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# The cortisol awakening response and autonomic nervous system activity during nocturnal and early morning periods

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## Abstract

The current study focuses on autonomic nervous system activity during sleep as a physiological aspect of sleep quality, and investigated the associations between the cortisol awakening response (CAR) and autonomic activity during sleep and after awakening.

Ambulatory electrocardiograms were obtained from 20 participants, who also provided saliva samples (at the time of awakening, and 30, 45, and 60 min after awakening) and rated the subjective quality of their sleep at home. Autonomic activity was assessed with the Lorenz plot indices, cardiac sympathetic index (CSI) and cardiac vagal index.

Total salivary cortisol secretion after awakening was calculated as area under the curve with respect to ground ($AUC_G$) and increase ($AUC_I$). After controlling for confounding factors, including sleep duration and awakening time, cortisol $AUC_G$ and $AUC_I$ were both found to be negatively correlated with CSI during the 30 min before and after awakening: before ($r$ = –0.526 and –0.601 respectively) and after ($r$ = –0.540 and –0.493 respectively). Self-reported sleep quality was not associated with the CAR.

These results suggest that the CAR is negatively affected by basal sympathetic activity immediately before and after awakening, but not affected by subjective sleep quality. Physiological arousals around the time of awakening might inhibit the CAR.

ORIGINAL ARTICLE

## INTRODUCTION

Free cortisol levels increase by 50–60% within the first hour after awakening. Recent studies have demonstrated that this acute increase in cortisol levels, the cortisol awakening response (CAR), can serve as a useful index of hypothalamus–pituitary–adrenal (HPA) axis activity. There are numerous reports of the CAR being associated with chronic stress, and with physical and psychiatric disease (Clow *et al*. 2004).

Cortisol secretion is closely linked to the sleep-wake cycle, and several studies have reported that sleep-related factors can affect the CAR. For example, a pronounced CAR has been observed in early awakeners and long sleepers (Kudielka & Kirschbaum, 2003; Späth-Schwalbe *et al.*1992). Other studies have reported an association between sleep quality and the CAR, including a negative correlation between cortisol levels after awakening and subjective ratings of sleep quality such as a higher frequency of nightly awakenings (Backhaus *et al*. 2004). Further, Lasikiewicz *et al*. (2008) found poorer sleep quality to be associated with a blunted CAR in middle-aged adults. However, experimental studies have found sleep disruption (forced nightly awakening) to not affect the CAR (Hucklebridge *et al*. 2000; Dettenborn *et al*. 2007). Therefore, the relationship between sleep quality and CAR remains to be resolved.

The current study focuses on autonomic nervous system activity during sleep as a physiological aspect of sleep quality. Increased sympathetic activity has been observed in people with poorer sleep quality (Bonnet & Arand, 1998). Some studies have reported a relationship between activity of the autonomic nervous system and activity of the HPA axis (Kizildere *et al*. 2003; Young *et al*. 2005). It is thus possible that autonomic activity contributes to any association between subjective sleep quality and the CAR. In line with this, the current study investigated the extent to which autonomic activity during nocturnal and early morning periods is associated with subjective sleep quality and the CAR.

## METHODS

*Participants*

The participants were 20 healthy students (12 males, 8 females); their mean age and body mass index (BMI) was 23.4 years and 20.8 kg/m$^2$, respectively. All participants were non-smokers, and none used medications or dietary supplements known to affect HPA axis activity. Any influence of sex hormones on HPA axis and autonomic activity was minimized by having the females participate during the late luteal or early follicular phase of their menstrual cycle. Written informed consent was obtained from all participants, and the study was approved by the University's ethical committee.

