The effect of an environmental stressor on gender differences on the awakening cortisol response

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**Summary**

**Objectives:** The aim of the current study was to investigate the effect of an environmental stressor, examination stress, on waking cortisol levels.

**Methods:** Sixty-two subjects were tested upon awakening during periods of low and high examination stress. Samples were collected on 4 sampling days total, two of these days were during a low examination period and two of these days were during a high examination period. During each day, subjects collected salivary samples at waking, 30 min after waking, and 60 min after waking. Subjects also completed three questions asking about their present mood.

**Results:** As a group, subjects had higher negative mood on the mornings during the high examination stress period than on the mornings during the low examination stress period. Furthermore, when the sex of the subject was considered, cortisol levels were found to be significantly higher in females during the high examination period, but not in males. However, the changes in waking cortisol across the two stress periods were not correlated with the changes in psychological stress across the same sessions for either sex. In conclusion, the waking cortisol was found to be sensitive to the examination stressor protocol, but only in females.

**Conclusions:** These findings, in conjunction with others, may help to build more comprehensive models of how the two sexes differ in hormonal and psychological stress responses.

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There is an abundance of evidence to suggest that changes in levels of psychological stress co-occur with a number of physiological changes. These include changes in: (i) stress hormone levels (e.g., Smyth et al., 1998; Cohen and Hamrick, 2003; Dickerson and Kemeny, 2005), (ii) immune functioning (e.g., Herbert and Cohen, 1994; Kiecolt-Glaser et al., 2002), (iii) neurophysiological measures (e.g., McEwen and Sapolsky, 1995), and (iv) neuropsychological functioning (e.g., Het et al., 2005; Lupien and Lepage, 2001). However, there have been significant inconsistencies with regard to the specific stressor used to trigger these stress responses, the time of day at which responses were measured, and whether individual and group differences have been investigated or observed. In the present study, we investigated the relationship between an environmental stressor, examination stress, and psychological and hormonal measures of stress at awakening. We also investigated sex differences in these responses.

Within approximately 30 min after wakening, most individuals experience a significant rise in cortisol excretion from 50% to 100% above waking levels (Pruessner et al., 1997; Hucklebridge et al., 1998). Numerous studies have suggested that this awakening cortisol response (ACR) serves as a marker of general hypothalamic–pituitary–adrenal (HPA) axis activity (see Clow et al., 2004 for a review). There has been consistent debate though, regarding the extent to which these awakening cortisol measures represent a stable individual characteristic or a transient, state-dependent effect of the environment (e.g., Hellhammer et al., 2006). More specifically, some studies suggest that ACR is relatively stable across time, and has significantly greater heritability coefficients than do cortisol levels taken later in the day (Wust et al., 2000; Kupper et al., 2005; see also Clow et al., 2004 for an extensive review).

However, other studies have shown ACR to be sensitive to several environmental factors (e.g., Kunz-Ebrecht et al., 2004; Lundberg and Hellstrom, 2002, Meinschmidt and Heim, 2003, Steptoe et al., 2005, Yehuda, 2002, Rasmusson et al., 2003). For example, enhanced ACR have been observed in individuals with high workload or chronic stress levels (Wust et al., 2000), whereas decreased ACR has been observed with burn-out (Pruessner et al., 1999). Furthermore, some studies have observed enhanced ACRs in depressed individuals (Pruessner et al., 2003; Bhagwagar et al., 2005, but see also Strickland et al., 2002; Burke et al., 2005; Huber et al., 2006). However, it is unclear whether these findings are actually state- or trait-dependent effects. That is, because most of these studies have been performed using within-subject analyses (e.g., Lundberg and Hellstrom, 2002), the extent to which these differences represent long-term characteristics of the individual (“trait” or long-term state) or transient, short-term characteristic of the individual’s reaction to the immediate environment (“state”) has not been well established.

