



Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults

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Summary

Objective: Saliva sampling has the advantage that it is non-invasive, making multiple sampling easy and stress free. We examined the effects of psychological stressor and soother on the salivary cortisol and amylase levels in young adults, and compared the characteristics of these parameters.

Design: The subjects completed the trait version of State-Trait Anxiety Inventory (STAI) to assess the predisposition to personal anxiety. The video of corneal transplant surgery was served as the stressor for 15 min. A scenic beauty video viewing was also used as the soother. Unstimulated whole saliva was collected every 3 min throughout the session.

Results: The amylase level was significantly increased just after the beginning of the stressful video viewing, and immediately returned to the pre-stress level just after the end of the video viewing. The cortisol level was also increased, but to a lesser extent compared with that of amylase. The latency time to the peak level for cortisol was longer than that of amylase. The carry-over effect was not observed in the amylase response but was in cortisol. Although the correlation between the amylase level and the STAI score was highly significant, cortisol level did not. In addition, soothing video viewing significantly decreased the amylase level, but did not affect the cortisol level.

Conclusion: Salivary amylase level was more significantly increased and reacted more rapidly than cortisol by psychological stressor, suggesting that it is a better index of stress. Furthermore, it is suggested that the enzyme is a soothing or relaxation index.

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Introduction

It is widely accepted that psychological stress could produce physiological effects that are similar to those produced by physical challenges in a variety of physiological systems. Two primary systems are particularly involved in setting on the stress response, hypothalamus–pituitary–adrenocortical axis (HPA) and sympatho-adrenomedullary (SAM) system. The activation of HPA causes an increase in cortisol secretion in adrenal cortex.^{1,2} Salivary cortisol concentrations are closely correlated to serum cortisol concentration.³ Thus, salivary cortisol reliably reflects the HPA activity, and is a more practical assessment tool than blood collection in stress research due to its potential to elicit spurious increases in cortisol secretion reflecting a hyper-stress component. Many reports have shown that various kinds of psychological stress activate the HPA of cortisol release, and consequently, induce significant increases in salivary cortisol level above a resting baseline level.³

In the SAM system, blood noradrenaline was considered to be derived from spillover of synaptic noradrenaline from the sympathetic nervous system, and its levels appear to be a useful index of overall sympathetic activity in the periphery.^{4,5} Blood adrenaline comes mainly from the adrenal medulla. As the blood catecholamines, noradrenaline and adrenaline are readily elevated by psychological stressor, if salivary noradrenaline and adrenaline come from the bloodstream, salivary catecholamines may be a useful index of SAM system activity. However, it was reported that salivary catecholamines concentrations are several fold lower than those of venous blood, and do not reflect the acute changes in the blood catecholamines.^{5–8} These studies suggested that catecholamine in the saliva is a poor index of the changes in sympathetic activity. Alpha-amylase is one of the major salivary enzymes in humans, and is secreted from the salivary glands in response to sympathetic stimuli.⁹ Chatterton et al.¹⁰ reported that there was a good association between the concentration of salivary amylase and blood levels of catecholamines. Currently, it is considered that measurement of this salivary enzyme is a useful tool for evaluating the SAM system.^{11,12}

The purpose of the present study was to examine the effects of acute psychological stress on the constituent of resting whole saliva in healthy young adults. Saliva sampling has the advantage that it is non-invasive, making multiple sampling easy and stress free. To rule out the confounding effects of additional stress induced by blood or urine collection, salivary cortisol and amylase were assayed

as indexes of the HPA and SAM system, respectively. The stressor utilised was stressful video viewing, which has been shown to elicit various stress responses.^{3,13} In addition, relaxation or soothing and saliva had not been directly investigated; we investigated the effect of soothing video showing on the salivary cortisol and amylase levels, and compared the characteristics of these parameters as the stress index.