*Procedure*

Ambulatory electrocardiograms were obtained with a Digital Holter Recorder (FM-120, Fukuda Denshi, Japan); this was fitted in the evening and removed the next morning at the participant's home. Saliva samples were collected with Salivette (Sarstedt Ltd.) at awakening, and 30, 45, and 60 min after awakening. Participants were instructed to maintain their regular life, including sleep patterns, but to not consume alcohol or take a bath whilst the electrocardiogram monitor was fitted. They were also asked to stay in bed for 30 min after waking, and to refrain from eating, drinking, or brushing their teeth during this period and for the 30 min thereafter.

After providing the first saliva sample, participants completed the OSA sleep inventory (Yamamoto *et al*. 1999). This questionnaire has 16 items, each with a 4-point bipolar response format, and is designed to measures 5 qualities of sleep: sleepiness at awakening, difficulty in getting to sleep and maintaining sleep, dreams, healing tiredness, and duration of sleep (with higher scores indicating better sleep quality). Participants also recorded their times for going to bed and awakening, and rated their acute stress level on a 4-point bipolar scale after providing the first saliva sample. The mean sleep duration was 5.91 ± 0.76 hours, and the mean time of awakening 6:47 am (± 52 min). We note that heart rate increased in close temporal proximity to self-reported awakening times (<10 min difference), suggesting that the participants provided their first saliva sample as per the study protocol.

*Cortisol assay*

Saliva samples were stored at –20 °C. Thawed samples were centrifuged at 3,000 rpm for 5 min, and the concentration of cortisol determined by an enzyme immunoassay using the EIA Kit (Salimetrics LLC., USA). The inter-assay and intra-assay variations were 6.9% and 6.2%, respectively.

*Assessment of autonomic activity*

Autonomic nervous system activity was assessed with the Lorenz plot indices, cardiac sympathetic index (CSI) and cardiac vagal index (CVI). Interbeat intervals were plotted as a function of the previous intervals, from which 2 components of the interbeat interval fluctuation were calculated, length of the transverse axis (T), and the longitudinal axis (L). Pharmacological experiments with sympathetic and parasympathetic antagonists have demonstrated that L/T ratio (CSI) and L × T (CVI) quantify sympathetic and vagal activity, respectively (Toichi *et al*. 1997).

*Data reduction and statistical analyses*

Cortisol concentrations were square root transformed before analysis. Total salivary cortisol secretion after awakening was calculated as area under the curve with respect to ground ($AUC_G$) and increase ($AUC_I$), using

the trapezoidal method (Pruessner *et al.* 2003). Values for CSI and CVI were determined for the total sleep period, and for each half-hourly period during the 2 hours before awakening and the hour after awakening (before awakening: 0–30 min [B1], 30–60 min [B2], 60–90 min [B3], 90–120 min [B4]; after awakening: 0–30 min [A1], 30–60 min [A2]).

Cortisol, CSI, and CVI data were analyzed with one-way analysis of variance (ANOVA) for repeated measures and degrees of freedom were adjusted with the Greenhouse-Geisser correction where appropriate. Post hoc comparisons were adjusted with the Bonferroni method. The effects of CSI or CVI on the CAR were analyzed by entering CSI or CVI as a continuous variable into the ANOVA for cortisol (analysis of covariance). Correlation analyses (Pearson correlation) were also conducted to examine the relationships between autonomic activity (CSI and CVI), self-reported sleep quality, and cortisol parameters (cortisol levels at awakening, $AUC_G$, and $AUC_I$). These relationships were further analyzed with partial correlations, adjusting for gender, time of awakening, sleep duration, and acute stress level.

## RESULTS

Cortisol levels at awakening and at 30, 45, and 60 min after awakening were 6.9, 14.1, 15.3, and 14.4 nmol/l, respectively. Cortisol levels at 30, 45, and 60 min were higher than at awakening ($F$ (1.6/29.7) = 21.4, $p$<0.01). CSI was significantly higher during the A2 period than during A1, B1, B2, and B4 ($F$ (3.4/65.4) = 6.50, $p$<0.01). When entering CSI during the B1 period into the ANOVA for cortisol, a repeated factor (0, 30, 45, 60 min) by CSI interaction was significant ($F$ (1.7/ 30.2) = 5.1, $p$<0.05), which indicate that CSI during B1 affected the increase in cortisol levels after awakening. The effects of CSI during B2, B3, B4, A1, and A2 periods or CVI on the CAR were not significant.

