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Salivary chromogranin A levels relate to fatigability after waking up

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ABSTRACT

This study aimed to assess fatigue using a noninvasive, quantitative, and simple method using salivary chromogranin A (CgA). In total, 171 adults participated in this study. We used the Cornell Medical Index (CMI) as a questionnaire that included a fatigability section. The high-fatigability group had significantly ($p < 0.01$, $d = 0.21$) lower levels of salivary CgA after waking up than those of the low-fatigability group. Before sleep, participants with high stress on the musculoskeletal system (CMI E, $p = 0.008$, $d = 0.18$), skin (CMI F, $p = 0.017$, $d = 0.16$), nervous system (CMI G, $p = 0.019$, $d = 0.16$), habit (CMI L, $p = 0.028$, $d = 0.16$), sensitivity (CMI P, $p = 0.022$, $d = 0.16$), and anger (CMI Q, $p = 0.011$, $d = 0.16$) had significantly lower CgA levels than those of low stress. In conclusion, we found that salivary CgA levels after waking up could reflect fatigability, and those before night sleep could reflect chronic physical complaints.

- Assessment of Salivary CgA levels using an enzyme-linked immunosorbent assay.
- Routine saliva analysis could enable allow detection of chronic fatigue or stress, leading to timely lifestyle adjustments or interventions to maintain overall well-being.

Specifications table

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Background

Chromogranin A (CgA) is a 49-kDa acidic hydrophilic secreted protein that exists abundantly in the endocrine and nervous systems. CgA levels are particularly high in the adrenal medulla and pituitary [1]. Plasma CgA levels increase in patients with tumors, rheumatoid arthritis, and other inflammatory diseases [2]. Salivary CgA is used as a sensitive and quantitative marker for monitoring the activity of the sympathetic nervous system and for reflecting the stress response [3,4]. Some studies have shown that salivary CgA levels increase with a performance of tasks or with acute stress [5]. Concentrations of salivary CgA peak shortly after waking up; however, they decline very quickly (within only 1 h) and remain constant throughout the day [4]. Den et al. (2011) have reported that salivary CgA levels peak upon waking up, quickly decrease thereafter, and finally increase again at night [6]. The study also showed that when awake, participants with high depression scores had significantly lower salivary CgA levels than those with low depression scores. However, very few reports have shown a relationship between salivary CgA levels and chronic symptoms.

Fatigue is broadly described as a feeling of weariness, tiredness, or lack of energy. Many people complain of fatigue, although 10 to 25 % prevalence is relative fatigue, according to population studies [7]. While previous studies had reported some quantitative methods to evaluate fatigue [8], most of them used invasive or complicated methods. A previous study found CgA levels unsuitable as diagnostic markers for occupational fatigue [9]. Sleep is closely associated with fatigue, and fatigue, especially in relation to a disease, is associated with an imbalance of sleep [10].

Cornell Medical Index (CMI), a questionnaire designed to collect a large amount of pertinent medical and psychiatric data with minimal resources, includes a section on fatigability [11]. We used this questionnaire in this study because it has been widely used in clinical practice as a screening procedure to assess neurotic tendencies.

Therefore, we considered that fatigue could be related to the levels of CgA before or after a night sleep. In this study, we aimed to analyze salivary CgA levels to evaluate fatigue using a simple, non-invasive, and quantitative method and quantitatively define fatigue by exploring the relationship between CgA and the CMI.

Method details

Participants and protocols

One hundred and ninety adults (age range, 20–85 years) volunteered to participate in this study. Nineteen participants were excluded due to lack of saliva. Finally, saliva samples from 171 participants were analyzed. The participants completed the questionnaire before or on the day of the assessment. They arrived at the laboratory and were lectured on how to collect saliva. They returned to their daily lives thereafter. They were asked to collect saliva samples before sleep and immediately after waking up (within 15 min), before drinking, eating or rinsing their mouths. They then placed the sample tube in a freezer overnight in their home and took it to the laboratory.

