The Effects of Physical Activity on Salivary Stress Biomarkers in College Students

Katarzyna Roberts

Rogers State University, School of Arts and Sciences, Department of Mathematics and Physical Sciences, Claremore, OK 74017

Abstract: Physical exercise has been proven to have a positive impact on one's physical and mental health. Today's fast- paced, high tension lifestyles have led to an increase of chronic stress in individuals of society. Chronic stress is associated with many diseases and disorders such as hypertension, depression, heart attack, stroke, immune suppression, diabetes, and obesity. Exercise has been found to play a significant role in stress reduction. This research is focused on evaluation of the stress relief effect of physical exercise by measuring the levels of salivary stress markers: cortisol and α-amylase. Cortisol is a steroid hormone secreted by the adrenal gland and associated with the stress response in the human body. Levels of cortisol are increased during stimulation of the sympathetic nervous system and regulated by the hypothalamic-pituitary-adrenal axis (HPA). Salivary α-amylase is a correlate of sympathetic activity under conditions of physical or psychological stress. Levels of salivary α-amylase increase under a variety of stressful conditions in human subjects. Three groups of college students were studied: individuals who exercise regularly (active, athletes), students who exercise two to four times a week (active, non-athletes) and students who do not exercise (non-active). Quantitative measurements of cortisol and α-amylase variations were done using salivary analysis in enzyme immunoassay kits. Cortisol levels overall were reduced in individuals who exercise regularly, whereas α-amylase appeared useful in observance of various lifestyle activity levels. The implications of these findings suggest means of reducing stress by regular physical exercise, thus promoting overall health and wellbeing.

Introduction

College students experience constant psychological stress from being away from home, dealing with costs of college, often working at a job during the school year, having a heavy workload from academics, feeling pressure to obtain high grades in connection with career aspirations, their involvement in different scholar activities and other [Pariat, 2014]. Chronic stress affects the cardiovascular, immune [Gouin, 2008], nervous and endocrine [Cox, 1984] systems and may lead, among others, to diabetes [Dallman, 2010], hypertension, depression [Hammen, 2005], substance abuse, and antisocial personality disorder [Dohrenwend, 2000]. Physiological markers of stress like cortisol and α-amylase, provide an objective measure of changes in the stress response. Cortisol is the main glucocorticoid hormone in humans and is most commonly associated with stress in the human body. It is released in response to many psychosocial stimuli processed in the hypothalamic-pituitary-adrenal axis. The level of free cortisol in the blood is accurately reflected by the level of cortisol in saliva [Kirschbaum 1989, 1994]. Cortisol is secreted more slowly than other adrenal hormones such as catecholamines (like adrenaline) and remains in the body longer after initial stress. Cortisol is beneficial in times of stress because of its many regulatory functions. It aids in breaking down of glucose and fatty acids for energy, it has anti-inflammatory activity, is involved in fat storage, and immune functioning. However, high and prolonged levels of cortisol have been shown to have negative effects leading to serious medical conditions including diabetes, hypertension, depression, cancer, obesity, improper thyroid functioning,

and decreased bone density. Cortisol is well known for its role in storing of abdominal fat and its association with heart attacks, strokes, increased susceptibility to autoimmune disease and infection [Buford, 2008]. Cortisol levels vary with the circadian cycles, peaking during the first hour after awakening [Pruessner 1997] and decreasing for the rest of the day. Thus, careful choosing of the testing time is crucial as cortisol levels are dependent on the time of day. Repeated measurement of free cortisol levels within 60 minutes after awakening in the morning is considered a reliable biological marker of adrenocortical activity [Prussner 1997]. Cortisol can be measured in urine, plasma, and saliva. Salivary measures of cortisol are considered valid and reliable and provide distinct advantages including their non-invasive nature [Gozansky 2005]. Nonetheless, saliva samples can be affected, among others, by food and caffeine consumption, smoking, and timing of collection, so the protocol compliance is crucial to obtaining valid data. Increasing the validity of the results includes the standardized saliva sampling, consistent collection materials and methods, controlling the effects of food, drinks, and medications [Hanrahan 2006]. Another non-invasive biomarker that can be used to study stress in the body is α-amylase. **α-Amylase** is the most abundant enzyme found in human saliva and is responsible for breaking down carbohydrates and starch. α-Amylase is secreted in response to stimulation of the sympathetic nervous system, has been found to increase in response to a psychological stressor [Takai 2004] and may be a useful parameter for the measurement of stress. Gland secretion of α-amylase is regulated by the Autonomic Nervous System and has been shown to increase under sympathetic stimulation in conditions of stress [Nater, 2005, 2006, Shimazaki, 2008]. It was found that salivary α-amylase activity was higher in subjects suffering from chronic psychosocial stress as compared to non-stressed individuals and therefore may be used as a biomarker of chronic stress [Vineetha, 2014]. Increased α-amylase levels have been associated with aggression, impaired memory, and immune system suppression. Substantial research has been conducted on methods of lowering stress

