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Impact of Physical Stress on Salivary Buffering Capacity

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Abstract

Background: Saliva has many properties and the buffering capacity is important for the neutralization of oral fluids. It is unclear whether stressful conditions directly affect salivary buffering capacity, and we investigated the impact of physical stress on salivary buffering capacity. **Methods:** Twelve participants were subjected to the physical stress of jogging and running. The salivary buffering capacity and flow rate of the participants were measured before and after exposure to stressful conditions. Salivary α-amylase activity was measured as a quantitative index of stress. **Results:** No change in buffering capacity was detected among each time point during the whole course under physically stressful conditions. Next, we examined the change in buffering capacity after jogging compared to baseline. Six participants showed an increase in buffering capacity (Group A), while the other six participants showed a decrease or no change (Group B) after jogging. Group B showed a decrease in flow rate and increases in α-amylase activity and protein level after jogging, whereas Group A showed no changes in these properties. **Conclusions:** The results suggest that salivary buffering capacity changes following exposure to physically stressful conditions, and that the changes are dependent on the stress susceptibility of individuals.

Keywords: α-amylase activity, physical stress, salivary buffering capacity, salivary flow rate

Introduction

People living in rapidly changing societies are liable to suffer from physical and psychological stress. Stress signals activate the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system, triggering physical responses involving the endocrine, nervous, and immune systems.[1] Stressful experiences increase HPA activity and are associated with depression and the risk of substance use disorder.[2] Also, the stimulation of sympathetic nerves can increase the number of granulocytes, which induce tissue damage leading to collagen diseases, inflammatory bowel disease, and cancer by producing superoxide.[3] Stress can also impact the oral cavity via immune suppression, promote the onset and progression of disease.[4] Furthermore, stress signals affect health behaviors, such as smoking, alcohol consumption, and physical exercise[5]; thus, coping with stress is important to maintain human health.

Saliva has many properties that keep the oral cavity healthy; in fact, it is said that saliva reflects the status of the body. Lee *et al*.[6] reported that saliva reflects the tissue levels of natural substances and a large variety of molecules introduced for therapeutic, dependency, or recreational purposes, an individual's emotional, hormonal, immunological, or neurological status, and nutritional and metabolic influences. The use of saliva to monitor the health status of an individual is a highly desirable goal for health promotion and health care research.

Salivary buffering capacity is important for the neutralization of oral fluids, and previous reports have demonstrated that a high buffering capacity is advantageous for the prevention of dental caries.[7,8] For this reason, utilizing saliva as a prediction tool for caries activity is recommended.[8,9] Previous studies have shown that the salivary flow rate tends to decrease due to stimulation of the sympathetic nervous system under stressful conditions and that the salivary buffering capacity tends to decrease as the flow rate declines. However, it is unclear whether stressful conditions directly affect salivary buffering capacity. In this study, we investigated the impact of physical stress including jogging and running on salivary buffering capacity. Jogging is a form of running at leisurely pace to increase physical fitness. Many reports have demonstrated that physical activities such as jogging and other kinds of sport are advisable and have a positive effect on our body.[10-12] According to the changes in buffering capacity detected after physical exercise of jogging, we divided the study participants into two groups and examined the relationship to other saliva properties including flow

rate, α-amylase activity, and protein content. Our results reveal an interesting relationship between physical stress and saliva properties.

Methods

Participants. Twelve healthy adults (6 males and 6 females; 29.2 ± 11.4 years of age) participated in this study. They completed a test to induce physical stress. Approval for this study was obtained from the ethics committee of the Kagoshima University Graduate School of Medical and Dental Sciences.

Physical exercise and saliva collection. Before starting experiments, participants were kept relaxed without stressful condition possibly. To induce physical stress, the participants completed 10 min of jogging (8 min/km) and 10 min of running (6 min/km) according to the exercise protocol shown in Figure 1. Saliva samples were collected before jogging (Baseline), after jogging (Jog), after resting (Rest), just after running (Run 1), and 5 min after running (Run 2). Stimulated whole saliva was collected at each time point by chewing a piece of gum with no taste (GC Corporation, Tokyo, Japan). After chewing the gum and swallowing saliva for the first 30 s, the whole saliva was expectorated into a tube on ice for 2 min.

Saliva analysis. The salivary flow rate (ml/min) was determined by weighing the collected saliva. Salivary buffering capacity was measured by using a handheld pH meter (Laqua twin model B-712, Horiba Ltd., Kyoto, Japan) according to a previous study[13] with modifications. An aliquot of each sample (0.5 ml) was applied to the pH sensor to determine the original pH value (a). After reading (a), 50 µl of 0.1 N HCl was titrated into the saliva sample and gently shaken to mix until the value stabilized (b). The buffering capacity was determined by calculating the inverse of [value (a) – value (b)].

