THE EFFECT OF SUBMAXIMAL EXERCISE ON SALIVARY α-AMYLASE ACTIVITY

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ABSTRACT


Purpose: Salivary α-amylase (sAA) has been suggested as a non-invasive biomarker of exercise intensity. Previous research has concluded differences in exercise intensities for stimulation of sAA. The aim of the present study is to determine the differences in sAA activity between pre- and post-submaximal exercise amongst various ages.

Methods: The present study recruited 12 apparently healthy individuals between the ages of 21-50 years. After completion of a 5-min warm-up, each participant would then exercise using a protocol at a submaximal intensity (70% HRmax) for 25 minutes using a treadmill modality. Salivary α-amylase was then collected pre- and post-exercise using a Salimetrics Oral Swab (SOS). Samples were shipped to Salimetrics for analysis.

Results: Individuals varied in age but a significant increase (t= -3.89, p < .05) was observed between pre- and post-exercise sAA values with a mean difference of 59.8 ±46.10 U/mL. Furthermore, similarities in exercise induced sAA secretion were seen amongst participants comparable in age.

Conclusion: Exercise has shown to increase sAA at specific submaximal intensities five minutes post exercise among a population varying in age.

Key Words: α-amylase, Saliva, Treadmill, Stress, Oral Swab

INTRODUCTION

Physiological stress responses are important health and disease markers. With an estimated rise of stress-related mental health conditions becoming second in prevalence to only that of ischemic heart disease in 2020, a greater understanding of exercise and its impact upon physiological and psychological health may be established through further research of salivary enzyme α-amylase (sAA). Salivary α-amylase is a digestive enzyme of the mouth and pancreas that aids in the digestion
of starches; however, current research has suggested sAA to be a possible biological marker of physiological and psychological stress. A variety of studies upon exercise and its physiological effects on sAA have been published; though further research is needed to gather a better understanding of sAA activity within various age groups.1,6,9

Salivary α-amylase has a wide array of psychological and physiological applications that can be used towards the study of exercise. Research conducted by Mohammad et al. (2011) demonstrated that various exercises such as treadmill, elliptical and cycle ergometers cause both physiological and psychological stress. Furthermore, Mohammad et al. (2011) suggests that the responses of exercise biomarkers, such as sAA, differ amongst exercise modes and intensities. Thus, measuring sAA in correlation to various exercises may be a simple, non-invasive measurement of exercise exertion. Based off these findings, a treadmill modality was considered most appropriate for both age groups within the present study. A treadmill modality using a submaximal intensity showed the most positive increase in sAA activity with the greatest ease of performance amongst participants.

Salivary α-amylase has been a topic of discussion throughout research in recent years.3,8 However, further information regarding the physiological effects of sAA and the factors that contribute towards its activity need to be investigated. Such findings may suggest new approaches to addressing a non-invasive measurement of exercise exertion6, sympathetic nervous system activity,8 maintenance of postprandial hyperglycemia,5 and establishment of a biological marker of psychological and psychosocial stress.4

Another reason sAA is discussed in relation to exercise is because of the fluctuating sAA activity due to exercise induced stress. As discussed previously, sAA has been studied as an indicator of physiological and psychological stressors; however, a definitive answer upon desired levels of sAA activity is currently uncertain. In some instances, such as depression, a low sAA activity level would be considered desirable and may even be induced through medication in years to come.10 However, research conducted by Mandel et al. (2012) with regards to postprandial hyperglycemia showed a diabetic individual may seek higher levels of sAA activity for better glucose utilization.5

As the prevalence of disease increases with age, further understanding of physiological and psychological stress differences over the life span is needed.8 A past study conducted by Strahler et al. (2009) has also looked at the differences of stress’ effects upon sAA activity and the changes that occurred between different age groups in which a response to acute stress did not uniformly change with age.8 Though the studies presented by Mohammed et al. (2011) and Strahler et al. (2009) conclude different cases as to why sAA activity increases, it has not been found that both areas of research were studied together. Furthermore, most research has dealt with sAA activity as an absolute measurement of sAA secretion released by an individual due to physiological or psychological stress. However, this study investigated the absolute secretion of sAA and the percent change of stimulated sAA between participants. Being previous research by Strahler showed psychological stress alluding to positive changes of sAA activity amongst individuals of various ages;6 it was hypothesized within this present study that an increase in post-exercise sAA activity would occur throughout both age groups. Thus, the present study looked to determine the differences in sAA activity amongst various ages after the completion of submaximal exercise.