levels [Gordis, 2010, Rudolph, 2010, Ju-Yang, 2019]. Suggested methods include exercise (aerobics, yoga, strength training), meditation, listening to music, healthy diet, proper sleep as well as avoiding alcohol, caffeine, and tobacco. Physical exercise and activity are important contributors to a healthy lifestyle and have a wide range of health-related and psychological benefits including reduction of stress [Fleshner 2005]. This research examines the influence of the intensity of physical exercise on the stress level in college students. While exercise induces a rise in cortisol level initially, the long term effect of lowering overall cortisol levels may be seen leading to reduction of the negative effects of chronic stress. The levels of intensity of physical activity may be an important factor in evaluating the benefits of exercise. Surprisingly there is very little study done specifically on the potential benefits of exercise and its effects on lowering cortisol levels. If exercise could indeed be proven to affect stress biology in a positive manner, many medical implications could be made and the importance of lifestyle choices could be more greatly emphasized. The purpose of this pilot study was to examine reduction in stress measured by the levels of salivary stress biomarkers in response to different levels of physical activity.

Methods

Participants were Rogers State University college students 18 to 25 years old. Participant exclusions included: habitual smoking (tobacco), caffeine dependency, drug use, diagnosed psychological disorders such as depression, anxiety, psychosis, alcohol dependency, endocrine metabolic disorders, autoimmune disorders, severe allergies, major medical conditions. Institutional Review Board approval was obtained and a written informed consent was taken from each participant. Subjects were divided into three groups: Active-athletes group which consisted of 19 individuals who exercise regularly and are part RSU basketball and soccer teams, Active-nonathletes group which consisted of 19 students who exercise two to four times a week and Non-active group which consisted of 19 students who do

not exercise. Subjective evaluation (Perceived stress questionnaire) of the stress level of the individuals participating in the study was elicited using the PSS Scale reprinted with permission of the American Sociological Association from the Journal of Health and Social Behavior [Cohen 1983]. Saliva samples were collected from all participants at the same time of the day, within 60 minutes after awakening to minimize the effects of circadian variation. Participants were asked not to eat or drink before sample collections and refrain from physical activity the day before. Participants were also asked to wash their mouth before saliva collection and approximately 1 mL of unstimulated saliva was collected in a disposable plastic test tube and immediately stored at -20°C. All samples were analyzed for the levels of cortisol and α-amylase with Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit and Salivary Alpha-Amylase Enzymatic Kit (Salimetrics LLC, State College, PA, USA). Cortisol concentrations were determined using a 4-Parameter Sigmoid Minus Curve Fit program from MyAssays.com. α-Amylase was calculated following Salimetrics kit instructions. All data were normally distributed and a single factor ANOVA was performed to detect intergroup differences. Values were considered to be statistically significantly different when p<0.05. Student's t-tests were computed for comparison of the means between the groups.

Results

Perceived stress questionnaire

The questionnaires completed by participants were used to evaluate perceived stress levels. The three groups were compared.

	Active-athlete	Active-nonathlete	Non-active
PSS level	17.50±6.23	12.88±6.14	16.55±7.61

Note: Data shown are means and standard deviations

It was found that the Active-athlete group had the highest scores in perceived stress, followed by the Non-active group, with the Active-nonathlete group showing the lowest scores of perceived stress. These differences however were not statistically significant (p=0.11)

Salivary stress markers analysis

	Active-athlete	Active-nonathlete	Non-active
Cortisol (µg/dL)	0.14±0.03	0.16±0.07	0.20±0.06
α-amylase (U/mL)	42.25±29.80	32.93±21.53	29.77±13.40

Note: Data shown are means and standard deviations

As shown by ANOVA analysis statistically significant difference (p=0.03) in cortisol concentrations between the three groups was observed. T-test computations showed statistically significant difference (p<0.05) in cortisol concentrations with lowest in the Active-athlete group (0.14 µg/dL) and the highest in the Non-active group (0.20 µg/dL). The Active-nonathlete group's mean cortisol concentration was 0.16 µg/dL.

In the α-amylase results, it was found that the enzyme activity in the saliva increased with participants increasing physical activity: Nonactive group (29.77U/mL), Active-nonathlete (32.93U/mL), Active-athlete (42.25U/mL). However, these results were not statistically significantly different (p=0.25).

