Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase

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Abstract

Recent evidence suggested that the free cortisol response to awakening is influenced by awakening time in healthy younger adults (Edwards et al., 2001). In order to investigate this association further, 179 community-dwelling subjects of a large age range (4–75 yrs) participated in the present study. The sample consisted of 99 women, 67 men and 13 children. Subjects were instructed to obtain saliva samples directly after awakening as well as at 15, 30, 45, and 60 minutes thereafter. A first analysis revealed that salivary cortisol profiles after awakening in healthy subjects differed from profiles in subjects who reported health problems or a chronic disease (p = 0.02) with healthy subjects showing a larger cortisol response. Therefore, only healthy subjects were included in the following analyses.

Subjects woke up between 0455 and 1203 h. Time of awakening strongly influenced the course of morning cortisol levels. Cortisol profiles differed significantly between two wake-up groups (p<0.001). Similarly, group differences for cortisol increase (p = 0.03) and area under the curve (p = 0.05) were also significant, with more pronounced responses in early awakeners compared to late awakeners. The findings are discussed with respect to the circadian cortisol rhythm and the effects of light exposure. Age was correlated with the cortisol levels immediately after awakening (r = 0.2, p = 0.04), the area under the cortisol curve (r = −0.20, p = 0.05), and with time of awakening (r = −0.21, p = 0.04), respectively. No differences were found between males and females, or between profiles obtained during the follicular or luteal menstrual cycle phase. Also, no differences were observed between habitual smokers vs. non-smokers.

These data suggest that the morning cortisol response is influenced by the awakening time.
but not by menstrual cycle phase. Moreover, health status and age appear to have an impact on this marker of adrenocortical activity. Wake-up time, health status and age should therefore be controlled for in future studies measuring cortisol responses to awakening.

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1. Introduction

Cortisol, the most important glucocorticoid in humans, is the end product of the hypothalamus–pituitary–adrenal (HPA) axis. It shows a marked circadian rhythm with peak levels usually found in the early morning hours with decreasing concentrations thereafter (Weitzman et al., 1971; Curtis, 1972). Kirschbaum & Hellhammer (2000) recently summarized that waking up in the morning is associated with a 50–100% increase in cortisol, peaking about 30 minutes after awakening. The awakening cortisol peak occurs at the time of maximal adrenocortical activity, as previously shown by Späth-Schalbe et al. (1992). It is well-documented that free (‘unbound’) cortisol levels, as measured in saliva, increase rapidly within the first hour of awakening and that the response magnitude and time course of salivary cortisol levels after awakening are significantly related to various psychological and physical conditions, e.g., persisting pain, chronic stress, and burn out (Geiss et al., 1997; Schulz et al., 1998; Pruessner et al., 1999).

Despite its potential as a marker of adrenocortical status, previous studies have shown that the awakening free cortisol increase is characterized by both inter- and intra-individual variability (Pruessner et al., 1997; Wüst et al., 2000). Among the possible confounders, gender, smoking, and use of oral contraceptives each account for approximately 1–4% of the total variance observed (Pruessner et al., 1997; Wüst et al., 2000). A recently conducted larger study including more than 500 subjects reported that waking up spontaneously versus timed wake up (by alarm clock) had no significant impact on the awakening salivary cortisol pattern (Wüst et al., 2000). However, Born et al. (1999) convincingly showed that the circadian plasma ACTH and cortisol rhythm as well as the awakening response can be significantly modulated by subjects’ expectation of waking time (expected vs. unexpected awakening). HPA axis awakening responses were significantly higher in an ‘unexpected wakening’ condition compared to both a long and a short sleep condition. While Wüst et al. (2000) observed no effect of wake-up time, Edwards et al. (2001) recently reported a significant impact of wake-up time on free cortisol responses after awakening in the morning. The earlier subjects woke up, the larger was their salivary cortisol response to awakening. Interestingly, the earlier awokeners also had higher cortisol levels for several hours after awakening compared to the late awakeners (Edwards et al., 2001).

Since these observation could have a profound impact on study protocols which include the early awakening cortisol response, we investigated this issue in a larger
sample of community-dwelling individuals over a wide age range. Moreover, since no studies have investigated the impact of the menstrual cycle phase on the awakening cortisol response so far, a second aim of the present work was to compare cortisol levels in the first hour after awakening in women in the follicular versus luteal phase of the menstrual cycle.

