Heliyon 8 (2022) e10506

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Baseline states of mind differentially affected diurnal salivary stress biomarkers: A preliminary study

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ARTICLE INFO

Keywords:
Saliva
Cortisol
Amylase
Chromogranin A
Mindfulness
Diurnal

ABSTRACT

The study aimed to determine how different baseline states of mind in each day (relaxed, ordinary, or stressful) affected the diurnal patterns of three commonly investigated salivary stress biomarkers: cortisol (sCort), alpha-amylase (sAA), and chromogranin A (sCgA). A total of 32 healthy volunteers collected saliva on three different mood days at six time points each day (awakening, 30 min after, 10:00, 12:00, 16:00, and 19:00 h). Pulse rates and subjective feeling of stress using a visual analog scale (VAS) were also recorded. The levels of sCort and sAA were highest on a stressful day at certain time points. The levels of sAA were lowest on a relaxing day in the afternoon. Surprisingly, sCgA levels showed an opposite pattern with the highest level seen on a relaxing day. Of note was that the majority of the participants chose a day during a meditation retreat as their relaxing day and participants practicing mindfulness manifested lower levels of sCort ($p = 0.003$) and sAA ($p = 0.043$) at 19:00 h compared with those choosing a general leisure day as their relaxing day. Different states of mind were associated with different courses of salivary stress biomarkers. sCort and sAA are the most reliable markers showing the expected trend with higher levels on a stressful day and lower levels on a relaxing day. While the current result cast doubt on the use of sCgA as a stress marker since it was the only marker that showed the opposite trend compared with those of the other two markers as well as pulse rates and VAS. Furthermore, this is the first study to demonstrate that mindfulness practice might have different effects on these biomarkers from just a general relaxed state of mind.

1. Introduction

The body's response to stress is primarily governed by the two major systems which are the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenomedullary (SAM) system [1]. Upon activation, the HPA axis produces cortisol which is a type of steroid hormone secreted by the adrenal gland. While the SAM system releases catecholamines (epinephrine and norepinephrine) [2]. Thus, measuring the levels of these hormones could indicate the activity of the stress response or the level of stress. Cortisol is present in saliva which is free cortisol that passively diffuses from the bloodstream [3]. The detection of salivary cortisol (sCort) levels has been widely used as a reliable stress marker in various kinds of stress studies including work stress, school stress, stress from noise, stress of maltreatment, and many others [4, 5]. Regarding stress research, the HPA axis is the most widely studied system with cortisol being considered as a gold standard biomarker of stress [6].

On the other hand, catecholamines' levels fluctuate a lot due to their short half-lives and various factors, so they are not very reliable indicators of stress [7, 8]. However, there are many surrogate markers of the SAM system, many of which are also present in the saliva. These include alpha-amylase and chromogranin A [9]. Salivary alpha-amylase (sAA) is the most abundant protein in human saliva and its secretion is regulated by the autonomic nervous system [10]. The situations that provoke the sympathetic response and direct application of norepinephrine increase sAA levels while decreased norepinephrine also results in decreased sAA amount [11]. Previous studies have consistently shown that the activation of the beta-adrenergic receptor stimulates the release of sAA while administration of beta-adrenergic antagonist led to a reduction of sAA levels [12]. These findings confirm the role of the autonomic nervous system in the regulation of sAA secretion and support the use of sAA as an indicator of autonomic activation and subsequently, stress. Indeed, sAA levels were found to be increased in a variety of stress

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https://doi.org/10.1016/j.heliyon.2022.e10506
Received 12 April 2022; Received in revised form 5 June 2022; Accepted 26 August 2022

models of physiological stress such as exercise and cold exposure, as well as psychological stress such as examination and Trier Social Stress Test [13]. Thus, sAA has emerged as a surrogate marker of the sympathetic activity over the past 15 years. These two markers (sCort and sAA) constitute the most studied biomarkers in stress [9].

For salivary chromogranin A (sCgA), there are increasing studies to indicate that it may be used as a stress marker as it is co-released with epinephrine or norepinephrine upon sympathetic stimulation [14]. Its gene and protein were found in submandibular glands but not other salivary glands in humans [15]. Many studies on various models of both physical and psychological stress have detected increased levels of sCgA in response to stress and that its level decreased with interventions to reduce stress such as music therapy, aromatherapy, laughter, medication, or tea consumption [16]. Hence, the general understanding that sCgA is representative of the SAM system [17].

