

Physiological Stress Assessments based on Salivary α -amylase Activity and Secretory Immunoglobulin A levels in Wheelchair-dependent Individuals with Congenital Physical Disabilities

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Abstract

We sought to assess the circadian rhythm of stress levels on the basis of the s-IgA/total protein ratio and α -amylase activity in the saliva in wheelchair-dependent individuals with congenital physical disabilities. We enrolled 11 individuals with congenital physical disabilities. Saliva samples were collected eight times in a day from each participant: on awakening, after breakfast, before lunch, after lunch, at 15:00 h, before dinner, after dinner and at bedtime. The above indices were measured and compared among the saliva samples obtained at the different time points. The mean s-IgA/total protein ratio from awakening to before lunch was higher than that after lunch and became slightly elevated again at bedtime. However, there were no significant differences among the mean value obtained for each time point. The mean α -amylase activity from before lunch to bed time was greater than that on awakening and after breakfast. The mean α -amylase activity after breakfast was significantly different from that before and after dinner. The values for s-IgA/total protein ratio and α -amylase activity after breakfast, before lunch and after dinner showed significant negative correlations. The circadian rhythm of stress levels in individuals with congenital physical disabilities showed large individual differences over one day.

Keywords: Congenital physical disabilities, stress, salivary s-IgA, salivary α -amylase.

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1 Introduction

Wheelchairs are used by individuals who cannot walk or feel that it is difficult to walk because of impairments in their lower limbs. Wheelchairs make it possible for these people to move around in daily life, and power-assisted wheelchairs provided developmentally disabled individuals with the freedom of movement based on their intentions. Therefore, means of transportation to enable the social participation of individuals with physical disabilities are focused on wheelchair movements using public transport systems.

Physical barriers that were initially obstacles for wheelchair movements have decreased dramatically with the increased use of barrier-free and universal designs as designated by law. However, in order to use wheelchairs, individuals with physical disabilities need to assume a sitting posture for long periods, and many of them find it difficult to maintain this posture. Even if wheelchairs are improved, it can be inferred that individuals with physical disabilities who regularly use wheelchairs will be subjected to physical stresses due to long periods of sitting. In addition, there are risks that may cause deuteropathies (secondary conditions) such as bed sores and oedema of the lower extremities and feet.

Many research reports on the relationship between physical disabilities and stress are available [1-6]. However, most were surveys on the relationship between living environments and stress in individuals with physical disabilities [7-15]. Very few studies on stress using physiological indices have been conducted [16-17].

Carneil et al [18] assessed stress using blood oxidative stress levels and chronic inflammation as indices in elderly individuals with intellectual impairments. They reported that both indices showed significantly higher values in elderly individuals with intellectual impairments than in those without. Johansson et al [19] examined circadian changes in salivary cortisol levels in 40 patients with lumbar vertebra herniated disks and reported that patients with low cortisol circadian rhythms were more sensitive to this stress compared with patients with high cortisol circadian rhythms.

Kalpajian et al [20] examined circadian changes in salivary cortisol levels in 25 individuals with cervical spinal cord injuries and 26 without any injuries and reported that circadian cortisol rhythms were comparable between the two groups. Cortisol levels have been used in various ways as a reflection of psycho-physiological stress [21-24]. However, reports have mentioned that cortisol is not suitable for evaluating this type of stress [25]. Rohleder et al [26] argued that the amylase activity was a better reflection of psycho-physiological stress compared with cortisol.

Secretory immunoglobulin A (s-IgA)/total protein levels and α -amylase activity in the saliva have recently been used as indices of physiological stress [26-27] because they can be easily measured and it is simple to acquire saliva samples. However, the circadian rhythm of stress levels in individuals with physical disabilities has not been examined using these parameters as measurement indices.

Therefore, in this study, we examined the circadian rhythm of stress levels in wheelchair-dependent individuals with congenital physical disabilities on the basis of the s-IgA/total protein ratio and α -amylase activity in the saliva.

2 Methods

2.1 Subjects

We enrolled 11 (5 males and 6 females; mean age, 40.5 ± 13.4 years; range, 20–57 years) wheelchair-dependent individuals with congenital physical disabilities. The disabilities included cerebral paralysis ($n = 5$), osteogenesis imperfecta ($n = 2$), trunk functional disorder ($n = 2$), cervical cord injury ($n = 1$) and rheumatism ($n = 1$; Table 1).