2. Methods

2.1. Subjects

The total sample consisted of 179 community-dwelling volunteers aged 4–75 yrs (mean: 29.6 ± 1.1 yrs ± SEM). One hundred and five subjects reported to be healthy and not taking any medication (except oral contraceptives), while 74 indicated various health problems, chronic diseases, or regular medication use. Of the 105 healthy subjects, 53 participants were females, 52 were males (including six boys and two girls). The subgroup of women consisted of 12 women in the follicular phase of the menstrual cycle, 11 women in the luteal phase, 25 women using oral contraceptives and 2 postmenopausal women. Due to missing data, the menstrual cycle phase of one woman remained unknown. Detailed information concerning different compounds of the ingested oral contraceptives was not recorded. Seventy-eight individuals of the healthy sub-sample reported to be non-smokers, whereas 27 reported to be habitual smokers consuming 3 to 30 cigarettes per day. In the sub-sample of non-healthy subjects, 29 participants were smokers while 45 were non-smokers. None of the study participants worked in evening or night shifts.

2.2. Study protocol

Salivette sampling devices (Sarstedt, Rommelsdorf, Germany) were used for saliva collections. Subjects were instructed to collect a single morning cortisol profile at home. They obtained saliva samples directly after awakening as well as 15, 30, 45, and 60 minutes thereafter. Subjects were free to use an alarm clock or wake up spontaneously. Subjects were instructed not to brush their teeth before completing saliva sampling to avoid contamination of saliva with blood caused by micro-injuries in the oral cavity. Subjects were asked to refrain from food intake and beverages containing alcohol, caffeine or fruit juices as well as smoking during the sampling period. Besides these restrictions, subjects were free to follow their normal morning routines on the sampling day. All participants filled out a demographics questionnaire to record age, gender, menstrual cycle phase, use of oral contraceptives, smoking habits, health problems, and time of awakening. To assess health status, subjects were asked if they had an acute or chronic health problem (‘general health question’). Additionally, specific questions assessed if subjects suffered from cardiovascular, autoimmune/atopic/allergic, psychiatric, or other diseases. Subjects who indicated that they had acute or chronic health problems, at least one specific disease, or that they were on medication (other than oral contraceptives), were coded as non-healthy.
Sleep quantity or quality was not further assessed in this study. All saliva profiles were sampled during winter time between 12/1/2000 and 1/18/2001. Subjects did not receive monetary compensation for study participation.

2.3. Cortisol analysis

Salivary cortisol was assayed with a time-resolved immunoassay with fluorometric detection (DELFIA) as described previously in Dressendörfer et al. (1992). Intra- and interassay variability were below 12%, respectively.

2.4. Statistical analysis

Two-way analyses of variance (ANOVA) for repeated measures (five saliva samples) were performed to assess cortisol patterns and group differences due to health status, menstrual cycle phase, sex, smoking, and use of oral contraceptives. Greenhouse–Geisser corrections were applied where appropriate and only adjusted results are reported (Greenhouse & Geisser, 1959; Vasey & Thayer, 1987). An ANCOVA procedure was applied to investigate the impact of female menstrual cycle phase using ‘time of awakening’ as a covariate.

The cortisol increase was defined as the difference between the individual cortisol peak value (e.g., sample 2, 3, 4, or 5) and the cortisol level immediately after awakening (sample 1). The area under the response curve (AUC_R) was computed including individual baseline cortisol levels. Student’s t tests were performed to assess group differences in the parameters’ cortisol increase and AUC_R as described elsewhere (Wüst et al., 2000).

A cluster analysis was performed on awakening times and the resulting dummy variable coded the individuals as ‘early’ or ‘late’ awakeners. Student’s t tests and Chi-square tests were computed to assess differences in sociodemographic variables between early and late awakeners. Pearson product–moment correlations were computed to test the associations between cortisol responses, wake-up time and age. Furthermore, multiple regression analyses were computed to assess the contribution of the different parameters simultaneously. Post-hoc power analyses were performed using GPower 2.0 by Faul & Erdfelder (1992). Effect sizes were chosen following the recommendations of Cohen (1988). The nominal significance level was p<0.05.

All results shown are the mean ± standard error of mean (SEM).