The intensity of physical stress was monitored according to the level of α-amylase activity. Saliva samples kept in an icebox were carried to the laboratory to measure the level of α-amylase activity using a commercial kit (α-Amylase Measuring Kit; Kikkoman, Tokyo, Japan). In the assay, α-amylase in the saliva sample reacts with the substrate, yielding 2-chloro-4-nitrophenol, which is yellow in color and can be detected using a spectrophotometer at 405 nm. The protein content in each sample was determined according to the method of Lowry *et al.*[14] with bovine serum albumin as a standard.

Data analyses. At first, changes in saliva properties following the complete course of physical exercise were examined. Next, we examined the change in buffering capacity after jogging compared to baseline, and divided participants into two groups (A and B) according to the results. Group A included six participants showing an increase in buffering capacity, while Group B included six participants showing a decrease or no change after jogging. Other saliva properties were examined for each group. Regarding statistical analyses, differences among time points were analyzed using Friedman test, Wilcoxon signed-rank test, and sequential step-down Bonferroni as appropriate. Statview version 5.0 (SAS Institute, Inc., Cary, NC, USA) was used and the significance level was set at < 0.05.

Results

Figure 2 shows the changes in buffering capacity, flow rate, and α-amylase activity for the participants following the complete course of physical exercise. No change in buffering capacity was detected among each time point during the whole course under physically stressful conditions (jogging and running). There were significant differences among several time points regarding flow rate; however, no differences were

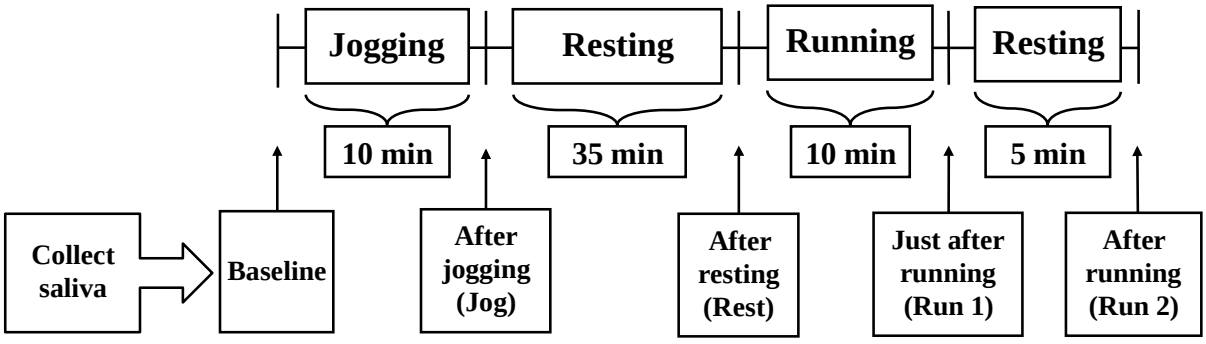

Figure 1. Protocol for Collecting Saliva after Physical Exercise. Participants Practiced Jogging and Running, and Stimulated Whole Saliva was Collected at Each Time Point

