Stress, symptoms of depression and anxiety, and cortisol patterns in working parents

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Summary
Whether stress or psychological distress is associated with alterations in diurnal cortisol secretion patterns in healthy adults is still uncertain. In this study, the authors tested for associations between stress or symptoms of depression or anxiety and changes in diurnal cortisol patterns in 91 working parents (57 women and 34 men) across the United States. Saliva samples were collected for cortisol analysis at six timepoints over 2 days. The diurnal cortisol slope and time-weighted average cortisol levels were analysed in relation to (1) two survey measures of stress, (2) diary reports of stress during cortisol sampling, and (3) self-reported depression and anxiety. Cortisol slopes and average cortisol levels varied widely across individuals and within individuals across days. Only two of the 24 associations between stress or psychological symptoms and cortisol were significant at the p < 0.05 level; men with more severe symptoms of anxiety had significantly higher average cortisol levels and women who reported that work was more frequently stressful had significantly lower average cortisol levels. These results highlight the variability of diurnal cortisol secretion patterns in healthy adults and suggest that neither stress nor psychological symptomatology is a strong determinant of differences in diurnal cortisol patterns in healthy individuals. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words
anxiety; depressive symptoms; hydrocortisone; stress

Introduction
Physiological change was one of the first ways in which researchers defined the experience of stress (Selye, 1950). Cortisol, a hormone produced by the adrenal gland, is the most important product of the hypothalamic–pituitary–adrenocortical (HPA) axis stress response. Accurate estimates of unbound cortisol can now be made easily from saliva samples (Kirschbaum & Hellhammer, 1992), which has led to strong interest in the use of cortisol as a convenient biomarker for stress (Theorell, 2003; Yehuda et al., 2003). Plasma cortisol levels have been described as following a relatively predictable diurnal pattern with an early morning peak following awakening, a rapid decrease over the next few hours and then a more
gradual decline over the course of the day to very low levels by bedtime (Désir et al., 1980; Kirschbaum & Hellhammer, 2000). One important question is whether stress or psychological symptoms (symptoms of depression or anxiety) can alter this pattern.

Interest in cortisol derives partly from the well-established relationship between stress and illness (Chrousos & Gold, 1992; Cohen & Herbert, 1996; McEwen, 1998) and the results of medical studies showing alterations in cortisol patterns in a variety of disease states (Tsigos & Chrousos, 1994). Patients with melancholic depression, for example, are frequently hypercortisolemic (Gold, Goodwin, & Chrousos, 1988; Halbreich, Asnis, Zumoff, Nathan, & Shindledercker, 1984) and have flattened cortisol patterns (Deuschle et al., 1997). Although these changes in cortisol secretion may be a consequence of illness, an important current hypothesis is that persistent activation of the stress system could contribute to the onset of these illnesses (Chrousos & Gold, 1992, 1998; McEwen, 1998).

A number of studies in both laboratory and natural settings have linked stress and cortisol at the momentary level (Kirschbaum, Wüst, & Hellhammer, 1992b; Smyth et al., 1998; van Eck, Berkhof, Nicolson, & Sulon, 1996). However, whether people who experience more stress or psychological symptoms have, on average, higher levels of cortisol has yet to be demonstrated unequivocally in natural settings. Studies of the relationship between stress or psychological symptoms and individual cortisol parameters (e.g. evening levels, morning levels, morning awakening response) have shown mixed results (Brantley, Dietz, McKnight, Jones, & Tulley, 1988; Caplan, Cobb, & French, 1979; Luecken et al., 1997; Ockenfels et al., 1995; Powell et al., 2002; Rose, Jenkins, Hurst, Herd, & Hall, 1982a; Schaeffer & Baum, 1984; Schulz, Kirschbaum, Prüßner, & Hellhammer, 1998; Sephton, Sapolsky, Kraemer, & Spiegel, 2000; Smyth et al., 1997; van Eck et al., 1996). Many of the study populations have consisted either of individuals selected on the basis of highly stressful events (e.g. marital disruption, Three Mile Island, metastatic breast cancer, unemployment) or of atypical convenience samples such as medical personnel or university students (Brantley et al., 1988; Ockenfels et al., 1995; Powell et al., 2002; Schaeffer & Baum, 1984; Schulz et al., 1998; Sephton et al., 2000). Much less is known about the relationship between stress or depression or anxiety and cortisol secretion patterns in healthy working adults.

Dual-earner families make up the majority of families with two parents in the United States today (Waite & Nielsen, 2001). In this study, we characterized the cortisol patterns of 91 healthy adults from dual-earner families and analysed these patterns in relation to a number of measures of stress and psychological symptomatology. We hypothesized that higher levels of stress and more symptoms of depression or anxiety would be associated with increased average cortisol levels and a flattened diurnal cortisol pattern.