Materials and methods

Subjects

A total of 83 healthy volunteers, 53 males and 30 females, ranged in age from 20 to 27 years (mean age: male, 23.3 years; female, 23.8 years), participated in the present study. Questionnaires administered prior to the experiment indicated that no volunteers had a physical or mental illness, were pregnant or taking corticosteroids or oral contraceptives. A dentist examined each of the subjects before the experiment to ensure that no subject was suffering from severe periodontal disease that has a gingival bleeding tendency by palpation. The subjects were instructed to abstain from eating, smoking, drinking any beverages except water and exercising 2 h before the experiment. The aims of the present study and the procedures involved were explained to the subjects before, and the written consent was obtained. The ethical research committee of Osaka Dental University approved the study protocol.

Collection of saliva

Experimental sessions were limited to the hours between 9:00 and 11:00 to minimise time of day effects. Subjects sat unrestrained in a comfortable chair with lumbar support opposite a 19-in. TV monitor placed 100 cm away at eye level after thoroughly rinsing their mouths. A video recording of corneal transplant surgery, which included scenes of injection into the eyeball and incision of the cornea with scissors, served as the psychological stressor for 15 min. A scenic beauty video viewing was also used as the soother. Forty-eight and 19 subjects viewed only the stressful video or the soothing video, respectively. Sixteen subjects viewed both the videos, but both the experimental sessions were not carried out on the same day. All subjects viewed each video for the first time. After the video ended, they remained quiet for 15 min.

Subjects were instructed to tilt their heads slightly forward without taking their eyes off the

TV monitor, and to accumulate the saliva in the floor of the mouth before spitting the saliva into a pre-weighed plastic vial to measure the weight. All the accumulated saliva was collected every 3 min throughout the session. Salivary flow was expressed in mg of saliva per min (mg/min). Then, the saliva samples were centrifuged and the supernatant was stored at -20° until analysed.

Psychological assessment

State-Trait Anxiety Inventory (STAI) was used to assess the personal anxiety.¹⁴ This test consisted of two separate, self-report scales for measuring the distinct concepts of state and trait anxiety. The STAI is one of the most commonly used scales to measure anxiety in student populations, and the trait anxiety reflects a predisposition to anxiety as determined by the personality pattern.¹⁵ We used the Y-version of trait section of STAI in Japanese translation (Japan UNI Agency, Inc., Tokyo) to assess the responsibility to anxiety. After informed consent was obtained, each subject completed the trait section of the STAI, and the trait anxiety score (STAI score) was calculated. It has been reported that mean \pm S.E. of Japanese university students for male and female are 48.819 ± 10.032 ($N = 1088$) and 47.657 ± 9.962 ($N = 1165$).¹⁶

Salivary analysis

Salivary α -amylase activity was assayed using a kit (Neo-Amylase test, Daiichi Pure Chemicals) which utilised blue-starch as the substrate. The saliva sample was diluted 1/500 with distilled water before addition to the substrate for the assay. Human pancreatic α -amylase (Humylase Control, Pharmacia & Upjohn, Sweden) was used as standard, and the enzyme activity was expressed as international units/ml of saliva (U/ml).

Salivary cortisol was assayed using ELISA kit (Cortisol Correlate-EIA kit, Assay Designs Inc., USA).

Statistical analysis

Data are presented as mean values and standard errors of the mean (\pm S.E.).

The unpaired *t*-test was used to assess the salivary parameters between males and females. To estimate differences in the salivary parameters with time, repeated measures ANOVA with significant differences were assessed by applying post hoc Tukey test. The Pearson's correlation coefficient (*r*) was determined for the relationship between STAI score and levels of salivary amylase or cortisol. Statistical significance was accepted at $P < 0.05$.

Result

Salivary parameters in the resting saliva

There were very wide ranges of the amylase and cortisol concentrations and flow rates in the resting saliva between different individuals. No significant difference between males and females was observed in any salivary parameters (Table 1).

Effect of stressful video viewing

The increase in salivary amylase and cortisol level by the video viewing was observed in 63 of 64 (98.4%) and in 61 of 64 (95.3%) subjects. Fig. 1 showed the normalised values of the percentage of pre-stress level for the amylase, cortisol and flow rate for every 3 min throughout the session. The amylase level was significantly increased ($P < 0.05$) just after the beginning of stressful video viewing with sustaining during the video viewing, and immediately returned to the pre-stress level just after the end of the video viewing. The cortisol level was

Table 1 Concentrations of amylase, cortisol and flow rate in the resting whole saliva.

