

Association between salivary alpha-amylase activity and stress-related characteristics

Yoshie NAGATA^{1,2)}, Fumio SAKAUCHI¹⁾, Hisako IZUMI³⁾,
Erhua SHANG¹⁾, Hirofumi OHNISHI¹⁾, Mitsuru MORI¹⁾

¹⁾Department of Public Health, Sapporo Medical University School of Medicine,

²⁾Department of Nursing, Hokkaido Bunkyo University School of Faculty of Human Science,

³⁾Department of Nursing, Sapporo Medical University School of Health Sciences

ABSTRACT

Introduction: Recently, salivary alpha-amylase activity has been suggested to be an index as a stress-related biomarker. In this study, we investigated possible associations between salivary alpha-amylase activity levels and lifestyle or stress-related characteristics.

Methods: In total, 476 subjects voluntarily participated in the survey and their levels of salivary amylase activity were measured by a noninvasive salivary amylase monitor. Every subject filled out a self-administered questionnaire just after the measurement.

Results: The results of these tests indicate the salivary alpha-amylase activity levels are significantly higher in males than those in females ($P<0.01$). Furthermore, the salivary alpha-amylase activity levels were significantly higher in the subjects aged 50 years or older than those of the subjects aged younger than 50 years ($P<0.01$). Scores of the behaviors of positively coping with stress as well as social support from relatives or friends against stress were inversely correlated to salivary alpha-amylase activity after being adjusted for sex and age with multiple linear regression analysis ($P=0.02$ and $P=0.03$, respectively). However, the perception of stress was not associated with changes in salivary alpha-amylase activity levels.

Conclusion: Our findings may suggest that positively stress coping and emotional supports reduce the physiological response to stress, as observed by the decrease in salivary alpha-amylase activity levels.

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Key words: Salivary alpha-amylase, Smoking, Alcohol drinking, Stress coping

1 INTRODUCTION

The stress response is controlled by two primary systems, namely, the hypothalamus-pituitary-adrenocortical axis system (HPA system) and sympathetic nervous-adrenomedullary system (SAM system). Psychological stress activates the HPA system and SAM system through secretion of cortisol and catecholamine, respectively¹⁻³⁾.

Salivary alpha-amylase is one of the major salivary enzymes in humans, which is secreted from the salivary glands in response to sympathetic stimulation. Currently, it has been proposed as an indicator for stress-induced activity of the sympathetic nervous system⁴⁻⁵⁾. In the SAM system, secretion of salivary alpha-amylase is regulated by both hormonal responses to stress via catecholamine

and direct nervous function. The salivary alpha-amylase activity regulated by direct nervous function might be considered as readily responding to psychological stress responses⁶⁻⁸⁾. Acute psychosocial stress, in the following procedure of the Trier Social Stress Test, from watching a stressful video, or social stress like giving a speech in front of audience^{6-7, 9)}, increases salivary alpha-amylase activity. However, chronic stress has not been reported to be associated with an increase in salivary alpha-amylase activity, except for in an article by Nater *et al.*¹⁰⁾ reported that chronic stress measured using the Chronic Stress Screening Scale was associated with an increase in salivary alpha-amylase activity.

Accordingly, we designed a cross-sectional study to determine whether there are any associations between

salivary alpha-amylase activity and lifestyles or stress-related characteristics.

2 METHODS

2.1 Questionnaire

The self-administered questionnaire contained inquiries about age, sex and lifestyle such as status of smoking (yes, no) and alcohol drinking (yes, no), perception of stress (good, poor), self-perceived health condition (yes, no), behaviors of escaping from stress, behaviors of positively coping with stress, and social support from relatives or friends against stress. These questions were developed by Munakata¹¹⁾. Behaviors of escaping from stress had 8 categories of 'behave with the feelings of tension and thrill,' 'behave merrily or brightly contrary to one's feeling,' 'eat something delicious and emotionally overeat,' 'go shopping for a change,' 'relieve one's feelings by throwing or breaking something,' 'get angry with the person who was involved in the problem and shift the blame onto him or her,' 'court the kindness of the opposite sex,' and 'dispel one's gloom by drinking and having a wild time with friends'. Behaviors of positively coping with stress had 6 categories of 'find out the way to relieve this problem by reading books and listening to others,' 'discern the cause of the problem and move toward a solution,' 'talk with the central person and the persons related to the problem to reach a solution for it,' 'change the attitudes and behaviors of oneself,' 'consult with someone that one can trust or make oneself heard,' and 'practice a hobby, amusement, exercise, sports, etc. for a change'. Social support of stress from relatives or friends had 3 categories of 'do you have someone with whom you can be relaxed?', 'do you have someone who supports and agrees with your behaviors and thinking?', and 'do you have someone whom you can privately confide your thinking and your secrets?'. Behaviors of escaping from stress, behaviors of positively coping with stress and social support of stress from relatives or friends were estimated by the total points by adding 1 point under each items. Therefore, the range of behaviors of escaping from stress, behaviors of positively coping with stress and social support of stress from relatives or friends were from 0 to 8, 0 to 6, and 0 to 3, respectively.

