

A Study into Salivary-based Measurement of Human Stress Subjected to Ellestad Stress Test Protocol

Y. K. Lee, A. Za'aba, N. K. Madzhi, A. Ahmad

Abstract—Previous works on the effects of salivary alpha amylase in respond to various stressors report encouraging findings on it being a good indicator of stress. Ellestad protocol is a clinical procedure to screen for coronary artery disease by introducing exercise induced physical stress. If a salivary based biomarker profile in accordance to a stress test protocol could be established, the critical stress state which disables rational decision making could be ascertained in a standardized procedure. This technique would serve to aid human resource management in times of critical events such as rescue, firefighting or even military, that would potentially prevent unnecessary sacrifice of human lives. In this pilot study with five healthy volunteers performing the Ellestad protocol treadmill, a measurement profile with physiologic and salivary based biomarker is obtained. It is found that the alpha amylase levels or the changes in it as workload changes from resting-walking-running at ease-exhaustive running, is relatively more significant in reflecting the stress state than heart rate and blood pressure. Moreover, it is strongly associated with mood state with correlation coefficient of 0.8 and significance of 0.01.

I. INTRODUCTION

STRESS is a natural instinct of human in reaction to threat with the consequent emotional response such as fear, aggression, generated in the limbic system for survival. The limbic system activates the hypothalamus of the brain which controls both the stress response systems, i.e. sympathetic adrenal medullary (SAM) response system and hypothalamic pituitary adrenal system (HPA). Both SAM and HPA regulate the cardiovascular system (heart rate and blood pressure) and the immune system. Even though heart rate and blood pressure are widely used as indices for measurement of stress, they are indirect, show small changes in comparison to their normal values and are influenced by homeostasis. It has been established that free cortisol in saliva marks the HPA activity while the salivary alpha amylase reflects the SAM activity [1, 2]. The use of hormonal regulation such as cortisol, suffers a delay of 20-30 minutes. On the other hand, it has been reported that the response of salivary alpha amylase is markedly quicker, within one to a few minutes since its secretion is stimulated by direct innervations [2]. Moreover, the concentration of

cortisol in blood or saliva is very much lower than that of salivary alpha amylase, between 10-20% of protein content produced by the salivary gland. Furthermore, analysis of cortisol requires high performance liquid chromatography or enzyme immunoassay (EIA), which is not portable and time consuming. On the other hand, the measurement of salivary alpha amylase is simple, instantaneous, non-invasive, dispense of cables and electrodes as well as can be repeated without pain and stress.

Previous works on the effects of salivary alpha amylase when challenged to various stressors, such as video viewing [2], driver fatigue [3], mental arithmetic [4], roller coaster ride [5], report encouraging findings on the use of salivary alpha amylase as good indicator of psychological stress. Ellestad protocol is one of the many clinical procedures [6-7] to screen for patients with coronary artery disease by introducing progressive exercise induced physical stress, which in turns trigger the cardiac stress. Normal practice uses ECG-based monitors to ascertain heart rate, blood pressure and arrhythmias pattern. However, these devices do not provide direct measurement of the associated stress due to physical and psychological distress. If a salivary based biomarker profile in accordance to stress test protocol could be established, perhaps the critical stress state due to physical and psychological distress which disables rational decision making could be ascertained in a standardized procedure. This would serve to aid human resource management in times of critical event such as rescue, firefighting or even military, that would potentially prevent unnecessary sacrifice of human lives.

Whereas most previous studies examine the effects of non-protocol stressors from different sources on salivary alpha amylase, this research is the very first attempt to produce a time course profile on heart rate (HR), blood pressure (BP), salivary alpha amylase (SaA) and mood state (MS), subjected to progressive increment in exercise induced physical stress, outlined by a standardized clinical procedure, Ellestad protocol. The influence of these physiologic and salivary based variables on the stress state, in terms of trend and magnitude of change, is studied. Association between them is investigated to determine the significance of alpha amylase, being a direct measurement, in reflecting the stress state, in comparison to the other physiologic variables. A subjective evaluation of the stress state of the subjects is also conducted.

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II. METHOD

As a preliminary study, a population of five male healthy volunteers between 19-25 years old without significant past medical history (from the health history form), participated in the study.