Saliva collection and CGA measurements

Saliva samples were collected in special sampling tubes using Salivette cotton swabs (Salivettes; Sarstedt Co., Nümbrecht, Germany). They placed the cotton under the tongue, without biting, for 1 min before night sleep and repeated the same after waking up. The tubes were frozen at −80 ˚C until further measurements. Before measurement, the Salivette cotton samples were centrifuged at 3000 rpm for 10 min at 4 ˚C. We used an enzyme-linked immunosorbent assay with a YK070 Human CgA EIA kit (Yanaihara Institute Inc., Shizuoka, Japan) to determine the concentration of CgA [12]. All samples were analyzed in duplicate and the average value was used. In the separate experiments, we confirmed that there was no significant variation within one individual over multiple days (cf. Supplementary Fig. 1).

Questionnaires

The CMI is a questionnaire aimed at collecting a large amount of pertinent medical and psychiatric data with minimum expenditure of resources [11]. The Japanese version of CMI (J-CMI) consists of 211 questions for males and 213 for females in 18 sections (A–R) divided into physical and psychological complaints.14 The J-CMI is used in clinical practice as a screening procedure to evaluate the following twelve physical complains (A–L), and six psychological complains (M–R) [13,14]. Participants answered each question with ‘yes’ or ‘no’ response. If they answered ‘yes’, it indicated that the participant had symptoms. Sections A to L (A: eyes and ears; B: respiratory system; C: cardiovascular system; D: digestive tract; E: musculoskeletal system; F: skin, G: nervous system; H: genitourinary system; I: fatigability, J: frequency of illness; K: miscellaneous disease; and L: habit) with 160 questions for males and 162 for females indicated physical complaints. Sections M to R (M: inadequacy, N: depression, O: anxiety, P: sensitivity, Q: anger, and R: tension), with 51 questions, indicated psychological complaints [13].

Statistical analysis

Data are expressed as means ± standard deviation. Statistical analysis was performed using the Student’s *t*-test and Mann–Whitney U-test using Excel and SPSS 21.0, as appropriate. For significant differences, we calculated Cohen’s d effect size. $p < 0.05$ was considered statistically significant.

Table 1
Comparison of the basic characteristics of age, body mass index, and physical and psychological complaint scores of J-CMI between fatigability score groups.

Group	I	II	d value
Fatigability score	< 3	≥ 3	
n	145	26	
Age	38.8 ± 21.1	51.5 ± 21.7*	0.60
Body mass index (Kg/m^2)	22.0 ± 3.0	23.1 ± 3.6	0.36
Physical complaints (J-CMI)	15.6 ± 10.3	37.3 ± 17.5#	0.64
Psychological complaints (J-CMI)	5.3 ± 4.9	13.0 ± 8.7#	0.71

J-CMI, Japanese version of Cornell Medical Index.
* $p < 0.05$, I vs. II (Student's *t*-test).
# $p < 0.05$, I vs II (Mann-Whitney *U* test.)

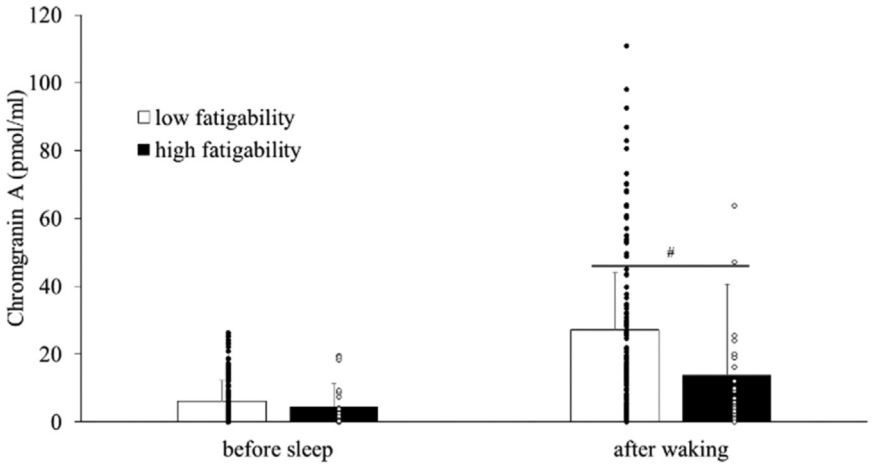

Fig. 1. Relationships between the salivary CgA levels before and after sleep and fatigability score.
# $p < 0.05$, low fatigability ($n = 145$) vs. high fatigability ($n = 26$) (Mann-Whitney *U test*).