It is well established that acute stress increases the levels of these biomarkers [17]. However, there are not many studies examining the impact of mood or expectation of stress on the diurnal patterns of these markers. A previous study has found that anticipated stress can affect the diurnal profile of sCort soon after awakening, when the presence of actual stress hadn't occurred yet [18]. However, there has not been any study examining if different baseline moods in each day affects the diurnal patterns of these stress markers. The aim of the present study was to compare the patterns of diurnal sCort, sAA, and sCgA among three different days where the same participants expected to feel different (relaxed, ordinary, or stressful) on each particular day. Furthermore, this study had a special aspect in that the majority of the participants chose a day during a meditation retreat as their relaxing day so we were able to compare the effect of mindfulness practice versus a general leisure day as a relaxing day on the levels of these biomarkers. This is of great interest since this is the first study to examine sCgA levels during mindfulness practice. The subjective feelings of the participants under different states of mind were also assessed to see if they agreed with the objective measurement of physiological indices (salivary markers and pulse rate). It would be interesting to see if subjective feeling may not last all day but objectively may persist beyond what one feels. We hypothesized that the diurnal patterns of all three biomarkers would be different among three moods with the highest levels on a stressful day and the lowest level on a relaxing day. This study would give more insight into the regulation of the HPA axis and the SAM system under different states of mind as well as during mindfulness practice.

2. Methods

2.1. Participants

The study recruited 36 healthy volunteers willing to participate in the experiment. The inclusion criteria were being at least 18 years old without underlying physical disease, mental disorder as well as a history of mental disorder, not taking medication, not pregnant, and not smoking. The exclusion criteria included inability to collect saliva at all assigned time points, consuming alcohol within 12 h, or eating/drinking (except plain water) within 1 h before saliva collection. Three of the volunteers were excluded due to the inability to collect saliva at all assigned time points and one was excluded because the subject no longer wished to participate in the study. The participants were graduate students at the Faculty of Dentistry. The study protocol was approved by the Human Research Ethics Committee of the Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2017/065.1812). The trial was registered in thaiclinicaltrials.org, registration number TCTR20180503002. The study was conducted according to the principles of the Declaration of Helsinki. All participants gave written informed consent.

2.2. Protocol

The design was to examine the diurnal profiles of sCort, sAA, and sCgA levels of individuals on three mood days (relaxing, ordinary, and stressful days). We asked the participants to subjectively choose three days where they considered each to be a relaxing, ordinary, or stressful day. The guideline was suggested that relaxing could be a day without any pressure such as a holiday, a day off, or during meditation retreat; an ordinary day could be a day with common chores and activities such as a routine job or going to classes; a stressful day could be a day with known or expected pressure such as an exam, an oral presentation, or a day with difficult job. There was no restriction on the sequence or the spacing among three mood days. The participants could choose any mood day to do the experiment first depending on their actual life events/situations/mood expectations in that period.

Each participant received a set of tubes and straws to collect saliva at six time points during the day: awakening (W), 30 min after awakening (W+30), 10:00, 12:00, 16:00, and 19:00 h. The participants were shown how to collect and store saliva, measure their pulse rates (PR), and assess their state of mind using a visual analog scale (VAS) in advance. Passive drooling technique was employed. The participants were instructed to pool the saliva for four minutes and then drool the saliva into a 2-ml plastic tube using a straw to guide the saliva into the tube. Then the saliva was kept at -20 °C. The next day, the participants returned the tubes to the researchers using an icebox where saliva was stored at -80 °C until further analysis. VAS was used to assess the current state of mind of the participants while collecting saliva at six time points. The scale ranges from 0 which indicates no stress at all to 10 which indicates maximal stress.