Materials and methods

Study population

Saliva samples for cortisol analysis, measures of stress and psychological symptoms, and data on medical conditions and health-related behaviours were collected from 43 men and 68 women ranging in age from 27 to 58 years. The individuals were a subset of the 500 Families Study, a large sociological study of dual-earner families in eight communities across the United States. Study families were recruited through solicitation by phone, mail, newspaper advertisements, and school-based advertisements. Individuals in the Sloan 500 Families Study who expressed interest in participating in a ‘Physical stress study’ were mailed full explanations of the study protocol. All participants gave their written informed consent to participate in the study, which was conducted between July 1999 and July 2000. The study was approved by the Institutional Review Boards of both the University of Chicago and NORC.

Medical conditions and health-related behaviours

Participants in the study filled out a health questionnaire assessing factors that could potentially affect cortisol levels, including health-related behaviours (caffeine consumption, smoking, alcohol consumption, exercise, hours of sleep), disease states (asthma, allergies, mental illness, other health conditions), height and weight, medications, and, for women, oral contraceptive use, pregnancy, menstrual regularity, and menstrual phase (Kirschbaum & Hellhammer, 1992; Kirschbaum, Pirke, & Hellhammer, 1995;
Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Twenty-four of the individuals in the study reported a serious health condition (e.g. arthritis, migraine headaches, hypertension), but the parameters describing their cortisol patterns were very similar to those without reported health conditions, and so they were included in the analysis. Two of the women reported being pregnant; they were excluded from the analysis.

Measures of stress and psychological symptoms

Overall stress. Overall stress was measured in the 500 Families Study questionnaire with five items from the Cohen's perceived stress scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). Overall stress scores were dichotomized into high overall stress (scores in top quartile) and all others for analysis.

Stress at work. Overall work-related stress in the 500 Families Study was measured with the question, ‘How often do you find your work stressful?’ Reports of stress at work were dichotomized into high stress (‘work is often/always stressful’) and all others (‘work is never/hardly ever/sometimes stressful’) for analysis.

Experience-Sampling Method (ESM) stress data. Participants wore watches with pre-set alarms that beeped four times on each of two days. The watch signals occurred at random moments within evenly spaced intervals across the day. When alerted by the beeps, as well as at waking and bedtime, participants filled out brief structured diaries describing their location, activities, thoughts, and mood state, including feelings of stress, which were rated on a scale from 0 (not at all) to 4 (very much). This method of collecting data about the subjective experience of peoples’ day-to-day lives is known as the ‘Experience-Sampling Method’ (Csikszentmihalyi & Larson, 1987). Data were aggregated for home and work separately and an average ESM stress value was calculated for each individual at each location. Average ESM stress scores for home and for work were each dichotomized into high stress (scores in top quartile) and all others for analysis.

Psychological symptoms. Depressive symptoms were measured with the Center for Epidemiological Studies Depression scale (CES-D; Radloff, 1977). Depression scores were dichotomized into substantial depressive symptoms (score ≥16) and all others for analysis. Symptoms of anxiety were measured with four items from the Taylor Anxiety Inventory (Taylor & Tomasic, 1996). Anxiety scores were dichotomized into high anxiety (scores in top quartile) and all others for analysis.

Cortisol sampling

Participants were directed to collect saliva samples 20 min following each ESM beep, since cortisol levels generally reach a peak 10–30 min following a stressor (Kirschbaum & Hellhammer, 2000), as well as upon waking and just before going to bed, for a total of six samples on each of two work days. Participants chewed a stick of gum to stimulate salivation and then expelled the saliva through a small straw into a plastic vial. Sample vials were refrigerated as soon as possible following sampling and were sent back to the University of Chicago via courier; tightly capped saliva samples can be kept without refrigeration for several days without affecting cortisol concentrations (Clements & Parker, 1998). Upon receipt at the University of Chicago, saliva samples were stored in a freezer and then all samples were forwarded together on dry ice to an analytical laboratory for cortisol analysis (Salimetrics, State College PA). Individuals who collected a minimum of four saliva samples, including a waking and bedtime sample, for at least one of the two days were eligible for inclusion in the analysis. Thirty-four (79 per cent) of the men in the study and 59 (87 per cent) of the women in the study met this criterion.