Furthermore, to the extent that waking cortisol levels are state dependent, there is some debate as to whether they are as sensitive to stress as are levels taken later in the day. Several findings suggest they are not. First, as described above, there is evidence to suggest that morning sensitivity may be affected by stable, trait differences (see Wust et al., 2000; Clow et al., 2004; Kupper et al, 2005) that are not observed later in the day. Second, numerous studies have suggested that elicitation of cortisol reactivity may be more successful in the afternoon because morning levels may “ceiling out” (e.g., Schulte et al., 1985; Dickerson and Kemeny, 2005). However, this diurnal effect may be dependent on whether the elicitor is a pharmacological agent, a physical activity (e.g., exercise), an environmental stressor or a laboratory stressor (see Kudielka et al., 2004 for a review of this issue).

Few studies have investigated sex differences in waking cortisol levels. Nevertheless, numerous studies have found greater ACRs in women than in men (Steptoe et al., 2000; Kunz-Ebrecht et al., 2004; Clow et al., 2004). Even here, though, state-dependent effects have also been observed, if only inconsistently so. For instance, Kunz-Ebrecht et al. (2004) found stronger sex differences in ACR during (higher stress) workdays with females showing greater ACRs than men. In contrast, during (lower stress) weekends, little evidence was found for such sex differences (Kunz-Ebrecht et al., 2004). In contrast, Lundberg and Frankenhaeuser (1999) have observed greater cortisol excretion in men on workdays than on non-workdays, but no such work-related difference in women. Furthermore, Steptoe et al. (2005) found that decreasing financial strain over a 3-year period was related to lower ACR in men but not in women. In interpreting these sex differences, it is important to note that the specific nature of the observed sex differences may be dependent on the nature of the stressor used. For instance, men and women have been shown to respond differently to work or task-related stressors and to personal life events or social rejection (Stroud et al., 2002). Therefore, these differences in the extent to which a stressor is actually stressful to the individual or sex group may confound the findings.

Regardless of the origin or sensitivity of the waking cortisol response, there is some evidence to suggest a connection between ACR and health outcomes (Clow et al., 2004). Moreover, there is evidence to suggest that the two sexes may differ in the relationship between stress and health (e.g., Handa et al., 1994; Weekes et al., 2006). Specifically, women have been shown to be more vulnerable to the health effects of psychological stress than have men (see Handa et al., 1994 for a review, though also see Benyamini et al., 2000). As such, it is critical to further investigate not only the direct relationship between ACR and health, but also how sex differences might moderate these relationships. While the present study did not look at health outcomes, it does help to further establish the relationships between sex and morning cortisol levels, as a preliminary step in this path.

Few studies have investigated the same subjects across different exposure levels to an environmental stressor that both sexes find similarly stressful (see Weekes et al., 2006). The present study accomplishes this with a within-subject design and with an examination stressor protocol. Such a design allows an investigation into the extent to which differences in ACR are related solely to chronic “wear and tear” on the HPA axis, as has been suggested by McEwen (1998), or also to state-dependent fluctuations in psychological stress (e.g., Hellhammer et al., 2006).

In summary, numerous studies have suggested that males demonstrate greater levels of cortisol reactivity in response to stressor than do females. However, the vast majority of these studies has been performed using laboratory stressors.
and has measured cortisol levels directly before and after an acute stressor. In the present study, cortisol levels were measured in response to a longer-term examination stressor and for the first hour following awakening. Sex differences in ACR have been far more equivocal than have cortisol measures taken at the time of an acute stressor. Nevertheless, it is predicted that the pattern of sex differences observed at awakening and in response to a longer-term stressor will parallel those in response to an acute stressor. Therefore, it is predicted that males will show a greater cortisol reactivity than will females when ACRs are compared during times of low and high examination stress.

1. Methods

1.1. Subjects

Sixty-six college students aged 18–21 years (31 males and 35 females) served as participants. Exclusion criteria included: (i) smokers, (ii) left-handers, (iii) non-native English speakers, (iv) those with vision that was not corrected to normal, (v) antihistamine, glucocorticoid or asthma medication users, (vi) those with exposure to general anesthesia in the last year, (vii) those with a personal or first-degree family diagnosis of a DSM-IV, Axis I disorder, and (viii) those with endocrine abnormalities. Originally, the intention was that all females would be tested during the midluteal stage of the menstrual cycle. However, this was not possible given the experimental demands of the examination stress paradigm (explained below). Therefore, only information regarding oral contraceptive usage was collected.