	Gender	Mean \pm S.E.	Range
Amylase (U/ml)	M	121.5 \pm 9.7	24.8–308.0
	F	132.1 \pm 13.5	19.0–283.1
	M + F	125.3 \pm 7.9	19.0–308.0
Cortisol (pg/ml)	M	966.6 \pm 57.2	182.2–2601.7
	F	1094.2 \pm 103.3	229.1–2989.5
	M + F	1014.6 \pm 52.8	182.2–2989.5
Flow rate (mg/min)	M	0.58 \pm 0.03	0.30–1.1
	F	0.54 \pm 0.04	0.26–0.8
	M + F	0.56 \pm 0.02	0.26–1.1

No statistical differences between male (M; $n = 53$) and female (F; $n = 30$) are observed in any salivary parameters (unpaired *t*-test).

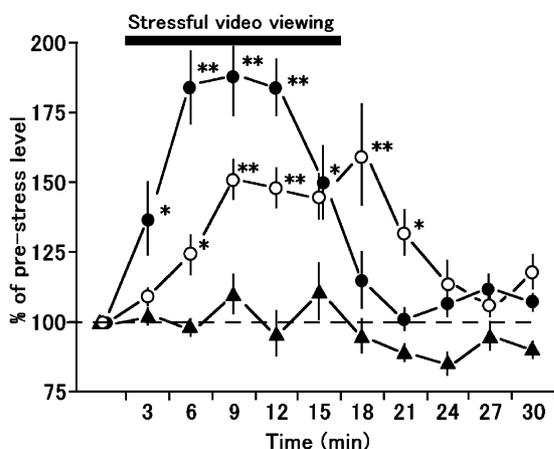


Figure 1 Effect of stressful video viewing on normalised values of percentage of resting level for the salivary amylase, cortisol and flow rate. Amylase (●) and cortisol (○) were significantly increased by video viewing (** $P < 0.01$, * $P < 0.05$). Salivary flow rate (▲) was not significantly changed. Error bars represent S.E. ($N = 64$).

also increased, but to a lesser extent compared with that of amylase (peak level of amylase, 188.0%; that of cortisol, 160.2%; both $P < 0.01$). The peak levels were obtained in the third and sixth samples in amylase and cortisol, respectively, that is, latency time to the peak level for cortisol was longer than that of amylase. In addition, the effect of video viewing on the cortisol level was lingering. Thus, the carry-over effect was not observed in the amylase response but was in cortisol. The salivary flow rate was also almost constant throughout the session.

Effect of soothing video viewing

The decrease in salivary amylase level by the video viewing was observed in 30 of 35 (85.7%) subjects. As shown in Fig. 2, the normalised values of percentage of pre-soothing level for the amylase were significantly decreased ($P < 0.05$) just after the beginning of soothing video viewing. Mean decreased level was 64.2% ranging from 82.5 to 42.8%. The amylase level was immediately returned to the pre-soothing level just after the end of the video viewing. In contrast to amylase, cortisol level was not influenced by the soothing video viewing. The salivary flow rate was also almost constant throughout the session.

Relation between the amylase or cortisol level and STAI score

There were no significant differences in the STAI score between males (45.1 ± 7.9 , $N = 53$) and females (45.2 ± 7.6 , $N = 30$), and both the salivary

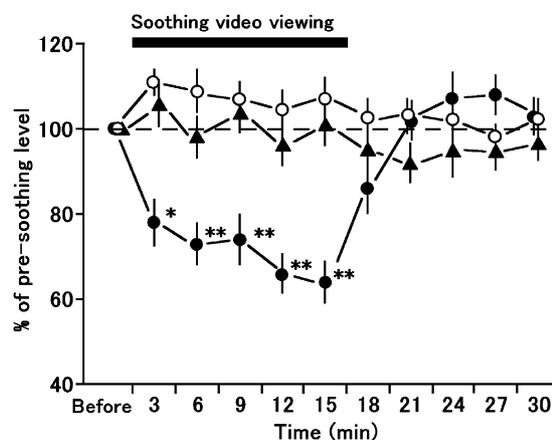


Figure 2 Effect of soothing video viewing on normalised values of percentage of resting level for the salivary amylase, cortisol and flow rate. Amylase (●) was significantly decreased by video viewing (** $P < 0.01$, * $P < 0.05$), but both cortisol (○) and flow rate (▲) were not significantly changed. Error bars represent S.E. ($N = 35$).

amylase and cortisol concentrations in the resting saliva did not correlate with the STAI score.

