.

DEDICATION

This work is dedicated to my wife, parents, and friends who have provided unwavering support throughout my entire scholastic career.

ACKNOWLEDGEMENTS

Dr. Michael Stone, for your guidance throughout this entire process, and for giving your time to create a program that I could study what I am passionate about.

Dr. Caleb Bazyler, for your assistance with the endocrinology lab and guidance on statistics.

Dr. Brad DeWeese, for your guidance and encouragement to think of the practical application of science.

Dr. Jeremy Gentles, for the guidance in the academic field, and advice for the next stages of my life.

Dr. Nick Fiolo, for the encouragement as a former student, and feedback into the possibilities of future research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Fatigue management is an important component of sport performance, especially when competitive seasons can last for many months. There are many ways to measure fatigue (i.e. session rate of perceived exertion (sRPE), vertical jump (VJ), subjective data, and biological markers) (Foster et al., 2001; Gleeson, 2002; Halson, 2014; Impellizzeri, Rampinini, Coutts, Sassi, & Marcora, 2004; Taylor, Chapman, Cronin, Newton, & Gill, 2012) However, each of these common fatigue measuring systems have inherent negative aspects. Subjective measures, although easily administered, rely on the athlete to give honest answers (Foster et al., 2001; Impellizzeri et al., 2004).Athletes may intentionally report either higher or lower answers if they understand that training and/or practice intensity may be altered due to their responses. Athletes may also become uninterested in continually answering surveys and just 'go through the motions,' providing the sport scientist and coach inaccurate data. The complications of subjective measures make biomarkers (hormone and immune) more appealing, but these markers require bodily fluids (blood, saliva, urine) and a lab to analyze. (Papacosta & Nassis, 2011; Rohleder & Nater, 2009). Many of the biomarkers that are monitored take anywhere from a few minutes (cortisol), to possibly a few days (CRP/CK) before peaking, leading to more time commitment from the athlete (waiting for blood draw). This type of monitoring is also reactive, requiring feedback from a training session (up to a week) before modifying training instead of being proactive. Analyzing biomarkers is both time consuming and expensive, but many of these biomarkers show good correlation with performance measures (vertical jump, hand dynamometer), making performance measures attractive, as they can be tested and analyzed quickly and can be proactive instead of reactive. Similar to subjective measures, athletes can

either unintentionally lose interest in performing optimally if tested consistently, or intentionally underperform if the athlete understands training loads and/or practice may be altered due to the performance measure reporting a false fatigued state. Due to these aforementioned issues, the optimal athlete monitoring tool would be an objective measure that the athlete cannot intentionally/unintentionally affect, that is proactive, and is quickly and cheaply analyzed.

Salivary alpha-amylase (sAA) is a biomarker that has show promise as a possible remedy to the issues mentioned above. Salivary alpha-amylase is produced and released directly from the salivary glands and demonstrates high responsiveness to both psychological and physiological stress. (Backes, Horvath, & Kazial, 2015; Calvo et al., 1997; Gill et al., 2013, 2014; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Ihalainen, Schumann, Häkkinen, & Mero, 2016; Li & Gleeson, 2004; Maruyama et al., 2012; Nater et al., 2005; Schaal et al., 2015; Takai et al., 2007; van Stegeren, Wolf, & Kindt, 2008; Vigil, Geary, Granger, & Flinn, 2010; Walsh, 1999). Since sAA is released from the salivary glands, the response to stress is much faster than cortisol and other salivary immune markers (Beltzer et al., 2010; Chicharro, Lucía, Pérez, Vaquero, & Ureña, 1998). However, using saliva for athlete monitoring has its own difficulties, such as requiring athletes to collect saliva using a swab or to drool into a collection tube, (Beltzer et al., 2010; Chicharro et al., 1998), which athletes may find uncomfortable to do in public. Recently a device has been invented that increases the viability of sAA for athlete monitoring (Shetty, Zigler, Robles, Elashoff, & Yamaguchi, 2011; Yamaguchi et al., 2006). A mobile analyzation device paired with under tongue saliva collectors that only requires 30 seconds of collection time, with another 30 seconds for analyzation. This device allows sport scientists to collect and analyze sAA in approximately one minute, meeting the demand for a quick, cheap, proactive way to measure a fatigue biomarker.

Although sAA may be a promising biomarker for sport science, the literature is limited to mainly acute aerobic exercise (Backes et al., 2015; Calvo et al., 1997; Gill et al., 2013, 2014; Walsh, 1999). and is nonexistent for resistance training (Gill et al., 2013; Ihalainen et al., 2016; Schaal et al., 2015).

Dissertation Purposes

1. To investigate the acute response to a single session of resistance training.
2. To investigate a handheld salivary a-amylase biosensor device as a tool for athlete monitoring during resistance training
3. To investigate the salivary alpha-amylase response to an over-reaching week of resistance training.

Operational Definitions

1. Fatigue: an increase in stress as measured by changes in mood state, changes in symptoms of stress, and changes in salivary alpha amylase concentrations.
2. Salivary Alpha-amylase: A protein produced and secreted by the salivary glands in response to norepinephrine.

CHAPTER 2

REVIEW OF THE LITERATURE

Salivary alpha-amylase: A potential biomarker for athlete monitoring

Asher Flynn[1] – Jonesborough, TN 37659

Joseph Walters[1] – Johnson City, TN 37614

Tara Whiton[1] – Jonesborough, TN 37659

Jake Bernards[1] – Johnson City, TN 37614

Mike Stone[1] – Johnson City, TN 37614

[1]Center of Excellence for Sport Science and Coach Education, Department of Sport, Exercise, Recreation, and Kinesiology, East Tennessee State University, Johnson City, TN

Corresponding author: Asher Flynn – East Tennessee State University, Johnson City, TN 37614

E-mail: AsherLFlynn@gmail.com

Key words: physical stress, exercise, training, overtraining, saliva

Prepared for submission to *Exercise and Sport Science Reviews*

ABSTRACT

A recent review by Koibuchi and Suzuki in 2014 provided detailed insight into the salivary alpha-amylase (sAA) response to exercise. Multiple research articles have been published since, investigating sAA response to different forms of exercise. The purpose of this review is to update and consolidate this research and examine the trends presented in the literature. Inclusion criteria were studies using healthy populations, a form of exercise, and athlete aged populations. The 31 studies included were then subcategorized based on $\dot{V}O_2$ demands into "high intensity, and "moderate intensity," another subcategory of "long term" was added for studies investigating sAA response to multiple days of training. The results of this review indicate that: 1) sAA concentrations increase in response to high intensity exercise, 2) Moderate intensity exercise results in a less consistent sAA response, with a threshold needing to be reached before sAA concentrations increase, and 3) During long term intense training, sAA baseline concentrations decline, which may be useful as a marker of training stress or an over-stressed state. In conclusion, sAA may be a useful biomarker for athletes that can indicate the stress of a single session, and may be able to provide insight into training strain.

INTRODUCTION

Our understanding of human performance is a consistently evolving science. Our means to reach human potential have evolved in recent decades assisted by technological advancements. However, these advancements are ineffective without a firm knowledge of the training process. The training process is an organized method of satisfying specific training methods in a timely manner (12). The training process is carefully formed to create the desired physiological adaptations for peak performance within the training or competition schedule. From the training process, athletes attain a level of fitness, but also accumulate fatigue. Therefore, it is in the athlete

and coach's best interest to monitor physical performance and psychological well-being to mitigate undesirable levels of stress and fatigue.

Athlete monitoring provides an organized approach to attain necessary feedback for athletes and coaches, gathered from physical, physiological, and psychological measures. The information collected from athlete monitoring is vital to enhance our understanding of performance and fatigue. The improvement in the techniques to monitor athletes continue to develop as scientists discover additional biomarkers that are useful to understand an athlete's physiological and psychological response to training. However, there are still many unknown variables contributing to both fitness and fatigue making it problematic to derive a specific physiological test sensitive to a wide range of athletes and sports. Thus, to improve our comprehension of physiological responses that accompany training, sports scientists have attempted to generate specific monitoring techniques that aid athletes in preparation. However, many of these tests, are impractically expensive, logistically unavailable, and take excessively long to analyze (35). Therefore, further exploration of specific biomarkers is needed to give quick and inexpensive feedback to athletes and coaches.

One commonly known biomarker related to physical training and psychological stress is cortisol. In response to a stressor, the hypothalamic-pituitary-adrenal (HPA) axis signals cortisol production, however, the cortisol response is slow and transient (26). Cortisol responds to a variety of stressors, functioning to maintain homeostats through a number of metabolic processes, including maintaining adequate blood glucose levels, as well as participating in the anti-inflammatory response. Due to cortisol's response to mulitple stressors, it has been used to detect an athlete's readiness for training and competition with varying levels of success (10,22,26), but

due to the slow and transient response, cortisol has not been viewed as a viable biomarker outside of a research setting.

Recently, salivary α-amylase (sAA) has captured noticeable attention from sports scientists as an effective biomarker for exercise(1,2,6,16,25). Benefits of testing salivary α-amylase are, it is noninvasive, relatively inexpensive, and easily analyzed (20). Salivary α-amylase is secreted by the parotid gland and functions to break down starch into maltose (29). It accounts for approximately one-half of all salivary proteins, and also functions to reduce the adherence of bacteria (46). Notably, sAA is secreted in response to adrenergic activity and is suppressed by beta-1 (β1) blockage, thus affirming its regulation by the sympathetic nervous system (25,29). Salivary α-amylase measurement has long been used in the psychological field to aid our comprehension of behavior, cognitive function, and cardiovascular psychophysiology (9).

Salivary α- amylase has been studied alongside cortisol to interpret the response to stress. Surprisingly, early research did not present any direct correlation of sAA and cortisol in response to psychological stress (9,38), and studies associating cortisol and sAA in response to exercise are minimal (44). Salivary α-amylase and cortisol are not well correlated in response to exercise (44), but this does not indicate these biomarkers cannot improve our understanding of exercise-induced stress. Cortisol works by way of the HPA axis; whereas, sAA works through the sympathetic adrenomedullary (SAM) system, providing researchers alternate means to investigate stress-induced responses (33). When subjects are exposed to different stressors, such as a Trier Social Stress Test (TSST), anticipation of competition, and exercise, salivary α-amylase increases substantially (20,29,33,38). These responses substantiate sAA as a likely biomarker for exercise-induced stress.

Currently, the scientific literature for α-amylase response to aerobic exercise is relatively abundant. This review aims to update the current understanding of sAA's response to aerobic exercise, shed light on the potential ways sAA could be used in athlete monitoring, expose areas lacking scientific research, and provide prospective practicality.

METHODS

Authors searched the PubMed database and Google Scholar. Inclusion criteria were studies using healthy populations, a form of exercise, and athlete aged populations (14 – 40 years old). Following the inclusion criteria, 31 articles were accepted and used in this review. Once accepted to be used for review, articles were then separated into sub-categories based on the dominate energy system used during exercise. Exercise protocols using 100% $\dot{V}O_{2max}$ or higher were categorized as "high intensity", exercise protocols prescribing a $\dot{V}O_2$ of under 100% $\dot{V}O_{2max}$ were categorized as "moderate intensity," and any exercise protocols that were not acute responses (longer than a typical training session) were categorized as "long term studies."

RESUTLS

Alpha amylase collection

Using saliva as a monitoring tool, although convenient, is not without intricacies. Saliva is a complex fluid, made up of many substances that are released from separate glands that are stimulated by autonomic activity (5). A review of the literature indicates various methods of collecting saliva. Stimulated and unstimulated are two distinct methods of saliva collection. Stimulated saliva refers to promoting saliva flow by use of chewing while unstimulated denotes collecting saliva in a passive manner (5). These two methods of collection result in significant differences in sAA concentrations, stimulated methods resulting in 152% higher concentrations than unstimulated (2). Another consideration when collecting sAA is the circadian rhythm, with

levels falling dramatically within the first hour of waking and steadily increasing throughout the day, potentially making this a confounding variable (39).

Instruments used in saliva collection are cotton and synthetic swabs, sugarless gum, and passive drool. Each method has pros and cons depending on the purpose of the investigation. Cotton and synthetic swabs are useful but may limit the amount of saliva available for analysis, while passive drool may be socially awkward in specific situations (5). For more information on saliva collection and analyzation methods, the authors suggest a review by Chicharro, Lucia, Pérez, Vaquero, & Ureña (1998).

Sex differences

The first investigation to study sex differences in sAA responses to exercise are works by Kivlighan & Granger (2006). In this study participants were subjected to a 2000 m rowing test that qualified them to participate in an upcoming regional competition. Kivlighan & Granger (2006) noted that females exhibited a statistically significant lower concentration of sAA (data not reported, $p < 0.001$) compared to men at the start of the test. However, during a time-matched sampling on a non-training day, there was no significant difference between sexes. In response to the 2000m rowing time trial, both males and females had statistically significant increases in sAA concentration compared to pre-test (156% increase). Interestingly, no statistical differences between sexes were reported after the time trial. The authors of this study note the delayed sampling (20-minutes) may have altered the sAA activity (24). Other literature has reported sAA responses as decreasing within the first 5 minutes post-stressor (5,41). The delay in measurement makes it challenging to understand sex differences in sAA responses. Another study investigating sAA activity, using an aerobic exercise protocol, are the works by Li, Hsu, Suzuki, Ko, & Fang, (2015). In this study, participants were highly trained endurance athletes (nine males and nine

females). Participants were subjected to a 5000m running competition. Saliva samples were collected 10 minutes prior and 10 minutes post competition. Unlike the results reported from Kivlighan & Granger (2006), women exhibited statistically higher sAA concentration compared to men (66 U/mL vs. 32 U/ml, respectively; $p < 0.05$) at baseline. While basal and post-race values of sAA differed between sexes, males and females did not differ in the rate of increase (28).

The current literature examining sex differences in sAA activity during exercise is scarce. Importantly, even though baseline levels are not well understood, the response to exercise is the same, making sAA a probable biomarker of exercise stress across sexes.

Exercise

Salivary α-amylase has been investigated in many types of aerobic exercise, ranging from interval training to multi-day ultra-endurance marathons (18,49). Salivary α-amylase activity responds to exercise in an intensity-dependent manner, as intensity increases, sAA activity also increases, following blood lactate responses (8). The high correlation with blood lactate levels ($r=0.95$, $p<0.05$) (8), while not specifically understood, provides insight into the differing responses resulting from different levels of intensity during aerobic exercise.