3. Results

In the total study sample, cortisol levels showed the typical response pattern with maximum cortisol levels at 30 minutes after awakening and a mean net increase of 13.4 ± 1.06 nmol/l (ANOVA time effect: F_{4,700} = 29.29, p<0.0001). Seventy-four subjects indicated health problems, a chronic disease and/or continuous medication intake compared to 105 subjects who reported to be healthy and drug-free (except oral contraceptives). Due to missing cortisol levels in two subjects, 103 subjects
remained in the sub-sample of healthy subjects. Analyses revealed a trend towards blunted cortisol responses in both the cortisol pattern and cortisol increase after awakening in subjects with health problems (ANOVA time by group effect: $F_{2,4,417.3} = 2.54, p = 0.07$; increase: $t_{175} = -1.8, p = 0.07$) while a significantly larger area under the curve ($AUC_R$) emerged for healthy subjects compared to the group of subjects suffering from health problems ($t_{174} = -2.4, p = 0.02$) (see Fig. 1A). Due to the heterogeneity of health problems and/or the drugs taken, more refined analyses were not performed on these subgroups (cardiovascular: $N = 11$, autoimmune/atopic/allergic: $N = 29$, psychiatric: $N = 7$, miscellaneous: $N = 27$). Thus, the cortisol profiles for four subgroups are shown in Fig. 1B for illustration purpose only. On a descriptive level, the individuals with cardiovascular problems/diseases ($N = 11$) stood out with a most unusual cortisol pattern. As a group, these individuals had largely increased wake-up levels and failed to show an increase within the first 30 minutes after awakening. The differences in cortisol awakening profiles among these four different groups approached statistical significance ($F_{7,5,173.5} = 1.95, p = 0.06$). Due to the differences in cortisol profiles between healthy and non-healthy subjects, only healthy subjects (mean age: $27.3 \pm 1.2$ yrs) were included in the following analyses.

Awakening time ranged between 0455 and 1203 h. One subject who woke up at 1544 h was excluded. A cluster analysis on wake-up time divided the remaining sample in 64 early awakeners (mean awakening time: 0649 h $\pm 0.06$) vs. 38 late awakeners (mean awakening time: 0943 h $\pm 0.09$). While early awakeners tended to be older than late awakeners ($p = 0.07$), the two groups were similar with respect to other demographic characteristics (i.e., sex, smoking status, pill use, cycle phase; see Fig. 2, Table 1). A two-way ANOVA revealed that the cortisol profiles differed significantly between wake-up groups ($F_{2.5,143.9} = 6.16, p = 0.001$). Group differences for cortisol increase and $AUC_R$ also reached statistical significance (increase: $t_{99} = 2.2, p = 0.029$; $AUC_R$: $t_{99} = 2.03, p = 0.05$), showing that the increase as well as the area under the curve were higher in early compared to late awakeners. Correlational analyses showed negative associations between wake-up time and cortisol increase and $AUC_R$, respectively (increase: $r = -0.22, p = 0.029$; $AUC_R$ $r = -0.23, p = 0.021$). Inclusion/exclusion of the children did not change the results.

ANOVA and t test procedures revealed no differences between women in the follicular phase versus luteal phase of the menstrual cycle (ANOVA time by group effect: $F_{4,84} = 0.24, p = 0.91$; increase: $t_{21} < 1, p = 0.64$; $AUC_R$: $t_{21} < 1, p = 0.99$). Likewise, no gender differences emerged in the sub-sample of healthy subjects (interaction gender by time: $F_{4,404} = 1.2, p = 0.27$; increase: $t_{101} < 1, p = 0.61$; $AUC_R$: $t_{101} < 1, p=0.41$). Controlling for time of awakening (using awakening time as a covariate) did not alter the reported results.

A compromise power analysis for the parameters cortisol increase and $AUC_R$ resulted in $1-\beta = 0.82$ (for large effects: $d = 0.8$) and $1-\beta = 0.72$ (for medium effects: $d = 0.5$), respectively, indicating that large differences between women in the follicular and the luteal phase of the menstrual cycle would have been detected with a probability of 82%. A post hoc power analysis with a nominal $\alpha = 0.05$ and $d = 0.08$ resulted in $1- \beta = 0.58$ (Fig. 3).
Fig. 1. A: Morning cortisol profiles (means ± SEM) in healthy (N = 103) versus non-healthy (N = 74) participants; *p<0.05. B: Morning cortisol profiles (means ± SEM) in non-healthy participants (cardiovascular: N = 11, autoimmune/ atopic/ allergic: N = 29, psychiatric: N = 7, miscellaneous: N = 27).
Fig. 2. Morning cortisol profiles (means ± SEM) in healthy early (N = 64) versus late (N = 38) awakeners; *p<0.05.