A day before the test, a pre-test instruction is given, which includes 2-hour restriction on eating, drinking (except for water), smoking and other restriction that would influence the results. On the day of the test, the patient consent is filled. Experiments are conducted in the morning only to minimize the effects of time of day. Prior to measurement, all the subjects were asked to rinse their mouth and dry out the water with cotton roll. In order to accentuate the psychological distress, only a brief explanation on the procedure is given and talking is strictly forbidden during the stress test. The treadmill-based Ellestad protocol stress test was used to induce exercise induced physical stress, according to a set change in speed and inclination as shown in Table 1.

TABLE 1
ELLESTAD STRESS TEST PROTOCOL [7]

Stage	Speed (km/h)	Grade (%)	D (Min)	WL (MET)	*2
0 ^{*1}					
1	2.72	10	3	4.8	5
2	4.83	10	2	6.4	5
3	6.44	10	2	8.8	5
4	8.05	10	2	10.0	5
5	8.05	15	2	12.0	4
6	9.66	15	2	15.2	3
7	11.27	15	2	17.2	1
8 ^{*1}					

*1: Stage 0 and 8 refers to Baseline (Standing) and Recovery respectively *2: Number of volunteers completed the stage (D: Duration; WL: Workload)

The subjects are exercised to volitional fatigue. As stress test progresses, condition of the subjects is scrutinized against a set of medical precautions detailed in [8], such as significant drop (20 mm HG) in systolic pressure. Measurements are taken at completion of each stage.

The heart rate and blood pressure are measured using IntelliSense BP meter (Model IA2) strapped to the forearm of the subjects. The salivary alpha amylase is measured using Cocoro salivary amylase activity monitor [3], which employs a salivary transfer device and an optical device. The optical device constitutes of a light emitting diode with a wavelength of 430 nanometres and a photo-detector, to measure reflectance on the reagent paper. The reagent paper contains a substrate for alpha amylase, 2-chloro-4-0- β -D-galactopyranosylmaltoside, which changes from white to yellow on hydrolysis [9]. The enzymatic activity is expressed as units per ml (U/ml) of saliva. This measurement is taken after heart rate and blood pressure since the salivary alpha amylase activity requires a reaction time of one to few minutes [9]. Data collected are processed statistically to produce the measurement profile. Correlations are computed as Pearson product-moment correlation.

III. RESULTS

Fig. 1 depicts the variation in heart rate, systolic and diastolic blood pressure, salivary alpha amylase and mood state with increase in exercise induced physical stress specified by the Ellestad protocol. Increment in the mean of all the measured variables is observed, except for blood pressure, with workload from stage 0 (standing, baseline) to stage 7, while decrement after stage 7 (recovery).

The subjective evaluation of the stress test is categorized into mood scale 1, 2 and 3, representing 'not at all', 'moderately' and 'extremely' [5]. From Fig. 1, it is found that most subjects found stage 1-2 relaxing while fatigue starts to set in as early as stage 4.

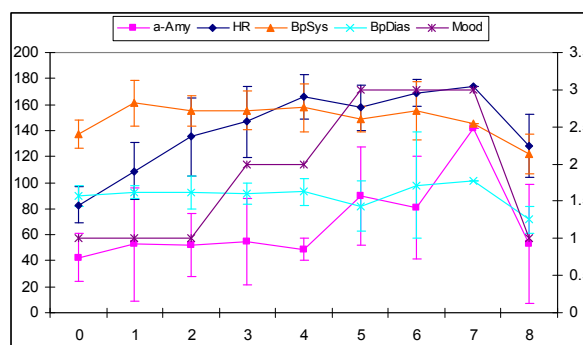


Fig. 1 Response of physiologic and salivary based biomarkers to Ellestad stress test protocol

The time course change in the salivary alpha amylase of the subjects is shown in Fig. 2. Even though a fixed trend pattern cannot be defined, the generic trend shows an increase in salivary alpha amylase with the increase in physical workload (stage 0 to 7) and a decrease on recovery (stage 7 to 8).