2.3. Saliva analysis

To assess the levels of salivary markers, the saliva was thawed and centrifuged at 1500g for 15 min prior to the measurement. The sCort levels were measured by the enzyme immunoassay kit (Salimetrics, State College, PA, USA) according to the manufacturer's protocol. The kit had a range of sensitivity from 0.007 to 3 μg/dl. The coefficient of variation was less than 5% for intra-assay and less than 10 % for inter-assay. For sCgA measurement, an enzyme-linked immunosorbent assay kit (MyBioSource, San Diego, CA, USA) was employed according to the manufacturer's instructions. The kit can measure between 30-9000 ng/l of sCgA with intra- and inter-assay coefficients of variation less than 10%. For sAA analysis, a hand-held biosensor (Nipro Co, Osaka, Japan) with the principle of enzyme kinetic assay was employed as explained in detail [19]. The biosensor could measure sAA levels correctly in the linear range between 0 and 200 Unit/ml with 10.2% coefficient of variation [20].

2.4. Statistical analysis

Data are presented as mean ± SD unless stated otherwise. Multivariate test with unstructured correlation was used to compare means of sCort, sAA, and sCgA levels, or VAS scores, and PR at different time points of each different mood day, and at the same time among three mood days, as well as duration of sleep among three mood days. Bonferroni's correction was applied to control overall type I error due to multiple comparisons. Related sample Cochran's Q test was used to compare the feeling of inadequate sleep, the presence of aphthous ulcer, and menstruation in the same subjects among three mood days. Statistical analysis was performed using SPSS statistics program version 20 (IBM, Armonk, NY, USA). The significant level was set at $p < 0.05$.

3. Results

3.1. Characteristics of the participants

A total of 32 participants completed the study with the age ranging from 26-34 years. There were only seven male participants. The BMI range of the participants was from 16.8 to 26.6 kg/m^2. Most participants (75%) were of medium built, 19% of the participants were underweight (BMI <18.5 kg/m^2) and only six percent were overweight (BMI ≥25 kg/m^2). No participant was obese (BMI ≥30 kg/m^2). The participants recorded the time of sleep the night before saliva collection for all three days. As shown in Table 1, the duration of sleep prior to a stressful day was approximately one hour less than those prior to an ordinary day ($p = 0.012$) or a relaxing day ($p = 0.01$). However, the percentages of participants that slept less than 6 h or had inadequate sleep were not different among three groups. The number of participants having menstruation during the experiment was not different among groups as well.

The participants also recorded the period of the day when they faced stressful events, 17 participants reported having a stressful event in the morning, 9 participants experienced stress in the afternoon, and only 6 participants faced stressful events both in the morning and afternoon.

3.2. Salivary markers

The diurnal patterns of sCort, sAA, and sCgA on three mood days are shown in Figure 1 (with mean ± SD in Supplementary Tables 1-5). For sCort, the levels ranged from 0.01 to 1.56 μg/dl and the daily mean ± SD of sCort levels on a relaxing day, an ordinary day and a stressful day were 0.24 ± 0.09, 0.25 ± 0.09 and 0.31 ± 0.12 μg/dl, respectively. The average daily output of sCort on a stressful day was significantly higher than that on an ordinary day ($p = 0.046$). Throughout the day, the levels of sCort on a stressful day were significantly higher than those on a relaxing day and an ordinary day at 10:00 h ($p = 0.000$) and an ordinary day at 12:00 h ($p = 0.039$). The diurnal patterns on a relaxing day and an ordinary day were identical.

For sAA, the levels ranged from 4 to 392 U/ml. The daily mean ± SD of sAA levels on an ordinary day was 120.82 ± 49.32 U/ml. While the average level on a relaxing day (105.92 ± 51.57 U/ml) was significantly lower than that on a stressful day (134.72 ± 56.91 U/ml; $p = 0.007$).

When looked at each time point, the levels of sAA on a stressful day were significantly higher than those on a relaxing day at 12:00, 16:00, and 19:00 h ($p = 0.045$, 0.001, 0.004, respectively) and on an ordinary day at 10:00 h ($p = 0.011$). The diurnal pattern of sAA on a relaxing day was different from others in that the usual high levels in the afternoon dropped down instead of showing a plateau pattern.

Table 1. Characteristics of participants (n = 32).