Fig. 3 shows the relation between the peak levels of the amylase or cortisol level by stressful video viewing and the STAI score. The correlation between the amylase level and the STAI score was highly significant ($r = 0.535$, $P < 0.01$). In contrast to that of amylase, the cortisol level did not statistically correlate with the STAI score. There were no significant correlations between pre-stress or -soothing level of the amylase or cortisol and the STAI score.

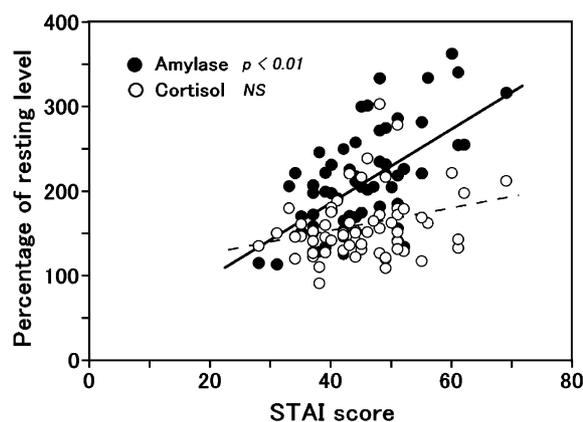


Figure 3 Relation between maximum levels of the salivary amylase or cortisol by stressful video viewing and STAI score. Correlation between the amylase level and the STAI score is highly significant ($Y = 4.458X + 8.494$; $r = 0.535$, $P < 0.01$, $N = 64$). However, no significant correlation between cortisol and STAI score was found ($Y = 1.325X + 97.42$; $r = 0.220$, NS, $N = 64$).

Discussion

Many studies have shown that psychological stressors, such as public speaking,¹⁷ academic examinations,¹⁸ dental procedures^{19–21} and suspense films viewing,^{13,22} can induce significant increases in salivary cortisol levels. Similar results using a stressful video viewing as a stressor were obtained in the present study, that is, reinforce the many previous findings showing that the salivary cortisol is a good stress index. Although there are numerous studies concerning the salivary cortisol response to HPA, only a few studies reported results according to the effect of the SAM system activities on salivary constituents such as amylase. Bosch et al.²³ reported a two-fold increase in the salivary amylase level by psychological stress. They assayed unstimulated whole saliva from 28 dental students, and used a written examination as the psychological stressor. A more recent study by Skosnik et al.¹² in which stressful video game playing served as the stressor, showed a 1.4-fold increase in the salivary amylase. In our time-course experiment, the amylase level was increased just after the beginning of stressful video viewing and immediately returned to the pre-stress level just after the end of the video viewing. On the other hand, the cortisol level was also increased, but to a lesser extent compared with that of amylase. The latency time to the peak level for cortisol was longer than that of amylase. In addition, the carry-over effect was not observed in the amylase response but was in cortisol. These results clearly showed that the psychological stressor increased in the amylase level, and the response and sensibility to the stressor were higher in amylase than those in cortisol.

It is considered that the major stress response can be conceptualised as occurring in two stages: a short latency catecholamine component which represents the first system, and a slower acting glucocorticoid response representing the second system. The first response depends upon the SAM system. The second response depends upon HPA, resulting in an increase in cortisol secretion. This cortisol response in HPA is the final step in the normal stress response, and has a longer latency of secretion than that of catecholamine in SAM system.^{1,2} Presumably, the differences in the response time to stress reactions between salivary amylase and cortisol may result from the differences in the time latency between the stress response of SAM system and that of HPA.