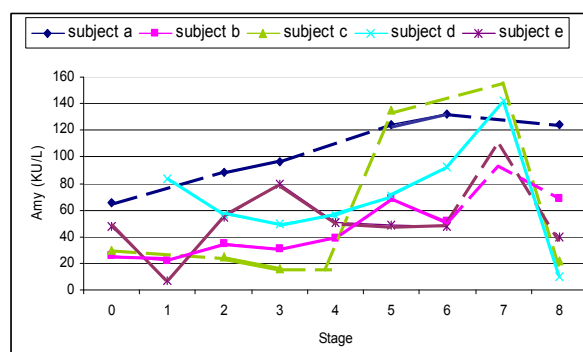


Fig. 2 Individual S α A profile

Amongst the measured variables, heart rate, alpha amylase and mood states are found to display significant changes. Fig. 3 shows the changes in the mean of heart rate and salivary alpha amylase with time. Gradual change is observed in heart rate while sudden change is observed in salivary alpha amylase.

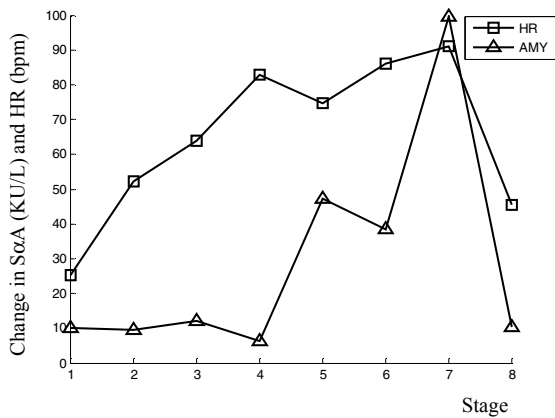


Fig. 3 Change in mean with workload

The Pearson correlation coefficients (r) and statistical significance (p) is used to investigate the relationship between the physiologic and salivary based biomarkers with mood state, which is summarized in Table 2. It is found that salivary alpha amylase activity is strongly correlated to mood states with p of 0.01 while r of 0.8, similar to heart rate, indicating that salivary alpha amylase is sufficient in reflecting the stress state due to physical and psychological distress experienced during the stress test.

TABLE 2
CORRELATION COEFFICIENT (R) AND STATISTICAL SIGNIFICANCE (P)

MS		HR	SBP	DBP	ScA
	r	0.86	0.21	0.43	0.8
p	0.003	0.59	0.25	0.01	

IV. DISCUSSION

Stress is a reaction to perceived psychological, emotional or physical distress. Here, treadmill-based Ellestad protocol, a standardized clinical procedure, is examined here to see if it could provide a procedure to determine the critical stress state, due to physical and psychological distress, which disables rational decision making during rescue work in a crisis. Even though Ellestad is said to reduce physical stress, it is thought that this is accompanied by psychological stress, as a natural response of one sees coping the oncoming stage as a threat, since they only know the workload will increase for the next stage, yet are ignorant of the degree of increment.

This is found to be true from Fig. 1. It can be observed that progressive increment in workload according to Ellestad protocol gives rise to corresponding increment in stress level, from the physiologic (heart rate, systolic BP, diastolic BP), psychological (mood state) and biochemical (salivary alpha amylase) measurements.

Stress incurs changes in the autonomic nervous system, which consists of the sympathetic and parasympathetic nervous system, that act in reciprocal. The former is active during stress while the other during relaxation. In response to stress, the human body activates the HPA of the sympathetic nervous system and releases stress hormones

(adrenaline, cortisol) into the bloodstream. These hormones speed up heart rate, breathing rate, blood pressure and metabolism. They also tense up the muscle, dilate pupils, release stored glucose from liver and produce sweat to cool the body [1].

Few of the physical changes as a result of the physiological adjustments as mentioned above are observed in Fig. 1. As workload is increased from stage 0 to stage 7, then from stage 7 to stage 8, the mean of heart rate is found to increase and then decrease. The change in heart rate with workload is almost linear. Systolic BP and diastolic BP display changes within 10 mm Hg, which are the normal physiologic response [6]. Even though linear elevation in systolic BP in response to workload is not observed, which could be attributed to motion artifacts while measurement is taken, a significant rise and drop are observed from stage 0 (Baseline, standing) to stage 1 and stage 7 to stage 8 (recovery), as expected. The accuracy is immaterial as accurate systolic BP is only crucial in the computation of maximum oxygen consumption.