All participants used manual or power-assisted wheelchairs. Those who used manual wheelchairs could not operate the wheelchair by themselves. We obtained informed consent from all participants after explaining the purpose and contents of this experiment in detail. This study was approved by the Ethics Committee on Human Experimentation of the Faculty of Human Science, Kanazawa University (approval No.2012-19).

2.1 Physiological Studies

Salivary samples for s-IgA and α -amylase activity analyses were collected eight times in a day from each participant: on awakening, after breakfast, before lunch, after lunch, at 15:00 h, before dinner, after dinner and at bedtime. These samples were collected from different individuals between October and November 2011 according to methods described by Nakata et al [28]. Saliva was collected both before and after lunch and supper to take into consideration the effects of consuming a meal.

Saliva was generally collected by placing a cotton ball in the mouth and allowing the subject to chew for several minutes. However, a care worker was asked to help subjects who could accidentally ingest the cotton ball or could not adequately chew on it by guiding them to directly deposit their saliva up to a level of approximately 1 ml in a sterile 50-ml centrifuge tube after gargling. Saliva samples were immediately frozen.

Prior to measurements, frozen saliva was thawed, transferred to a 1.5-ml tube and centrifuged. A part of the saliva supernatant was used for analysis. Saliva s-IgA levels were measured by a sandwich enzyme-linked immunoabsorbent assay (ELISA) using a Human IgA ELISA Quantitation Kit (Bethyl/Funakoshi, E80-102) according to the manual instructions and the method of Sakai et al [29]. Total protein levels were determined using a Modified Lowry Protein Assay Kit (Thermo Scientific, #23240).

The s-IgA level per total protein level (s-IgA/total protein ratio) was determined using the following equation: $\text{s-IgA/total protein ratio} = (\text{s-IgA level/total protein level}) \times 100 (\%)$.

α -amylase activity was determined using Salivary α -Amylase Assay Kit 96 Well Plate (Salimetrics Co. Ltd., Burlington, VT). In addition, the awakening time, bedtime and sleep duration were evaluated at the time of saliva collection because some reports [30-31] have suggested that these timings influence the s-IgA/total protein ratio and α -amylase activity (Table 2).

2.2 Statistical Analysis

Significant differences among the means of values obtained for the s-IgA/total protein ratio and α -amylase activity at each time point from the 11 participants were determined using paired one-way analysis of variance (ANOVA). If a significant difference was found, multiple comparisons were made using Tukey's honestly significant difference method. The level of significance was set at 0.05.

3 Main Results

3.1 Features and Life Styles of the Subjects

Table 1 shows the subjects' demographic (gender, age) and physical [height, body weight and body mass index (BMI)] characteristics and their disabilities as well as the summary statistics for all participants' physical characteristics. Table 2 shows the awakening time, bedtime and sleep duration of each participant in addition to the summary statistics for all these parameters.

Table 1: Participants' demographic and physical characteristics

Participants	Gender	Age	Height	Weight	BMI	Disability
K.I.	female	38	152	49	21.2	cerebral paralysis
Y.W.	male	56	160	65	25.4	cerebral paralysis
H.M.	male	42	158	50	20.0	cerebral paralysis
Y.M.	female	43	148	38	17.3	cerebral paralysis
H.Y.	male	46	172	60	20.3	cerbicalcord injury
S.N.	female	21	112	28	22.3	osteogenesis imperfecta
N.F.	male	54	130	30	17.8	osteogenesis imperfecta
S.F.	female	57	152	46	19.9	cerebral paralysis
K.M.	female	20	158	54	21.6	trunk functional disorder
A.H.	female	21	155	44	18.3	trunk functional disorder
S.K.	male	48	165	50	18.4	rheumatism
Mean		40.5	151.1	46.7	20.2	
SD		13.4	16.0	10.9	2.2	
CV		0.3	0.1	0.2	0.1	

CV, co-efficient of variation; SD, standard deviation

Table 2: Participants' awakening time, bedtime and sleep duration

Participants	awakening time	bedtime	sleeping duration
K.I.	7:30 AM	11:00 PM	8 hour 30 min
Y.W.	8:00 AM	10:30 PM	9 hour 30 min
H.M.	7:00 AM	11:00 PM	8 hour 00 min
Y.M.	8:00 AM	11:30 PM	8 hour 30 min
H.Y.	7:00 AM	10:30 PM	8 hour 30 min
S.N.	7:30 AM	11:00 PM	8 hour 30 min
N.F.	8:00 AM	12:30 AM	7 hour 30 min
S.F.	9:30 AM	1:00 AM	8 hour 30 min
K.M.	7:30 AM	11:30 PM	8 hour 00 min
A.H.	7:00 AM	11:00 PM	8 hour 00 min
S.K.	9:00 AM	1:00 PM	8 hour 00 min
Mean	7:49 AM	11:30 PM	8 hour 19 min
SD	47 min	53 min	29 min
CV			0.1