Parameters			Values (mean ± SD)
Age (years)			28.84 ± 1.71
BMI (kg/m^2)			21.1 ± 2.5
Female			78%
	Relaxing day	Ordinary day	Stressful day
Duration of sleep the previous night			
Mean (h:mm)	6:34 ± 0:56	6:44 ± 1:08	5:40 ± 1:43*
Min	5:00	4:00	1:00
Max	8:20	8:40	9:10
Sleep less than 6 h	28%	22%	47%
Feeling of inadequate sleep	50%	44%	69%
Presence of aphthous ulcer	0%	3%	16%**
Menstruation	16%	9%	22%

* $p < 0.05$ compared with both relaxing and ordinary days.
** $p < 0.05$ compared with relaxing day.

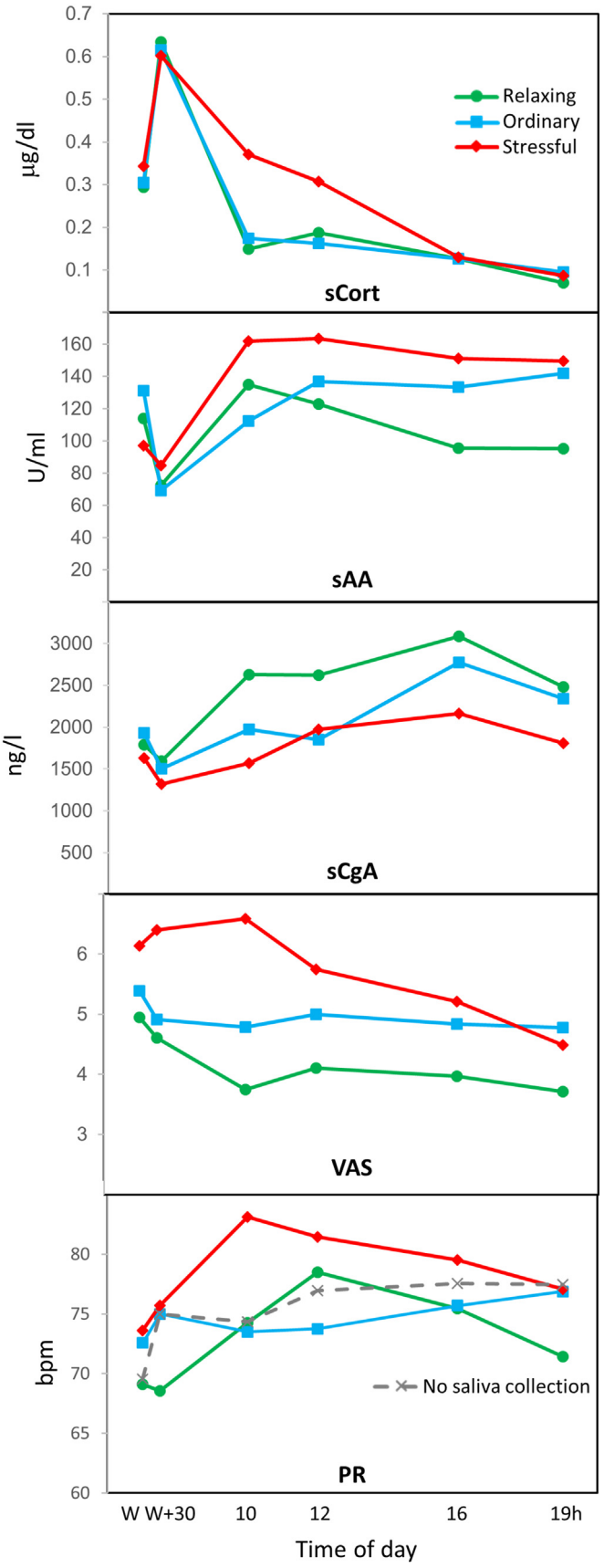

Figure 1. Diurnal patterns of sCort, sAA, sCgA levels, and VAS scores, on three mood days as well as PR on three mood days and the day without saliva collection. (n = 32).

For sCgA levels, they ranged from 453.5 to 10,670 ng/l with the daily mean ± SD of 2,367 ± 1,165.70 ng/l for a relaxing day, 2,062.73 ± 642.89 ng/l for an ordinary day, and 1,742.36 ± 481.55 ng/l for a stressful day which was significantly lower compared with a relaxing day ($p = 0.009$) and an ordinary day ($p = 0.010$).