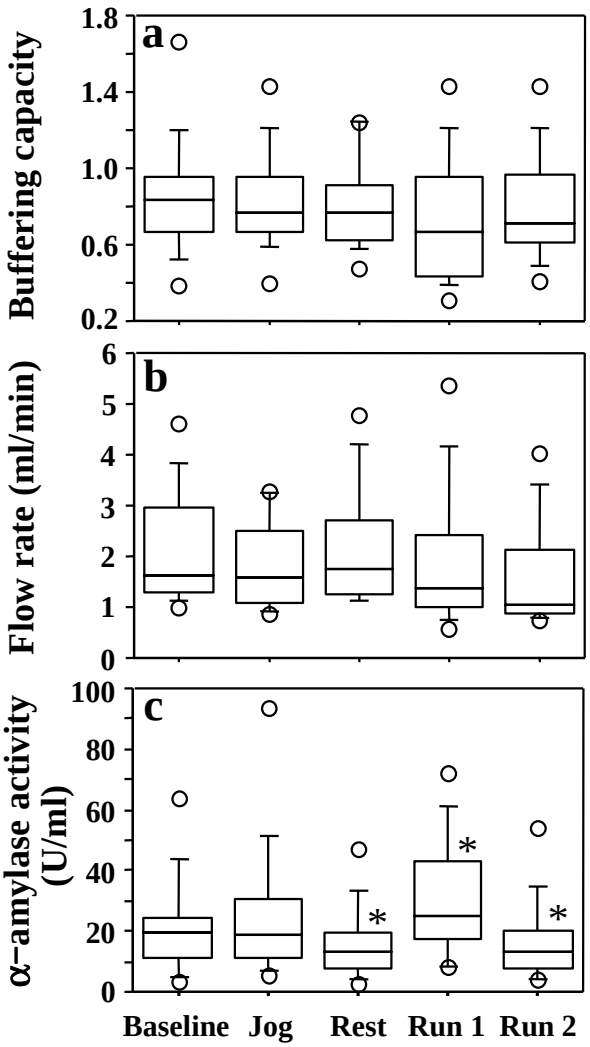

Figure 2. Changes of Salivary Buffering Capacity (a), Flow Rate (b), and α-amylase Activity (c) after taking Physical Exercise. The Results are presented as Box Blots (n = 12). The Boxes extend from the 25th Percentile to the 75th Percentile, with a Horizontal Line at the Median. Additional Symbols Indicate the 10th and 90th Percentiles (bars) and Outliers (circles). *$p < 0.05$ Compared with Baseline. Statistical Analysis was Performed using Friedman Test Followed by Wilcoxon Signed-rank Test and Sequential Step-down Bonferroni

observed compared to baseline. The α-amylase activity level decreased significantly after resting (Rest) and increased just after running (Run 1) compared to

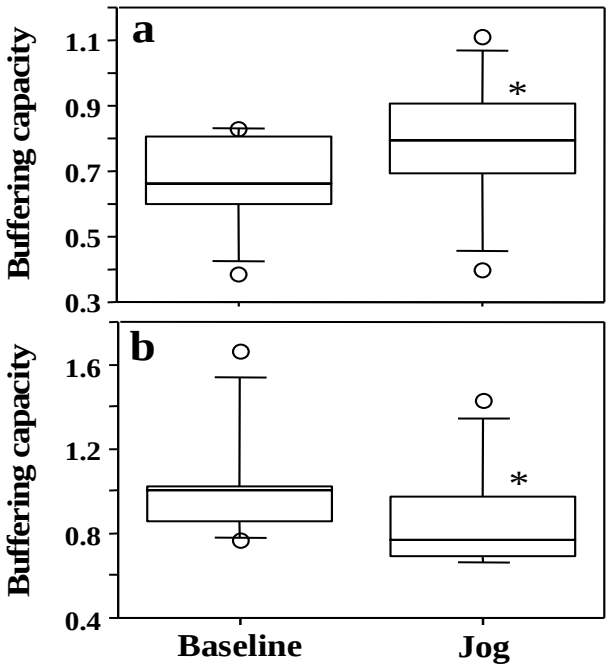

Figure 3. Changes of Salivary Buffering Capacity in Group A (a) and Group B (b) after Jogging. The Results are Presented as Box Blots (n = 6 for Each Group). The Boxes Extend from the 25th Percentile to the 75th Percentile, with a Horizontal Line at the Median. Additional Symbols Indicate the 10th and 90th Percentiles (bars) and Outliers (Circles). *$p < 0.05$ Compared with Baseline. Statistical Analysis was Performed Using Wilcoxon Signed-rank Test

Table 1. Changes of Salivary Flow Rate, α-amylase Activity, and Protein Concentration in Group A and B

	Flow rate (mL/min)		α-amylase activity (U/mL)		Protein concentration (mg/mL)	
	Baseline	After jogging	Baseline	After jogging	Baseline	After jogging
Group A	2.1 ± 1.3	1.7 ± 0.8	17.5 ± 11.7	17.9 ± 11.0	2.2 ± 0.8	2.6 ± 1.0
Group B	2.2 ± 1.1	1.9 ± 1.0*	25.2 ± 19.8	32.4 ± 31.3*	2.4 ± 0.9	3.0 ± 1.7*

Data represent means ± standard deviations of 6 participants in each group. *$p < 0.05$ compared with baseline using Wilcoxon signed-rank test

baseline. Next, we examined the buffering capacity at two time points (Baseline and Jog) and divided the participants into two groups (A and B) as described in the materials and methods section. Participants in the Group A showed an increase in buffering capacity, while participants in the Group B showed a decrease or no change after jogging (Figure 3). Regarding other saliva properties, Group B showed a decrease in flow rate and increases in α-amylase activity and protein level after jogging, whereas Group A showed no changes in these properties (Table 1).