METHODS

Participants
The participants of the study consisted of 12 apparently healthy individuals (six males, six females) between the ages 21-50 years that were divided into two groups based upon age, and all participants
were randomly asked to participate. Each group consisted of an equal ratio of participants and gender. Guidelines for the present study excluded individuals with any of the following criteria: metabolic, cardiovascular or pulmonary disease, physical limitations, current tobacco use (within last six months) or participation in physical activity or ingestion of alcohol in the past 48 hours. It was assumed that participants arrived after an overnight fast; however, participants were allowed to drink water up to 10 minutes prior to saliva collection to maintain hydration. Prior to participation, all participants had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Wisconsin – Eau Claire.

Procedures

Study Design and General Procedure
The purpose and procedure of the study was explained to the participants before written consent was obtained. In addition to an informed consent, participants completed a PAR-Q and health history form prior to arriving to the lab to ensure all compliances were met before testing. Upon arrival, a Polar wireless heart rate monitor (Polar Electro, Woodbury, NY) was placed on the participant to record their resting heart rate and heart rate during exercise. A Salimetrics oral swab (Salimetrics, State College, PA) was used to collect sAA prior to and following testing. Placement of the swab was done by the experimenter between the participant’s cheek and top right gum for two minutes. Protocol for sAA collection for this study was determined by Salimetrics (Salimetrics, State College, PA). Each swab was then placed in a tube insert, categorized using a numerical barcode label, and then refrigerated till testing completion. Samples were then transferred and kept in a freezer (at or below -20 °C) until sent to the Salimetrics’ lab for analysis.

A 5-minute low intensity warm-up was conducted to begin each test in order to familiarize the participants with the exercise modality of the treadmill while also gradually increasing their heart rate to approach 70% of heart rate (HRmax) maximal. After completion of the 5-min warm-up, each participant would then exercise using an exercise protocol of a submaximal intensity (70% HRmax) for 25 minutes. The exercise protocol for this study was established by Mohammad et al. (2011) to show positive increases in sAA activity compared to that of other higher treadmill intensities. To determine 70% HRmax of the participants, the equation from Tanaka et al. (2001) was used [208-(.7xage)]. This HRmax formula has a ±10 beat per minute margin of error and has been used in previous research by Mohammad et al. (2011). Throughout each individual test, the exercise intensity (mph and % incline) was adjusted to maintain 70% HRmax which was recorded once per minute. After completion of the test, participants would again have their mouth swabbed; however, unlike immediate sampling of sAA taken pre-exercise, post-exercise sAA sampling occurred five minutes after testing. The literature showed that waiting five minutes after exercise gave the highest increase in sAA activity.

Lab Analysis Procedure
Salimetrics sAA determination process is as follows: samples were warmed to room temperature and a kinetic reaction assay was used to determine the amount of sAA. The reaction of 8ml of diluted sAA (1:200) and 320ml of chromogenic substrate at 37 degrees Celsius gives 2-chloro-p-nitrophenol. The sAA and chromogenic substrate were mixed, transferred to a 96-well microtiter plate and spun for three minutes between 500 and 600 revolutions per minute. The optical density is measured using a laboratory plate reader with a 405nm filter, and measurements were taken at minute one and three. The amount of sAA is directly proportional to the absorbance of 2-chloro-p-nitrophenol (refer to formula below).
Amylase and Submaximal Exercise

\[
sAA \ (U/ml) = \frac{\Delta \text{ absorbance}}{2\text{min}} \times 328 \ (\text{assay total volume}) \times 200 \ (\text{dilution factor}) \\
12.9 \ (\text{millimolar absorptivity of 2-chloro-p-nitrophenol} \times 0.008 \ (\text{sample volume}) \times 0.97 \ (\text{light path})
\]

Delta absorbance (change in absorbance) is the reading at minute one minus the reading at minute two.

**Statistical Analyses**

Data were analyzed using SPSS (19.0) and Microsoft Excel (2010). A dependent t-test was used to compare groups pre- and post-exercise. Mean sAA and the corresponding standard deviation were calculated for all subjects pre- and post-exercise. An alpha level of P<.05 was used to show a statistical significance.