High intensity aerobic exercise

The high correlation of blood lactate and sAA provides a logical basis that sAA is a viable biomarker to consider for athlete monitoring during high-intensity exercise. Walsh (1999) provided the first study to examine sAA responses to high-intensity training. In this study, eight subjects performed 60 minutes of interval training consisting of 1 minute at 100% $\dot{V}O_{2max}$ followed by 2 minutes of recovery at 30% $\dot{V}O2max$. Immediately post-exercise, sAA activity had increased 5 fold (188±62 U/mL – 1085 ±384 U/mL, $p<0.01$), and was still elevated 1 hour after cessation of exercise (428±118 U/mL, $p<0.05$), and returned to baseline levels by 2.5 hours post exercise (49).

Furthering the work of Walsh (1999), four studies have investigated the response of sAA during graded exercise tests (3,7,17,41). Bocanegra et al. (2012), had 12 professional swimmers perform eight 100m trials of increasing intensity based on predetermined time intervals. Salivary α-amylase concentrations increased from pre to post test (9,167±3194 – 17,949±3,138 pixel density from a western blot) and were highly correlated with blood lactate ($r=0.81$, $p<0.05$) (7). De Oliveira et al. (2010), investigated the sAA response to a maximal cycling test. In this study, 12 elite cyclists performed an incremental cycle test to voluntary exhaustion. During the first few stages, sAA activity remained unchanged, but increased statistically during the last three stages prior to exhaustion, and returned to baseline levels within 5 minutes post exercise (41). Gallina et al. (2011), had 21 active men perform a maximal treadmill test to exhaustion. Salivary α-amylase activity increased statistically from 45.9 ± 13.7 U/mL at baseline to 279±26.7 U/mL at the cessation of exercise (17). In another investigation of sAA response to a maximal treadmill test, Backes et al. (2015) had 15 college students perform 18-minutes of moderate intensity exercise followed immediately by an incremental test to exhaustion. Salivary α-amylase activity was statistically different between baseline, post moderate intensity, and cessation of high intensity, with sAA increasing from 85±10 U/mL to 204±32 U/mL and 284±30 U/mL, after moderate intensity and high-intensity treadmill running, respectively (3).

In a similar study to Walsh (1999), Kilian (2016) investigated the sAA response of 12 adolescent cyclists (14±1 years old) performing four 4-minute intervals at 90-95% peak power output (derived from a graded exercise test) with 3 minutes of active recovery between high-intensity segments. In contrast to Walsh (1999), sAA levels were not statistically different post-exercise compared to pre-exercise levels even though blood lactate and heart rate both increased statistically (23). Ligtenberg, Brand, Van Den Keijbus, and Veerman (2015) reported similar sAA

responses as Kilian (2016) with twenty-nine subjects (21.6±1.5 years) participating in a moderate and high-intensity exercise protocol. Subjects ran on a track for 10 minutes at approximately 130bpm (moderate intensity) followed by 10 minutes of "all-out" running. Salivary α-amylase activity did not change statistically from baseline (58±28 U/mL) after either the moderate intensity (59±23 U/mL) or high intensity (55±29 U/mL) running, although heart rate increased statistically at each level of intensity (30). These results are inconsistent with the previously mentioned literature. Ligtenberg et al. (2015) did not define the population in greater detail than as collegiate students and faculty with a mean age of 21.6±1.5 years.

Unlike the previously mentioned studies, Kivlighan and Granger (2006), De Pero et al. (2016), and Lin et al. (2014) investigated the sAA response to sport situations. These studies are particularly important, providing ecologically valid results for athlete monitoring. Kivlighan and Granger (2006) investigated the sAA response to a rowing time trial for a collegiate crew team. Forty-two team members competed in a 2000m rowing time trial (6-8 min). Following the time trial, sAA increased 156% from pre-trial to 20 minutes post-trial. Opposed to methods used in most studies, no immediate post-exercise samples were taken (24). De Pero et al. (2016) investigated the sAA response of 11 gymnasts performing at an international caliber gymnastics competition. Gymnasts performed in 3 events (tumbling, trampette, and floor), each event lasted approximately 3 minutes. Salivary α-amylase activity increased from pre-competition levels after each event and returned to baseline levels by 30 minutes post competition (43). To study the stress response to a combat sport, Lin et al. (2014) investigated the effect of a 2-hour taekwondo training session on sAA activity. The athletes (13 male 20.5±1.2 years, 9 female 19.9±1.5 years) performed 1-hour of technique practice and simulated matches, followed by 1-hour of physical training.

Salivary α-amylase activity statistically increased for both males (26.2±15.7 pre, 41.76±22.3 post, $p<0.05$) and females (22.2±11.7 pre, 39.8±21.8 post, $p<0.05$) after exercise (31).

The sAA response to high intensity aerobic exercise has been well studied, with the majority of research agreeing on a meaningful increase. This evidence indicates that, during high-intensity exercise, at least for certain sports, sAA is an effective biomarker and could be used to monitor athletes stress responses.

Moderate intensity aerobic exercise

With reference to high-intensity aerobic exercise, sAA activity appears to show a distinctive increase in concentration following exercise. However, as intensity decreases, the response of sAA to exercise is less consistent. Seven studies have investigated sAA response to moderate intensity running (11,19,28,32,34,40,45). Li et al., (2015) investigated sAA activity in a 5000m race using 18 (9 males and 9 females) highly trained endurance runners. Salivary α-amylase activity showed a statistically significant increase from pre-race (32.3±5.5 males; 66.2±16.3 females) to post-race (59.2±9.5 males; 88.6±18.0 females) (28). During a cross country race (2 hours), 25 participants with a minimum of 5 years cross country experience statistically increased sAA concentrations from pre to post-race (24-502 kU/L – 155-5030 kU/L) (40). The first study to explore sAA activity responses to a competitive marathon was performed by Ljungberg, Ericson, Ekblom, & Birkhed (1997). Their investigation examined the response of sAA in 20 well-trained endurance runners (24-62 years), sAA values were sampled pre-race, immediately post-race, and 1h post-race. Consistent with previous research, sAA values were statistically higher immediately post-race (1113±217 U/mL) and 1 hour post marathon (765±129 U/mL) compared to pre-race values (496±66 U/mL) (32). In the longest acute exercise study, sAA responses to 24 h of continuous running were investigated by Gill et al. (2014). The distance covered by the subjects

during the 24hrs race ranged from 122 to 208 km. Consistent with previous findings, sAA activity post-exercise increased by 85% compared to pre-exercise concentrations (19). In a study investigating downhill running, 11 active males performed two 60-minute downhill running sessions at 75% vV̇O2max with two weeks separating testing sessions. Immediately post run, sAA activity had statistically increased above baseline and remained elevated for two hours post exercise (34). In contrast to the reported increases in sAA, Costa, Fortes, Richardson, Bilzon, & Walsh (2012) reported no change in 11 male runners (27 years ± 7) who ran for 2 hours at 75% V̇O2max (14 ± 10 U/min pre, 14 ± 14 U/min post). Rosa et al. (2014) also reported no statistical increase in sAA activity following 60 min of running at 70% V̇O2max for trained male runners (not reported pre, 422.93 ± 17.58 U/µg post), unfortunately, no resting sAA concentrations were reported.

In addition to investigations on running, three studies have investigated the sAA response to cycling. Kunz et al. (2015) had 17 subjects cycle for 30 min at 3 different workloads (-5%, +5%, +15% of lactate threshold). Subjects were sub-grouped as high fit (9) and low fit (8) individuals. Salivary α-amylase activity increased from pre-to post at all 3 workloads regardless of fitness sub-group (-5% 44.9±41.9 to 105±62U/mL, +5% 49.3±39.3 to 141±123U/mL, +15% 47.7±40.9 to 191±146U/mL). A statistical difference in sAA levels was discovered between the -5% trial and the +15% trial, regardless of fitness sub-group, indicating an intensity dependent sAA response. Interestingly, a fitness dependent response was reported, with high fit subjects having lower resting sAA levels and a more pronounced exercise response (27).

In 2004, Li & Gleeson investigated the sAA response to multiple workouts per day in a fasted state. Eight recreationally active males performed two different cycling protocols. After fasting since 23:00 the night before, participants cycled at 60% $\dot{V}O_{2max}$ for 2 hours starting at

14:00. Following this protocol, sAA concentrations increased statistically. Following the same fasting protocol, participants cycled for 2 hours at 60% V̇O2max starting at 0900, resting for 3 hours, and then cycling to exhaustion at 14:00. In this study, sAA activity increased from pre to post exercise and returned to baseline levels within 1 hour after exercise, indicating that sAA activity is not confounded by prior exercise (29). In contrast to the other studies, Kilian et al. (2016) reported no increase in sAA activity in 12 male cyclists (14 ± 1 years) performing a 90 minute cycle at 60% peak power output (23). This contrasting result may have been because 60% PPO did not reach the intensity threshold needed to elicit a sAA response in a non-fasted state.

To elucidate the effects of sAA response to different exercise equipment (cycle ergometer, elliptical, and treadmill), Fatolahi, Rasaee, & Peeri (2011) had ten subjects perform two protocols using each piece of equipment. One protocol required subjects to exercise at 70% of max heart rate (MHR), and the second protocol was at 85% MHR. Surprisingly, sAA decreased at 85% using the elliptical and treadmill. All other sessions resulted in nonsignificant changes in sAA activity (14). While this is at odds with other research, and 85% MHR should have reached the probable intensity threshold, the authors handling of the saliva was poorly executed. Typically, Saliva is stored at -80 C until analyzed (2,3,24,27,29–34,37,38,4,39,41–43,49,7,8,15,17–19,23). However, in this study saliva was held at 4 C (39 F), which could have altered sAA even though saliva was reported to have been sent to the lab within two hours.

The current evidence is reasonably conclusive that sAA concentration increases with moderate-intensity exercise. The results from the works by Azarbayjani et al., (2011) and Kilian et al., (2016) indicates that 60% V̇O2max (in a fed state) is the threshold of sAA's intensity response. Sport scientists can use this information for athlete monitoring to ensure adequate intensity levels during training.

Long-term studies

Long-term studies can provide us with additional evidence of the efficacy of sAA as a potential biomarker. Unfortunately, long-term studies for sAA are practically nonexistent, with only four studies lasting longer than one week (13,18,21,47) with only two of those studies lasting 12 weeks or longer (13,21). Schaal et al. (2015) investigated the effects of two 14-day intensified overreaching periods in 10 elite synchronized swimmers separated by 9 days of light swimming, using a cross over design. These findings indicate that during intensified training (overreaching), compared to normal swim training, sAA concentration changes due to training decreased statistically ($p = 0.031$), which also corresponded with decreased performance, and increased RPE during a 400m swimming time trial. The results from this study, combined with results from Le Meur et al. (2013), who reported that a decrease in HR with an increase in RPE can differentiate between over-reached athletes, suggest these athletes may be reaching a fatigued state (36,47) and that sAA may be able to be used to determine over-reaching state of an athlete.

Investigating the sAA response to a multi-day event, 23 ultra-endurance runners completed a 230km 5-day race that averaged about 28-hours in total running time among the participants (18). Post-exercise sAA concentrations were measured after each day of the race and compared to pre-exercise concentrations and the prior day's concentration. Unsurprisingly, sAA response was statistically higher than pre-exercise levels during every stage of the race ($p< 0.001$). Notably, resting concentrations of sAA trended upward from day 2 to day 5 (22±27 – 75±89 U/ml) and the post-exercise response continually decreased after the second day (148±126 – 96±82). This suggests resting sAA activity may increase and the sAA response to exercise may decrease as athletes conduct multiple intense days of training, such as during an overreaching phase.

To elucidate the effect of chronic training on sAA concentrations, Diaz, Bocanegra, Teixeira, Soares, and Espindola (2013) investigated alpha amylase concentrations during 21 weeks of training in 11 elite male swimmers (13). Salivary alpha-amylase concentrations were recorded every four weeks. Strong negative correlations were observed between resting sAA concentrations and training intensity ($r=-0.78$ $p<0.05$), with statistically significant decreases from baseline (30.9% week 6, 38.6% week 11, 54.2% week 16, 37.6% week 21) as the intensity increased (13). Interestingly, intensity, volume, and training load all decreased for the week 11 measurement, but sAA activity increased, and intensity was the highest during week 21 with training load and volume decrease along with a concomitant increase (returning toward baseline) in sAA activity. These findings indicate that resting sAA levels may be more sensitive to the accumulated training fatigue and not the acute training load.

Another study investigating sAA response to long-term training was performed by Ihalainen, Schumann, Häkkinen, & Mero (2016). This study examined salivary proteins in recreational endurance runners over the course of 12 weeks. The subjects were divided into groups based on having experienced upper respiratory symptoms (URS) or having no signs of URS (healthy) during the 12 weeks. Participants engaged in endurance running 4 to 6 times per week in a polarized model (continuous training with interval training). Before the 12-week endurance training, all participants were subjected to an incremental treadmill run to exhaustion to measure initial sAA values. The results from pre-training values indicate that both groups increased in sAA concentration, but only the healthy group was statistically significant (69±15 – 210±54 U/mL healthy $p < 0.05$; 79±28 – 200±100 U/mL URS). After the 12 weeks of endurance training, subjects completed the same treadmill test, and as expected sAA increased in both groups; however, only the healthy group was statistically significant (71±15 – 198±46 U/mL healthy; 61±27 – 130±36

U/mL URS $p < 0.05$). Interestingly, in the URS group, resting sAA concentrations were 23% lower post-training than during pre-training; however, these finding did not reach statistical significance, but had a medium effect size (Cohens $d = 0.65$) (21).

The results from Gill et al. (2013), Diaz et al. (2013), and Ihalainen (2016) indicate that a chronic increase in training stress or accumulation of training fatigue can decrease resting sAA concentrations. Monitoring resting sAA concentrations may give coaches and athletes useful insight to detect overreaching and prevent nonfunctional overreaching or overtraining.