1.2. Methods and procedures

All subjects participated in 7 days of home waking cortisol sampling during a low examination stress period, 2 days of home waking cortisol sampling during a high examination stress period and two afternoon behavioral sessions, one during the low examination stress period and one during the high examination stress session. The findings from the home waking cortisol sampling on Tuesday and Thursday of each of the two weeks will be described. Findings from the afternoon behavioral sessions have been described elsewhere (Lewis et al., 2007; Volkmann and Weekes, 2006; Weekes et al., 2006).

The order of the low and high examination phases of the study were counterbalanced across subjects, such that approximately half of the subjects (Group A) had their low examination periods during the Summer of 2003 and their high examination periods during Fall 2003 examinations. The other half of the subjects (Group B) had their high examination periods during Spring of 2004 and their low examination periods during Summer of 2004. Assignment into Groups A and B occurred solely based on the timing of the prospective subject’s response to the lab’s recruitment requests. The counterbalancing was done primarily to control possible practice effects on behavioral tasks that will be explained elsewhere.

After completing an online informed consent and exclusion criteria survey, subjects who met the criteria were invited to attend two home sampling meetings, one before the low examination phase and the other before the high examination phase of the experiment. Subjects were asked in advance of their “high examination” meeting to bring documentation of their examination schedule for that academic term.

1.2.1. Home sampling meetings

During these meetings, subjects were given an informed consent form to complete, followed by a second exclusionary criteria questionnaire to confirm the responses of the preliminary, online questionnaire. Those who met criteria were then given 21 salivettes during the low examination period meeting and six salivettes during the high examination period meeting (Sarstedt, Rommelsdorf, Germany). Subjects were instructed to obtain three morning salivary samples on 7 consecutive days during the low exam period and on Tuesday and Thursday during the high exam period. The other mornings of the high examination week were used to investigate other salivary markers (e.g., salivary immunoglobulin-A) and used a different sampling technique (i.e., passive drool as opposed to salivettes). The results of these markers have been described elsewhere (Volkmann and Weekes, 2006). Each day, a sample was required (i) at the time of awakening, (ii) 30 min after awakening, and (iii) 60 min after wakening. Subjects were asked to take nothing by mouth except for water, and not to clean their teeth before completion of the morning samples that day (see Clow et al. (2004), for greater description of these methodological practices). Subjects were asked to document in a provided logbook the time and date of each sampling. They also were asked to provide information about (i) actual time of waking, as well as exercise, diet, hours of sleep and mood in the same logbook.

Finally, subjects were asked to complete three single likert scale questions regarding their current mood. The scales were 1 “not at all” to 3 “extremely”. The mood items included how (i) stressed, (ii) happy, and (iii) anxious the subject was at the time of waking sampling.

Samples were returned to the laboratory each day and were immediately placed in a −20°C freezer. After all samples had been collected, they were sent to Salimetrics, Inc in State College, PA to be analyzed via ELISA.

All procedures were approved by the Pomona College Human Subjects Committee.

1.3. Analysis

Preliminary hypotheses (effect of order and compliance) and mood data were tested through analyses of variance (ANOVA) for males and females using SPSS for Macintosh, Version 11.5. Pearson product correlations were performed in order to test the significance of the relationships between cortisol and mood variables. Major predictions were analyzed using multi-level modeling that allowed for more flexible tests of nested designs, and mixed random and fixed designs. Previous studies have demonstrated the advantages of this analysis over multivariate and repeated measures analyses of cortisol data (e.g., Hruschka et al., 2005). We conceptualized our models to test random individual difference effects, random effects of day, and fixed effects of time, sex, session, and their interactions.
2. Results

2.1. Inclusion of subjects

Sixty-two subjects were included in all cortisol-related analysis. Fifty-three of these subjects were included in analyses of mood and in analyses of the correlations between mood and morning cortisol because of missing mood data for nine subjects.