**RESULTS**

Table 1 and 2 show the characteristics of the participants. One individual was an influential case and was excluded due to excessive values. Two individuals were also excluded due to insufficient saliva production post-exercise which resulted in an inability for Salimterics to collect data. These exclusions resulted in a smaller population for analysis (n=9).

Table 1. Descriptive summary of older males and females and pre- versus post-exercise sAA statistical data.

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age</th>
<th>Gender</th>
<th>Pre-Exercise sAA (U/mL)</th>
<th>Post-Exercise sAA (U/mL)</th>
<th>HRmax</th>
<th>Testing HR</th>
<th>% Difference of sAA</th>
<th>Mean sAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>F</td>
<td>126.9</td>
<td>227.6</td>
<td>178.6</td>
<td>125</td>
<td>56.81</td>
<td>156.3</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>F</td>
<td>82.3</td>
<td>137.8</td>
<td>175.8</td>
<td>123</td>
<td>50.43</td>
<td>110.1</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>F</td>
<td>118.7</td>
<td>236.5</td>
<td>175.1</td>
<td>122.5</td>
<td>66.33</td>
<td>177.6</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>M</td>
<td>81.3</td>
<td>158.8</td>
<td>177</td>
<td>124</td>
<td>64.56</td>
<td>120.1</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>M</td>
<td>223</td>
<td>290.9</td>
<td>175</td>
<td>123</td>
<td>26.43</td>
<td>257.0</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>M</td>
<td>272.6</td>
<td>271.3</td>
<td>173</td>
<td>121</td>
<td>-0.478</td>
<td>272.0</td>
</tr>
</tbody>
</table>

Table 2. Descriptive summary of younger males and females and pre- versus post-exercise sAA statistical data.

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age</th>
<th>Gender</th>
<th>Pre-Exercise sAA (U/mL)</th>
<th>Post-Exercise sAA (U/mL)</th>
<th>HRmax</th>
<th>Testing HR</th>
<th>% Difference of sAA</th>
<th>Mean sAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>21</td>
<td>F</td>
<td>259.1</td>
<td>267.6</td>
<td>193.3</td>
<td>135</td>
<td>3.228</td>
<td>263.4</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>M</td>
<td>85</td>
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<td>192.6</td>
<td>134</td>
<td>5.603</td>
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<tr>
<td>9</td>
<td>23</td>
<td>M</td>
<td>76.8</td>
<td>183.4</td>
<td>191.9</td>
<td>134</td>
<td>81.94</td>
<td>130.1</td>
</tr>
</tbody>
</table>

An increase was observed between pre- and post-exercise sAA values with a mean difference of 59.8 ±46.10 U/mL. Overall, sAA levels were increased between pre- versus post-exercise (t= -3.89, p < .05). The overall percent change (28.5%) between all participants was found from the mean difference (59.8 ±46.10 U/mL) ÷ mean post-exercise sAA (207.1 ±68.49 U/mL).

Figure 1 shows the % difference of sAA secretion ∆ between each participant. Salivary α-amylase increased for the majority (n=8) of the population (n=9) (Figure 1).
Figure 1. % Difference sAA secretion per subject.

Figure 2 shows the difference in absolute sAA secretion between pre- and post-exercise.

Figure 2. Mean sAA pre- versus post-exercise secretion (n=9)
DISCUSSION

It has been suggested that sAA may be an indicator of exercise exertion.\textsuperscript{1,7} From the current study, sAA measurements showed significant absolute change (59.8 ±46.10 U/mL) after the completion of a 5 minute warm-up and a 70% HRmax treadmill modality of 25 minutes. These findings corresponded to previous data of Mohammad et al\textsuperscript{7} with the effects of various exercise modalities and the positive impact of submaximal exercise upon sAA secretion. In the 2011 study of Mohammad et al,\textsuperscript{7} it has been suggested that intensities < 70% HRmax for exercise relate to a positive increase in sAA activity; the opposite may be seen for intensities >85% HRmax. Data in the current study showed that submaximal exercise intensities do relate to increases in sAA activity; however, other research has proposed intensities reaching maximal exertion show positive changes to sAA secretion.\textsuperscript{12} Thus, a discrepancy is seen amongst the measurement of sAA activity as a viable biomaker of exercise exertion. However, in all cases, findings of previous and present research show variation of sAA secretion during or after physical activity.\textsuperscript{2,7,12}