Discussion

In this study, jogging and running were used to induce physical stress. No significant changes compared to baseline were observed in buffering capacity or flow rate during the whole course of physical exercise. Therefore, we picked two time points and examined the changes in buffering capacity after jogging compared to baseline, and divided the participants into two groups. In Group A, the buffering capacity increased significantly, whereas other saliva properties including flow rate, α-amylase activity, and protein concentration showed no changes after jogging. In Group B, both the buffering capacity and flow rate decreased, while α-amylase activity and protein concentration increased significantly. The observed effects of physical exercise on saliva properties are not consistent with those in previous reports. Ljungberg *et al.*[15] found no significant changes in buffering capacity, a decrease in salivary secretion, and an increase in amylase activity after a marathon race. Nakano *et al.*[16] reported decreases in buffering capacity and flow rate after treadmill running. Ligtenberg *et al.*[17] demonstrated increases in salivary flow rate and amylase activity after 20-minute running with moderate or high intensity. The salivary flow rate is affected by the autonomic nervous system.[18] If sympathetic stimulation occurs due to physical stress, the salivary flow rate decreases. Prolonged exercise like marathon may induce strong stress and dehydration, which result in a decrease in salivary secretion. The differences observed in these studies may be attributable to differences in the type of physical stress-inducing activity. In our study, participants practiced jogging for only 10 min, and the stress level under the circumstances seems not strong. Actually, the participants in Group A were not obviously affected by jogging. The α-amylase activity level in the participants in Group A did not change, indicating that jogging was not stressful for them. In contrast, the level of α-amylase activity increased significantly in Group B after jogging compared to baseline. Several participants in Group B looked pale and exhausted, indicating that the stress intensity was enough to increase α-amylase activity. It has been reported that jogging has a stress-relieving effects.[19,20] Interestingly, jogging was apparently sufficient regarding intensity to induce stimulation of the sympathetic nervous system for participants in Group B, but not for those in Group A. Individual differences in response might be due to their susceptibility to stress.

A positive association between salivary buffering capacity and flow rate has been reported.[21,22] In this study, the buffering capacity and flow rate decreased in Group B. However, the buffering capacity increased and salivary flow rate did not change in Group A. Physical stress might affect buffering capacity and flow rate independently.

To clarify the level of physical stress, we measured α-amylase activity as a stress marker. Previous studies have reported that salivary α-amylase is an effective stress marker of the autonomic/sympathetic nervous system,[23] and associations of salivary α-amylase with cardiovascular function, wide range of behaviors including infant attachment, academic performance, and adolescent aggregation have been demonstrated.[24] It was also reported that the response of salivary α-amylase to a stressor occurs more quickly than changes in salivary cortisol,[25] and there are wide-ranging differences between individuals in salivary α-amylase activity; therefore, we utilized this parameter to assess the susceptibility of the participants to the stress. The results of this study suggest that the buffering capacity of saliva may be altered via stimulation of the autonomic nervous system by physical stress and that the degree is dependent on the subject's susceptibility to physical stress.

Salivary protein is secreted in response to sympathetic nervous stimulation, and several reports have demonstrated that salivary proteins can affect the buffering capacity of saliva.[26] In this study, the salivary protein level was measured to investigate how salivary gland function is affected by the autonomic nervous system and its contribution to the buffering capacity of saliva. The protein level increased after jogging in Group B, but not in Group A. Dawes[27] reported that physical exercise caused a marked rise in total salivary protein concentration. Speirs *et al.*[28] noted that the high concentration of salivary proteins following physical exercise may be due to increasing β-sympathetic activity in the salivary glands. The increased protein level in Group B may have been due to the decrease in flow rate, but no effect on buffering capacity was observed. Further studies are necessary to determine the precise mechanism underlying these phenomena.

This study has a limitation that we evaluated only 12 participants; therefore, it may be necessary to interpret the findings with caution. However, there have been no studies reporting the relationship between physical stress and salivary buffering capacity after dividing the participants into groups based on the change of

buffering capacity, with evaluation of other saliva properties including flow rate, α-amylase activity, and protein content. Examination of a large number of participants is expected to reveal more distinct effects of physical stress on buffering capacity and also gender differences in the effects.

Conclusions

The results suggest that salivary buffering capacity changes following exposure to physically stressful conditions, and that the changes are dependent on the stress susceptibility of individuals.

Acknowledgments

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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