2.2. Log transformation of cortisol data

As is typical for cortisol data, the distribution was positively skewed. Therefore, we used a natural log transformation on cortisol (LNCORT), which reduced the skewness and kurtosis of the distribution.

2.3. Preliminary analyses: analysis for confounding group variable

A preliminary ANOVA was performed to exclude season of high stress session as a significant factor in the findings. Order of session (i.e., whether the subject was a member of Group A—high examination period during the fall or Group B—high examination period during the spring) had no effect on the findings, and therefore, there was no evidence to suggest a confound of seasonality of the high exam session on the present findings.

Preliminary analyses were also performed to check for compliance to waking sampling procedure. Similar to Kunz-Ebrect et al. (2004), we investigated the number of subjects who had at least a 10 min interval between waking and first (“waking”) sampling for possible exclusion. Of the 62 subjects included in the analyses, only four met this criterion for exclusion. Of the 62 subjects, six had no intervals of 10 min or more between actual waking and “waking” sample. Furthermore, when analyses were run excluding the subjects who met this criterion, the same pattern of results was found as described below.

2.4. The influence of sex on cortisol responsivity

The variance in LNCORT due to waking fluctuation was first examined, and subsequently controlled. Time of day was a significant predictor of LNCORT, $F(2,676) = 91.5, \ p < .001$ (see Figure 1 for expected rise in waking cortisol). Next, we calculated the intraclass correlation (ICC), which determines the proportion of the total variance attributable to between-individual differences. After controlling for time of day, and entering subjects as a random variable, the ICC was .21. Thus, the proportion of the total variance attributable to between-individual differences was 21%, which is comparable to other datasets (see e.g. Hruschka et al., 2005).

The appropriateness of models with random and fixed effects was then compared. Entering Day of Sample as a random factor did not improve the model (i.e., final Hessian matrix was not positive definite; $-2$ restricted log likelihood increased). Therefore, day of sample was not used in subsequent models. The fixed effects of time, sex, session, and the interactions were tested (see Table 1 for significance tests). Interestingly, a sex by session interaction

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### Table 1: Type III tests of fixed effects.

<table>
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<th>Source</th>
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<th>Sig.</th>
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<td>.161</td>
<td>.688</td>
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<td>Session</td>
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<td>.303</td>
<td>.582</td>
</tr>
<tr>
<td>Time*sex</td>
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<td>.425</td>
<td>.654</td>
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<tr>
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<td>728</td>
<td>.644</td>
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</table>
emerged such that LNCORT values were higher for women during the high stress session, than during the low stress session (t = 3.61; p < .001) (see Figure 1 for this interaction). Time and sex by session accounted for 18.6% of the variance of LNCORT. Since sleep can affect cortisol values, amount of sleep was entered into the final model as a covariate. This analysis did not change the main effects or interactions.

2.5. Repeated measures analysis of variance (ANOVA) for effects of examination stress on the three mood items

Three ANOVAs were performed to investigate the effect of examination stress session on each of the three emotional measures. In each case, a 2 x 2 mixed ANOVA was performed with Sex (female, male) as the between-subject factor and with Session (low examination period, high examination period) as the within-subject factor. The mood item happiness, anxiety and stress served as the dependent variable in three respective ANOVAs. In all three analyses, session differences were observed. Specifically, as predicted, subjects were less happy F(1, 53) = 6.75; p = .02, more anxious F(1, 44) = 17.17; p = .000, and more stressed F(1, 53) = 46.12; p = .000 during the high examination stress period than during the low examination stress period. The only ANOVA to show an interaction between Sex and Session was when stress was used as the dependent variable F(1, 53) = 7.57; p = .008. In this case, females reported a larger increase in stress between the low and high examination sessions t(25) = -6.90; p = .001, than did males t(28) = -2.82, p = .01.