2.2 Measurement of salivary alpha-amylase activity

Level of salivary alpha-amylase activity was measured by a Salivary Amylase Monitor (Nipro Co.,

Japan), which consisted of a testing-strip, a salivary transcription device and an optical analyzer. By adding maltose, a testing-strip becomes able to detect the salivary amylase activity from 0 to 200 KU/L during a time of less than 150 seconds, and a salivary transcription device controls the reaction time of the enzyme¹²⁾.

In order to evaluate the Salivary Amylase Monitor, salivary amylase activity was measured. In a range of salivary amylase activity between 0 and 200KU/L, the calibration curve for the Salivary Amylase Monitor obtained a coefficient of R² value of 0.988 and coefficient of variation of 10.2%¹³⁾. Although gargling with water before measurement is recommended, we could not follow this recommendation.

2.3 Participants

A total of 476 participants (139 males and 337 females) voluntarily participated in our study, after including 330 participants (107 males and 223 females) and 146 participants (33 males and 113 females) recruited from care prevention and a health festival in Sapporo, respectively. Saliva of each participant was measured once during the festival from 10:00 am to 4:00 pm at a space in a shopping center with a Salivary Amylase Monitor. Just after measurement, each participant filled out a self-administered questionnaire. The mean ages (\pm standard deviation or SD) of the male and female participants were 58.0 \pm 17.3 years and 55.3 \pm 17.0 years, respectively. All participants were informed about this study and informed consent was obtained from each participant. This study had been approved by the Ethics Committee of Sapporo Medical University.

2.4 Statistical analysis

We calculated the quartile of salivary alpha-amylase activity levels in the subjects. Proportional changes were examined by the Mantel-Haenszel chi-square test for trends. Correlations between sex, age, behaviors of positively coping with stress and social support from relatives or friends against stress were tested using the Spearman's correlation coefficient or the Cramer's coefficient of association. Finally, we also conducted a multiple linear regression analysis to assess the relationship between salivary alpha-amylase activity and the behaviors of positively coping with stress, and social support from relatives or friends against stress. Logarithmic-transformation was performed for levels of salivary alpha-amylase activity in the multiple linear regression analysis, because the distribution of the levels

was positively skewed. The statistical significance level was set at 0.05 of the P value. The analyses were performed with a commercially available statistical package (SPSS Ver. 16.0).

3 RESULTS

Status of smoking and alcohol drinking as well as the distributions of stress-related characteristics are shown in Table 1. Regarding the behaviors of positively coping with stress, the female subjects with 6 points were more frequent than the male subjects with the same points (36.5 vs. 28.1%). Concerning social support from relatives or friends against stress, the female subjects with 3 points were more frequent than the male subjects with the same points (71.2 vs. 51.8%).

As shown in Table 2, salivary alpha-amylase activity

levels were significantly higher in males than in females ($P<0.01$). Salivary alpha-amylase activity levels were significantly higher in the subjects aged 50 years or older than those aged younger than 50 years ($P<0.01$).

Salivary alpha-amylase activity levels were significantly higher in the subjects with behaviors of positively coping with stress scored from 4 to 6 than those that scored from 0 to 3 ($P<0.01$). Furthermore, salivary alpha-amylase activity levels were significantly higher in the subjects with social support from relatives or friends against stress from relatives or friends scored from 2 to 3 than those that scored from 0 to 1 ($P<0.01$). However, salivary alpha-amylase activity levels did not show a significant association with the status of smoking or alcohol drinking, perception of stress, self-perceived health condition, or behavior of escaping from stress.