Samples were assayed for salivary cortisol using a high-sensitivity enzyme immunoassay. Regression analysis comparing the cortisol levels from the split samples in this study showed that laboratory measurement error was negligible ($R^2 = 0.994$). The test’s sensitivity ranges from 0.19 to 47.9 mol l$^{-1}$; average intra- and inter-assay coefficients of variation are 4.13 and 8.89 per cent respectively (Eve Schwartz, Salimetrics LLC, personal communication, 2002).

Statistical analysis

We computed two summary measures of the diurnal cortisol pattern for each individual in the
study—slope and time-weighted average. As described in the Introduction, plasma cortisol values generally show a morning peak following awakening, a steep decline over the next few hours, and then a slower decline thereafter to an evening nadir. As our data include only the waking portion of the morning awakening response, a log-transformation of the cortisol values resulted in approximately linear patterns of cortisol across the day. We expected the log-transformed cortisol values of most individuals to show a relatively strong negative slope over the course of the day (Kirschbaum & Hellhammer, 2000). Individuals who had either low morning values or high evening values, or who did not show the expected morning drop—all indications, potentially, of dysregulation of the HPA axis—would have flatter slopes. Individuals with either a larger number of secretory episodes or more intense secretory episodes would have higher average levels of cortisol across the day, an indication, perhaps, of overactivity of the HPA axis. We chose these parameters to capture differences both in the pattern and the amount of cortisol secreted.

We calculated cortisol slopes (versus time of day) for each individual, for each day, using ordinary least squares regression of the log-transformed cortisol values. The time-weighted log cortisol average for an individual was calculated as follows, where \( n \) equals the number of samples in a given day (ranging from four to six, as described above), \( x_i \) equals the log cortisol value for sample number \( i \) and \( t_i \) is the corresponding sampling time, in 24-h decimal notation:

\[
\frac{1}{2} \sum_{i=1}^{n-1} (x_i + x_{i+1}) \cdot (t_{i+1} - t_i) \quad (1)
\]

We calculated a mean slope and a mean time-weighted cortisol average for those individuals with two complete days of data (\( N = 66 \)). Slopes and averages for the remaining individuals (\( N = 25 \)) were based on the single complete day of data collected.

We first used simple, ordinary least squares regression, stratified by sex (Van Cauter, Leproult, & Kupfer, 1996), to detect individual associations between stress, depression, or anxiety (all dichotomous, independent variables) and cortisol slope or average (both continuous variables). For the bivariate associations significant at the \( p < 0.05 \) level, we used multiple regression analysis to control for regularity of exercise, which was significantly associated with cortisol slope, for use of oral contraceptives, which was significantly associated with cortisol average in women, and for age (Halbreich et al., 1984; Van Cauter et al., 1996) and total household income. None of the other health-related factors were significantly related either to cortisol slope or cortisol average.

**Results**

**Description of study population**

The 91 participants in this study who met the criteria for analysis included both parents from 30 families and individual parents from a further 31 families (the second spouses in these families chose not to participate in the study; Table I). The participants were nearly all Caucasian (94 per cent), with the remainder evenly divided among African American, Asian American, and biracial individuals.

Self-reported stress in this population was somewhat lower than expected (Table I). Mean scores on the PSS subscale were slightly lower, proportionally, than scores on the full PSS in the original populations tested (Cohen et al., 1983). The distributions of ESM stress scores were skewed, with low values much more common than high values at both home and work. A large fraction of the sample (35 per cent), however, did report that ‘work is stressful’ often or always.

A substantial fraction of the population also reported strong psychological symptomatology. Scores on the Taylor Anxiety Inventory subscale were comparable, proportionally, to those of the original population tested, which consisted principally of low-income, single African American mothers (Taylor & Tomasic, 1996). On the CES-D scale, 21 per cent of the women and 16 per cent of the men scored 16 or higher (an indication of substantial depressive symptomatology), approximately what would be expected in the general population (Radloff, 1977; Weissman & Myers, 1978).

**Cortisol patterns**

The diurnal cortisol values showed the expected pattern across the population of higher values in
the morning and lower values in the evening, although there was strong variation across individuals and days in that pattern (Figure 1). Cortisol slopes (Figure 2) and time-weighted log cortisol averages (Figure 3) were both distributed approximately normally across the population. For those individuals with 2 days of cortisol data (N = 66), we also examined intra-individual variation in the cortisol parameters. Both slope and average cortisol levels were significantly correlated within individuals across the two days (Pearson’s r = 0.569, p < 0.001 for slope and Pearson’s r = 0.433, p < 0.001 for average), however the majority of the variance in the cor-