DISCUSSION

The evidence supports an increase in sAA activity from many types of aerobic exercise. This response gives the sport scientist an alternate method of monitoring sympathetic responses to programmed exercises. Since chronic stress alters sAA baseline concentrations and responses to additional stress, there is some evidence that sAA may be useful to help monitor and diagnose overreaching/overtraining (13,21,47,48). Although most of these studies involve aerobic exercise, De Pero et al. (2016) and Lin et al. (2014) both give insight into what might occur in a strength/power sport (31,43). Future areas of research should entail measuring sAA responses to chronic aerobic training, how sAA activity adapts as fitness is obtained, and measure sAA responses to both acute and chronic resistance training.

CONFLIC OF INTEREST

The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of this study do not constitute endorsement by ACSM..

REFERENCES

1. Allgrove, JE, Gomes, E, Hough, J, and Gleeson, M. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci* 26: 653–661, 2008.
2. Allgrove, JE, Oliveira, M, and Gleeson, M. Stimulating whole saliva affects the response of antimicrobial proteins to exercise. *Scand J Med Sci Sport* 24: 649–655, 2014.
3. Backes, TP, Horvath, PJ, and Kazial, KA. Salivary alpha amylase and salivary cortisol response to fluid consumption in exercising athletes. *Biol Sport* 32: 275–280, 2015.
4. Bellar, D, Murphy, KA, Aithal, R, Davis, GR, and Piper, T. The effects of a 36-hour mixed task ultraendurance race on mucosal immunity markers and pulmonary function. *Wilderness Environ Med* 28: 10–16, 2017.
5. Beltzer, EK, Fortunato, CK, Guaderrama, MM, Peckins, MK, Garramone, BM, and Granger, DA. Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiol Behav* 101: 289–296, 2010.
6. Bishop, NC, Walker, GJ, Scanlon, GA, Richards, S, and Rogers, E. Salivary IgA responses to prolonged intensive exercise following caffeine ingestion. *Med Sci Sports Exerc* 38: 513–519, 2006.
7. Bocanegra, OL, Diaz, MM, Teixeira, RR, Soares, SS, and Espindola, FS. Determination of the lactate threshold by means of salivary biomarkers: Chromogranin A as novel marker of exercise intensity. *Eur J Appl Physiol* 112: 3195–3203, 2012.
8. Calvo, F, Chicharro, JL, Bandres, F, Lucia, A, Perez, M, Alvarez, J, et al. Anaerobic threshold determination with analysis of salivary amylase. *Can J Appl Physiol* 22: 553–561, 1997.

9. Chatterton, RT, Vogelsong, KM, Lu, Y, Ellman, AB, and Hudgens, GA. Salivary α-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 16: 433–448, 1996.

10. del Corral, P, Mahon, AD, Duncan, GE, Howe, CA, and Craig, BW. The effect of exercise on serum and salivary cortisol in male children. *Med Sci Sports Exerc* 26: 1297–1301, 1994.

11. Costa, RJS, Fortes, MB, Richardson, K, Bilzon, JLJ, and Walsh, NP. The effects of postexercise feeding on saliva antimicrobial proteins. *Int J Sport Nutr Exerc Metab* 22: 184–191, 2012.

12. DeWeese, BH, Hornsby, G, Stone, M, and Stone, MH. The training process: Planning for strength-power training in track and field. Part 1: Theoretical aspects. *J. Sport Heal. Sci*. 4: 308–317, 2015.

13. Diaz, MM, Bocanegra, OL, Teixeira, RR, Soares, SS, and Espindola, FS. Salivary nitric oxide and alpha-amylase as indexes of training intensity and load. *Int J Sports Med* 34: 8–13, 2013.

14. Fatolahi, H, Rasaee, MJ, and Peeri, M. The effect of exercise mode and intensity of submaximal physical activities on salivary testosterone to cortisol ratio and α-amylase in young active males. *Int J Exerc Sci* 4: 9, 2011.

15. Fortes, MB, Diment, BC, Di Felice, U, and Walsh, NP. Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. *Appl Physiol Nutr Metab* 37: 850–859, 2012.

16. Fortes, MB and Whitham, M. Salivary Hsp72 does not track exercise stress and caffeine-stimulated plasma Hsp72 responses in humans. *Cell Stress Chaperones* 16: 345–352, 2011.

17. Gallina, S, Di Mauro, M, D'Amico, MA, D'Angelo, E, Sablone, A, Di Fonso, A, et al. Salivary chromogranin A, but not α-amylase, correlates with cardiovascular parameters during high-intensity exercise. *Clin Endocrinol (Oxf)* 75: 747–752, 2011.

18. Gill, SK, Teixeira, AM, Rama, L, Rosado, F, Hankey, J, Scheer, V, et al. Salivary antimicrobial protein responses during multitstage ultramarathon competition conducted in hot environmental conditions. *Appl Physiol Nutr Metab* 38: 977–987, 2013.

19. Gill, SK, Teixeira, AM, Rosado, F, Hankey, J, Wright, A, Marczak, S, et al. The impact of a 24-H ultra-marathon on salivary antimicrobial protein responses. *Int J Sports Med* 35: 966–971, 2014.

20. Granger, DA, Kivlighan, KT, El-Sheikh, M, Gordis, EB, and Stroud, LR. Salivy α-amylase in biobehavioral research. In: *Annals of the New York Academy of Sciences* 1098: 122–144, 2007

21. Ihalainen, JK, Schumann, M, Häkkinen, K, and Mero, AA. Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. *Appl Physiol Nutr Metab* 41: 96–102, 2016.

22. Jacks, DE, Sowash, J, Anning, J, McGloughlin, T, and Andres, F. Effect of exercise at three exercise intensities on salivary cortisol. *J Strength Cond Res* 16: 286–289, 2002.

23. Kilian, Y, Engel, F, Wahl, P, Achtzehn, S, Sperlich, B, and Mester, J. Markers of biological stress in response to a single session of high-intensity interval training and high-volume training in young athletes. *Eur J Appl Physiol* 116: 2177–2186, 2016.

24. Kivlighan, KT and Granger, DA. Salivary a-amylase response to competition: Relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology* 31: 703–714, 2006.

25. Koibuchi, E and Suzuki, Y. Exercise upregulates salivary amylase in humans. *Exp Ther Med* 7: 773–777, 2014.

26. Kraemer, WJ, Fleck, SJ, and Deschenes, MR. Exercise physiology : integrated from theory to practical applications. Wolters Kluwer/Lippincott Williams & Wilkins Health, 2012

27. Kunz, H, Bishop, NC, Spielmann, G, Pistillo, M, Reed, J, Ograjsek, T, et al. Fitness level impacts salivary antimicrobial protein responses to a single bout of cycling exercise. *Eur J Appl Physiol* 115: 1015–1027, 2015.

28. Li, CY, Hsu, GS, Suzuki, K, Ko, MH, and Fang, SH. Salivary immuno factors, cortisol and testosterone responses in athletes of a competitive 5,000 m race. *Chin J Physiol* 58: 263–269, 2015.

29. Li, TL and Gleeson, M. The effect of single and repeated bouts of prolonged cycling on leukocyte redistribution, neutrophil degranulation, IL-6, and plasma stress hormone responses. *Int J Sport Nutr Exerc Metab* 14: 501–516, 2004.

30. Ligtenberg, AJM, Brand, HS, Van Den Keijbus, PAM, and Veerman, ECI. The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme. *Arch Oral Biol* 60: 1639–1644, 2015.

31. Lin, SP, Li, CY, Suzuki, K, Chang, CK, Chou, KM, and Fang, SH. Green tea consumption after intense taekwondo training enhances salivary defense factors and antibacterial capacity. *PLoS One* 9, 2014.

32. Ljungberg, G, Ericson, T, Ekblom, B, and Birkhed, D. Saliva and marathon running. *Scand J Med Sci Sports* 7: 214–9, 1997.

33. Maruyama, Y, Kawano, A, Okamoto, S, Ando, T, Ishitobi, Y, Tanaka, Y, et al. Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the trier social stress tests. *PLoS One* 7, 2012.

34. Mckune, AJ, Bach, CW, Semple, SJ, and Dyer, BJ. Salivary cortisol and α-amylase responses to repeated bouts of downhill running. *Am J Hum Biol* 26: 850–855, 2014.

35. Meeusen, R, Duclos, M, Foster, C, Fry, A, Gleeson, M, Nieman, D, et al. Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. *Med Sci Sports Exerc* 45: 186–205, 2013.

36. Le Meur, Y, Hausswirth, C, Natta, F, Couturier, A, Bignet, F, and Vidal, PP. A multidisciplinary approach to overreaching detection in endurance trained athletes. *J Appl Physiol* 114: 411–420, 2013.

37. Nater, UM, La Marca, R, Florin, L, Moses, A, Langhans, W, Koller, MM, et al. Stress-induced changes in human salivary alpha-amylase activity - Associations with adrenergic activity. *Psychoneuroendocrinology* 31: 49–58, 2006.

38. Nater, UM, Rohleder, N, Gaab, J, Berger, S, Jud, A, Kirschbaum, C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol* 55: 333–342, 2005.

39. Nater, UM, Rohleder, N, Schlotz, W, Ehlert, U, and Kirschbaum, C. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology* 32: 392–401, 2007.

40. Nexø, E, Hansen, MR, and Konradsen, L. Human salivary epidermal growth factor, haptocorrin and amylase before and after prolonged exercise. *Scand J Clin Lab Invest* 48: 269–273, 1988.

41. De Oliveira, VN, Bessa, A, Lamounier, RPMS, De Santana, MG, De Mello, MT, and Espindola, FS. Changes in the salivary biomarkers induced by an effort test. *Int J Sports Med* 31: 377–381, 2010.

42. Papacosta, E and Nassis, GP. Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. J. Sci. Med. Sport. 14: 424–434, 2011.

43. De Pero, R, Cibelli, G, Cortis, C, Sbriccoli, P, Capranica, L, and Piacentini, M. Stress related changes during TeamGym competition. *J Sports Med Phys Fitness* 56: 639–647, 2016.

44. Rohleder, N, Nater, UM, Wolf, JM, Ehlert, U, and Kirschbaum, C. Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? In: Annals of the New York Academy of Sciences 1032: 258–263, 20004

45. Rosa, L, Teixeira, AAS, Lira, FS, Tufik, S, Mello, MT, and Santos, RVT. Moderate acute exercise (70% VO2 peak) induces TGF-β, α-amylase and IgA in saliva during recovery. *Oral Dis* 20: 186–190, 2014.

46. Scannapieco, F a, Torres, G, and Levine, MJ. Salivary alpha-amylase: role in dental plaque and caries formation. *Crit Rev Oral Biol Med* 4: 301–307, 1993.

47. Schaal, K, Le Meur, Y, Louis, J, Filliard, JR, Hellard, P, Casazza, G, et al. Whole-body cryostimulation limits overreaching in elite synchronized swimmers. *Med Sci Sports Exerc* 47: 1416–1425, 2015.

48. Vigil, JM, Geary, DC, Granger, DA, and Flinn, M V. Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following Hurricane Katrina. *Child Dev* 81: 1228–1240, 2010.

49. Walsh, NP. The effects of high-intensity intermittent exercise on saliva IgA, total protein and a-amylase. *J Sports Sci* 17: 129–134, 1999.

CHAPTER 3

THE RESPONSE OF SALIVARY ALPHA AMYLASE TO SINGLE RESISTANCE TRAINING SESSIONS: A PRELIMINARY STUDY

Authors: Asher L. Flynn[1], Joseph A. Walters[1], Jeremy Gentles[1], Caleb Bazyler[1], Brad H. DeWeese[1], Michael H. Stone[1]

[1]Center of Excellence in Sport Science and Coaching Education, East Tennessee State University

Prepared for submission to *Journal of Strength and Conditioning Research*

ABSTRACT

The ability to understand the physiological stress created by a training session is essential to athlete monitoring. There are a plethora of monitoring tools, a new monitoring tool that may be of use is salivary alpha-amylase (sAA). Salivary alpha-amylase concentrations have been proven to increase in response to both psychological and aerobic training stress, but no research has been conducted on resistance training. Eight subjects were recruited to participate in two resistance training days, consisting of 5 sets of 10 repetitions of squat and bench press. Saliva samples were obtained pre- and post-training. A t-test used to determine if there was a change in sAA concentrations from pre- to post-training indicated a statistically significant increase on training day one($p= 0.045$, $d= -0.954$, 95% CI= -1.838 – -0.019) and training day two ($p= 0.020$, $d= -1.184$, 95% CI= -2.146 – -0.170). There results indicate that sAA has similar responses as reported from psychological and aerobic training stress. Salivary alpha-amylase can be used as a biomarker to measure physiological stress from resistance training.

INTRODUCTION

Quantifying training related stress is an essential component of an athlete monitoring program (5,7,8). A number of measures may be used to quantify training and non-training related stress including, but not limited to, physical performance tests (e.g., vertical jump, sprint, change of direction) (22) self-report questionnaires (e.g., Daily Analysis of Life Demands for Athletes, Short Recovery Stress Survey) (12,18,22), and biomarkers (e.g., cortisol, testosterone, CRP, CK) (12). While these measures have been shown to be valid and reliable indicators of stress in athletic populations, there are a variety of limitations associated with these monitoring tools. For instance, when completing self-report questionnaires, athletes may not provide true responses or they may over-report stressful symptoms in hopes of less intense practices (12). There is also the chance that athletes underperform on physical performance tests, intentionally or unintentionally,

that may be misinterpreted as being indicative of high fatigue. Biomarkers, while not prone to the same shortcomings of the aforementioned tests, may require time, resources and expertise that most athletes, teams, and coaches do not have access to.

