

Hypothalamic-pituitary-adrenal axis differentially responses to morning and evening psychological stress in healthy subjects

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Abstract

Aim: The hypothalamic-pituitary-adrenal (HPA) axis responds to changing environmental demands including psychological stressors. The aim of the present study was to assess whether the time of day effects on the acute response of HPA axis activity to acute psychological stress.

Method: We studied 27 healthy young subjects. The subjects were participated two experiments as follows. In the first experiment, subjects were instructed to keep their regular sleep schedule for 2 weeks which were measured by using a wrist-worn activity monitor. Afterward, to evaluate a diurnal rhythm of salivary cortisol, eight saliva samples were collected during waking period every 2 hours from when the subjects woke up. In the second experiment, the subjects were randomly assigned to two groups. The Trier Social Stress Test (TSST) was performed either in the morning (n = 14) or in the evening (n = 13). We measured diurnal rhythm of salivary cortisol and stress response of salivary cortisol and heart rate by the TSST. Morning and evening tests were started at 2 hours and 10 hours after woke up, respectively.

Results: All subjects showed a normal diurnal rhythm of salivary cortisol concentration, with a peak in the morning immediately after awaking and a minimum in the evening. The salivary cortisol response after the TSST was significantly increased from the prestress level in the morning but not in the evening.

Conclusion: The HPA response to acute psychological stress was more pronounced in the morning than in the evening, correlating with the circadian regulation of cortisol synthesis.

KEYWORDS

circadian rhythm, hypothalamic-pituitary-adrenal axis, salivary cortisol, time of day, Trier Social Stress Test

1 | INTRODUCTION

The Hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenal medullary (SAM) system play a central role in stress

response. Firstly, the SAM system quickly reacts to stress and thereby elicits increase of heart rate. Secondary, the HPA axis reacts more slowly by secreting the glucocorticoid hormone, including corticosterone in rodents and cortisol in humans. The cortisol is the

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primary stress hormone in humans as an index of stress response of HPA axis activity. The cortisol response to acute psychological stress is influenced by subject's age,^{1,2} sex,^{3,4} and mood states.^{5,6} With respect to the effect of sleep on the cortisol level, partial and total sleep deprivation increase basal level of HPA axis activity.^{7,8} There is also evidence that shorter sleep duration and lower sleep efficiency are associated with higher cortisol response to acute psychological stress.⁹

Regarding circadian rhythm of the HPA axis activity, cortisol has a distinct circadian rhythm with a higher level in the early morning and lower in the evening in humans.^{10,11} This rhythm is regulated by the central circadian pacemaker of the suprachiasmatic nucleus (SCN) via the preautonomic paraventricular nucleus (PVN) neurons projecting to the sympathetic preganglionic intermediolateral neurons of the spinal cord and splanchnic nerve innervation of the adrenal cortex.^{12,13}

The Trier Social Stress Test (TSST) is the most widely used standardized laboratory stressor for human psychological stress research.¹⁴ As for the salivary cortisol levels, the TSST leads to two- to fourfold increase from the prestress baseline levels.¹⁴ In addition, the HPA axis activity response to the TSST is modulated by the time of day when the test is performed.¹⁵ Most of previous studies using the TSST were conducted in the morning and afternoon.^{16–20} Previously, Kudielka et al²¹ have reported that the ACTH and cortisol responses to the TSST are higher in the morning (begun between 09:00 hours and 10:00 hours) compared to the afternoon (begun between 15:00 hours and 16:00 hours). The time of day effect of HPA axis response to TSST is limited comparing morning vs afternoon but not evening (after 17:00 hours). As acknowledged by the authors, a limitation of the previous study²¹ is that the times of TSST were determined by the local clock time and sleep efficiency in individual subjects before the TSST were not measured. This may have obscured the influence of circadian rhythm and sleep duration on the HPA axis activity. In the present study, we examined whether the HPA axis differentially responds to morning and evening psychological stress by TSST in healthy subjects. The times of TSST were determined based on the individual differences in circadian rhythms of salivary cortisol and sleep-wake cycle.