In line with ANOVA results, there was a significant association between cortisol $AUC_I$ and CSI during B1 ($r$ = –0.558, $p$<0.05; Figure 1). As shown in Table 1, partial correlations indicate negative associations between each of cortisol $AUC_G$ and $AUC_I$ with CSI during both the B1 and A1 periods. The only significant association between autonomic activity and OSA sleep inventory scores was for the "Sleepiness at awakening" subscale and CSI during B1 ($r$ = 0.445, $p$<0.05), indicating that sleepiness increased as sympathetic activity decreased. There were no significant associations between OSA sleep inventory scores and cortisol parameters. The partial correlation analyses revealed no consistent associations of OSA sleep inventory scores with cortisol parameters and autonomic activity.

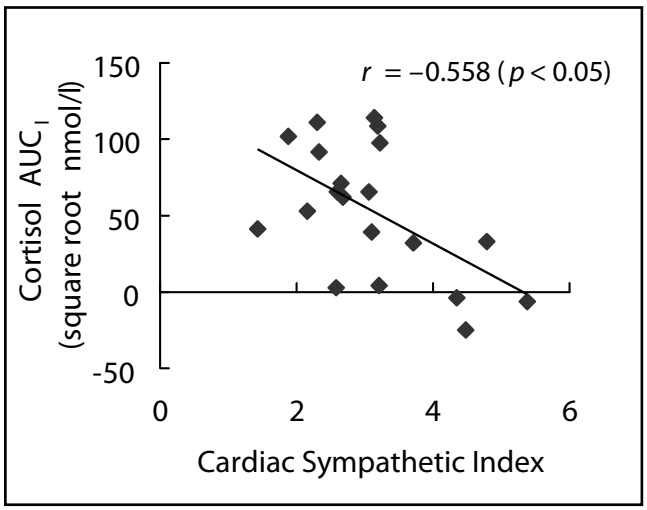


**FIGURE 1.** Sympathetic activity during a 30-min period before awakening and the cortisol awakening response ($AUC_I$)
$AUC_I$: Area under the curve with respect to increase.

## DISCUSSION

The main finding of the current study was that sympathetic activity during 30-min periods before and after awakening was moderately and inversely related to the CAR. Cortisol secretion shows a marked circadian

**TABLE 1.** Partial correlations between autonomic activity before and after awakening and cortisol awakening secretion [a)]

| | | Cortisol | | |
|---|---|---|---|---|
| | | **Awakening levels** | **$AUC_G$** | **$AUC_I$** |
| CSI | B4 | 0.424 | 0.309 | –0.154 |
| | B3 | –0.235 | –0.181 | 0.077 |
| | B2 | 0.013 | 0.039 | 0.028 |
| | Bl | 0.042 | –0.526 * | –0.601 * |
| | Al | –0.072 | –0.540 * | –0.493 † |
| | A2 | 0.287 | 0.279 | –0.032 |
| **total sleep period** | | **0.067** | **0.008** | **–0.068** |
| CVI | B4 | –0.122 | 0.042 | 0.180 |
| | B3 | –0.247 | –0.141 | 0.131 |
| | B2 | –0.170 | –0.027 | 0.166 |
| | Bl | –0.093 | –0.066 | 0.037 |
| | Al | –0.178 | –0.259 | –0.072 |
| | A2 | –0.388 | –0.178 | 0.251 |
| **total sleep period** | | **–0.180** | **–0.118** | **0.079** |