In light of the limitations of the previously mentioned athlete monitoring tools, researchers are interested in identifying biomarkers that are not only valid and reliable markers of stress, but also low cost and require little technical expertise. A new biomarker testing procedure has become available in recent history that may alleviate many of the previously mentioned issues. Salivary alpha-amylase (sAA) is a protein, released from the parotid saliva glands, that is highly responsive to stress and has been used extensively in psychological research (3,13,21,23). Recently sAA has been investigated as a marker of training stress, and has shown consistent increases in concentration as a result of aerobic exercise (2,6,24)

The 'gold standard' of saliva testing is through passive drool (67), which obviates some of the limitations of other biomarkers (e.g. non-invasive), these tests still require time and technical expertise to analyze. In an attempt to make sAA testing more practical, obviating the complications previously mentioned with testing biomarkers, a handheld 'point of care' biosensor (NIPRO Corporation) has been developed (19). The biosensor consists of a handheld device with a digital display along with under-the-tongue disposable test strips. The test strip is placed under the tongue to collects saliva on a cotton pad on the end of the test strip for approximately 30 seconds, then the test strip is inserted into the biosensor and a digital read-out is displayed in approximately 30 more seconds. The entire process is completed about one minute. This biosensor has shown good validity and reliability (R^2 0.989, CV 9%) with non-athletic populations when compared with laboratory measures (19), but this device has not been used in an investigation with an athletic population.

While there is a preponderance of evidence of the sAA response to psychological stress, and there is beginning to be an understanding of the sAA response to aerobic exercise, there has yet to be an investigation of the sAA response to resistance training. Therefore, the purposes of this investigation were to determine the sAA response to an acute (single session) resistance training protocol and secondarily, to investigate the biosensors accuracy in an athletic population.

METHODS

Participants

Eight participants with a minimum of 12 months of consistent resistance training were recruited to be a part of this study (91.25 ± 8.46 kg, 24.00 ± 3.30 years old). Participants had a mean squat 1RM of 145.17 ± 41.86 kg (range = 72 – 204 kg), relative squat strength of 1.59 ± 0.47 x BW (0.89 – 2.16 x BW) and a bench press 1RM of 99.72 kg ± 23.91 (54 – 127 kg), 1.09 ± 0.27 x BW (0.67 – 1.47 x BW). Prior to participation, each participant answered the NSCA Medical History questionnaire (CITE) to provide estimated one repetition max for the squat and bench press exercises and to ensure they were free from any exclusion conditions before performing high volume resistance training. Participants provided written informed consent as approved by the East Tennessee State University IRB.

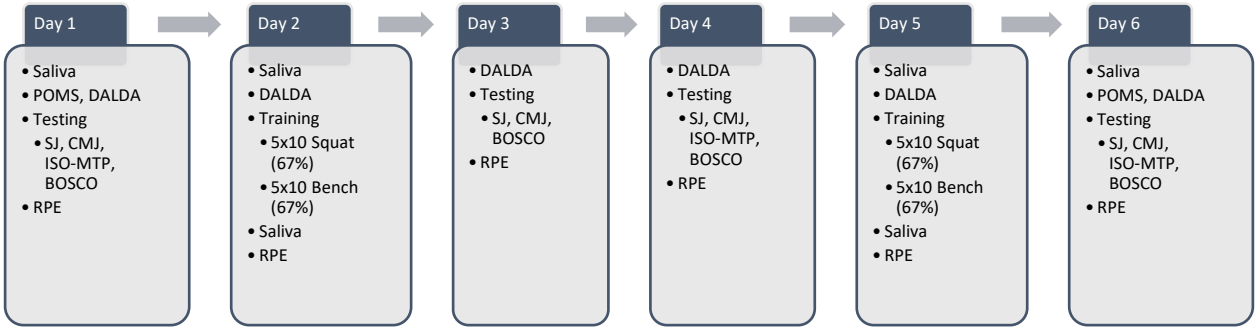
Procedures

This investigation was part of a much larger research study. Participants were instructed to arrive in the lab each day of the study in a fasted (8 hour) and hydrated state. Verbal confirmation was obtained to ensure each participant had fasted for at least 8 hours and urine specific gravity was measured to assess hydration status(4,20). After arrival to the lab, participants filled out two psychological questionnaires, profile of mood states (POMS) and daily analysis of life demands for athletes (DALDA). After filling out the questionnaires, participants provided saliva samples, then performed a general standardized warmup consisting of 25

jumping jacks, 1 set of 5 repetitions (1x5) of the mid-thigh pull exercise with 20kg and a 3x5 mid-thigh pull with 60kg. After the general standardized warm-up, participants performed several performance tests including static and counter-movement jumps (VJ), isometric-mid thigh pull, and the Bosco repeated jumps test (30s). On day two, participants provided saliva samples (passive drool (D2) and biosensor (BSD2)), filled out the DALDA questionnaire, and then performed the same general standardized warm-up. After the general warm-up, participants performed a specific warm up that included 1x10 back squat at 20kg, 1x5 back squat with 70% of their prescribed weight and 1x3 back squat with 85% of prescribed weight. After the specific warm-up, participants performed 5x10 back squats with 67% of their self-reported 1RM. After squats, participants performed the same specific warm-up protocol for bench press. All sets were separated by 2-3 minutes of rest.

Within two minutes of finishing the last set of bench press, participants provided another saliva sample (D2Post, BSD2Post) in order to determine if there was a difference in sAA concentration due to the resistance training protocol. Participants provided their rating of perceived exertion (RPE) 15 minutes after finishing the last set of bench (BORG CR-10) (16). On day three and four, participants performed the same performance testing as on day one, with at least 24 hours between testing sessions. On day five, participants repeated the same protocol as day two, providing saliva samples before (D5, BSD5) and after training (D5Post, BSD5Post). On day six, participants provided a saliva sample and then performed the same performance testing as on day one. Figure 1 provides a flow chart of the study.

FIGURE 1:



SALIVA

Participants arrived after an 8 hour fast to eliminate the sAA response to caffeine, eating, and drinking (except water) prior to providing saliva samples, and were only allowed to drink water while training, except during the last set of bench press, until after providing the post-session saliva sample (17). All saliva samples were collected within 5 minutes after the last set of bench press (1,14). After collection, saliva was frozen at -80° C until analyzed.

Saliva alpha-amylase samples were assayed at the Salimetrics' SalivaLab (Carlsbad, CA) using the Salimetrics Salivary Alpha-Amylase Assay Kit (Cat. No. 1-1902), without modifications to the manufacturers' protocol. Samples were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3,000 RPM (1,500 x g) immediately before performing the assay. Samples were tested for salivary alpha-amylase using a kinetic enzyme immunoassay (Cat. No. 1-1902). Sample test volume was 8 µl of 200x diluted saliva per determination. The assay has a lower limit of sensitivity of 0.4 U/mL, samples exceeding 400 U/mL needed further dilution, an average intra-assay coefficient of variation of 5.47% and an average inter-assay coefficient of variation of 4.7%, which meets the manufactures' criteria for accuracy and repeatability in Salivary Bioscience, and exceed the applicable NIH guidelines for Enhancing Reproducibility through Rigor and Transparency.

BIOSENSOR

The biosensor consists of a handheld device with a digital display along with under-the-tongue disposable test strips. A test-strip is placed under the tongue to allow the test strip collect saliva for 30 seconds. The test-strip is has a collector pad which collects a fixed amount of saliva (25uL), which is then analyzed by the handheld device (19). The entire process is completed in less than one minute. This hand-held device has shown good validity and reliability (R^2 0.989, CV 9%) with non-athletic populations when compared with laboratory measures (19).

STATISTICAL ANALYSES

In order to determine the change in sAA concentration from baseline to post resistance training, two paired t-tests were used to compare pre- and post-training passive drool sAA concentrations (D2 – D2Post, D5 – D5Post). Holms sequential adjustment post hoc tests were used as required. Pearson's correlations were used to compare laboratory sAA results with the biosensor. All statistical analyses were performed using JASP (0.9.1.0). Effect size is reported as trivial <0.2, small 0.2-0.6, moderate 0.6-1.2, large 1.2-2.0, and very large 2.0-4.0 (9).

RESULTS

All data were screened for normality and outliers, with one participant being removed from analysis due to baseline sAA levels being outside of normal ranges (300% higher). Both t-tests were statistically significant (D2 – D2Post, p= 0.045, *d*= -0.954, 95% CI= -1.838 – -0.019; D5 – D5Post, p= 0.020, *d*= -1.184, 95% CI= -2.146 – -0.170). All correlations were statistically insignificant, ranging from r= 0.06 – 0.49 (Table 2). Descriptive statistics from the laboratory analysis are provided in Table 1. Individual responses are provided in Table 3 and Figures 1 & 2.

TABLE 1: Descriptive Statistics

	N	MEAN	SD	RANGE
D2	7	34.23	±23.20	1.50 – 64.60
D2Post	7	63.79*	±24.24	30.30 – 98.60
D5	7	34.11	±18.26	12.00 – 59.00
D5Post	7	75.89*	±42.51	13.90 – 128.90

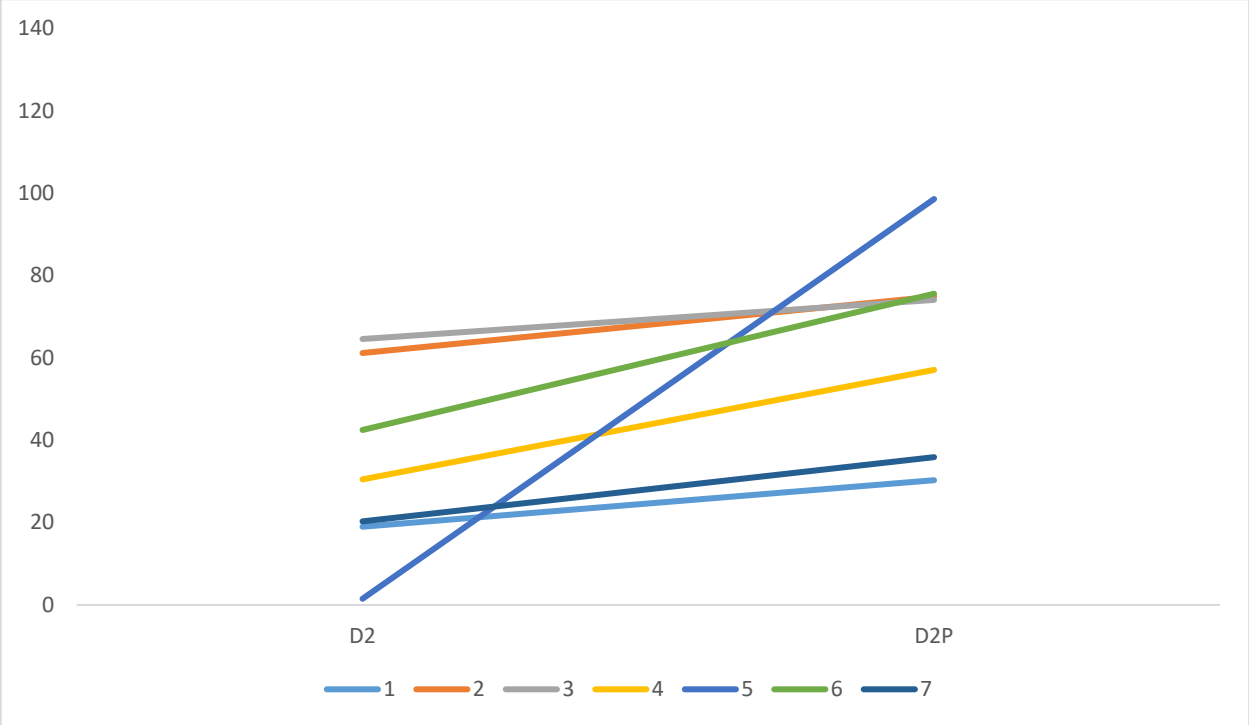
NOTE: Salivary Alpha-Amylase concentrations pre- and post-resistance training (U/ml).
D2: Resting sAA concentrations day two
D2Post: Post-training sAA concentrations on day two
D5: Resting sAA concentrations day five
D5Post: Post-training sAA concentrations on day five.
* p<0.05 D2-D2P, D5-D5P

TABLE 2: Correlations Between Laboratory and Biosensor sAA Analysis

D2 – MD2	D2P – MD2P	ΔD2 – ΔMD2	D5 – MD5	D5P – MD5P	ΔD5 – ΔMD5
r= 0.27	r= 0.54	r= 0.54	r= 0.06	r= 0.13	r= 0.44
p= 0.57	p= 0.21	p= 0.24	p= 0.90	p= 0.78	p= 0.33

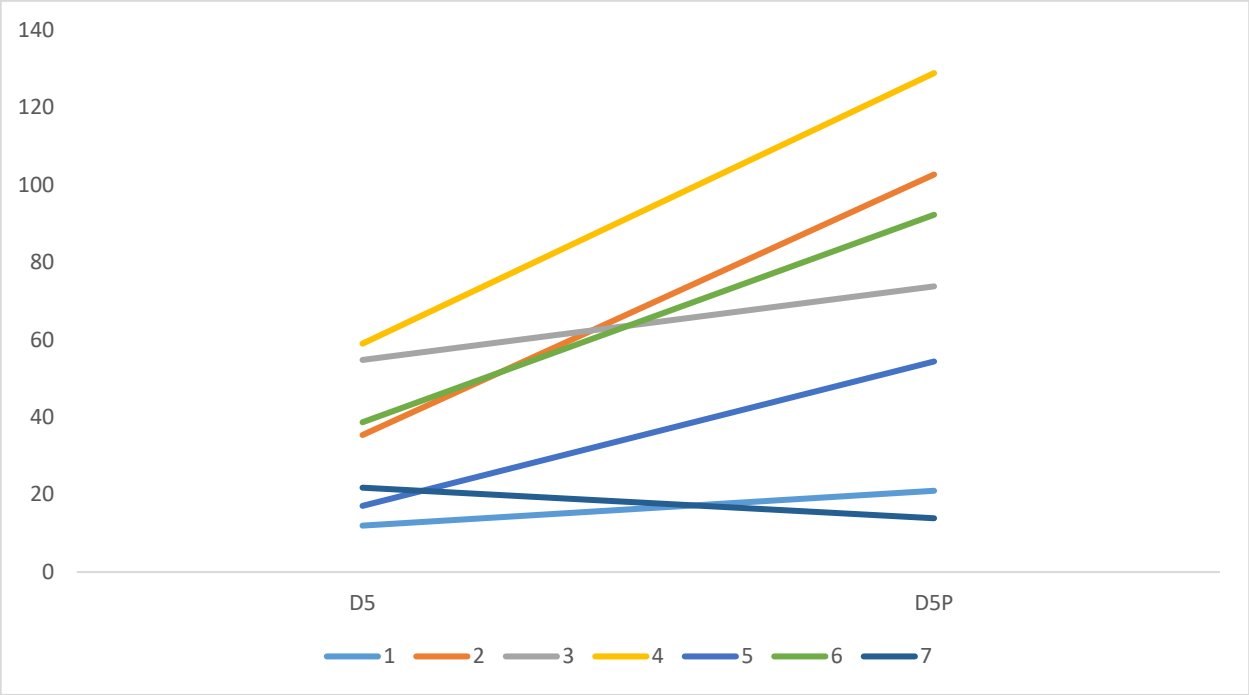
Note: Correlations between laboratory and biosensor salivary alpha-amylase analysis.
D2: Pre-training sAA concentrations, MD2: Biosensor pre-training sAA concentrations, D2P: Post-training sAA concentrations, MD2P: Biosensor post-training sAA concentrations, ΔD2: Percent change in pre- and post-training sAA concentrations, ΔMD2: Biosensor percent change in pre-and post-training concentrations. D5: Pre-training sAA concentrations, MD5: Biosensor pre-training sAA concentrations, D5P: Post-training sAA concentrations, MD5P: Biosensor post-training sAA concentrations, ΔD5: Percent change in pre- and post-training sAA concentrations, ΔMD5: Biosensor percent change in pre- and post-training concentrations.