2 | METHODS

2.1 | Subjects

A total of 27 healthy young subjects, 7 females and 20 males, completed the study (mean age \pm SD, 20.5 \pm 1.9 years) as paid volunteers. All subjects were recruited through advertisements at the Hokkaido University in Japan. They were nonobese (mean body mass index \pm SD, 21.2 \pm 2.4 kg/m²) and were in good physical condition. They did not have jobs in the early morning or late night and rotating night shift. All subjects had no personal history of psychiatric, endocrine, or sleep disorders. Before starting the experiment, the subjects completed a questionnaire of morningness-eveningness preference (Morningness-Eveningness questionnaire: MEQ)²² and mood states (Profile of Mood States 2nd Edition: POMS2).²³ The

mean of MEQ score in all subjects was 53.5 \pm 5.9 (mean \pm SD), and total mood disturbance (TMD) score of POMS2 was 46.2 \pm 5.1, respectively. The mean and distribution of MEQ and TMD scores were essentially normal.^{22,23} All subjects gave written informed consent prior to entering the study, which allowed them to withdraw from the experiment whenever they wanted. This study was approved by the ethical committee of Hokkaido University Graduate School of Education (no. 17-41) and conducted according to the Declaration of Helsinki.

2.2 | Experimental protocol

Beginning 2 week prior to the experiment (baseline), the subjects were instructed to keep their regular sleep-wake cycle at night and to conduct a self-recording of a sleep diary. To evaluate objective sleep and sleep quality, they were instructed to wear a data collection device (MotionWatch 8; Camtech Co. Ltd., Actiwatch-L; Minimitter, Bend, OR, USA), which recorded wrist activity and light intensity. On the last day of the baseline period, diurnal rhythm of salivary cortisol level was estimated. The subjects were instructed to collect saliva samples at 2 hours intervals from right after waking up and repeated eight times. In addition, the subjects were instructed to prohibit taking caffeine and performing exercise throughout the day which are known to influence the cortisol level via activating the sympathetic system. Saliva samples were collected with a cotton swab (Sarstedt, Numbrecht, Germany), placed in the subject's mouth for 3 minutes. After sampling, saliva samples were immediately frozen in -30°C until the assay.

Within one week after the baseline period, all subjects underwent the TSST for investigating the psychological stress response of HPA axis in laboratory setting.¹⁴ A slight modification was made to the standard TSST protocol.¹⁴ Figure 1 illustrates the experimental protocol of TSST. After arrival at the laboratory, subjects rested for 30 minutes in room A (prestress period). The subject was taken to a second room (room B) by the experimenter where three trained experts were already sitting at a table and video camera was installed. In room B, the subject sat on a chair in front of the experts and was interviewed about personal information by the experts, and then the experts explained task the subject would have to perform subsequently for 5 minutes (anticipation period). Next, the subjects were asked to step in front of the video camera and instructed to start free speech for 5 minutes and subsequent performance of a mental arithmetic for 5 minutes (test period). Afterward, the subject returned to room A and rested for 30 minutes (poststress recovery period). Saliva samples were taken at each phase of the TSST (30 minutes after arrival at the laboratory, 0, 10, 20, 30 minutes after the TSST) by using a cotton swab. Beat-to-beat heart rate (HR) was monitored continuously throughout the experiment.

To determine the time of day effect of acute stress response on the HPA axis, the subjects were randomly assigned to two groups by using random allocation software GraphPad Software (GraphPad Software Inc., CA, USA), morning group ($n = 14$; 10 males, 4 females) and evening group ($n = 13$; 10 males, 3 females) (Table 1). The subjects