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<tr>
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<th>Women (N = 57)</th>
<th>Men (N = 34)</th>
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<td>Oral contraceptive use (% women)</td>
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*Perceived stress was measured with an abbreviated Cohen’s perceived stress scale (Cohen et al., 1983). Scores can range from 0 to 20.
†Scores can range from 0 to 3.
‡Scores can range from 0 to 4.
§Depressive symptoms were measured with the 20-item CES-D scale (Radloff, 1977). Scores can range from 0 to 60; a score of 16 is traditionally used as a cut-off point to describe substantial depressive symptoms.
||Anxious symptoms were measured with an abbreviated Taylor Anxiety Inventory (Taylor & Tomasic, 1996). Scores can range from 0 to 16.
tisol parameters remained unexplained. Neither cortisol slope nor cortisol average varied significantly between men and women (\(F = 0.757, p = 0.39\) for slope and \(F = 0.091, p = 0.76\) for average).

**Bivariate analyses of cortisol parameters versus stress and psychological symptoms**

Of the 24 comparisons made, two associations between the cortisol parameters and stress or psychological symptoms were significant at the \(p < 0.05\) level, approximately what would be expected by chance. Women who reported that work was often or always stressful had lower time-weighted average cortisol levels than those who did not and men who reported more frequent symptoms of anxiety had higher time-weighted average cortisol levels than other men (Table II).

**Multivariate analyses of cortisol parameters versus stress and psychological symptoms**

The two significant associations detected in the bivariate analyses persisted in the multiple regression models controlling for age, household income, oral contraceptive use and regular exercise (Tables III and IV), although the overall models were weak.
The two major findings of this study were the variability of the diurnal cortisol slopes and average cortisol values both between and within individuals and the generally weak evidence for an association between the diurnal cortisol parameters and stress or psychological symptoms. The two findings are almost certainly linked.

In our analysis we employed two simple parameters to describe individual-level cortisol secretion in terms of pattern and amount. Prior research into cortisol patterns suggested two types of diurnal cortisol patterns: (1) typical, characterized by a strong morning peak followed by a first steep and then slower decline over the course of the day or (2) flattened, with either a strong morning peak and little decline over the day (resulting in hypercortisolemia) or with no morning peak and low levels across the day (resulting in hypocortisolemia). We observed, however, that both cortisol slopes and average cortisol levels were approximately normally distributed across individuals in the population (Figures 2 and 3). Describing an individual’s cortisol secretion pattern as normal or disordered,
then, or even of dividing individuals into sensible groups on the basis of differences in their cortisol patterns, is problematic.

Smyth and colleagues (1997) were the first to report on diurnal cortisol cycles in a relatively healthy population. Only half of the participants in their study showed ‘typical’ cortisol patterns across the two days of sampling while the rest showed either no pattern (17 per cent) or an inconsistent pattern (31 per cent). Patterns were defined as typical or atypical on the basis of visual inspection. No association was found between cortisol cycle type, defined either as typical, absent, or inconsistent over a 2-day period and any of the numerous psychosocial measures employed in their study. The findings on the heterogeneity of cortisol cycle type were confirmed by a subsequent re-analysis of data from four studies in which cortisol was sampled in natural settings; in at least 10 per cent of each sample no significant diurnal cycles were detected (Stone et al., 2001).

Variation in cortisol patterns across individuals consists of a number of components, including genetic differences in baseline cortisol secretion (Hellhammer & Wade, 1993; Kirschbaum, Wüst, Faig, & Hellhammer, 1992a), exposure to day-to-day environmental factors affecting cortisol secretion, responsiveness to environmental factors affecting cortisol secretion (e.g. coping strategies; Rose, 1980) and random variation. Environmental factors affecting cortisol secretion include not only stressors, but also eating, physical exercise, sleep loss, and reproductive factors (Kirschbaum & Hellhammer, 1992; Leproult, Copinschi, Buxton, & Van Cauter, 1997; Luger et al., 1987). Our observations of extensive within-individual variability in cortisol slope and average cortisol level suggest that any stress- or psychological symptom-related component of an individual’s cortisol pattern may be simply overwhelmed by the noise resulting from the number of other factors affecting cortisol secretion.