FIGURE 2: Individual sAA responses



Note: Salivary alpha-amylase response to a single training day.
D2: Pre-training on sAA concentrations, first training day
D2P: Post-training sAA concentrations, first training day

FIGURE 3: Individual sAA responses



Note: Salivary alpha-amylase response to the second training day.
D5: Pre-training sAA concentrations, second training day
D5P: Post-training sAA concentrations, second training day

TABLE 3: Salivary Alpha-Amylase Concentrations

	D2	D2P	ΔD2	D5	D5P	ΔD5
Participant 1	19.0	30.3	59%	12.0	21.0	75%
Participant 2	61.2	74.9	22%	35.4	102.7	190%
Participant 3	64.6	74.1	15%	54.8	73.8	35%
Participant 4	30.5	57.1	87%	59.0	128.9	118%
Participant 5	1.5	98.6	6473%	17.1	54.4	258%
Participant 6	42.5	75.6	78%	38.7	92.3	139%
Participant 7	20.3	35.9	77%	21.8	13.9	-36%

NOTE: Individual responses of salivary alpha-amylase concentrations (U/mL) of all participants on both training days.
D2: Pre-training sAA concentrations, first training day
D2Post: Post-training sAA concentrations, first training day
ΔD2: Percent change from pre- to post-training sAA concentrations on the first training day
D5: Pre-training sAA concentrations, second training day
D5Post: Post-training sAA concentrations, second training day
ΔD5: Percent change from pre- to post-training sAA concentrations on the second training day

DISCUSSION

Research examining the sAA response to acute aerobic exercise has indicated there is an increased concentration following high intensity (above lactate threshold) exercise (2). This study

indicates that there is a similar response due to high volume, high intensity resistance training. There was a moderate to large effect size between pre- and post-sAA concentrations from both training days, and individual differences were qualitatively similar, indicating that sAA concentrations can be used to monitor the stress response from this resistance training protocol. The increased standard deviation noted on the second training day indicates that there may be an individual response associated with the accumulated fatigue. This may be due to training age, or the training the participants were performing prior to participating in this study. Due to this study being a part of a larger study there is a possibility that participants may have been anxious of that blood draw on day two. Koh (2014) reported an increase in sAA concentrations in anticipation of venous blood draw, which may have been partially responsible for the decreased response due to training observed from the first training day (11).

There may be a different response if the participant was performing high volume resistance training prior to participating in this study, prior training was not controlled. Similar to Gill (2013), where well-trained ultra-endurance runners exhibited an decreased sAA change as fatigue from running a multi-day 120k race, the three participants that had different sAA concentrations post training on day 5 may have started to become over-reached, possibly due to a substantial increase in training volume.

The insignificant and poor correlations between laboratory and biosensor sAA concentrations indicate that the biosensor is unable to be used in this population. This result is corroborated by the lack of good correlations between the biosensor and assay methods reported during field exercises in the military ($r= 0.113$) (15).

PRACTICAL APPLICATIONS

The data indicates there is an increase in sAA concentrations following a single high volume, high intensity resistance training session. There may also be a decreased response

observed if there is not a significant change in training volume, or as reported by Gill (2013) and Ihalainen (10), a decreased response could indicated a fatigued state. Salivary alpha-amylase concentration changes may indicate whether the change in volume between training blocks was too severe. This research provides a basis to continue to investigate the sAA response to resistance training and elucidate how this information can best be used as an athlete monitoring tool.

.

REFERENCES

1. Beltzer, EK, Fortunato, CK, Guaderrama, MM, Peckins, MK, Garramone, BM, and Granger, DA. Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiol Behav* 101: 289–296, 2010.
2. Calvo, F, Chicharro, JL, Bandres, F, Lucia, A, Perez, M, Alvarez, J, et al. Anaerobic threshold determination with analysis of salivary amylase. *Can J Appl Physiol* 22: 553–561, 1997.
3. Chatterton, RT, Vogelsong, KM, Lu, Y, Ellman, AB, and Hudgens, GA. Salivary α-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 16: 433–448, 1996.
4. Cheuvront, SN, Kenefick, RW, and Zambraski, EJ. Spot Urine Concentrations Should Not Be Used for Hydration Assessment: A Methodology Review. *Int J Sport Nutr Exerc Metab* 25: 293–297, 2015.
5. DeWeese, BH, Hornsby, G, Stone, M, and Stone, MH. The training process: Planning for strength-power training in track and field. Part 1: Theoretical aspects. *J. Sport Heal*. Sci. 4: 308–317, 2015.
6. Gill, SK, Teixeira, AM, Rosado, F, Hankey, J, Wright, A, Marczak, S, et al. The impact of a 24-H ultra-marathon on salivary antimicrobial protein responses. *Int J Sports Med* 35: 966–971, 2014.
7. Haff, G and Triplett, NT (eds.). Essentials of strength training and conditioning. Fourth edition. Champaign, IL: Human Kinetics, 2015.
8. Halson, SL. Monitoring training load to understand fatigue in athletes. Sport. Med. 44: 139–147, 2014.
9. Hopkins, W. New View of Statistics: Effect Magnitudes. , 2002. Available from: http://www.sportsci.org/resource/stats/effectmag.html

10. Ihalainen, JK, Schumann, M, Häkkinen, K, and Mero, AA. Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. *Appl Physiol Nutr Metab* 41: 96–102, 2016.

11. Koh, D, Ng, V, and Naing, L. Alpha amylase as a salivary biomarker of acute stress of venepuncture from periodic medical examinations. *Front Public Heal* 2: 121, 2014.

12. Meeusen, R, Duclos, M, Foster, C, Fry, A, Gleeson, M, Nieman, D, et al. Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. *Med Sci Sports Exerc* 45: 186–205, 2013.

13. Nater, UM, Rohleder, N, Gaab, J, Berger, S, Jud, A, Kirschbaum, C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol* 55: 333–342, 2005.

14. De Oliveira, VN, Bessa, A, Lamounier, RPMS, De Santana, MG, De Mello, MT, and Espindola, FS. Changes in the salivary biomarkers induced by an effort test. *Int J Sports Med* 31: 377–381, 2010.

15. Peng, HT, Savage, E, Vartanian, O, Smith, S, Rhind, SG, Tenn, C, et al. Performance evaluation of a salivary amylase biosensor for stress assessment in military field research. *J Clin Lab Anal* 30: 223–230, 2016.

16. Pierce, KC, Rozenek, R, and Stone, M. Effects of high volume weight training on lactate, heart rate, and perceived exertion. *J. Strength Cond. Res*. 7(4): 211–215, 1993.

17. Rohleder, N and Nater, UM. Determinants of salivary α-amylase in humans and methodological considerations. *Psychoneuroendocrinology* 34: 469–485, 2009.

18. Rushall, BS. A tool for measuring stress tolerance in elite athletes. *J Appl Sport Psychol* 2: 51–66, 1990.

19. Shetty, V, Zigler, C, Robles, TF, Elashoff, D, and Yamaguchi, M. Developmental validation of a point-of-care, salivary α-amylase biosensor. *Psychoneuroendocrinology* 36: 193–199, 2011.

20. Sommerfield, LM, McAnulty, SR, McBride, JM, Zwetsloot, JJ, Austin, MD, Mehlhorn, JD, et al. Validity of urine specific gravity when compared with plasma osmolality as a measure of hydration status in male and female ncaa collegiate athletes. *J Strength Cond Res* 30: 2219–2225, 2016.

21. van Stegeren, AH, Wolf, OT, and Kindt, M. Salivary alpha amylase and cortisol responses to different stress tasks: Impact of sex. *Int J Psychophysiol* 69: 33–40, 2008.

22. Taylor, K-L, Chapman, DW, Cronin, JB, Newton, MJ, and Gill, N. Fatigue monitoring in high performance sport: a survey of current trends. *J Aust Strength Cond* 20: 12–23, 2012.

23. Vigil, JM, Geary, DC, Granger, DA, and Flinn, M V. Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following Hurricane Katrina. *Child Dev* 81: 1228–1240, 2010.

24. Walsh, NP. The effects of high-intensity intermittent exercise on saliva IgA, total protein and a-amylase. *J Sports Sci* 17: 129–134, 1999.

CHAPTER 4

THE RESPONSE OF SALIVARY ALPHA AMYLASE TO AN OVER-REACHING RESISTANCE TRAINING PROTOCAL

Authors: Asher L. Flynn[1], Joseph A. Walters[1], Jeremy Gentles[1], Caleb Bazyler[1], Brad H. DeWeese[1], Michael H. Stone[1]

[1]Center of Excellence in Sport Science and Coaching Education, East Tennessee State University

Prepared for submission to *Journal of Strength and Conditioning Research*

ABSTRACT

Monitoring accumulated fatigue in athletes is necessary in order to ensure proper adaptation and stress application at appropriate times in the training cycle (such as when to over-reach). There are many monitoring tools that provide insight into the accumulated fatigue of athletes (POMS, DALDA, T:C). Salivary alpha-amylase (sAA) is a biomarker that is receiving interest to monitor accumulated psychological and aerobic training fatigue. There has been no research conducted on the usefulness of sAA as a tool to monitor accumulated fatigue from resistance training. Eight subjects were recruited to participate in two resistance training days, consisting of 5 sets of 10 repetitions of squat and bench press. Resting sAA concentrations along with POMS and DALDA surveys were obtained in the morning before and the day after the training week. T-tests used to determine if there was a change in any of the variables. No statistically significant changes were observed ($p>0.05$). The results from this study indicate that resting sAA levels did not change as a result of two days of resistance training.

INTRODUCTION

The necessity to monitor the athlete training process has been well established (1,10,16). The use of monitoring data can help the sport science and coaching staff make informed decisions about training and practice plans to ensure the appropriate adaptation to training is occurring and decrease the possibility of non-functional over-reaching and over-training (16,27). There are a plethora of techniques used to monitoring for over-training, such as physical performance (vertical jump, sprint, change of direction) (5,27,28), self-report questionnaires (Profile of Mood States, REST-Q) (1,27), and biomarkers (Testosterone, Cortisol, CRP, CK) (10,15).

While each of these monitoring tools are useful, there are inherent problems with each of them. Self-report questionnaires have the inherent issue of the athlete consistently taking the

questionnaire seriously (loss of interest) and answering honestly (27). There is the also possibility of an athlete thinking there could be negative repercussions due to certain responses and therefore not providing true responses. Physical performance tests reduce the possibility of reported false recovered states, but there is the possibility of underperforming, either intentionally (to reduce training load) or unintentionally (loss of interest) that could be interpreted as an under-recovered athlete.

Biomarkers can obviate some of the limitations of the aforementioned tests, since athletes cannot easily intentionally nor unintentionally alter test results. Biomarkers are not without limitations though, taking at least 30 minutes (salivary cortisol) up to 72 hours (CRP, CK) to peak (10,15).

A new biomarker has received increased interest recently that may alleviate many of the previously mentioned issues. Salivary alpha-amylase (sAA) is a protein released directly from the parotid saliva glands and is highly responsive to stress and has been used extensively in psychological research (3,22,26,29). Due to sAA being stored in the saliva glands, there is an almost immediate response to stress, obviating the delay observed in other salivary measures (18).

Recently sAA has been investigated as a fatigue monitoring tool during aerobic exercise (8,13,30). Over-reaching protocols have consistently resulted in increased resting baseline sAA concentrations for both running and swimming (7,12,24). While there is beginning to be an understanding of the sAA response to over-reaching aerobic exercise protocols, the sAA response to a resistance training over-reaching protocol has yet to be investigated. Therefore, the purpose of this study was to investigate the sAA response associated with a one week high-volume resistance training protocol.

METHODS

Subjects

Eight participants, with a minimum of 12 months of consistent resistance training, were recruited to be a part of this study (91.25±8.46 Kg, 24.00±3.30 years old). Participants had a squat 1RM of 145.17 Kg ±41.86 (range: 72 – 204 Kg), relative squat strength of 1.59±0.47xBW (range: 0.89 – 2.16xBW) and a bench press 1RM of 99.72 Kg±23.91 (range: 54 – 127 Kg), 1.09±0.27xBW (range: 0.67 – 1.47xBW). Each participant completed the NSCA medical history questionnaire (CITE) several days prior to study initiation, to ensure participants were free from any exclusion conditions before performing high volume resistance training. Participants provided written informed consent as approved by the East Tennessee State University IRB.