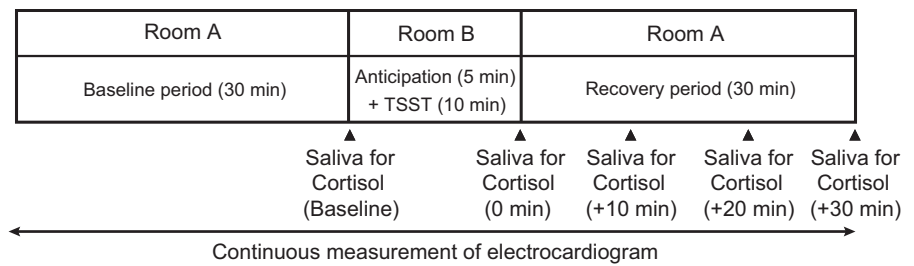


FIGURE 1 Experimental protocol of Trier Social Stress Test (TSST)

performed TSST only one time either in the morning or in the evening, because there is a high degree of habituation of the HPA axis response with repeated TSST.^{20,24} The time of day is standardized with respect to the habitual sleep-wake cycle, where the wake-up time was defined as zeitgeber time 0 (ZT0). Therefore, the morning test was started at ZT2 (begun between 08:00 hours and 10:00 hours) and the evening test was at ZT10 (begun between 17:30 hours and 20:00 hours), respectively. For seven female subjects, the TSST was only done during their follicular phase, since the significant cortisol response to the TSST was only demonstrated during the follicular phase but not the luteal phase.²⁵

2.3 | Sleep measurement

Subject's sleep quality and quantity during the 2-week baseline period was determined from the sleep diary and the Actiware-Sleep version 3.4 software (Minimitter, Bend, OR, USA), at 1 minutes epochs, and medium sensitivity. We analyzed the mean of actiwatch based sleep period time (SPT), total sleep time (TST), and percent of sleep efficiency. The SPT was defined as the length of the sleep interval from the first epoch counted as sleep to the last epoch counted as sleep in the main sleep interval. The TST was defined as total number of minutes counted as sleep in the main sleep interval, in hours. The sleep efficiency was defined as the ratio of TST to SPT, as a percentage.

2.4 | Salivary cortisol

Salivary free cortisol concentrations were measured by using a salivary cortisol ELISA kit (kit No. 1-3002, Salimetrics LLC, State

TABLE 1 Descriptive statics for the morning and evening test group subjects

	Morning test group	Evening test group	P
N	14 (10 men, 4 women)	13 (10 men, 3 women)	
Age, y	20.9 ± 1.9	21.2 ± 2.6	0.941
Body mass index	21.3 ± 2.4	20.9 ± 2.3	0.645
MEQ score ^a	52.1 ± 6.7	55.4 ± 4.8	0.287
TMD score ^b	47.6 ± 5.2	44.0 ± 4.3	0.163

Values are means ± SD.

^aMorningness-eveningness questionnaire (MEQ) score (higher scores = more morningness).

^bTotal mood disturbance (TMD) score of POMS2 (higher score = greater mood state disturbances).

College, PA, USA). The lowest detection limit of the assay is 0.33 nmol/L. Inter-assay and intra-assay variance were 3.0% and 2.6%, respectively. The incremental area under the curve (iAUC) was calculated by the trapezoid method using 0, 10, 20, 30 minutes salivary cortisol level after the TSST.

2.5 | Statistical analysis

Statistical calculations were performed using a nonparametric test, since the salivary cortisol data were classified as not normally distributed by the Shapiro-Wilk normality test. Analysis of time series data was tested with Friedman test and post hoc Wilcoxon signed-rank test. Comparison of two independent values was analyzed by Mann-Whitney *U* test. Significant correlation between two values was analyzed by Spearman rank order correlation test. Graph Pad Prism version 7 (GraphPad Software Inc., San Diego, CA) was used for all statistical analysis. A *P* value <0.05 considered presence of a statistically significant difference.