Although data in the present study suggested submaximal intensities of exercise promote a positive influence upon sAA activity, corresponding variables between exercise and sAA activity, such as age, were also examined upon evaluation. A great deal of variability can be seen for absolute sAA values throughout all participants of the study for both pre- and post-exercise sAA secretion. However, when looking at the percent change of sAA secretion that occurred, subjects alike in age (subjects 1-4) showed similar change (Δ =54.2\%) of sAA activity; exclusion for subjects 5-6 may be seen in the following paragraph. Findings such as these would suggest that individuals of the same age, exercising at the same intensity, would see similar changes in their sAA secretion regardless of baseline values. This idea is comparable to findings from Strahler et al which suggested that age and BMI were the strongest predictors of sAA increases and that no difference for sAA secretion should be seen between genders.\textsuperscript{8}

In a few instances within the present study (subjects 5-7), large baseline sAA values (251.7 ± 28.57 U/mL) showed small variability in overall sAA secretion after completion of exercise. These small variabilities in sAA secretion may be explained by the ceiling effect of sAA described by Strahler et al.\textsuperscript{8} In this study, children experienced much larger baseline values of sAA compared to that of older and younger adults. These large baseline values of sAA then showed smaller variability in sAA secretion after stress, which was then associated with a ceiling affect that hindered the production of sAA.

Rationale for sAA variation with exercise may be explained by sympathetic nervous system stimulation through an increasing heart rate or by response to catecholamine.\textsuperscript{2,7,9} Salivary α-amylase is secreted upon stimulation of the autonomic nervous system which is affected by both sympathetic and parasympathetic nervous system activity.\textsuperscript{4,9,10} Until recently, it was thought that these two branches of the autonomic nervous system resulted in an inhibitory effect upon one another; however, it has been shown that these branches may work together to control the heart and salivary glands.\textsuperscript{9} As sAA is secreted from both the epithelial acinar cells of the salivary glands and stored areas within the parotid gland granules during sympathetic and androgenic stimulation,\textsuperscript{2,7,13} variability seen in sAA secretion within the present study may be due to neural stimulation from anticipation or performance of exercise. Variability of sAA secretion due to anticipation have been noted in previous research such as Walsh et al where decreases of sAA were significant immediately prior to testing.\textsuperscript{9}
There were several limitations that may have affected the sAA results such as the small population size. Other limitations included using a swab technique compared to a drooling technique. Though no significant difference is seen amongst saliva collection technique, the passive drooling technique may have provided a larger quantity of measurable saliva. Collecting saliva using passive drooling may have contributed to the prevention of insufficient quality of saliva (QNS) that was seen for two participants. Another possible limitation of the present study included participants varying in exertion prior to testing. In some instances individuals walked to the facility where testing was conducted, while other participants drove. Being there was different exertion levels experienced by participants prior to the pre-exercise sAA collection, possible baseline sAA measurements variability may have occurred. As the present study used submaximal exercise for its testing protocol, further limitation may have resulted due to fluctuation of the heart rate above or below the desired 70% HRmax.

CONCLUSIONS

Due to insufficient data within the younger population of the present study, a conclusion upon the differences of sAA activity in various ages after completion of submaximal exercise can’t be drawn. However, exercise has shown to increase sAA at specific submaximal intensities five minutes post-exercise among a population varying in age. A large mean difference (59.8 ±46.10 U/mL) was seen between pre- and post-exercise sAA values to indicate this claim. Similar findings of sAA activity post-exercise may be further seen from Mohammad et al. (2011) in which submaximal exercise showed an increased in sAA activity five minutes after completion. Protocol for the current study was based upon the findings of Mohammad et al. (2011) to elect a positive environment for sAA activity with using a treadmill modality. Within the present study, individuals who were also comparable in age showed a similar percent increase in sAA which may suggest the need for future research on sAA secretion for individuals of the same age exercising at the same intensity. Furthermore, being that individuals varied in pre-exercise sAA baseline values, additional research may be needed upon exercise’s effect on sAA secretion at rest and its variability over a length of time. If baseline values of sAA did indeed vary over time due to exercise, sAA could serve as a possible biomarker for exercise’s effect on psychological health.

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