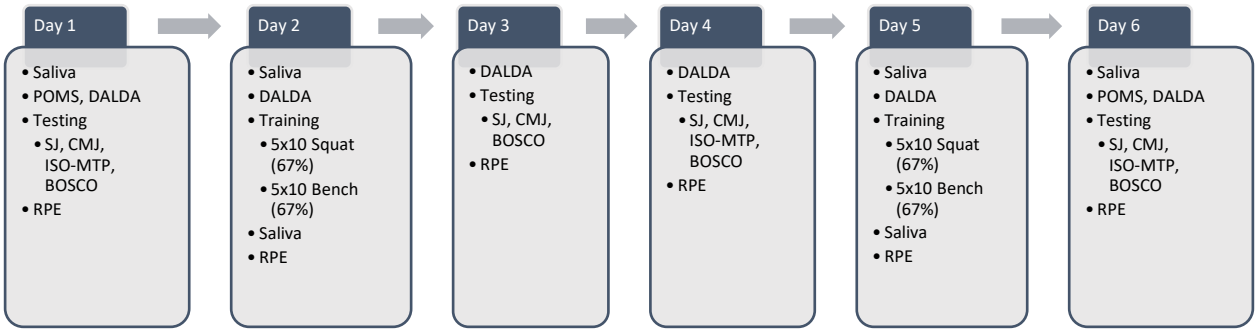
Procedures

This investigation was part of a much larger research study. Participants were instructed to arrive in the lab each day of the study in a fasted (8 hour) and hydrated state (4,25). Verbal confirmation was obtained to ensure each participant had fasted for at least 8 hours and urine specific gravity was measured to assess hydration status. After arrival to the lab, participants completed two psychological questionnaires, the Short Form Profile of Mood States (SF - POMS) (9) and the Daily Analysis of Life Demands for Athletes (DALDA) (23). After completing the questionnaires, participants provided a saliva sample, then performed a general standardized warmup consisting of 25 jumping jacks, 1 set of 5 repetitions (1x5) of the mid-thigh pull exercise with 20kg and a 3x5 mid-thigh pull with 60kg. After the general standardized warm-up, participants performed several performance tests including static and counter-movement jumps (VJ), isometric-mid thigh pull, and the Bosco repeated jumps test (30s). On day two, participants provided saliva samples (passive drool (D2), filled out the DALDA questionnaire, and then performed the same general standardized warm-up. After the general

warm-up, participants performed a specific warm up that included 1x10 back squat at 20kg, 1x5 back squat with 70% of their prescribed weight and 1x3 back squat with 85% of prescribed weight. After the specific warm-up, participants performed 5x10 back squats with 67% of their self-reported 1RM. After squats, participants performed the same specific warm-up protocol for bench press. All sets were separated by 2-3 minutes of rest. Participants provided their rating of perceived exertion (RPE) 15 minutes after finishing the last set of bench (BORG CR-10) (20). On day three and four, participants performed the same performance testing as on day one, with at least 24 hours between testing sessions. On day five, participants repeated the same protocol as day two. On day six, participants provided a saliva sample (D6) and then performed the same performance testing as on day one. Figure 1 provides a flow chart of the study.

On day six, participants provided a saliva sample (D6), completed second DALDA and SF-POMS questionnaire, and then performed the same performance testing as on day one. Figure 1 provides a flow chart of the study.

FIGURE 1:



QUESTIONNAIRES

The POMS-SF questionnaire is a 40 item survey that can be used to measure five negative (Anger, Confusion, Depression, Fatigue, Tension) and one positive (Vigor), affect scales. Participants answer how they are feeling "right now" on adjectives such as "Tense", "Unhappy", and "Active", ranking that adjective on a Likert-scale from a 0 "not at all" to 4

"extremely" (9,17). The participants answers can then be used to calculate total mood disturbance (TMD) by adding all negative sub-scales and subtracting the positive from that (TMD = 100 + (Anger + Confusion + Depression + Fatigue +Tension) – Vigor) (9).

The DALDA survey is a 34 item, two part questionnaire. Part A consists of 9 items that examine sources of stress (Diet, Training), while Pat B consists of 25 items examining symptoms of stress (Muscle pains, Congestion) (23). Participants respond to each item by selecting if that item is either A – worse than normal, B – normal, or C – better than normal. "A" scores from Part B are summed to calculate changes in stress symptoms (17,23).

SALIVA

Participants were instructed to avoid caffeine, eating, and drinking (except water) for an hour prior to providing saliva samples, and were only allowed to drink water while training, except during and immediately (up to 6 minutes) after the last set of bench press (21). All saliva samples were collected immediately (within 5 minutes) after the last set of bench press (2,19). After collection, saliva was frozen at -80 C until analyzed, as suggested by the Salimetrics protocol.

Saliva alpha-amylase samples were assayed at the Salimetrics' SalivaLab (Carlsbad, CA) using the Salimetrics Salivary Alpha-Amylase Assay Kit (Cat. No. 1-1902), without modifications to the manufacturers' protocol. Samples were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3,000 RPM (1,500 x g) immediately before performing the assay. Samples were tested for salivary alpha-amylase using a kinetic enzyme immunoassay (Cat. No. 1-1902). Sample test volume was 8 µl of 200x diluted saliva per determination. The assay has a lower limit of sensitivity of 0.4 U/mL, samples exceeding 400 U/mL needed further dilution, an average intra-assay coefficient of variation of 5.47% and an average inter-assay coefficient of variation of 4.7%, which meets the

manufactures' criteria for accuracy and repeatability in Salivary Bioscience, and exceed the applicable NIH guidelines for Enhancing Reproducibility through Rigor and Transparency.

STATISTICAL ANALYSIS

A t-test was used to compare resting sAA concentrations on the first day of training (D2) and the last day of the study (D6) to determine if there was a change in resting sAA concentrations following a week of high-volume resistance training. Two more t-tests were used to compare TMD scores (as calculated from the SF-POMS questionnaire) on day one of the study (TMD1) and on the last day (TMD2), and DALDA scores on day one (DALDA1) and on the last day (DALDA2), to determine if there was an increase in perceived stress. The DALDA scores were calculated by adding the "A" (worse than normal) responses in Part B (6,12). Holms sequential adjustment post hoc tests were used as required. All statistical analyses were performed using JASP (0.9.1.0). Effect size (Cohen's d) is reported as trivial <0.2, small 0.2-0.6, moderate 0.6-1.2, large 1.2-2.0, and very large 2.0-4.0 (11).

RESULTS

Data was screen for normality and outliers with one participant being removed due to baseline sAA concentrations on day two being outside normal physiological ranges. Another participant had scheduling conflicts on day 6 of the study, therefore all results are based on six participants (23.18±2.86 years, 89.82±7.06 Kg, bench press 1RM – 99.24±27.52 Kg, 1.10±0.28xBW, squat 1RM 141.67±47.38 Kg, 1.57±0.50xBW).

The change in resting sAA concentrations after one week of high-volume training was statistically insignificant with a moderate effect size (p=0.357, *d*= -0.414, 95% CI= -1.234 – 0.443). The change in perceived stress, from both the POMS and DALDA questionnaires, were also statistically insignificant (POMS: p= 0.085, *d*= -0.876, 95% CI= -1.806 – 0.111; DALDA:

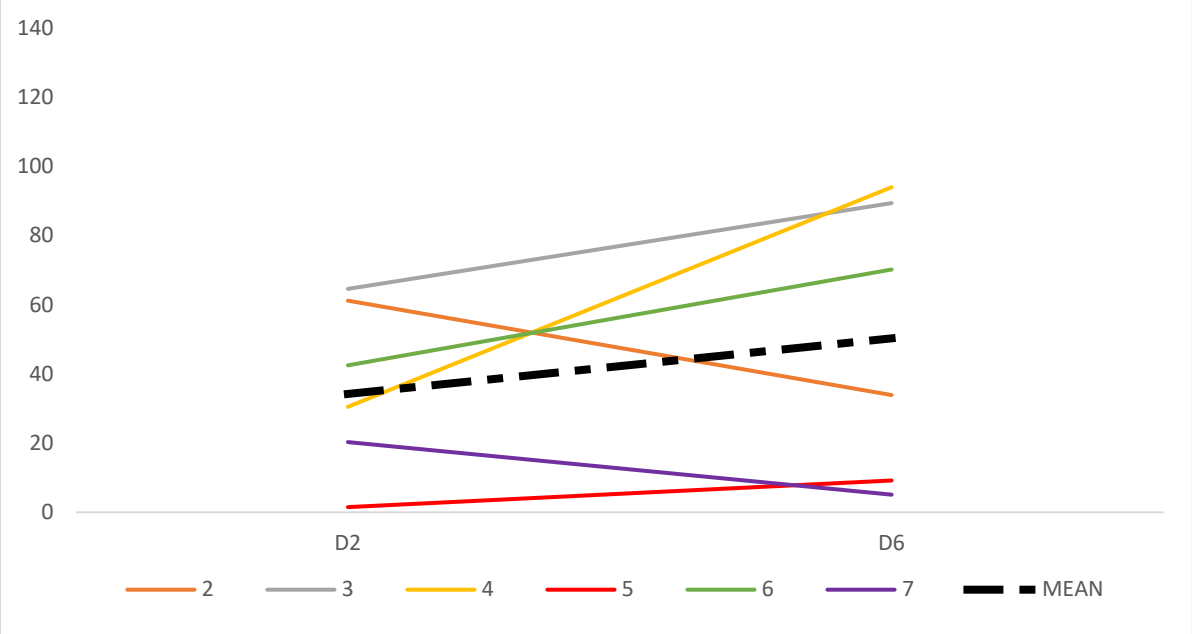
p=0.765, *d*= 0.129, 95%CI = -0.681 – 0.927). Descriptive statistics are provided in Table 1, and individual responses are provided in Figures 2 & 3.

TABLE 1: Descriptive Statistics

	N	MEAN	SD	RANGE
D2	6	36.77	±24.33	1.50 – 64.60
D6	6	50.33	±39.59	5.10 – 94.00
TMD1	6	88.33	±5.65	81.00 – 97.00
TMD2	6	93.67	±5.35	87.00 – 101.00
DALDA2	6	2.50	±2.67	0.00 – 7.00
DALDA6	6	2.17	±1.94	0.00 – 5.00

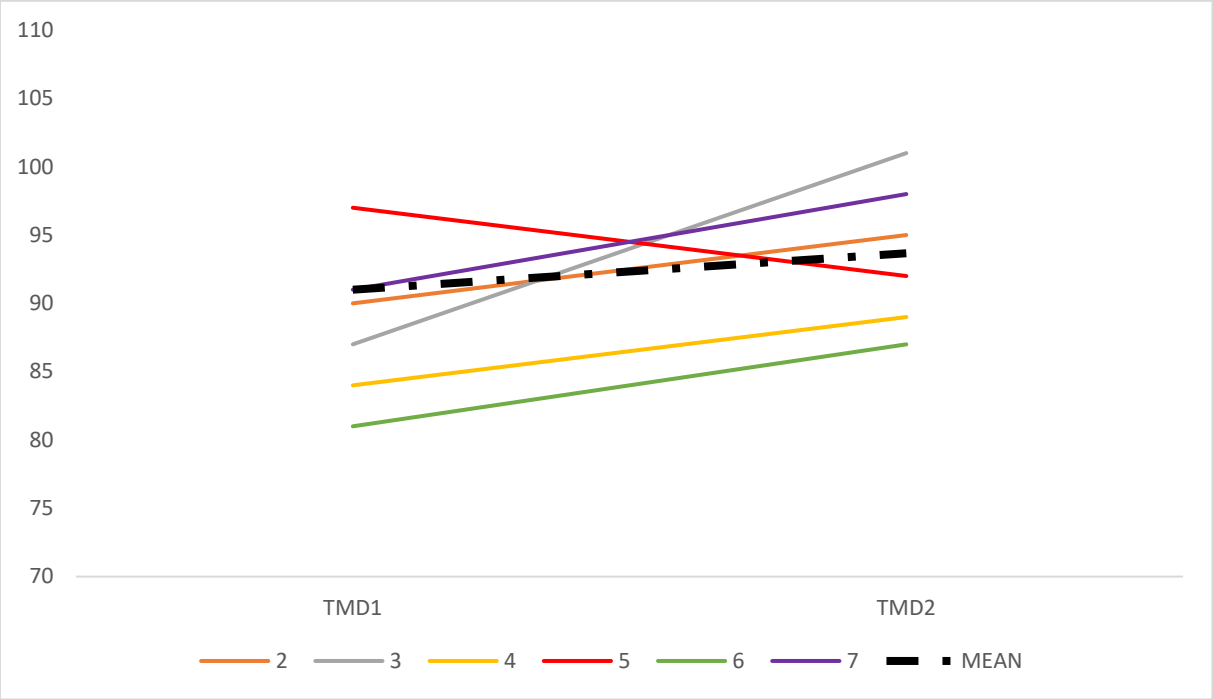
NOTE: Salivary Alpha-Amylase concentrations (U/ml).
D2 –Resting sAA concentrations day 2, D6 – Resting sAA concentrations day 6
TMD1 – Total mood disturbance score day 1, TMD2 – Total mood disturbance score day 6
DALDA2 – Daily analysis of life demands for athlete's score day 2
DALDA6 – Daily analysis of life demands for athlete's score day 6

FIGURE 2: Individual sAA Responses



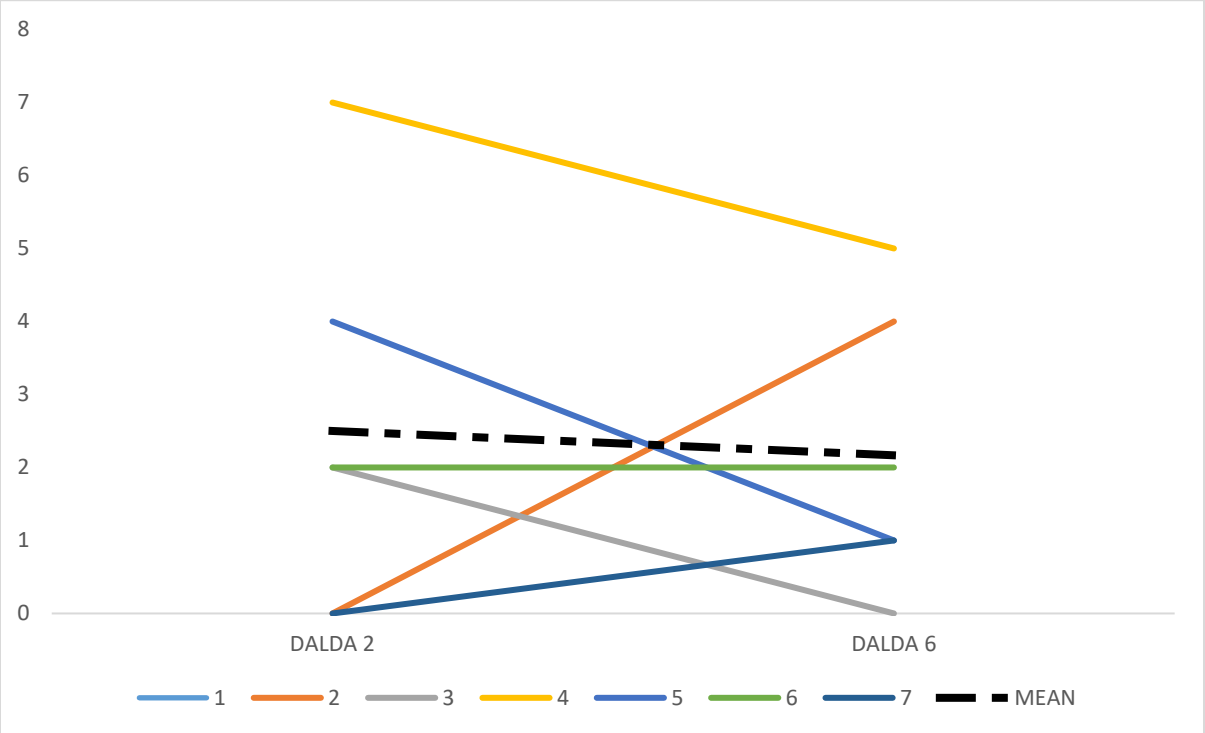
Note: Resting sAA concentration changes on day 2 (D2) too day 6 (D6)

FIGURE 3: Individual Changes in TMD Scores



Note: Individual changes in Total Mood Disturbance from day 1 (TMD1) too day 6 (TMD2).