Another possible explanation can be considered. Cortisol is highly lipid-soluble with relatively small molecules (362 Da). This property enables the molecule to diffuse through the cell membranes via

passive intracellular diffusion. Once carried to the secretory cells of the salivary glands by the blood stream, cortisol can pass through these cells into saliva with osmotic pressure due to the concentration gradient. In that case, cortisol must pass through the cell membranes twice, both the basal and luminal membranes. These membranes act as a rate-limiting barrier. In contrast, the origin of amylase is the acinar cells of the salivary gland, that is, amylase is secreted from the secretory granules.⁹ It seems likely that these differences result in the difference of time latency between the response of salivary amylase and cortisol.

Many studies tried to reveal possible associations between cortisol stress responses and personal variables.^{24–30} However, the relation between HPA stress responses and personal traits was less consistent, and some studies failed to show effects of personal traits on HPA stress responses.^{25,27,28,30} We also found that the increasing rate for cortisol tended to become so high that the STAI score, or susceptibility of stress, was high, but there was no significant difference between cortisol and STAI score. Meanwhile, the amylase which is an index of the SAM system was significantly correlated to the STAI score. This may suggest that salivary amylase, as a SAM index, is more precise than cortisol as a HPA index. In the present study, only healthy young subjects with a narrow age range were examined. Further investigations will be necessary to validate and extend these associations in detecting personal trait-related amylase stress response dispositions.

In contrast to results obtained in stress research which clearly suggest increasing salivary cortisol levels following various stressful stimuli, only a few results are available on the possible effects of anti-stress, that is, relaxation or meditation. Jin³¹ reported no significant effect of meditation on the cortisol level. Field et al.³² found minimal differences in salivary cortisol using a questionable control group and inadequate statistical method. Thus, the effect of anti-stress on the salivary cortisol remains obscure. In the present study, the soothing video viewing did not affect the salivary cortisol level. However, the salivary amylase level was significantly decreased by soothing video viewing. The detailed mechanism of the amylase decrease is unclear. A greater decrease in SAM activity may occur than that in resting condition.

We clearly showed that the salivary amylase level was more significantly increased and reacted more rapidly than cortisol by psychological stressor, suggesting that it is a better index of stress. Furthermore, it is suggested that the enzyme is a soothing or relaxation index. As mentioned above, the sali-

vary amylase measurement will be powerful tool for psychological research.