The level of sCgA on a relaxing day was higher than that on a stressful day at 10:00 h ($p = 0.016$). Overall, the levels of sCgA on a stressful day appeared lower throughout the day compared with other days; however, these were not significantly different.

3.3. VAS and PR

The VAS scores on three mood days ranged from 0 to 10 and the daily mean ± SD of VAS on a relaxing day, an ordinary day and a stressful day were 4.18 ± 0.98, 4.95 ± 0.90, and 5.76 ± 1.04, respectively. The participants reported the highest VAS scores upon awakening on relaxing and ordinary days, although the scores were about 5 indicating a neutral state of mind. On a stressful day, the score was highest at 10:00 h and later declined in the afternoon, in line with the report of stressful events in the morning. The mood on an ordinary day was quite stable throughout the day with the relaxing day showing stable mood later after awakening period. While on a stressful day, VAS score was highest in the morning and lower in the afternoon.

VAS scores reported from participants on a relaxing day showed significantly lower scores than a stressful day at all time points except at 19:00 h ($p = 0.01$ (W), 0.000 (W+30), 0.000 (10:00 h), 0.000 (12:00 h), 0.014 (16:00 h)), and lower than an ordinary day at 10:00, 12:00, 16:00, and 19:00 h ($p = 0.012$, 0.005, 0.004, 0.018, respectively). VAS score on a stressful day was significantly higher than on an ordinary day only in the morning at W, W+30, and 10:00 h ($p = 0.023$, 0.000, 0.000 respectively) as shown in Figure 1.

For PR, they ranged from 48 to 121 bpm among three mood days. The daily mean ± SD was 79.38 ± 9.26 bpm on a stressful day which was significantly higher than on a relaxing day (73.63 ± 8.19 bpm; $p = 0.002$) and on an ordinary day (74.96 ± 8.57 bpm; $p = 0.008$). Throughout the day, PR on a stressful day were higher than those on a relaxing day at W+30, 10:00, and 19:00 h ($p = 0.011$, 0.004, 0.018, respectively) and on an ordinary day at 10:00 and 12:00 h ($p = 0.001$, 0.005, respectively).

To test whether saliva collection impacted stress or PR, the comparison of PR on a day without saliva collection and an ordinary day was also performed. The daily average of PR on a day without saliva collection (76.25 ± 7.38 bpm) was not different from an ordinary day with saliva collection ($p = 0.273$). However, PR upon awakening on a day without saliva collection was significantly lower than on an ordinary day with saliva collection ($p = 0.040$) but no difference was observed at all other time points as shown in Figure 1. This implied that saliva collection might affect the level of stress at first time of saliva collection but once the participant got used to the process, it no longer affected stress. Overall the process of saliva collection did not likely interfere with the level of stress.

3.4. The impact of mindfulness practice

For a relaxing day, some participants ($n = 23$, 71.88%) chose a day during a meditation retreat as their relaxing day where they did a one-week walking and sitting meditation. All participants were novices who did not regularly practice meditation in daily life. During a retreat, the participants had to follow the rules and schedules generally not observed outside a retreat such as waking up at 5:00 h or withholding dinner. However, drinks were allowed, only solid food was not served. The saliva was collected on the fourth day of a retreat. The rest of the participants ($n = 9$) chose a general leisure day as their relaxing day. Subgroup analysis found that these two groups differed in the levels of stress markers.

As shown in Figure 2 (with mean ± SD in Supplementary Tables 6-10), sCort levels at W, W+30, and 19:00 h were significantly different between meditation retreat and general leisure day ($p = 0.038$, 0.045, 0.003, respectively), while sAA levels differed at 19:00 h ($p = 0.043$) but not at other time points. For sCgA, the levels were not significantly different at all time points.

On the other hand, VAS scores were higher in a meditation retreat group at W, W+30, 12:00 h compared with a general leisure group ($p = 0.008$, 0.001, 0.019, respectively). PR at 12:00 h was also higher in the retreat group compared with the leisure group ($p = 0.047$); however, the daily mean of PR was not significantly different ($p = 0.165$).