3 | RESULTS

3.1 | Sleep measurements prior to the TSST

The mean of SPT, TST, and sleep efficiency were 6.9 ± 0.8 hours (mean ± SD), 6.1 ± 0.7 hours, and 89.5 ± 5.2% in all subjects for 2 weeks prior to the TSST. These parameters were not significantly different between the morning test group (SPT, 6.8 ± 1.1 hours; TST, 6.1 ± 0.8 hours; sleep efficiency, 89.7 ± 4.8%) and evening test group (SPT, 6.9 ± 0.5 hours; TST, 6.2 ± 0.7 hours; sleep efficiency, 89.2 ± 5.9%).

3.2 | Diurnal rhythm of salivary cortisol

To determine the time of TSST at two different circadian phases, diurnal rhythm of salivary cortisol was examined eight times a day every 2 hours from ZT0 (the time of woke up) to ZT14 on the last day of the baseline period. Figure 2 indicates the diurnal rhythm of salivary cortisol measured on the last day of baseline period in the all subjects (*n* = 27). Friedman test revealed significant diurnal rhythmicity of salivary cortisol (*P* < 0.01). The salivary cortisol level showed a peak value at ZT0 (10.2 ± 0.8 nmol/L, mean ± SEM) and lowest value at ZT14 (1.0 ± 0.2 nmol/L) on average (Figure 2A). There was a significant difference in between ZT2 (7.0 ± 0.7 nmol/L) and ZT10 (3.1 ± 0.4 nmol/L) when the TSST was undertaken in the

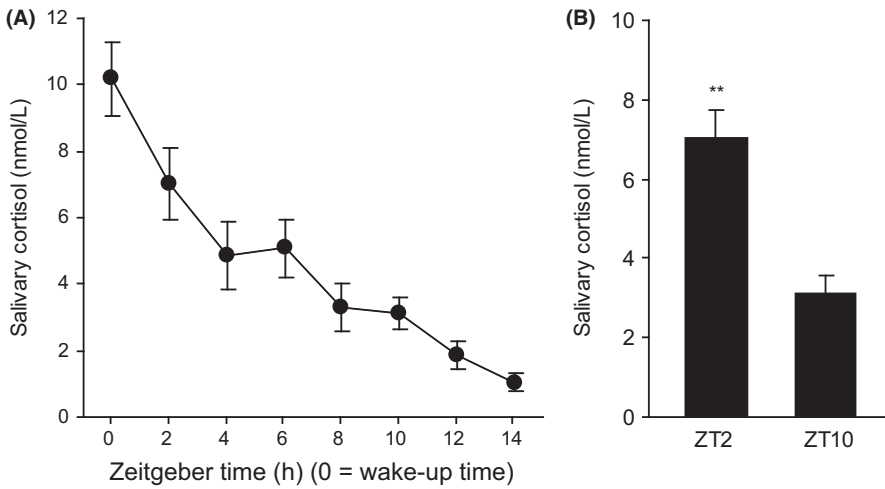


FIGURE 2 Mean diurnal rhythm of salivary cortisol on the baseline condition. Panel A indicated diurnal rhythm of salivary cortisol level measured on the last day of baseline condition (Mean \pm SEM, $n = 27$). Panel B indicates the superimposed salivary cortisol levels at ZT2 and ZT10. $**P < 0.01$ vs ZT10 by Wilcoxon signed-rank test followed by Friedman test