[a)] Adjusting for gender, time of awakening, sleep duration, and acute stress level.
CSI: cardiac sympathetic index; CVI: cardiac vergal index
$AUC_G$: Area under the curve with respect to ground
$AUC_I$: Area under the curve with respect to increase
B4: 90-120 min before; B3:60–90 min before; B2: 30–60 min before; B1: 0–30 min before; Al: 0–30 min after; A2: 30–60 min after
*$p$<0.05; †$p$<0.10

rhythm: typically lowest during the first half of nighttime sleep, with an abrupt elevation during the second half of this period, and peak levels after awakening. While a negative correlation between nocturnal cortisol levels and post awakening cortisol secretion was recently reported (Wilhelm *et al.* 2007), we believe the current study to be the first to suggest that sympathetic activity immediately before and after awakening affects the CAR.

Our finding may contribute to a better understanding of the associations between sleep quality and the CAR. Previous studies have reported a blunted CAR as being associated with poorer sleep quality (Backhaus *et al.* 2004; Lasikiewicz *et al.* 2008), but not with experimentally-disrupted sleep (Hucklebridge *et al.* 2000; Dettenborn *et al.* 2007). Chronically poor sleep quality has been related to increased sympathetic activity during sleep (Bonnet & Arand, 1998), suggesting that it is sympathetic activity that influences the CAR. The results of the current study support this idea, with sympathetic activity, but not subjective sleep quality, being associated with the CAR. Our findings also suggest that it is sympathetic activity immediately before and after awakening, rather than sympathetic activity during the whole night, which influences the CAR. Born *et al.* (1986) reported an association between cortisol secretion in the night and particular sleep stages, including rapid eye movement (REM) sleep. REM sleep is well known to involve changes in autonomic activity. In addition, it has been reported that arousals that are induced experimentally during sleep are followed by a temporary inhibition of nocturnal cortisol secretion (Späth-Schwalbe *et al.* 1991). In the current study, less sleepiness at awakening was associated with greater sympathetic activity. Considering this evidence, it seems that sleep-related physiological arousals around the time of awakening might inhibit the CAR.

There is reason to think that sympathetic activity inhibits HPA axis activity. Young *et al.* (2005) reported that the basal brain noradrenergic activity of humans, as estimated by clonidine challenge, was related to a decreased ACTH response to psychosocial stress. Another study (Kizildere *et al.* 2003) found an increased cortisol response to hCRH testing in participants administered a beta-adrenoceptor antagonist (propranolol). These results suggest that cortisol secretion is negatively regulated by basal sympathetic activity. Therefore, in the current study, an elevated sympathetic tone before and after awakening could have facilitated a decreased CAR.

While the current study identified an association between sympathetic activity and the CAR, it must be considered whether this could have been influenced by a difference in the time between actual awakening and provision of the first saliva sample. It has been previously reported that a delay in collecting the first saliva sample results in a lower value for the CAR: the response had already begun by the time of the first sample, and no further increase in cortisol levels was observed (Kunz-Ebrecht *et al.* 2004). It is possible that participants of the current study who had higher levels of sympathetic activity were awake for some time before providing their first saliva sample; this could have resulted in lower CAR values. However, we found that sympathetic activity was not correlated with awakening cortisol values (the first sample values), indicating that the CAR had not yet begun when saliva was first sampled by these participants. Given this, and the evidence for compliance with the study protocol, any effects of a difference in time between awakening and provision of the first saliva sample are likely to be small.

The findings of the current study are to be interpreted only within the context of certain limitations, one of these being the small number of participants from whom the data were obtained. A second limitation arises from the collection of saliva and recording autonomic activity occurring in the participants' home rather than controlled laboratory conditions. This is because in the previous studies saliva samples were collected at the participants' home and cortisol responses to spontaneous awakening were investigated, this design, however, has several disadvantages, including an inability to control the time of awakening or sleep duration. Thirdly, we did not establish a causal association between sympathetic activity and the CAR; thus, there is the possibility that other physiological or environmental factors could be involved. Pharmacological studies are needed to further, and more fully, explore the effects of sympathetic activity on the CAR.

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