Table 1
Differences in demographic variables in healthy early versus late awakeners (means ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Early Awakeners (N = 64)</th>
<th>Late Awakeners (N = 38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awakening Time (h)</td>
<td>0649 h ± 0.06</td>
<td>0943 h ± 0.09</td>
<td>0.0001</td>
</tr>
<tr>
<td>Awakening Time (h): range / median</td>
<td>0455–0803 / 0700 h</td>
<td>0824–1203 / 0915 h</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>29.0 ± 1.7</td>
<td>24.6 ± 1.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Age (yrs): range / median</td>
<td>4–64 / 24</td>
<td>5–54 / 22</td>
<td></td>
</tr>
<tr>
<td>Females (n)</td>
<td>33</td>
<td>20</td>
<td>0.69</td>
</tr>
<tr>
<td>Males (n)</td>
<td>31</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>10 (15.6%)</td>
<td>14 (36.8%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Pill Users</td>
<td>14 (42.4%)</td>
<td>11 (55.0%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Follicular Phase</td>
<td>09 (27.3%)</td>
<td>03 (15.0%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Luteal Phase</td>
<td>07 (21.2%)</td>
<td>04 (20.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Finally, possible associations between chronological age and cortisol levels were tested. Correlations revealed that age tended to be negatively associated with the cortisol increase (r = –0.17, p = 0.08), while the cortisol AUCR was negatively associated with age (r = –0.20, p = 0.05). Furthermore, chronological age was posi-
Fig. 3. Morning cortisol profiles (means ± SEM) in women in the luteal (N = 11) versus follicular (N = 12) phase of the menstrual cycle; *p<0.05.

4. Discussion

The present study focused on the impact of health status, awakening time, and menstrual cycle phase on the salivary cortisol pattern in the first hour after awakene-
ing. Elevated cortisol concentrations were observed upon wake-up in subjects reporting health problems or a chronic disease. In the present study, the elevated initial cortisol levels seemed to result in an altered salivary cortisol profile with a smaller increase and a significantly smaller area under the curve. The increased morning cortisol levels in combination with a reduced cortisol increase after awakening may indicate a generally altered HPA axis activity in the early morning in subjects suffering from a wide range of health problems. It should be noted, however, that these findings are merely correlational in nature, thus other factors might as well have caused this effect (e.g., altered sleep patterns). While it is tempting to speculate that an increased awakening cortisol level might be a biological marker for general health problems, the present data are derived from small samples and should thus be viewed as very preliminary. However, this finding underscores the importance of exact definitions of exclusion/inclusion criteria for study designs with community-dwelling volunteers.

The present data strongly support the idea that time of awakening has a significant impact on the cortisol pattern after awakening. Subjects who woke up early in the morning showed larger cortisol increases compared to subjects who woke up later in the morning. The finding of an effect of awakening time on free cortisol levels agrees with and extends recent data reported by Edwards et al. (2001) and Federenko et al. (2001), respectively. Edwards et al. reported that early awakeners show a significantly higher cortisol increase after awakening compared to late awakeners. Federenko et al. (2001) investigated cortisol profiles after awakening in nurses working in a three-shift system (early shift: awakening 0400–0530 h, late shift: awakening 0600–0900 h, night shift: awakening 1100–1400 h). Their results show that a significant increase of cortisol occurred in the first hour after awakening in all three groups of nurses. In line with the findings reported by Edwards et al., further analyses revealed a trend that the later the time of awakening, the lower was the subsequent mean increase of cortisol levels. A fourth study group of 22 students was instructed to sleep two hours in the evening (awakening between 1845 h and 2030 h). In this group, no increase of cortisol levels after awakening could be observed.

It must be noted that all of these studies (including the present) cannot exactly determine the relationship between the cortisol awakening response and the course of the circadian cortisol rhythm. A study by Späth-Schwalbe et al. (1992) impressively showed that the cortisol response to awakening is strongly influenced by the circadian rhythm and the length of sleep. The authors reported that short sleepers had significantly higher plasma cortisol levels during their sleep period compared to long sleepers during the same time period, although the cortisol increase following awakening was more pronounced in the long-sleep group than in the short-sleep group. The authors argue that the lower cortisol levels prior to awakening in the long sleepers have possibly led to higher awakening responses in this group, suggesting that the awakening response was closely related to the circadian rhythm. In the present study, the impact of sleep length could not be determined. The cortisol levels upon awakening (first sample) did not differ between early vs. late awakeners, only the response was more pronounced in early awakeners.

In contrast to the results by Späth-Schwalbe et al. (1992), Wüst et al. (2000)
reported on a small but significant negative correlation between sleep duration and free cortisol awakening responses \((r = -0.16)\), suggesting a slightly larger cortisol increase in subjects who reported a shorter sleep duration.