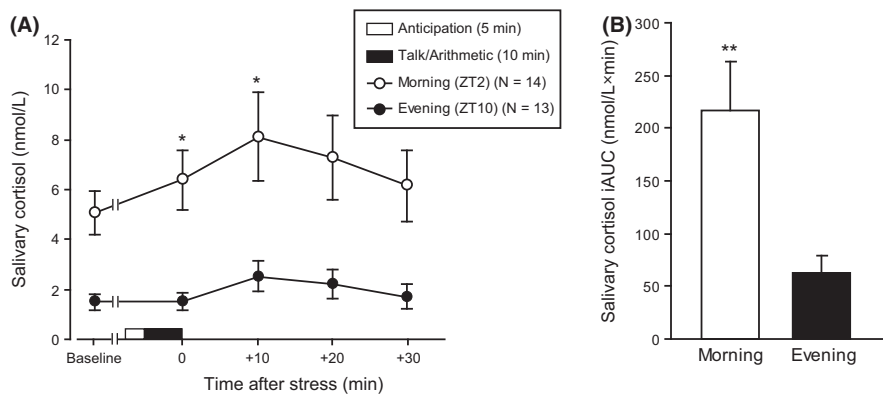


FIGURE 3 Salivary cortisol responses before and after the Trier Social Stress Test (TSST) in the morning and evening. Panel A indicates the mean salivary cortisol levels before and after the TSST in the morning (open circles) and evening (closed circles). Open and closed horizontal bars indicate 5 min anticipation and 10 min TSST periods, respectively. $*P < 0.05$ vs baseline levels by Wilcoxon signed-rank test followed by Friedman test. Panel B indicates an incremental area under the curve of cortisol (iAUC_{0-30 min}) in the morning (open bar) and evening (closed bar) test groups. $**P < 0.01$ vs evening test by Mann-Whitney U test

morning and evening tests, respectively ($P < 0.01$, Wilcoxon signed-rank test) (Figure 2B).

3.3 | Salivary cortisol and heart rate responses to TSST

Salivary cortisol levels of mean time course during the TSST and incremental area under the curve of cortisol (iAUC_{0-30 minutes}) from the morning (ZT2) test and evening (ZT10) test groups are shown in Figure 3. In the morning test group, Friedman test revealed significant changes of salivary cortisol level on the course of TSST ($P = 0.015$). After the TSST, the salivary cortisol levels at 0 minutes (6.4 ± 1.2 nmol/L) and 10 minutes (8.1 ± 1.8 nmol/L) were significantly increased from that at the pretest level (5.1 ± 0.9 nmol/L) ($P < 0.05$, Wilcoxon signed-rank test, Figure 3A). On the other hand, in the evening test group, salivary cortisol levels showed a similar trend with the morning test group but did not show the significant changes on the course of TSST ($P = 0.08$, Friedman test, Figure 3A). Incremental area under the curve of cortisol (iAUC_{0-30 minutes}) in the morning test group was significantly higher value than that in the

evening test group (morning vs evening, 217 ± 47 nmol/L \times minutes vs 63 ± 19 nmol/L \times minutes, $P = 0.0014$, Mann-Whitney U test, Figure 3B). During the TSST, the means of heart rate were significantly increased by ca. 15 beats/min on average from those at the pretest values in each group, whereas there was no significant difference between the two groups (Figure 4). There was no significant relationship between the subject's sleep efficiency and iAUC_{0-30 minutes} of salivary cortisol ($n = 27$, $r = -0.183$, $P = 0.218$, Spearman rank order correlation test).

4 | DISCUSSION

We could demonstrate that acute stress response to the HPA axis induced by the TSST showed the significant time of day difference (morning vs evening) in normal young subjects. The HPA axis activity has a powerful response to acute psychological stress in the morning rather than in the evening (Figure 3), whereas the sympatho-adrenal medulla system in terms of increase of HR did not show a significant time of day difference between the two groups (Figure 4).

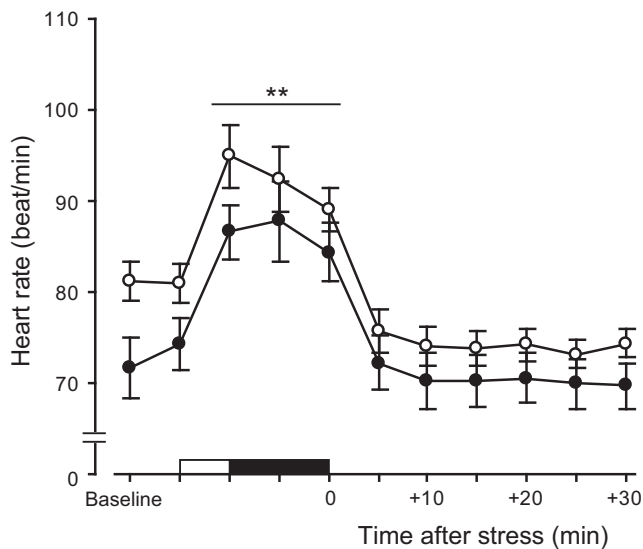


FIGURE 4 Heart rate responses before and after the Trier Social Stress Test (TSST) in the morning and evening. $**P < 0.01$ vs baseline in each group by Wilcoxon signed-rank test followed by Friedman test