As shown in Table 3, female gender was significantly positively correlated with scores of behaviors of positively coping with stress (Cramer's $V = 0.16$, $P<0.01$) as well as scores of social support from relatives or friends against stress (Cramer's $V = 0.16$, $P<0.01$). Scores of behaviors of positively coping with stress were significantly positively related to scores of social support from relatives or friends against stress ($r=0.28$, $P<0.01$). However, age was not related to either behaviors of positively coping with stress or social support from relatives or friends against stress.

We did multiple linear regression analysis in association with salivary alpha-amylase activity levels with stress-related characteristics, to evaluate confounding factors such as sex, age, behavior of positively coping with stress, and perception of stress. We showed the results of 3 analyses; sex and age for the first analysis; sex, age, behaviors of positively coping with stress, and perception of stress for the second analysis; and sex, age, social support from relatives or friends against stress, and perception of stress for the third analysis. As shown in Table 4, scores of behavior of positively coping with stress as well as social support from relatives or friends against stress were inversely correlated to salivary alpha-amylase activity after being adjusted for sex and age by multiple linear regression analysis ($P=0.02$ and $P=0.03$, respectively). However, when scores of behavior of positively coping with stress and social support from relatives or friends against stress were simultaneously involved in multiple linear regression model, significance levels elevated as much as $P=0.08$ and $P=0.07$, respectively. Perception of stress was not associated with

Table 1 Lifestyle and stress-related characteristics of the study subjects

Variables	Male (n=139)	Female (n=337)
Age (yr)		
Mean±SD	58.0±17.3	55.3±17.0
Smoking status (%)		
Yes	42.4	12.8
No	57.6	87.2
Alcohol drinking (%)		
Yes	65.5	33.4
No	34.5	66.6
Perception of stress (%)		
Yes	38.1	43.6
No	61.9	56.4
Self-perceptive health condition (%)		
Good	73.2	73.2
Poor	26.8	26.8
Behavior of escaping from stress (%)		
0 point	20.9	17.8
1 point	28.8	25.8
2-3 points	34.5	40.1
4-8 points	15.8	16.3
Behavior of positively coping with stress (%)		
0-1 point	9.4	5.6
2-3 points	24.5	13.6
4-5 points	38.1	44.2
6 points	28.1	36.5
Social support from relatives or friends against stress (%)		
0 point	12.2	7.4
1 point	16.5	7.7
2 points	19.4	13.6
3 points	51.8	71.2

Data are mean±SD, or percentages.

Table 2 Association of quartile levels in salivary alpha-amylase activity with lifestyle and stress-related characteristics

Variables	Levels of salivary alpha-amylase activity KU/L				Total(%)	P-value for trend ¹
	<17.0	17.0-37.9	38.0-80.4	>80.4		
Sex n=476						
Male	26(18.7)	32(23.0)	30(21.6)	51(36.7)	139(100%)	
Female	86(25.5)	93(27.6)	90(26.7)	68(20.2)	337(100%)	<0.01
Age (yr) n=476						
<50	43(26.1)	58(35.2)	42(25.5)	22(13.3)	165(100%)	
≥50	69(22.2)	67(21.5)	78(25.1)	97(31.2)	311(100%)	<0.01
Smoking Status n=475						
Yes	19(18.6)	30(29.4)	22(21.6)	31(30.4)	102(100%)	
No	93(24.9)	95(25.5)	97(26.0)	88(23.6)	373(100%)	0.21
Alcohol drinking n=474						
Yes	49(24.1)	53(26.1)	52(25.6)	49(24.1)	203(100%)	
No	61(22.5)	72(26.6)	68(25.1)	70(25.8)	271(100%)	0.66
Perception of stress n=476						
Yes	41(20.5)	59(29.5)	58(29.0)	42(21.0)	200(100%)	
No	71(25.7)	66(23.9)	62(22.5)	77(27.9)	276(100%)	0.84
Self-perceptive health condition n=474						
Good	87(25.1)	91(26.2)	84(24.2)	85(24.5)	347(100%)	
Poor	23(18.1)	34(26.8)	36(28.3)	34(26.8)	127(100%)	0.17
Behavior of escaping from stress n=476						
0-1 point	53(24.5)	52(24.1)	46(21.3)	65(30.1)	216(100%)	
2-8 points	59(22.7)	73(28.1)	74(28.5)	54(20.8)	260(100%)	0.34
Behavior of positively coping with stress n=476						
0-3 points	20(17.9)	28(25.0)	24(21.4)	40(35.7)	112(100%)	
4-6 points	92(25.3)	97(26.6)	96(26.4)	79(21.7)	364(100%)	0.01
Social support from relatives or friends against stress n=476						
0-1 point	11(12.1)	26(28.6)	24(26.4)	30(33.0)	91(100%)	
2-3 points	101(26.2)	99(25.7)	96(24.9)	89(23.1)	385(100%)	0.01