Discussion

The purpose of the present pilot study was to examine reduction in stress in response to varying levels of physical activity in college students and to evaluate the usefulness of salivary cortisol and α-amylase as a biomarker of stress in chronically stressed individuals. Chronic stress, rather than acute stress, usually results in damage to the physical and mental wellbeing of an individual and may cause a number of pathologies. Individual's verbal or self-reporting questionnaires in stress evaluation usually provide inconsistent results and heavily depend on subject's mood and attitude at the time of testing. As shown here, by the results of "perceived stress questionnaire", there are no statistically significant differences in the stress level between the three groups studied. A more objective method in stress evaluation is necessary. Salivary cortisol and α-amylase provide a simple and non-invasive method in assessment of stress. In this study it was hypothesized that increased level of physical activity leads to the

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significant overall decrease in amounts of stress biomarkers, cortisol and α-amylase in saliva. The findings partially support this hypothesis. Cortisol levels were statistically significantly different in all three groups studied with the lowest level in the most physically active group and the highest in the non-active group. It is well known that the salivary cortisol level increases under psychological stress [Hargreaves1990, Biondi 1990, Kirschbaum 1994], which college students are exposed to on regular basis. In the stressed individual, the cortisol secretion is elevated regardless of the time of day as stress overrides the circadian rhythm [Chaudhuri 1991]. The results presented show that salivary cortisol is a useful indicator of chronic stress and that intense physical activity leads to overall decrease in cortisol amounts in saliva. This indicates long term stress reduction and shows a great benefit of physical activity on individuals health and wellbeing. It is possible however, that the reduction in stress indicated by the cortisol data is not solely the result of physical exercise. Other aspects may play role in stress reduction, such as being in a group of similar individuals creating the atmosphere of acceptance, positive reinforcement, sense of accomplishment, and success. Nevertheless, the overall positive effect of physical activity on stress reduction is clearly shown. According to Noto [Noto 2005] the levels of salivary α-amylase change during exercise and psychological stress and increase after exposure to acute mental stress. However, the results of other studies of α-amylase reactivity to psychological stimuli have been inconsistent. Long term effect of the intensity of physical activity on the levels of salivary α-amylase was investigated in this study. It was found that the enzyme activity in saliva increased with participants increasing physical activity, the opposite effect to the one seen for cortisol, but the differences were not statistically significant. It has been suggested [Nater 2004] that α-amylase reflects the reaction of a different stress system than the Hypothalmic-pituitary-adrenal (HPA) axis which functioning and reactivity is assessed by the measurement of free cortisol in saliva. Salivary α-amylase has become established as a new biomarker of the psychosocial stress response within the sympathetic-adrenal-medullary (SAM) system. Even though SAM and HPA axis are closely intertwined, α-amylase and cortisol are not significantly correlated in the response to psychosocial stress [Nater 2004]. According to this study, α-Amylase activity can not be used to measure reduction of stress as an effect of intense physical exercise. Salivary α-amylase is used as one of the physiological parameters that are indicative for stress reactions in the body, however the mechanisms that lead to changes of the enzyme's activity due to stress are not entirely understood.

Conclusions

The findings of the present study show that the intense physical activity leads to the reduction of stress level in college students and is beneficial to their health and wellbeing. Cortisol, but not α-amylase, is a useful biomarker in measurements of stress reduction in college students as an effect of intense physical exercise. Some of the limitations of this study include a small sample size of 19 individuals in each group. Larger groups of participants should be studied in the future. The salivary biomarker levels were measured at the same time of day, however, there are typically individual differences in the diurnal cycle of cortisol and α-amylase, which were not controlled in this study. These results should be replicated in future studies before being generalized.

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Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Date _____Age _____ Gender (*Circle*): **M F**

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset

because of something that happened unexpectedly?...................................... **0 1 2 3 4**

2. In the last month, how often have you felt that you were unable

to control the important things in your life?.. **0 1 2 3 4**

3. In the last month, how often have you felt nervous and "stressed"? **0 1 2 3 4**

4. In the last month, how often have you felt confident about your ability

to handle your personal problems?.. **0 1 2 3 4**

5. In the last month, how often have you felt that things

were going your way?.. **0 1 2 3 4**

6. In the last month, how often have you found that you could not cope

with all the things that you had to do? **0 1 2 3 4**

7. In the last month, how often have you been able

to control irritations in your life?.. **0 1 2 3 4**

8. In the last month, how often have you felt that you were on top of things?..... **0 1 2 3 4**

9. In the last month, how often have you been angered

because of things that were outside of your control? **0 1 2 3 4**

10. In the last month, how often have you felt difficulties

were piling up so high that you could not overcome them?................................. **0 1 2 3 4**