With respect to time of day effect of cortisol response to the TSST, the cortisol response to the TSST in the morning and afternoon showed a similar and significant increases, but the AUC was significantly higher in the morning than in the afternoon.²¹ In the present study, the TSST in the evening showed no significant increase of cortisol response (Figure 3). Previous and present observations suggest that the HPA axis response to acute psychological stress attenuates in the evening compared to the morning and afternoon.

The HPA axis activity in terms of cortisol level in humans shows a distinct circadian rhythm with higher level in the morning and lower in the evening.²⁶ In mammals including humans, the circadian pacemaker is located in the SCN of anterior hypothalamus. The SCN entrains to an environmental LD cycle, and then the time signals are transmitted to the adrenal gland via the central sympathetic intermediolateral cell column of the spinal cord.¹³ As shown in Figure 2, circadian rhythm of salivary cortisol concentration showed a normal diurnal pattern, suggesting that their circadian pacemaker could entrain to an external LD cycle and drive the HPA axis rhythmicity.

Regarding the present findings that salivary cortisol concentrations during the TSST and $iAUC_{0-30 \text{ minutes}}$ were higher in the morning than in the evening (Figure 3). Although the underlying mechanism about time of day effect of HPA axis response to stress is still unknown, cortisol level at the prestress baseline would be related to net increase in cortisol after exposure to stressors. In humans, the response of the adrenal cortex to ACTH is a major source of variation in the cortisol response to stressors.²⁷ The animal studies also demonstrated that adrenal responsiveness to the ACTH exhibits a daily rhythm, with a higher sensitivity leading to higher corticosterone release in the evening than in the morning.^{28,29} If humans and rodents had the same circadian rhythm of adrenal responsiveness to ACTH, the present finding that psychological stress in the morning produced a greater increase of cortisol

secretion rather than in the evening is consistent with the previous studies in rodents. Taken together previous studies in rodents^{28,29} and in humans,²¹ we believe that the HPA axis activity response to acute psychological stress is under the control of circadian rhythm of ACTH and glucocorticoid hormone which are strictly regulated by circadian pacemaker in the SCN. In addition to a recent study,³⁰ the salivary cortisol concentration after high intensity exercise as an index of exercise stress is higher in the morning than in the evening. Collectively, the HPA axis activity responses to exercise and psychological stresses depend on time of day when subjects were exposed to stressors. With respect to the subject's chronotype of MEQ, we attracted the subjects whose chronotypes were intermediate type. There are in line of studies demonstrate that cortisol awakening response, total cortisol secretion over the day, and cortisol secretion after exercise stress are associated with an individual chronotype.³⁰⁻³³ Further studies would be needed to assess whether the HPA axis activity response to psychological stressors depends on the time of day and individual chronotypes in humans.

In summary, we could demonstrate that the HPA axis activity response to acute psychological stress is higher in the morning than in the evening, relating with circadian rhythm of cortisol concentration. In clinically, it is important to concern the subject's circadian rhythm and time of day when assess individual HPA axis activity responses to various stressors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

AUTHOR CONTRIBUTION

Y.Y designed the study and wrote the first draft of the manuscript. Y.Y, H.M, K.U undertook the study. Y.Y undertook analysis. All authors contributed to and have approved the final manuscript.

DATA REPOSITORY

Supporting Information in Table S1.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

All study protocols were approved by the ethical committee of Hokkaido University Graduate School of Education.



INFORMED CONSENT

Written informed consent was obtained from all subjects before starting the experiment.

REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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