The observation that the time of awakening has a significant impact on the cortisol response to awakening appears also to contradict recent findings on the effect of light exposure. While here we report increased cortisol responses with earlier wake-up times, it has been shown that exposure to light has a stimulatory effect on the HPA axis. Scheer & Buijs (1999) could show that the cortisol increase after awakening in the morning was greater when individuals woke up with 800 lux as opposed to waking up at 0 lux. No such effect was observed in the evening. Likewise, a transition from dim light to bright light appears to stimulate the HPA axis in the early morning hours while again this effect was absent in the afternoon (Leproult et al., 2001). Accordingly, awakening later in the morning should be associated with exposure to brighter light and thus result in larger cortisol increases in late awakeners. This was obviously not the case in the present study. It should be noted however, that under naturalistic conditions as in the previous study (which was conducted during the winter season), most individuals will turn on the (electric) lights upon awakening and thus receive similar (additional) stimulation of their HPA axis over a wide range of wake-up times. The cortisol rise to awakening is not simply a response to light exposure. As shown in the Scheer & Buijs (1999) study, even in complete darkness, the HPA response to awakening is very prominent. Future ambulatory studies including awakening cortisol measures may need to instruct the participants to turn on the electric lights immediately after awakening to control for this possible confounder.

Menstrual cycle phase, sex, or smoking habits did not alter morning cortisol profiles in the sub-sample of healthy participants significantly, although there was a trend towards a postponed cortisol peak in females in the total study sample \((p = 0.08)\). These results are largely in accordance with data from a study which included 509 subjects (Wüst et al., 2000). They reported a significant but rather small sex difference, with women showing a virtually identical cortisol increase after awakening but a delayed decrease compared to men. This effect explained 3% of the total variance. The cortisol increase after awakening appears to be not influenced by the menstrual cycle phase. However, it can not be ruled out if different components of oral contraceptives could have an effect on free cortisol profiles. A compromise post hoc power analysis confirmed a satisfactory test power for large effects of the present investigation. Thus, smaller group differences between follicular and luteal phase profiles could have been missed due to insufficient statistical power here. Other studies which investigated possible effects of menstrual cycle phases on basal adrenocortical activity also showed no or only minor effects (Abplanalp et al., 1977; Carr et al., 1979; Kanaley et al., 1992; Leibenluft et al., 1994; Altemus et al., 2001). In contrast, using a psychosocial stress test (TSST), we recently reported that salivary cortisol responses differ significantly between women in the luteal or follicular phase, respectively (Kirschbaum et al., 1999).

In line with other studies, the effect of age on morning cortisol responses seemed to be small in the present study. In this study, correlational analyses revealed only
4% explained variance due to age. This finding appears to support the idea that there is only a minor change in morning cortisol levels across the lifespan in healthy subjects. This assumption is supported by several studies in men and women (Gray et al., 1991; Seeman and Robbins, 1994; Kudielka et al., 1999, 2000; Wüst et al., 2000), although there seems also to be evidence for an altered cortisol response to awakening at older age. A reanalysis of seven studies revealed that the morning cortisol acrophase was significantly related to chronological age in older women but not men (Van Cauter et al., 1996). According to our present findings, it should therefore be expected that the time of awakening effect on morning cortisol responses is even more significant in age-advanced women than in men. However, the multiple regression procedure confirmed a significant impact of age on the cortisol awakening response. While the present study covered a wide age range, only 20 participants of the total sample were children or elderly people over 60 years and the majority of the elderly subjects had to be assigned to the non-healthy group.

A note of caution must be given here: Most recently we have observed that ambulatory saliva sampling is performed in the majority of individuals quite accurately. However, a significant number of subjects failed to comply with the sampling protocol even when applying rather low standards for compliance. As a result, noncompliant individuals were found to have flatter cortisol responses to awakening compared to compliant subjects (Kudielka et al., 2002). One therefore needs to be very cautious in interpreting differences in morning cortisol patterns unless compliance with the sampling protocol is assured. Finally, Pruessner et al. (1997) and Wüst et al. (2000) have reported that the salivary free awakening response shows moderate to high stability across days. However, to increase data reliability in future studies, we recommend collecting morning cortisol profiles after awakening, at least, at two different test days.

In sum, we report here community-dwelling individuals with health problems had elevated cortisol concentrations after awakening with a relatively blunted cortisol response. From a methodological point of view, the time of awakening should be standardized or statistically controlled for in future studies while apparently no special precautions need to be taken with regard to menstrual cycle phase.

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References