¹Mantel-Haenszel chi-square test for trend**Table 3** Correlations between sex, age, behavior of positively coping with stress and social support from relatives or friends against stress

	Age	Behavior of positively coping with stress	Social support from relatives or friends against stress
Sex (Females vs. males)	–	0.16 ^{1*}	0.16 ^{1*}
Age	–	-0.03 [‡]	-0.02 [‡]
Behavior of positively coping with stress	–	–	0.28 ^{‡*}

¹Cramer's V [‡]Spearman's correlation coefficient **P*< 0.01

changes in the levels of salivary alpha-amylase activity.

4 DISCUSSION

The results of this study showed a significant association between lower salivary alpha-amylase activity levels and higher scores of behaviors of positively coping with stress as well as higher scores of social support from relatives or friends against stress. Although the relationship between salivary alpha-amylase activity

levels and stress coping behaviors has not been well documented, the association of salivary alpha-amylase activity levels with response to psychological stress has been well known. For example, previous studies have indicated that salivary alpha-amylase activity levels rise in response to both physical and psychological stress^(14,15). Moreover, recent studies have shown that salivary alpha-amylase levels increase in response to the Trier Social Stress Test^(6,7,9,16,17). Levels of salivary alpha-amylase

Table 4 Multiple linear regression analysis in association of salivary alpha-amylase activity levels with stress-related characteristics.

Variables	Coefficient(95%CI)	P-value
Intercept	3.54	
Sex	-0.32 (-0.53- -0.11)	<i>P</i> <0.01
Age ¹	0.25 (0.13-0.38)	<i>P</i> <0.01
Intercept	3.86	
Sex	-0.28 (-0.50- -0.07)	<i>P</i> <0.01
Age ¹	0.26 (0.14-0.38)	<i>P</i> <0.01
Behavior of positively coping with stress ²	-0.27 (-0.49- -0.04)	<i>P</i> =0.02
Perception of stress ³	0.05 (-0.14-0.25)	<i>P</i> =0.61
Intercept	3.94	
Sex	-0.29 (-0.50- -0.08)	<i>P</i> <0.01
Age ¹	0.25 (0.12-0.37)	<i>P</i> <0.01
Social support from relatives or friends against stress ⁴	-0.28 (-0.53- -0.04)	<i>P</i> =0.03
Perception of stress ³	0.04 (-0.16-0.23)	<i>P</i> =0.71
Intercept	4.07	
Sex	-0.24 (-0.46- -0.04)	<i>P</i> <0.01
Age ¹	0.01 (0.01-0.02)	<i>P</i> <0.01
Behavior of positively coping with stress ²	-0.21 (-0.44- -0.02)	<i>P</i> =0.07
Social support from relatives or friends against stress ⁴	-0.22 (-0.47- 0.03)	<i>P</i> =0.08
Perception of stress ³	-0.06 (-0.25-0.14)	<i>P</i> =0.58

¹<40, 40-59 and ≥60 years²0-3 and 4-6 points³0-1 and 2-3 points⁴Yes or no

activity have also been reported to significantly increase just after the beginning of watching a stressful video¹⁶. In contrast, chronic stress measured using the Chronic Stress Screening Scale (CSSS) was associated with an increase in salivary alpha-amylase¹⁰. The CSSS is a 12-item scale derived from a larger chronic stress questionnaire. However, perception of stress was not associated with salivary alpha-amylase activity levels in our study. The significant inverse association of salivary alpha-amylase activity levels with positively coping with stress disappeared, if social support from relatives or friends against stress was simultaneously involved in the multiple linear regression model. Insufficient power caused by small sample size may induce this disappearance of statistical significance.