Method validation

Comparison of the basic characteristics between fatigability score groups

The 171 participants were divided into two groups, with fatigability scores either of 3 or more (high-fatigability group) or 2 or less (low-fatigability group). The basic characteristics of age, body mass index, and physical and psychological complaint scores of the J-CMI between the fatigability score groups are summarized in Table 1. The high-fatigability group had significantly higher age and physical and psychological complaint scores than the low-fatigability group (CMI I, $p < 0.001$, $d = 0.21$).

Relationships between each J-CMI items and CgA levels

Table 2 shows the relationships between each CMI item and CgA levels before sleep and after waking up. Before sleep, participants with high stress on the musculoskeletal system (CMI E, $p = 0.008$, $d = 0.18$), skin (CMI F, $p = 0.017$, $d = 0.16$), nervous system (CMI G, $p = 0.019$, $d = 0.16$), habit (CMI L, $p = 0.028$, $d = 0.16$), sensitivity (CMI P, $p = 0.022$, $d = 0.16$), and anger (CMI Q, $p = 0.011$, $d = 0.16$) had significantly lower CgA levels than those with low stress.

Relationships between the salivary CgA levels and fatigability score

The high-fatigability group had significantly lower levels of salivary CgA after waking up than the low-fatigability group ($p < 0.01$, $d = 0.21$); however, there was no significant difference in salivary CgA levels before night sleep (Fig. 1).

Fatigue and autonomic nervous system

High fatigability caused low levels of salivary CgA after waking up. The autonomic nervous system controls the saliva activity. An increase in saliva CgA secretion is related to an increase in epinephrine and norepinephrine and reflects changes in blood pressure

Table 2
Relationships between each J-CMI items and CgA levels before sleep and after waking up.

item	n (low/high)	CgA before sleep		CgA after waking up	
		low stress	high stress	low stress	high stress
A	113/58	5.8 ± 6.8	6.3 ± 7.6	24.8 ± 24.2	27.2 ± 34.9
B	134/37	6.0 ± 7.0	4.9 ± 6.5	24.6 ± 24.3	28.1 ± 34.1
C	132/39	6.1 ± 7.0	5.0 ± 6.5	26.6 ± 25.0	23.7 ± 29.3
D	130/41	6.2 ± 7.2	4.8 ± 5.8	26.0 ± 26.3	22.5 ± 25.1
E	150/21	6.3 ± 7.1	2.9 ± 4.0[#]	26.0 ± 26.4	18.6 ± 22.6
F	134/37	6.3 ± 7.0	4.4 ± 6.4[#]	25.2 ± 24.7	24.9 ± 30.6
G	136/35	6.5 ± 7.4	3.2 ± 3.7[#]	26.1 ± 26.4	21.5 ± 24.4
H	124/47	6.1 ± 7.0	5.2 ± 6.5	26.6 ± 26.8	21.3 ± 23.4
I	145/26	6.1 ± 6.2	4.5 ± 7.0	27.2 ± 16.8	13.7 ± 26.8[*]
J	151/20	6.1 ± 7.1	4.3 ± 5.4	25.5 ± 26.2	22.1 ± 24.9
K	121/50	6.1 ± 7.0	5.2 ± 6.7	25.3 ± 25.4	24.8 ± 27.1
L	136/35	6.3 ± 7.1	4.3 ± 5.7[#]	25.5 ± 26.3	23.7 ± 24.8
M	131/40	6.7 ± 7.2	6.3 ± 6.0	25.5 ± 26.7	24.0 ± 23.6
N	134/37	5.9 ± 7.1	5.8 ± 6.2	24.4 ± 26.5	27.9 ± 25.9
O	130/41	6.0 ± 6.9	5.3 ± 6.9	26.4 ± 26.9	21.0 ± 22.4
P	145/26	6.1 ± 6.9	4.8 ± 6.8[#]	24.3 ± 24.9	30.0 ± 31.2
Q	138/33	6.3 ± 6.9	4.1 ± 6.5[#]	26.3 ± 27.1	20.4 ± 20.4
R	152/19	6.1 ± 7.1	4.3 ± 4.8	25.8 ± 26.7	20.1 ± 19.5