At the same time the HPA is reacting, activation of the autonomic nervous system also innervates the salivary gland leading to high activity of the salivary enzyme alpha amylase [1].

With reference to Fig. 1, the progressive increment in workload gives rise to a corresponding increment in the mean of the salivary alpha amylase activity. SAM triggers more secretion of alpha amylase from the adrenal gland. Small rise in the mean of alpha amylase level is noted from stage 0 to stage 4, where the pace is at ease and inclination is lower. A steeper rise is noted after stage 4 to stage 7, where the increase in workload is in step of 2-3 MET (metabolic equivalent). On recovery from stage 7 to stage 8, the mean of salivary alpha amylase activity is reduced to the initial baseline.

Similar behavior is observed in the time course changes in salivary alpha amylase in Fig. 2. On the whole, the trend depicted here is a relatively slow rate of increase from stage 0 to stage 4 while a higher rate of increase from stage 4 to stage 7. Slope is positive from stage 0 to stage 7 showing the SAM system is activated. Slope is negative from stage 7 to stage 8 showing the SAM system is deactivated. The initial high reading in alpha amylase activity could be due to the influence of initial tensions towards the stress test. Alpha amylase baseline for different individual is observed to be different, which explains the use of change in these values from baseline to be taken in the computation for the mean values in Fig. 1.

The alpha amylase activity in response to stress test as shown in Fig. 2 draws similar conclusion to that in response to mental stress as in video viewing [2], driver fatigue [3], mental arithmetic [4], roller coaster ride [5] and so on. This implies physical stress evokes the same reaction as mental stress. The difference is that Ellestad follows a protocol while the others are non-protocol stressors. As such, Ellestad protocol is useful to provide a profile on stress state through

salivary alpha amylase, the index for SAM activity.

Amongst variables as shown in Fig. 1, changes of significance are observed in heart rate and salivary alpha amylase against mood state. This change is more obviously shown in Fig. 3. The increment in heart rate as workload progressively changes from resting – walking - running at ease - exhaustive running, is gradual. The salivary alpha amylase level displays sudden jump at stage 4, as the mood state expressed by the subjects is stepped up. This sudden change makes it relatively better in reflecting the change in stress state than heart rate and also to profile the stress state to identify the critical one.

On inquiry into the mood state, the progressive increment in workload gives rise to the corresponding increment in stress level, as acknowledged by the subjects when interviewed. As shown in Fig. 1, subjects found stage 0-2 consisting of walking mainly, to be relaxing; stage 2-4 consisting of fast pacing, to be moderately physically taxing; stage 5-7 which requires running to be physically demanding. All the subjects completed the Ellestad protocol with the distribution of completed stages as shown in Table 1. Four subject runs up to stage 5; three up to stage 6 while one up to stage 7.

The Pearson correlation coefficients (r) and statistical significance (p) are used to investigate the relationship between the physiologic, biochemical and psychological measurements, as summarized in Table 2. The nearer the value of r to one, the closer the two variables is correlated. A p -value of less than 0.05 represents statistical significance. It is found that salivary alpha amylase shows strong association with mood state, with r of 0.8 and p of 0.01, similar to heart rate, indicating that salivary alpha amylase is sufficient in reflecting the stress state experienced. The systolic and diastolic blood pressure shows weak correlation with the mood states. The trends showing mean in these values in Fig. 1 draw similar conclusion.

V. CONCLUSION

A profile of physiologic and salivary based biomarkers in accordance to the Ellestad stress test protocol, a very first attempt, is obtained. Salivary alpha amylase is found to elevate with increasing workload and to have the strong association to mood states, with a correlation coefficient of 0.8 and significance of 0.01, similar to heart rate. In fact, it is found that salivary alpha amylase or the changes in it as workload is increased, is relatively more significant in reflecting the stress state than others. While being a standard clinical procedure to elicit cardiac stress through ECG, it is shown that the Ellestad protocol could also provide a profile on stress state through salivary alpha amylase. This could then be used to determine the stress state that disables rational decision making in critical situations, such as rescue or military operations that would potentially prevent unnecessary sacrifice of human lives. Furthermore, being simple, instantaneous, non-invasive, dispense of cables and electrodes as well as can be repeated without pain and stress,

the measurement of alpha amylase activity offers a promising automatic detection method for such purpose.

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