In this cross-sectional study, salivary alpha-amylase activity levels were significantly higher in the males than in the females, and higher in the older subjects than in the younger subjects. These sex and age differences in stress reactivity may be associated with the hypothalamic-pituitary-adrenal axis and autonomic nervous system responses. It is suggested that between puberty and menopause, females show a lower hypothalamic-pituitary-adrenal axis and autonomic response than males of the

same age¹⁸. Particularly, estrogen has been shown to regulate sympathetic responses and the hypothalamic-pituitary-adrenal axis^{19,20}, however, differences in salivary alpha-amylase activity due to age or sex have not been observed in other studies^{10,21,22}.

Our study needs to be interpreted in light of the following limitations. For this cross-sectional study, salivary alpha-amylase activity levels were measured only once in the daytime. However, secretion of endogenous substances such as salivary alpha-amylase levels is characterized daily oscillations. In previous studies, levels of salivary alpha-amylase activity were reported to be lower in the morning and higher in the afternoon^{23,24}. In another study, a peak of salivary alpha-amylase activity was observed not only in the early evening but also in the morning at 8 o'clock am²⁵. A recent study showed that levels of salivary alpha-amylase activity decreased within 60 min after awakening and steadily increased through activity during the course of the day¹⁰, although another study showed that there were no distinct changes in salivary alpha-amylase activity levels over the course of the day²⁶. Therefore, further study is necessary to investigate the levels of salivary alpha-amylase activity with regard to its circadian pattern.

Another limitation of this study is that the reliability and validity of the Salivary Amylase Monitor have not been established yet. Especially, we did not assess volume of salivary flow, which might have affected the measurements of the Monitor. Moreover, there are several limitations in the questionnaire, which have been developed by Munakata¹¹⁾. Therefore, another questionnaire is required to be utilized in the future study.

In conclusion, our findings may suggest that better behavior of stress coping as well as better social support would reduce the physiological response to stress observed by the decrease in salivary alpha-amylase activity levels. However, further study needs to be conducted to establish an association between salivary alpha-amylase activity levels and stress-related characteristics.

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Correspondence:

Yoshie NAGATA

Department of Public Health, Sapporo Medical University School of Medicine,

Nishi-17, Minami-1, Chuo-ku, Sapporo, Hokkaido, 060-8556, Japan.

Tel: +81-(0)11-611-2111

Fax: +81-(0)11-641-8101

E-mail: ynagata@sapmed.ac.jp

唾液 α -アミラーゼ活性とストレス関係因子との関連性

長多好恵^{1, 2)}, 坂内文男¹⁾, 和泉比佐子³⁾

尚 爾華¹⁾, 大西浩文¹⁾, 森 満¹⁾

¹⁾ 札幌医科大学医学部公衆衛生学

²⁾ 北海道文教大学人間科学部看護学

³⁾ 札幌医科大学保健医療学部看護学

近年、唾液 α -アミラーゼ活性はストレスと関連する生体指標になることが示唆されている。今回の研究で、われわれは唾液 α -アミラーゼ活性レベルと生活習慣との関連、またストレスのような刺激を受けたときの対処行動および人間関係を主体とした情緒的支援網との関連性について検討した。

476人のボランティアの対象者がこの研究に参加した。同意が得られた対象者には自記式質問票に答えてもらい、同時に対象者の唾液 α -アミラーゼ活性値は、非侵襲的な唾液 α -アミラーゼモニター（ニプロ社製）によって測定された。各質問項目と唾液 α -アミラーゼ活性レベルの関連性について Mantel-Haenszel の検定を行った。重回帰分析により、ストレス対処行動および情緒的支援網と唾液 α -アミラーゼ活性レベルとの関連を調べた。

唾液 α -アミラーゼ活性レベルは、女性より男性において有意に高い傾向を示した ($P < 0.01$)。さらに、唾液 α -アミラーゼ活性レベルは 50 歳以上の対象者では、50 歳未満の対象者より有意に高い傾向を示した ($P < 0.01$)。

ストレスに対しての積極的対処行動および人間関係における情緒的支援網のスコアが高い方が低い場合に比べて、唾液 α -アミラーゼ活性レベルは有意に低かった（それぞれ $P = 0.02$ と $P = 0.03$ ）。その他の生活習慣と唾液 α -アミラーゼ活性レベルの間に関連はみられなかった。

今回の結果から、ストレスに対して積極的に対処しようとする行動や人間関係において情緒的支援網があることと唾液 α -アミラーゼ活性レベルの間には、何らかの関連性があることが示唆された。