[#] $p < 0.05$, low stress vs high stress (Mann-Whitney *U* test), CgA (pmol/mL), n (low/high) indicates the number of the participants with low or high stress for each item, A: eyes and ears 10 items cut-off value is 3; B: respiratory system 21 items cut-off value is 4; C: cardiovascular system 14 items cut-off value is 4; D: digestive tract 28 items cut-off value is 5; E: musculoskeletal system 10 items cut-off value is 3; F: skin 9 items cut-off value is 3, G: nervous system 19 items cut-off value is 3; H: genitourinary system 11 items for men and 13 items for women cut-off value is 3; I: fatigability 7 items cut-off value is 3; J: frequency of illness 9 items cut-off value is 2; K: miscellaneous disease 15 items cut-off value is 2; L: habit 7 items cut-off value is 3; M: inadequacy 12 items cut-off value is 3; N: depression 6 items cut-off value is 1; O: anxiety 9 items cut-off value is 2; P: sensitivity 6 items cut-off value is 2; Q: anger 9 items cut-off value is 2; and R: tension 9 items cut-off value is 3.

and pulse [15]. The findings suggested that salivary CgA levels may relate to the fatigue level that may further affect the autonomic nervous system.

Saliva CgA could be an index of fatigue

In this study, participants in the high-fatigability group were significantly older than those in the low-fatigability group. However, there was no significant correlation between age and salivary CgA levels both before sleep ($p = 0.38$, Mann-Whitney *U test*) and after waking up ($p = 0.52$, Mann-Whitney *U test*). Thus, we considered that the levels of salivary CgA after waking up could be considered an index of fatigue as a non-invasive and simple method of diagnosis.

Stress and fatigue

Generally, chronic stress results from post-traumatic stress disorder, psychological distress from depressive and anxiety symptoms, and acute stress from a psychological response to a traumatic or surprising experience [16]. Additionally, chronic activation of the stress system causes adaptation of the hypothalamic-pituitary-adrenal axis, which ultimately blunts the acute stress response [17]. Most former studies between salivary CgA and stress considered acute stress response in the daytime [18–20].

Stress and CgA

In this study, before a night sleep, participants with high musculoskeletal system (CMI E), skin (CMI F), nervous system (CMI G), habit (CMI L), sensitivity (CMI P), and anger (CMI Q) complaints had significantly lower CgA than participants with low stress. Additionally, the high-fatigability group had significantly lower levels of salivary CgA after waking up than the low-fatigability group. We considered that saliva CgA before a night sleep might reflect many physical or psychological stress, whereas saliva CgA after waking could specifically reflect fatigue. Many studies have investigated the relationships between salivary CgA and complaints; however, to the best of our knowledge, almost all of them targeted acute stress in daytime, and only few targeted it after waking up; no study targeted it before a night sleep. Our results also suggested that salivary CgA before a night sleep can be a marker for chronic physical complaints.

Future study

In this study, there was a larger variety of CgA in our participants especially in the non-fatigue group. We considered that the fatigue group may consist of subjects only feeling obvious fatigue, however non-fatigue group may include subjects under borderline area. Further studies are needed to address this issue while taking into account the relationship between stress level and CgA.

Conclusions

In conclusion, considering the results, it is possible that salivary CgA levels after waking up reflect fatigability, whereas salivary CgA levels before a night sleep reflect chronic physical complaints.

Limitations

Not applicable.

Ethics statements

This study was approved by the Ethics Committee of the National Cerebral and Cardiovascular Research Center (M18-19-2, M26-158), Chubu University (280031), and the Nagahama Institute of Bio-Science and Technology (006). All participants provided written informed consent prior to participating in the study.

CRediT author statement

Kentaro Taniguchi: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing - Original Draft, Funding acquisition, **Naoya Okumura:** Investigation, **Naoya Jinno:** Validation, Investigation, Visualization, **Akitoshi Seiyama:** Formal analysis, Writing - Review & Editing, **Akito Shimouchi:** Conceptualization, Validation, Resources, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2024.103085.

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