FIGURE 4: Individual Changes in DALDA scores



Note: Changes in perceived stress calculated from the DALDA questionnaire

DISCUSSION

The statistically insignificant changes in both perceived stress (TMD, DALDA) and sAA concentrations suggests that this training protocol was not extensive enough to create an over-reached stated in this population. The data from this study does provide evidence that sAA as a biomarker for physiological stress may follow the same trends as psychological stress, as measured by the POMS survey, but longer over-reaching protocols need to be implemented in order to better determine this relationship.

Individual responses to the training produced some interesting results. Four participants who had elevated sAA baselines, also had increased TMD scores, leading to the possibility that these participants may have been in an over-reached state but with the small sample size and the two participants who did not follow the same pattern altered the group means to statistical insignificance. Due to this study being a part of a larger study that required blood draws, baseline sAA concentrations may have been artificially elevated prior to the first training day. A study by Koh (2014) reported an increased sAA concentration in anticipation of venous blood draw and may have caused an increase in baseline sAA concentrations on day two (14). The group trend observed in these two time points does tend to follow the same trends observed from aerobic over-reaching training as reported by Walsh (2013) (7,24).

These results also may provide evidence of a delayed physiological response (increased sAA concentrations) compared with perceived stress (TMD scores). Rushall (2003) argues that the psychological perceived state is the first factor to be altered in an athlete's capacity to adapt to stress (22), which can be observed by the large effect size of increased TMD scores along with moderate effect size of increased sAA concentrations.

PRACTICAL APPLICATION

Salivary alpha-amylase is a potentially useful athlete monitoring tool, increasing practicality in many situations by obviating many of the limitations of other monitoring tools. Monitoring resting sAA concentrations may provide unique insight into the fatigue state of athletes, but more research needs to be performed, with longer over-reaching cycles and with larger sample sizes in order to better elucidate the sAA responses to chronic resistance training

REFERENCES

1. Austin, KG and Deuster, P. Monitoring training for human performance optimization. *J Spec Oper Med* 15: 102–8, 2015.
2. Beltzer, EK, Fortunato, CK, Guaderrama, MM, Peckins, MK, Garramone, BM, and Granger, DA. Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiol Behav* 101: 289–296, 2010.
3. Chatterton, RT, Vogelsong, KM, Lu, Y, Ellman, AB, and Hudgens, GA. Salivary α-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 16: 433–448, 1996.
4. Cheuvront, SN, Kenefick, RW, and Zambraski, EJ. Spot urine concentrations should not be used for hydration assessment: A Methodology Review. *Int J Sport Nutr Exerc Metab* 25: 293–297, 2015.
5. Claudino, JG, Mezncio, B, Soncin, R, Ferreira, JC, Couto, BP, and Szmuchrowski, LA. Pre vertical jump performance to regulate the training volume. *Int J Sports Med* 33: 101–107, 2012.
6. Coutts, AJ, Wallace, LK, and Slattery, KM. Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *Int J Sports Med* 28: 125–134, 2007.
7. Gill, SK, Teixeira, AM, Rama, L, Rosado, F, Hankey, J, Scheer, V, et al. Gill_2013_Salivary antimicrobial protein responses during multitstage ultramarathon competition conducted in hot environmental conditions. *Appl Physiol Nutr Metab* 38: 977–987, 2013.
8. Gill, SK, Teixeira, AM, Rosado, F, Hankey, J, Wright, A, Marczak, S, et al. The impact of a 24-H ultra-marathon on salivary antimicrobial protein responses. *Int J Sports Med* 35: 966–971, 2014.

9. Grove, J. R.; Prapavessis, H. Preliminary evidence for the reliability and validity of an abbreviated profile of mood states. *Int. J. Sport Psychol*. 93–109, 1992.

10. Halson, SL. Monitoring training load to understand fatigue in athletes. *Sport. Med*. 44: 139–147, 2014.

11. Hopkins, W. New view of statistics: effect magnitudes. , 2002. Available from: http://www.sportsci.org/resource/stats/effectmag.html

12. Ihalainen, JK, Schumann, M, Häkkinen, K, and Mero, AA. Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. *Appl Physiol Nutr Metab* 41: 96–102, 2016.

13. Kilian, Y, Engel, F, Wahl, P, Achtzehn, S, Sperlich, B, and Mester, J. Markers of biological stress in response to a single session of high-intensity interval training and high-volume training in young athletes. *Eur J Appl Physiol* 116: 2177–2186, 2016.

14. Koh, D, Ng, V, and Naing, L. Alpha amylase as a salivary biomarker of acute stress of venepuncture from periodic medical examinations. *Front Public Heal* 2: 121, 2014.

15. Maso, F, Lac, G, Filaire, E, Michaux, O, and Robert, A. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. *Br J Sport Med* 38: 260–263, 2004.

16. Meeusen, R, Duclos, M, Foster, C, Fry, A, Gleeson, M, Nieman, D, et al. Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. *Med Sci Sports Exerc* 45: 186–205, 2013.

17. Nässi, A, Ferrauti, A, Meyer, T, Pfeiffer, M, and Kellmann, M. Psychological tools used for monitoring training responses of athletes. *Perform. Enhanc. Heal*. *5*(4), 125-133, 2017.

18. Nater, UM, Rohleder, N, Gaab, J, Berger, S, Jud, A, Kirschbaum, C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol* 55: 333–342, 2005.

19. De Oliveira, VN, Bessa, A, Lamounier, RPMS, De Santana, MG, De Mello, MT, and Espindola, FS. Changes in the salivary biomarkers induced by an effort test. *Int J Sports Med* 31: 377–381, 2010.

20. Pierce, KC, Rozenek, R, and Stone, M. Effects of high volume weight training on lactate, heart rate, and perceived exertion. *J. Strength Cond. Res*. 7(4): 211–215, 1993.

21. Rohleder, N and Nater, UM. Determinants of salivary a-amylase in humans and methodological considerations. *Psychoneuroendocrinology* 34: 469–485, 2009.

22. Rohleder, N, Nater, UM, Wolf, JM, Ehlert, U, and Kirschbaum, C. Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? In: *Annals of the New York Academy of Sciences* 1032: 258–263, 2004.

23. Rushall, BS. A tool for measuring stress tolerance in elite athletes. *J Appl Sport Psychol* 2: 51–66, 1990.

24. Schaal, K, Le Meur, Y, Louis, J, Filliard, JR, Hellard, P, Casazza, G, et al. Whole-body cryostimulation limits overreaching in elite synchronized swimmers. *Med Sci Sports Exerc* 47: 1416–1425, 2015.

25. Sommerfield, LM, McAnulty, SR, McBride, JM, Zwetsloot, JJ, Austin, MD, Mehlhorn, JD, et al. Validity of urine specific gravity when compared with plasma osmolality as a measure of hydration status in male and female ncaa collegiate athletes. *J Strength Cond Res* 30: 2219–2225, 2016.

26. van Stegeren, AH, Wolf, OT, and Kindt, M. Salivary alpha amylase and cortisol responses to different stress tasks: Impact of sex. *Int J Psychophysiol* 69: 33–40, 2008.

27. Taylor, K-L, Chapman, DW, Cronin, JB, Newton, MJ, and Gill, N. Fatigue monitoring in high performance sport: a survey of current trends. *J Aust Strength Cond* 20: 12–23, 2012.

28. Twist, C and Highton, J. Monitoring fatigue and recovery in rugby league players. *International Joural of sports physiology and performance* 8: 467-474, 2013

29. Vigil, JM, Geary, DC, Granger, DA, and Flinn, M V. Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following Hurricane Katrina. *Child Dev* 81: 1228–1240, 2010.

30. Walsh, NP. The effects of high-intensity intermittent exercise on saliva IgA, total protein and a-amylase. *J Sports Sci* 17: 129–134, 1999.

CHAPTER 5

SUMMARY AND FUTURE INVESTIGATIONS

The purposes of this dissertation were to investigate the salivary alpha-amylase response to both acute (single training session) and a week-long over-reaching cycle of resistance training. In order to investigate these responses, the following research projects were conducted: 1) an investigation of the change in salivary alpha-amylase concentration from pre- to post-training following 5 sets of 10 repetitions of squat and bench press, 2) an examination of the change in resting salivary alpha-amylase concentrations between pre and post following two training days of 5 sets of 10 repetitions of squat and bench press.

The results of study one indicated that there is a statistically significant increase in salivary alpha-amylase following a single training day consisting of 5 sets of 10 repetitions of squat and bench press. This study provides evidence that high-volume high-intensity resistance training evokes similar salivary alpha-amylase responses as aerobic training.

The results of study two indicated that there was not a statistically significant change in resting salivary alpha-amylase following an over-reaching training week consisting of two days of 5 sets of 10 repetitions of squat and bench press. The lack of change in resting salivary alpha-amylase coincided with two surveys (POMS, DALDA) to measure perceived stress that also showed no change from the over-reaching training week. This study provides evidence that there is an individualized response to an over-reaching week of high-volume high-intensity resistance training. These results can be interpreted as a need to monitor both the psychological perceived stress and the physiological stress of athletes as well as more evidence that individual fatigue monitoring is of more importance than group mean.

While this dissertation provided the first evidence for the salivary alpha-amylase response to resistance training, further research is needed. Future research should include the

salivary alpha-amylase response to different set x repetition protocols, such as high volume low intensity training (3x10 at 70% intensity) or high intensity low volume training (1RM testing). Future research should also include longer over-reaching cycles such as two or three weeks and monitor the resting salivary alpha-amylase along with changes in how salivary alpha-amylase responds to the individual training session in that over-reaching cycle, such as was done by Ihalainen (2016) and Gill (2013), while also monitoring performance and psychological measures.

REFERENCES

Allgrove, J. E., Gomes, E., Hough, J., & Gleeson, M. (2008). Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *Journal of Sports Sciences*, *26*(6), 653–661.

Allgrove, J. E., Oliveira, M., & Gleeson, M. (2014). Stimulating whole saliva affects the response of antimicrobial proteins to exercise. *Scandinavian Journal of Medicine and Science in Sports*, *24*(4), 649–655.

Austin, K. G., & Deuster, P. (2015). Monitoring training for human performance optimization. *Journal of Special Operations Medicine : A Peer Reviewed Journal for SOF Medical Professionals*, *15*(2), 102–108.

Backes, T. P., Horvath, P. J., & Kazial, K. A. (2015). Salivary alpha amylase and salivary cortisol response to fluid consumption in exercising athletes. *Biology of Sport*, *32*(4), 275–280.

Bellar, D., Murphy, K. A., Aithal, R., Davis, G. R., & Piper, T. (2017). The Effects of a 36-Hour Mixed Task Ultraendurance Race on Mucosal Immunity Markers and Pulmonary Function. *Wilderness & Environmental Medicine*, *28*(1), 10–16.

Beltzer, E. K., Fortunato, C. K., Guaderrama, M. M., Peckins, M. K., Garramone, B. M., & Granger, D. A. (2010). Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiology and Behavior*, *101*(2), 289–296.

Bishop, N. C., Walker, G. J., Scanlon, G. A., Richards, S., & Rogers, E. (2006). Salivary IgA responses to prolonged intensive exercise following caffeine ingestion. *Medicine and Science in Sports and Exercise*, *38*(3), 513–519.

Bocanegra, O. L., Diaz, M. M., Teixeira, R. R., Soares, S. S., & Espindola, F. S. (2012). Determination of the lactate threshold by means of salivary biomarkers: Chromogranin A as novel marker of exercise intensity. *European Journal of Applied Physiology*, *112*(9), 3195–3203.

Calvo, F., Chicharro, J. L., Bandres, F., Lucia, A., Perez, M., Alvarez, J., … Legido, J. C. (1997). Anaerobic threshold determination with analysis of salivary amylase. *Can J Appl Physiol*, *22*(6), 553–561.

Chatterton, R. T., Vogelsong, K. M., Lu, Y., Ellman, A. B., & Hudgens, G. A. (1996). Salivary α-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology*, *16*(4), 433–448.

Cheuvront, S. N., Kenefick, R. W., & Zambraski, E. J. (2015). Spot urine concentrations should not be used for hydration assessment: A methodology review. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(3), 293–297.

Chicharro, J. L., Lucía, A., Pérez, M., Vaquero, A. F., & Ureña, R. (1998). Saliva composition and exercise. *Sports Medicine*, *26*(1), 17–27.

Claudino, J. G., Mezncio, B., Soncin, R., Ferreira, J. C., Couto, B. P., & Szmuchrowski, L. A. (2012). Pre vertical jump performance to regulate the training volume. *International Journal of Sports Medicine*, *33*(2), 101–107.