4. Discussion

The current study examined the effects of three different baseline moods in each day on the levels of sCort, sAA, and sCgA throughout the day and found that mood had subtle association with the diurnal patterns of these biomarkers. The participants subjectively chose the day where they expected to feel relaxed, ordinary, or stressful in a day. The majority of the participants chose a day during a meditation retreat as a relaxing day and this was compared with a general leisure day. For an ordinary day, the diurnal patterns of sCort, sAA, and sCgA were similar to those from previous studies [21, 22, 23]. The hypothesis was that on a stressful day, the levels of all three biomarkers would be highest and that their levels would be lowest on a relaxing day with an ordinary day showing the levels in between.

For sCort, a stressful day showed increased levels at two time points (10:00 and 12:00 h) only, not throughout the day when compared with an ordinary day. This likely corresponded with the period of time the participants reported having stressful events in that day which reflected the acute nature of stress that bounced back to normal levels when there was no stress present. A previous study found that the expectation of stress affected the levels of sCort before the stressful event occurred as well as after the event was over but such increased levels quickly fell back to baseline [18]. It was suggested that the change around cortisol awakening response (CAR) might prepare the participants for the stress ahead. That study was performed with the manipulated stressful event, not the expected naturalistic stress as in the current study. We also found no difference in CAR among the three days as well as no difference between an ordinary day and a relaxing day. Previous studies have found that poor sleep quality or short sleep duration resulted in flatter cortisol profile, lower waking sCort level, and increased evening sCort secretion [24, 25, 26]. The current study also found flatter sCort pattern but no significant difference in waking or evening sCort levels.

For sAA, the results exhibited the predicted patterns of a stressful day showing the highest sAA levels and a relaxing day showing the lowest levels, albeit only at some time points. Most previous studies focus on the determinants such as age, sex, BMI, but not on different moods [27, 28, 29, 30]. A previous study by Van Lenten [24] found no association between prior night sleep duration or efficiency and sAA levels suggesting that this factor should not play a significant role in the current study.

Surprisingly, sCgA levels on a stressful day was lower than those on a relaxing day, though the value was significant only at one time point. The results of sCgA implied that it might not be a sensitive marker of stress or that its interpretation was not straightforward. The diurnal pattern of sCgA was similar to a previous study by our group with some slight but nonsignificant differences. More studies are needed regarding the use of sCgA as a stress marker. A previous study from our group also found sCgA to be not as sensitive as sCort or sAA to indicate stress [31].

Among the three biomarkers, sCort and sAA remain the most reliable markers. The results of sCgA were difficult to interpret as it has the largest SD with sCort showing the smallest SD. By and large, sCgA was not as sensitive and as easily interpretable as sCort or sAA levels. Our results cast doubt on the use of sCgA level as a proxy of the SAM system activity. There has to be more studies regarding the physiology of sCgA as little is known about sCgA, for example, there was only one study