2.6. Correlations between waking, psychological measures of stress and waking cortisol levels

Pearson product–moment correlations were performed between percentage change values from the three mood items (happiness, anxiety, and stress) and the percentage change values from the three waking cortisol levels and percentage change values from the average cortisol waking level for the two sessions. Following Bonferroni corrections, no significant correlations were observed.

3. Discussion

There is an abundance of evidence to suggest that waking cortisol serves as an important marker of HPA-axis activity. While there is evidence to suggest that this marker may represent a relatively stable, individual difference variable (e.g., Clow et al., 2004), there is also evidence to suggest that this cortisol response can be affected by proximate characteristics, such as transient stress levels. In the present study, we tested the effect of examination stress on morning cortisol levels and whether these levels were dependent on the sex of the subject. Three major findings were observed. First, examination stress proved to be a significant trigger of elevations in negative mood states and declines in positive mood states. That is, both negative affect mood items (i.e., anxiety and stress) were higher during the high stress period than during the low stress period. Furthermore, during the same high stress period, happiness levels were found to diminish. Second, sex differences were observed in both cortisol and the mood item measure of stress. However, the specific pattern of the sex difference was opposite from the pattern typically observed following acute laboratory stressors and the pattern we originally predicted. Only females showed significant elevations in cortisol across the two examination periods. Males showed no such elevation across the two periods in cortisol and showed a smaller psychological stress response (relative to females) across the same two examination periods. Third, these elevations in cortisol were not found to correlate with reports of mood in either sex.

We have observed rises in cortisol and psychological measures of stress in response to an examination stress paradigm in the past (Weekes et al., 2006). However, the present findings differ from the previous findings in the specific nature of the sex differences observed. While we previously reported higher cortisol responses in the afternoon in males than in females, we are now reporting higher cortisol responses at awakening in females than in males. Finally, and consistent across the two findings, both male and female subjects showed higher psychological stress during the high examination period.

While the sex difference in cortisol is in the opposite direction of the original prediction, this data is consistent with previous findings suggesting greater ACR in women than men (e.g., Kunz-Ebrecht et al., 2004; Pruessner et al., 1997; Shirtcliffe et al., 2005; see also Clow et al., 2004). For example, Shirtcliffe et al. (2005) found a much greater variance in day-to-day cortisol levels in females than in males, suggesting a greater role for situational factors in the morning release of cortisol in females.

One explanation for this sex difference is that while genetic factors seem to be more involved in setting morning cortisol levels than afternoon cortisol levels, the extent and specific nature of this heritability may differ in the two sexes. Consistent with this model, Kurina et al. (2005) have found sex differences in the genetic component of the morning cortisol levels. However, inconsistent with this model is the finding that specific loci correlated with morning cortisol levels were observed in females but not in males. Nevertheless, Kurina et al. (2005) findings were based on a single morning cortisol measure, and the level of stress of the individual on that day was not recorded. Therefore, we cannot establish the extent to which these findings suggest sex differences in the heritability of stable, morning cortisol excretion or stress-related, morning cortisol excretion. As such, future studies are necessary to directly investigate the proposed model.

In conclusion, the present results suggest that the specific nature and direction of sex differences in cortisol responses may depend on whether those cortisol responses are measured at awakening or later in the day, and whether they are triggered by a laboratory or environmental stressor. While previous studies have suggested that waking levels of cortisol may be more stable across time and may represent a stable individual characteristic, other studies suggest a transient component of the waking response to stress and other environmentally triggered factors.
Finally, these relationships have clear implications for the study of stress and health. More specifically, there has been debate in the literature as to the extent to which different measures of cortisol might selectively predict negative health symptoms (e.g., Kajantie and Phillips, 2006). One explanation for these discrepancies may be whether the sex of the subject was considered as a moderating variable, whether stress levels (and sex differences therein) were assessed in response to a laboratory or environmental stressor, and whether samples were collected in the morning or in the afternoon. Together with the previous literature, the present findings suggest a need for further investigation into the disassociation between sexes differences in distinct cortisol response to awakening during examination stress if we are to better understand the relationships between sex, stress and health.

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Conflict of interest

None of the authors have any conflicts of interest.

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References