CV, co-efficient of variation; SD, standard deviation

3.2 Physiological Results

Table 3 shows the results of statistical analysis of the s-IgA/total protein ratio and α -amylase activity measured at each time point. The mean s-IgA/total protein ratio from awakening to before lunch was higher than that after lunch and became slightly elevated at bedtime. However, no significant differences were found among the mean s-IgA/total protein ratios at the different time points. The mean α -amylase activity from before lunch to bedtime was higher than that on awakening and after breakfast. In addition, there was a significant difference between the mean α -amylase activity after breakfast and that before and after dinner.

The co-efficient of variation (CV) for the s-IgA/total protein ratio was used as an index of individual differences. The CV was the highest after lunch (1.7) and lowest on awakening (0.8). The CV for α -amylase activity was the highest at bedtime (0.8) and lowest after breakfast and dinner (0.5). Overall, the CV for the s-IgA/total protein ratio was higher than that for α -amylase activity. In addition, the CV's for both parameters were higher than those for age, physical variables, and sleep duration (Tables 1 and 2).

Table 4: shows the correlations among the values for the s-IgA/total protein ratio and α -amylase activity at the eight measurement points and their levels of significance. Values for the s-IgA/total protein ratio and α -amylase activity after breakfast, before lunch and after dinner showed significant negative correlations.

Table 3: Results of statistical analysis of the s-IgA/total protein ratio and α -amylase activity measured at eight time points in a day

		1	2	3	4	5	6	7	8	F	effect size	p	post-hoc
		awakening	after breakfast	before breakfast	after lunch	15:00 h	before dinner	after dinner	bedtime				
s-IgA/protein	M	13.5	11.1	14.4	8.0	9.8	8.8	4.3	8.0	1.42	0.12	0.21	
	SD	10.6	9.0	14.6	13.3	9.5	5.8	4.4	8.9				
	CV	0.8	0.8	1.0	1.7	1.0	0.7	1.0	1.1				
	MAX	36.0	29.8	49.4	49.6	30.4	20.9	13.3	34.3				
	MIN	2.0	0.6	1.8	0.8	1.1	0.8	0.1	0.5				
α -amylase	M	58.4	40.8	128.2	112.1	116.3	148.3	142.5	109.5	2.84	0.22	0.01*	2<6,7
	SD	38.4	20.4	101.4	80.7	92.9	81.6	71.9	90.7				
	CV	0.7	0.5	0.8	0.7	0.8	0.6	0.5	0.8				
	MAX	148.3	70.2	390.3	309.3	319.8	301.1	280.1	356.5				
	MIN	21.3	10.5	5.6	28.9	22.0	46.6	31.5	2.3				

*p < 0.05

Table 4 Correlations among values for the s-IgA/total protein ratio and α -amylase activity obtained at eight time points in a day

	1	2	3	4	5	6	7	8	
	awakening	after breakfast	before lunch	after lunch	15:00 h	before dinner	after dinner	bedtime	mean
Coefficient correlation	-0.125	-0.332*	-0.348*	0.070	0.041	0.115	-0.393*	-0.134	-0.138

*p < 0.05

5 Discussion

Stress responses are mediated by at least two systems: the limbic - hypothalamic - pituitary - adrenal axis (LHPA) and the sympathetic-adrenal-medullary axis (SAM) [32-33]. Therefore, in this study, we examined the stress levels in wheelchair-dependent individuals with physical disabilities on the basis of salivary s-IgA/total protein ratio and α -amylase activity, both of which are related to these systems.

The s-IgA/total protein ratio of saliva is an index of activities in the sympathetic nervous system, the parasympathetic nervous system, and the immune system, which play essential roles in whole body defence and local immune system function [32]. It has been reported that this ratio decreases because of both psychological stress and physiological stress [30-34]. It has been shown that during daily life, the s-IgA/total protein ratio is high on awakening and decreases with time, with no apparent gender-related differences [35-37].

Nakata et al [28] collected saliva samples five times every two hours from 09:00 h to 17:00 h for five consecutive days and assessed the circadian rhythm of s-IgA levels. They reported that the mean level for five days was significantly higher at 09:00 h than at other time points during the day, with no significant change from 11:00 h to 17:00 h.