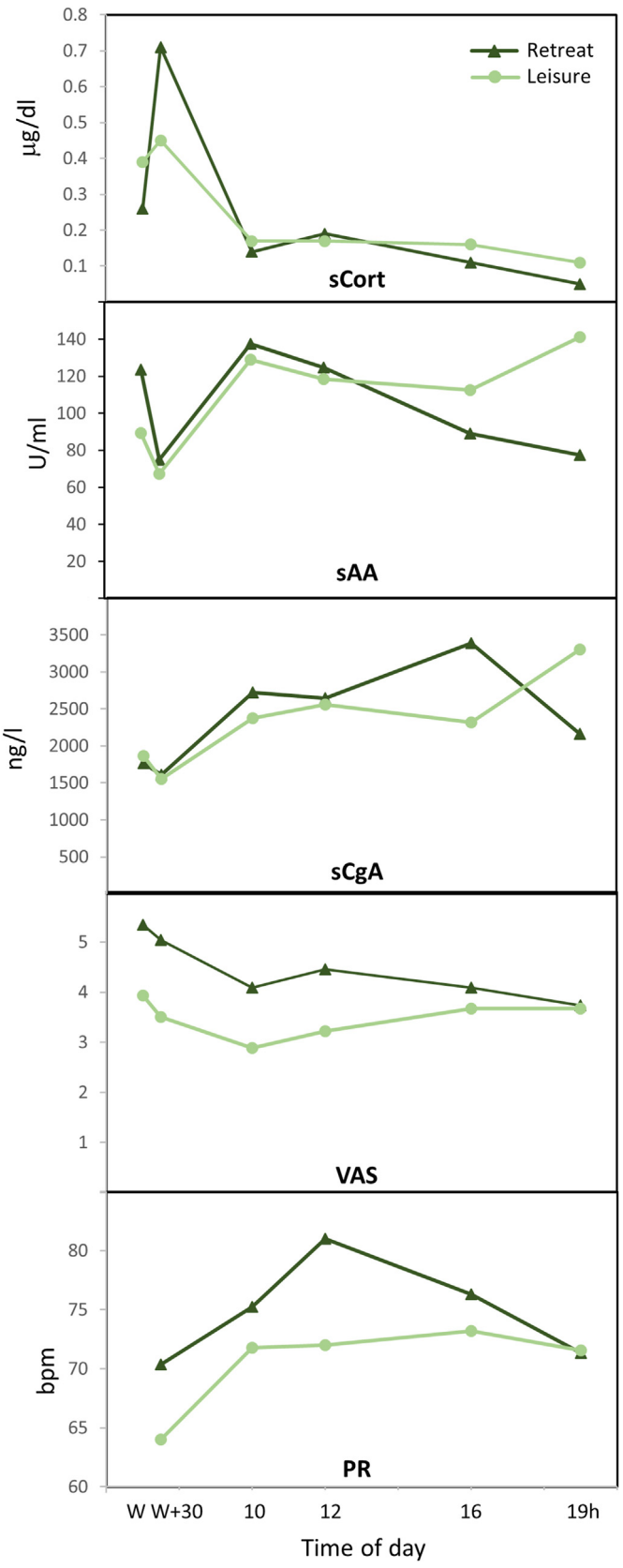

Figure 2. Comparison of sCort, sAA, sCgA levels, VAS scores, and PR between participants during meditation retreat or a general leisure day. (n = 32).

indicating that sCgA was present in the submandibular gland and not the other salivary glands [15]. Also, there are only a few studies on the diurnal pattern of sCgA [22, 23].

VAS was used to confirm the subjective feeling of the participants. The results showed that VAS scores corresponded with the mood of the day as the scores were higher on a stressful day and lower on a relaxing day as well as being in line with sCort and sAA, but not with sCgA. The average VAS scores on each day, though significant, suggested that the mood might not be drastically different since VAS scores centered around 5 which was suggestive of neutral feeling. This is an ecological momentary assessment type of study, so the stressful event or the relaxing event might not be that intense. Still, it provided insight into the naturalistic trend of the biomarkers.

For PR, the results were as expected as the daily mean of PR on a stressful day was higher than that on a relaxing day. PR on an ordinary day without saliva collection was similar to PR recorded on a day with saliva collection suggesting that the saliva collection process did not interfere with mood or increase stress.

Of highly interesting note in our work is the effect of mindfulness practice during a one-week meditation retreat for a novice. The results showed that in participants practicing mindfulness the levels of sCort were lower in the evening compared with participants on a general leisure day. This is in line with a recent meta-analysis which has concluded that meditation decreased blood cortisol [32]. CAR also showed more fluctuation during a meditation retreat. Previous studies have suggested that more fluctuation of CAR indicated better adaptive nature to stress response [33, 34]. However, no conclusion has been reached regarding this aspect.