References

1. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrinol Rev* 1986;7:284–301.
2. al' Absi M, Arnett DK. Adrenocortical responses to psychological stress and risk for hypertension. *Biomed Pharmacother* 2000;54:234–44.
3. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 1994;19:313–33.
4. Lake CR, Chernow B, Feuerstein G, Goldstein DS, Ziegler MG. The sympathetic nervous system in man: its evaluation and the measurement of plasma NE. In: Ziegler MG, Lake CR., editors. *Norepinephrine*. Baltimore: Williams and Wilkins; 1984. p. 1–26.
5. Kennedy B, Dillon E, Mills PJ, Ziegler MG. Catecholamines in human saliva. *Life Sci* 2001;69:87–99.
6. Schwab KO, Heubel G, Bartels H. Free epinephrine, norepinephrine and dopamine in saliva and plasma of healthy adults. *Eur J Clin Chem Clin Biochem* 1992;30:541–4.
7. Mitome M, Shirakawa T, Kikui T, Oguchi H. Salivary catecholamine assay for assessing anxiety in pediatric dental patients. *J Clin Pediatr Dent* 1997;21:255–60.
8. Okumura T, Nakajima Y, Matsuoka M, Takamatsu T. Study of salivary catecholamines using fully automated column-switching high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997;694:305–16.
9. Gallacher DV, Petersen OH. Stimulus-secretion coupling in mammalian salivary glands. *Int Rev Physiol* 1983;28:1–52.
10. Chatterton RT, Vogelsong KM, Lu Y, Ellman AB, Hudgens GA. Salivary α -amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 1996;16:433–48.
11. Walsh NP, Blannin AK, Clark AM, Cook L, Robson PJ, Gleeson M. The effects of high-intensity intermittent exercise on saliva IgA, total protein and α -amylase. *J Sports Sci* 1999;17:129–34.
12. Skosnik PD, Chatterton RT, Swisher T, Park S. Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *Int J Psychophysiol* 2000;36:59–68.
13. Nejtek VA. High and low emotion events influence emotional stress perceptions and are associated with salivary cortisol response changes in a consecutive stress paradigm. *Psychoneuroendocrinology* 2002;27:337–52.
14. Spielberger CD. *Manual for the State-Trait Anxiety Inventory: STAI (Form Y)*. Palo Alto, CA: Consulting Press; 1983.
15. Gotlib IH, Cane DB. Self-report assessment of depression and anxiety. In: Kendall PC, Watson D, editors. *Anxiety and depression; distinctive and overlapping features*. San Diego: Academic Press; 1989. p. 131–70.
16. Hidano N, Hukuhara M, Iwawaki M, Soga S, Spielberger CD. *Manual for the State-Trait Anxiety Inventory-Form JYZ*. Tokyo: Jitsumu Kyoiku-Shuppan; 2000.
17. Bassett JR, Marshall PM, Spillane R. The physiological measurement of acute stress (public speaking) in bank employees. *Psychophysiology* 1987;5:265–73.
18. Maes M, Van der Planken M, Van Gastel A, Bruyland K, Van Hunsel F, Neels H, et al. Influence of academic examination stress on hematological measurements in subjectively healthy volunteers. *Psychiatry Res* 1998;80:201–12.
19. Millar CS, Dembo JB, Falace DA, Keplan AL. Salivary cortisol response to dental treatment of varying stress. *Oral Surg Oral Med Oral Pathol* 1995;79:436–41.
20. Akyuz S, Pince S, Hekim N. Children's stress during a restorative dental treatment: assessment using salivary cortisol measurements. *J Clin Pediatr Dent* 1996;20:219–23.
21. Hill CM, Walker RV. Salivary cortisol determination and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth. *Br Dent J* 2001;191:513–5.
22. Hubert W, de Jong-Meyer R. Emotional stress and saliva cortisol response. *J Clin Chem Clin Biochem* 1989;27:221–2.
23. Bosch JA, Brand HS, Ligtenberg TJ, Bermond B, Hoogstraten J, Nieuw Amerongen AV. Psychological stress as a determinant of protein levels and salivary-induced aggregation of *Streptococcus gordonii* in human whole saliva. *Psychosom Med* 1996;58:374–82.
24. Arnetz BB, Fjellner B. Psychological predictors of neuroendocrine responses to mental stress. *J Psychosom Res* 1986;30:297–305.
25. Salmon P, Pearce S, Smith CC, Manyande A, Heys A, Peters N, et al. Anxiety, type A personality and endocrine responses to surgery. *Br J Clin Psychol* 1989;28:279–80.
26. Hubert W, de Jong-Meyer R. Saliva cortisol responses to unpleasant film stimuli differ between high and low trait anxious subjects. *Neuropsychology* 1992;25:115–20.
27. Lehmann J, Tennigkeit M, Haschke R, Haschke W, Rosahl S. Differences in mental task performance and slow potential shifts in subjects differing in cortisol level. *Int J Psychophysiol* 1992;13:1–8.
28. Seeman TE, Berkman LF, Gulanski BI, Robbins RJ, Greenspan SL, Charpentier PA, et al. Self-esteem and neuroendocrine response to challenge: MacArthur studies of stressful aging. *J Psychosom Res* 1995;39:69–84.
29. van Eck M, Berkhof H, Nicolson N, Sulon J. The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. *Psychosom Med* 1996;58:432–46.
30. Vedhara K, Miles J, Bennett P, Plummer S, Tallon D, Brooks E, et al. An investigation into the relationship between salivary cortisol, stress, anxiety and depression. *Biol Psychol* 2003;62:89–96.
31. Jin P. Efficacy of Tai Chi, brisk walking, meditation, and reading in reducing mental and emotional stress. *J Psychosom Res* 1992;36:361–70.
32. Field T, Morrow C, Valdeon C, Larson S, Kuhn C, Schanberg S. Massage reduces anxiety in child and adolescent psychiatric patients. *J Am Acad Child Adolesc Psychiatry* 1992;31:125–31.