In our study, the mean s-IgA/total protein ratio was high during the morning period, decreased with time after lunch and was the lowest after dinner. However, we found no significant differences in these values over time, a finding inconsistent with that of Nakata et al [28]. We also found that the awakening time, bedtime, and sleep duration differed among our participants (Table 2). These factors may have affected our results.

Leicht et al [16] examined mucosal immune responses on the basis of the secretion speed of s-IgA and α -amylase activity in 23 elite wheelchair runners who were made to undergo a treadmill exercise test. They reported that secretion speed increased significantly during exercise, although large individual differences were found. Although our participants were different from those in the study by Leicht et al [16], both studies noted large individual differences in the s-IgA/total protein ratio and α -amylase activity.

The α -amylase activity is a part of the SAM system activity and is an index of stress mediated primarily by a sympathetic nervous activity. In addition, it was reported that the α -amylase activity was influenced by the time of meal consumption because it is a digestive enzyme [38]. Nater et al [39] collected hourly saliva samples from 76 healthy individuals from the time of awakening (09:00 h) until 20:00 h and assessed the circadian rhythm of α -amylase activity. They found a remarkable decrease within 60 min after awakening and a significant increase during periods of activity during the day and at night. Therefore, they argued that this was parameter was useful for evaluating sympathetic nervous activity.

Yamaguchi et al [17] examined the relationship between heart rate and pain induced by medical manipulation using a saliva amylase monitor in 10 individuals with serious psychosomatic disabilities. They found that the amylase showed an average increase of 70% after medical manipulation and that this increase was 4 times greater than that in the heart rate. In addition, because there was a significant correlation between the salivary amylase activity and the degree of pain induced by medical manipulation, they argued that the salivary amylase activity may be useful for nonverbal evaluation of pain in psychosomatically disabled individuals.

In this study, the α -amylase activity in the saliva tended to be low on awakening and after breakfast, high from before lunch to 15:00 h and even higher before and after dinner. In

addition, the α -amylase activity after breakfast was significantly different from that before and after dinner. Therefore, the sympathetic nervous activity becomes active particularly during the period before and after dinner, indicating that stress levels are low in the morning and become high from before lunch to bedtime.

Shiba et al [40] collected saliva before and after lunch from 47 elementary school children and assessed the effects of mealtime duration (10 min, 15 min and 20 min) on amylase and peroxidase levels and saliva amounts. They found that salivary amylase levels were higher when the mealtime was shorter, albeit not significantly.

Our present results also showed no significant difference among the mean α -amylase activity values before and after lunch and dinner. The individuals with physical disabilities in this study could not eat or prepare meals by themselves. Therefore, they did not need to think about meals because of the complete assistance provided by care workers. Because of this, they experienced little stress for a meal, which was probably a reason why no significant differences were found among values obtained before and after meals.

The values for the s-IgA/protein ratio and α -amylase activity after breakfast, before lunch and after dinner showed significant negative correlations. In general, the relationship between both these indices showed that as the stress level increased, α -amylase activity increased and the s-IgA/total protein ratio decreased. However, both these indices do not always show complete negative correlations in their circadian rhythms [41]. The s-IgA production is affected by the immune system of the oral cavity and is regulated by the sympathetic and parasympathetic nervous systems, whereas α -amylase activity is regulated primarily by the former system during stress periods. Therefore, the mechanisms that control the secretion of these indicators are different. In the future, it will be necessary to clarify stress conditions from many perspectives by using other indices such as chromogranin A, active oxygen (reactive oxygen species; ROS), biological antioxidant potential and others.

6 Conclusion

From the above considerations, we identified the following trends on examining the daily stress levels in wheelchair-dependent individuals with congenital physical disabilities on the basis of the s-IgA/total protein ratio and the α -amylase activity in the saliva. Although the s-IgA/total protein ratio did not show any significant circadian changes, α -amylase activity was low on awakening and after breakfast and was high from before lunch to bedtime. Both indices showed large individual differences and were negatively correlated with each other after breakfast, before lunch and after dinner.

In conclusion, our study demonstrated that the stress level was higher before dinner and after dinner than after breakfast. Values for the s-IgA/total protein ratio and α -amylase activity exhibited an inverse relationship after breakfast, before lunch and after dinner. These findings indicate that the circadian rhythm of stress levels in individuals with congenital physical disabilities shows large individual differences over one day.

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