For sAA, the levels were lower only at one time point in the evening. This pattern was clearly different from a general leisure day or an ordinary day. Normally, the diurnal sAA would showed a higher level as time passes towards the evening. While practicing mindfulness obviously brought this trend downward in an opposite direction. This is the first study showing the effect of mindfulness practice on the diurnal pattern of sAA in healthy individuals. There are only a few studies on mindfulness and sAA levels. A previous workplace-based mindfulness intervention found decreased sAA levels after an 8-week program in surgical intensive care unit personnel [35]. That study measured sAA levels at one time point. Another experiment found that sleep-focused mind-body intervention but not mindfulness meditation attenuated waking sAA levels but not at other time points in cancer survivors with sleep problems [36]. Also, the study did not find sCort to change after mindfulness practice. A recent ecological momentary assessment study on three-month mindfulness intervention found total sAA levels to decrease compared with a control group after the intervention but no change in the sCort levels [37]. In the current study, we measured sAA levels during a meditation retreat not after the practice so it may reflect the acute effect of mindfulness practice better. Having no dinner during a retreat did not likely affect the sAA levels since a previous study has found that fasting had no effect [38], and one study even found fasting to increase sAA levels [39].

There was no difference between mindfulness practice and a general leisure day on sCgA levels. No previous study was performed in this aspect. More studies are needed. It's interesting to note that the impact of mindfulness was observable since day 2 (data not shown for sAA) or day 4 of a retreat, suggesting that the benefit of mindfulness practice might be evident earlier than expected. Most previous studies investigated a full course of generally at least 2 months of the program.

In contrast to the results on three mood days, VAS scores during a meditation retreat were higher than those on a general leisure day. This is where subjective feelings were incongruent with the objective measure of salivary markers. It could be that the participants in a meditation retreat found the practice not that relaxing, they had to behave and stick to the schedule during the practice such as waking up at 5:00 h. PR

during a meditation retreat was higher than on a general leisure day only at one time point (12:00 h). We are not certain why this is the case but it could be that this was the period after rest where the participants had to hurry back to practice meditation in a group after an individual break. However, the daily mean of PR was not different between the two types of relaxing days.

There are some limitations to this study. First, the sample size was rather small especially in subgroup analysis (only 23 vs 9 participants in the meditation and the leisure groups, respectively). Second, we compared only one day of each mood. Repeating measurements for several days of each mood might give more prominent results. Third, the participants got to choose the day they expected to have a certain state of mind in advance. In actuality, there could be many factors that influence the mood each day. This might not truly match the mood that day. Also, the sequence and the spacing among three mood days were varied. This could further confound the results. However, we tried to control this factor with VAS. Still, the mood appeared to not be intensely relaxed or stressful as VAS scores centered around 5 on all three days. This is the limitation of the naturalistic study. Fourth, as mindfulness practice is very subjective, we did not control for the efficiency of mindfulness practice like using a standard questionnaire. Lastly, the long-term effect of mindfulness practice was not followed. Also, our study more likely reflected the short-term effect of the current state of mind on a particular day. The presence of chronic stress was not explored in the current study. It should be further investigated if chronic stress would affect the diurnal patterns of these markers in a similar manner.

5. Conclusion

Different states of mind were associated with different diurnal patterns of the salivary markers. The levels of sCort and sAA at certain time points were higher on a stressful day. Our results confirm the validity of using sCort and sAA as stress markers. VAS scores and PR gave a similar trend. While the results on sCgA levels were ambiguous and not in line with those of sCort and sAA, casting doubt in its usability as a stress marker. Moreover, a general relaxed state of mind did not affect sCort levels but different types of relaxation were differentially associated with sCort levels as during a general leisure day, sCort levels were higher in the evening with flatter CAR compared with those during mindfulness practice. The levels of sAA also decreased in the evening during mindfulness practice. Interestingly, even in new beginners who had been practicing mindfulness for only a few days, the biological responses were different from those on a general leisure day. Moreover, subjective and objective feelings may not go hand in hand. Even though the participants might not feel that relaxed, their bodies showed responses different from those not practicing mindfulness as evinced from the levels of biomarkers. Future studies should seek to answer the basic physiology of sCgA regulation as well as the long-term effect of chronic stress and mindfulness practice.

Declarations

Author contribution statement

Nattinee Jantaratnotai: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Thi Kim Anh Do; Manita Tammayan: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Praewpat Pachimsawat: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This study was supported by the CIF and CNI grant, Faculty of Science, Mahidol University as well as a grant from Mahidol University and the Office of National Higher Education Science Research and Innovation Policy Council through Program Management Unit for Competitiveness (C10F630097).

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e10506.

Acknowledgements

We thank Assist. Prof. Chulaluk Komoltri for statistical consultation.

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