Costa, R. J. S., Fortes, M. B., Richardson, K., Bilzon, J. L. J., & Walsh, N. P. (2012). The effects of postexercise feeding on saliva antimicrobial proteins. *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(3), 184–191.

Coutts, A. J., Wallace, L. K., & Slattery, K. M. (2007). Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *International Journal of Sports Medicine*, *28*(2), 125–134.

De Oliveira, V. N., Bessa, A., Lamounier, R. P. M. S., De Santana, M. G., De Mello, M. T., & Espindola, F. S. (2010). Changes in the salivary biomarkers induced by an effort test. *International Journal of Sports Medicine*, *31*(6), 377–381.

De Pero, R., Cibelli, G., Cortis, C., Sbriccoli, P., Capranica, L., & Piacentini, M. (2016). Stress related changes during TeamGym competition. *The Journal of Sports Medicine and Physical Fitness*, *56*(5), 639–647.

del Corral, P., Mahon, A. D., Duncan, G. E., Howe, C. A., & Craig, B. W. (1994). The effect of exercise on serum and salivary cortisol in male children. *Medicine and Science in Sports and Exercise*, *26*(11), 1297–1301.

DeWeese, B. H., Hornsby, G., Stone, M., & Stone, M. H. (2015). The training process: Planning for strength-power training in track and field. Part 1: Theoretical aspects. *Journal of Sport and Health Science*, 4(4), 308-317.

Diaz, M. M., Bocanegra, O. L., Teixeira, R. R., Soares, S. S., & Espindola, F. S. (2013). Salivary nitric oxide and alpha-amylase as indexes of training intensity and load. *International Journal of Sports Medicine*, *34*(1), 8–13.

Fatolahi, H., Rasaee, M. J., & Peeri, M. (2011). The effect of exercise mode and intensity of submaximal physical activities on salivary testosterone to cortisol ratio and α-amylase in young active males. *International Journal of Exercise Science*, *4*(4), 9.

Fortes, M. B., Diment, B. C., Di Felice, U., & Walsh, N. P. (2012). Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. *Applied Physiology, Nutrition, and Metabolism*, *37*(5), 850–859.

Fortes, M. B., & Whitham, M. (2011). Salivary Hsp72 does not track exercise stress and caffeine-stimulated plasma Hsp72 responses in humans. *Cell Stress and Chaperones*, *16*(3), 345–352.

Foster, C., Florhaug, J. A., Franklin, J., Gottschall, L., Hrovatin, L. A., Parker, S., … Dodge, C. (2001). A new approach to monitoring exercise training. *J. Strength Cond. Res*, *15*(151).

Gallina, S., Di Mauro, M., D'Amico, M. A., D'Angelo, E., Sablone, A., Di Fonso, A., … Di Baldassarre, A. (2011). Salivary chromogranin A, but not α-amylase, correlates with cardiovascular parameters during high-intensity exercise. *Clinical Endocrinology*, *75*(6), 747–752.

Gill, S. K., Teixeira, A. M., Rama, L., Rosado, F., Hankey, J., Scheer, V., … Costa, R. J. S. (2013). Salivary antimicrobial protein responses during multitstage ultramarathon competition conducted in hot environmental conditions. *Applied Physiology, Nutrition, and Metabolism*, *38*, 977–987.

Gill, S. K., Teixeira, A. M., Rosado, F., Hankey, J., Wright, A., Marczak, S., … Costa, R. J. S. (2014). The impact of a 24-H ultra-marathon on salivary antimicrobial protein responses. *International Journal of Sports Medicine*, *35*(11), 966–971.

Gleeson, M. (2002). Biochemical and immunological markers of overtraining. *Journal of Sports Science and Medicine*, *1*, 31–41.

Granger, D. A., Kivlighan, K. T., El-Sheikh, M., Gordis, E. B., & Stroud, L. R. (2007). Salivy α-amylase in biobehavioral research: Recent developments and applications. In *Annals of the New York Academy of Sciences* 1098: 122–144, 2007.

Grove, J. R.; Prapavessis, H. (1992). Preliminary evidence for the reliability and validity of an abbreviated profile of mood states. *International Journal of Sport Psychology*.

Haff, G., & Triplett, N. T. (Eds.). (2015). *Essentials of strength training and conditioning* (Fourth edition). Champaign, IL: Human Kinetics.

Halson, S. L. (2014). Monitoring training load to understand fatigue in athletes. *Sports Medicine 44* 139-147.

Hopkins, W. (2002). New view of statistics: Effect Magnitudes. Retrieved November 7, 2017, from http://www.sportsci.org/resource/stats/effectmag.html

Ihalainen, J. K., Schumann, M., Häkkinen, K., & Mero, A. A. (2016). Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. *Applied Physiology, Nutrition, and Metabolism*, *41*, 96–102.

Impellizzeri, F. M., Rampinini, E., Coutts, A. J., Sassi, A., & Marcora, S. M. (2004). Use of rpe-based training load in soccer. *Med. Sci. Sports Exerc*, *36*(6), 1042–1047.

Jacks, D. E., Sowash, J., Anning, J., McGloughlin, T., & Andres, F. (2002). Effect of exercise at three exercise intensities on salivary cortisol. *Journal of Strength and Conditioning Research*, *16*(2), 286–289.

Kilian, Y., Engel, F., Wahl, P., Achtzehn, S., Sperlich, B., & Mester, J. (2016). Markers of biological stress in response to a single session of high-intensity interval training and high-volume training in young athletes. *European Journal of Applied Physiology*, *116*(11–12), 2177–2186.

Kivlighan, K. T., & Granger, D. A. (2006). Salivary a-amylase response to competition: Relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology*, *31*(6), 703–714.

Koh, D., Ng, V., & Naing, L. (2014). Alpha amylase as a salivary biomarker of acute stress of venepuncture from periodic medical examinations. *Frontiers in Public Health*, *2*, 121.

Koibuchi, E., & Suzuki, Y. (2014). Exercise upregulates salivary amylase in humans. *Experimental and Therapeutic Medicine*, *7*(4), 773–777.

Kraemer, W. J., Fleck, S. J., & Deschenes, M. R. (2012). *Exercise physiology : integrated from theory to practical applications*. Wolters Kluwer/Lippincott Williams & Wilkins Health.

Kunz, H., Bishop, N. C., Spielmann, G., Pistillo, M., Reed, J., Ograjsek, T., … Simpson, R. J. (2015). Fitness level impacts salivary antimicrobial protein responses to a single bout of cycling exercise. *European Journal of Applied Physiology*, *115*(5), 1015–1027.

Le Meur, Y., Hausswirth, C., Natta, F., Couturier, A., Bignet, F., & Vidal, P. P. (2013). A multidisciplinary approach to overreaching detection in endurance trained athletes. *Journal of Applied Physiology*, *114*(3), 411–420.

Li, C. Y., Hsu, G. S., Suzuki, K., Ko, M. H., & Fang, S. H. (2015). Salivary immuno factors, cortisol and testosterone responses in athletes of a competitive 5,000 m race. *Chinese Journal of Physiology*, *58*(4), 263–269.

Li, T. L., & Gleeson, M. (2004). The effect of single and repeated bouts of prolonged cycling on leukocyte redistribution, neutrophil degranulation, IL-6, and plasma stress hormone responses. *International Journal of Sport Nutrition and Exercise Metabolism*, *14*(5), 501–516.

Ligtenberg, A. J. M., Brand, H. S., Van Den Keijbus, P. A. M., & Veerman, E. C. I. (2015). The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme. *Archives of Oral Biology*, *60*(11), 1639–1644.

Lin, S. P., Li, C. Y., Suzuki, K., Chang, C. K., Chou, K. M., & Fang, S. H. (2014). Green tea consumption after intense taekwondo training enhances salivary defense factors and antibacterial capacity. *PLoS ONE*, *9*(1).

Ljungberg, G., Ericson, T., Ekblom, B., & Birkhed, D. (1997). Saliva and marathon running. *Scandinavian Journal of Medicine & Science in Sports*, *7*(4), 214–219.

Maruyama, Y., Kawano, A., Okamoto, S., Ando, T., Ishitobi, Y., Tanaka, Y., … Akiyoshi, J. (2012). Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the trier social stress tests. *PLoS ONE*, *7*(7).

Maso, F., Lac, G., Filaire, E., Michaux, O., & Robert, A. (2004). Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. *Br J Sports Med*, *38*, 260–263.

Mckune, A. J., Bach, C. W., Semple, S. J., & Dyer, B. J. (2014). Salivary cortisol and α-amylase responses to repeated bouts of downhill running. *American Journal of Human Biology*, *26*, 850–855.

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., … Urhausen, A. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the european college of sport science and the American College of Sports Medicine. *Medicine and Science in Sports and Exercise*, *45*(1), 186–205.

Nässi, A., Ferrauti, A., Meyer, T., Pfeiffer, M., & Kellmann, M. (2017). Psychological tools used for monitoring training responses of athletes. *Performance Enhancement and Health, 5*(4), 125-133.

Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M., & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity - Associations with adrenergic activity. *Psychoneuroendocrinology*, *31*(1), 49–58.

Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*, *55*(3), 333–342.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, *32*(4), 392–401.

Nexø, E., Hansen, M. R., & Konradsen, L. (1988). Human salivary epidermal growth factor, haptocorrin and amylase before and after prolonged exercise. *Scandinavian Journal of Clinical and Laboratory Investigation*, *48*(3), 269–273.

Papacosta, E., & Nassis, G. P. (2011). Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. *Journal of Science and Medicine in Sport, 14*, 424-434.

Peng, H. T., Savage, E., Vartanian, O., Smith, S., Rhind, S. G., Tenn, C., & Bjamason, S. (2016). Performance Evaluation of a Salivary Amylase Biosensor for Stress Assessment in Military Field Research. *Journal of Clinical Laboratory Analysis*, *30*(3), 223–230.

Pierce, K. C., Rozenek, R., & Stone, M. (1993). Effects of high volume weight training on lactate, heart rate, and perceived exertion. *J. Strength Cond. Res*. 7(4): 211–215.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary α -amylase in humans and methodological considerations. *Psychoneuroendocrinology*, *34*(4), 469–485.

Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? In *Annals of the New York Academy of Sciences* 1032: 258–263.

Rosa, L., Teixeira, A. A. S., Lira, F. S., Tufik, S., Mello, M. T., & Santos, R. V. T. (2014). Moderate acute exercise (70% VO2 peak) induces TGF-β, α -amylase and IgA in saliva during recovery. *Oral Diseases*, *20*(2), 186–190.

Rushall, B. S. (1990). A tool for measuring stress tolerance in elite athletes. *Journal of Applied Sport Psychology*, *2*(1), 51–66.

Scannapieco, F. a, Torres, G., & Levine, M. J. (1993). Salivary alpha-amylase: role in dental plaque and caries formation. *Critical Reviews in Oral Biology and Medicine : An Official Publication of the American Association of Oral Biologists*, *4*(3–4), 301–307.

Schaal, K., Le Meur, Y., Louis, J., Filliard, J. R., Hellard, P., Casazza, G., & Hausswirth, C. (2015). Whole-body cryostimulation limits overreaching in elite synchronized swimmers. *Medicine and Science in Sports and Exercise*, *47*(7), 1416–1425.

Shetty, V., Zigler, C., Robles, T. F., Elashoff, D., & Yamaguchi, M. (2011). Developmental validation of a point-of-care, salivary α-amylase biosensor. *Psychoneuroendocrinology*, *36*(2), 193–199.

Sommerfield, L. M., McAnulty, S. R., McBride, J. M., Zwetsloot, J. J., Austin, M. D., Mehlhorn, J. D., … Utter, A. C. (2016). Validity of urine specific gravity when compared with plasma osmolality as a measure of hydration status in male and female ncaa collegiate athletes. *Journal of Strength and Conditioning Research*, *30*(8), 2219–2225.

Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., & Nishikawa, Y. (2007). Gender-specific differences in salivary biomarker responses to acute psychological stress. In *Annals of the New York Academy of Sciences* 1098, 510–515.

Taylor, K.-L., Chapman, D. W., Cronin, J. B., Newton, M. J., & Gill, N. (2012). Fatigue monitoring in high performance sport: a survey of current trends. *J. Aust. Strength Cond*, *20*(1), 12–23.

Twist, C., & Highton, J. (2013). Monitoring fatigue and recovery in rugby league players demands of rugby league. *International Journal of Sports Physiology and Performance* 8: 467-474, 2013.

van Stegeren, A. H., Wolf, O. T., & Kindt, M. (2008). Salivary alpha amylase and cortisol responses to different stress tasks: Impact of sex. *International Journal of Psychophysiology*, *69*(1), 33–40.

Vigil, J. M., Geary, D. C., Granger, D. A., & Flinn, M. V. (2010). Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following Hurricane Katrina. *Child Development*, *81*(4), 1228–1240.

Walsh, N. P. (1999). The effects of high-intensity intermittent exercise on saliva IgA, total protein and a-amylase. *Journal of Sports Sciences*, *17*(2), 129–134.

Yamaguchi, M., Deguchi, M., Wakasugi, J., Ono, S., Takai, N., Higashi, T., & Mizuno, Y. (2006). Hand-held monitor of sympathetic nervous system using salivary amylase activity and its validation by driver fatigue assessment. *Biosensors and Bioelectronics*, *21*(7), 1007–1014.

VITA

ASHER FLYNN

Education:	Kickapoo High School, Springfield, Missouri 2004
	B.S. Exercise Science, Evangel University, Springfield, Missouri 2011
	M.S. Kinesiology, University of Central Arkansas, Conway, Arkansas 2016
	Ph.D., Sport Physiology & Performance, East Tennessee State University, Johnson City, Tennessee 2019
Professional Experience:	Student Instructor, East Tennessee State University; Johnson City, Tennessee, 2017-2018
	Adjunct Instructor, Central Baptist College; Conway, Arkansas, 2015 – 2016
Publications:	Flynn, Asher. (2018). "Preliminary Analysis - Moderating the Stress Perception of Collegiate Distance Runners Using Branched-Chain Amino Acids." ACSM, 2018
	Flynn, Asher. (2018). "Validation of the SRSS Questionnaire During Women's Volleyball In-Season Training." Sport Science & Coach Education, 2017
Honors and Awards:	Dean's List, Evangel University. 2010-2011