Despite our use of multiple measures of stress and the inclusion of the well-validated CES-D depressive symptoms scale and a Taylor Anxiety Inventory subscale, we found only two significant relationships between felt stress or psychological symptoms and either cortisol parameter, approximately what would be expected by chance. These results suggest one of two possible interpretations. First, there is no relationship between cortisol secretion patterns and stress or psychological symptoms; the two significant associations were false positives. Alternatively, true relationships between stress or psychological symptoms and changes in cortisol patterns were obscured by the noise in cortisol secretion patterns due to the factors described above; this study, and by extension many other cortisol studies with similar sample sizes, is limited by power. To check for power limitations, we plotted the cumulative distribution of observed p-values from the bivariate regression analyses together with the distribution under the null hypothesis (Figure 4). Under conditions of limited power but true effect, we would expect an excess of small (but not necessarily significant) p-values, as evidenced by a substantial deviation from the diagonal, something akin to a U-shaped curve. Figure 4 shows that the observed p-values track those expected under the null hypothesis very closely and thus argues against power limitation as the primary explanation for our findings. If cortisol studies are generally power-limited, however, it is clear that the impact of stress or psychological symptoms on cortisol secretion patterns must be relatively weak. Both interpretations of the results (false positives or power limitations) are consistent with the mixed results of prior studies of the relationship between stress and cortisol in natural settings.

Figure 4. Cumulative distribution of observed p-values from the bivariate regression analyses. The observed p-values are ordered according to size (x-axis) and their values are plotted on the y-axis. The graph is plotted in such a way that under the null hypothesis, the observed p-values (shown as connected squares) should lie along the diagonal, as shown by the solid line. In fact, they do not deviate significantly from this line.
Several studies have shown no significant relationship between cortisol patterns and stress levels across individuals. Two decades ago in a study of air traffic controllers, Rose and colleagues (Rose et al., 1982a; Rose, Jenkins, Horst, Livingston, & Hall, 1982b) found no correspondence between psychological states and overall average cortisol levels across individuals. The authors’ interpretation was that individuals adapt to repeated stressors fairly quickly and that HPA activation occurs in response only to novel or unfamiliar stimuli. Ockenfels and colleagues (1995) compared the psychological states and cortisol levels of employed and unemployed individuals and observed no associations between either depressive disorders or anxiety and cortisol (either overall levels or diurnal rhythm). Further, they saw no difference in cortisol parameters between the two study groups despite strong differences in levels of perceived stress in the two groups. An earlier hospital-based study (Czeisler et al., 1976) compared the cortisol secretory patterns of patients awaiting cardiac surgery with a set of healthy controls and found no evidence of chronic HPA activation in the patients (who had been anticipating this operation over a period of weeks) or of a relationship between felt anxiety or apprehension and cortisol secretion.

Some studies, however, have linked psychological symptoms with increased cortisol levels. Van Eck and colleagues (1996) showed that trait anxiety and depressive symptoms were associated with small but significant increases in overall cortisol levels in male, white-collar workers in Germany. Our results, too, hinted at a positive association between anxiety and overall cortisol levels in the men in our study, who are largely professionals. A follow-up study of individuals residing near Three Mile Island found that relative to control subjects, the Three Mile Island residents had higher levels of cortisol (measured in 15-h urine samples) and that their cortisol levels correlated with self-reported physical and mental symptoms, although these correlations were modest (Schaeffer & Baum, 1984). Interestingly, no such relationship was observed among the control subjects. Schulz and colleagues (1998) compared the morning awakening response between ‘chronically stressed’ and ‘unstressed’ university students (as defined by chronic work overload) and found that the chronically stressed students had larger increases in cortisol following awakening than the unstressed students. In contrast, lower morning cortisol values (and flatter slopes) were observed in white collar male workers in the US who reported higher workloads (Caplan et al., 1979). Similarly, Adam and Gunnar (2001) showed an inverse association between hours of maternal employment and morning cortisol values in working mothers. In contrast, Luecken and colleagues (1997) observed higher levels of 24-h cortisol excretion in working women with at least one child at home compared with working women without children at home, and suggested that role strain might be the cause of the heightened HPA activity. Finally, in their study of potential biomarkers of stress, Powell et al. (2002) observed an association between stress-associated marital disruption and elevated evening cortisol levels in middle-aged women.

It is difficult to compare across cortisol studies given the diversity of the populations studied and the cortisol parameters employed. It is possible, however, that in situations of novel and relatively severe ongoing stressors (Three Mile Island residents, divorce) HPA over-activation does indeed occur, accounting for the associations found between subjective stress and increased cortisol levels. As noted, subjective diary reports of stress levels in our study were relatively low, which may have limited our ability to detect a relationship between stress and cortisol. It is also possible that some cortisol parameters (e.g. the morning awakening response) may be more stable within individuals and more closely connected with persistent psychological states (Pruessner et al., 1997; Schulz et al., 1998).

Our data do hint at a possible sex difference in the relationships between stress and cortisol in these working parents. We observed a curiously inverse relationship between perceived stress at work and average cortisol levels in women, but not in men. In their review of endocrine responses to stress, Rose and colleagues (Rose, 1980) briefly discussed the downregulation of the HPA axis observed in response to stress in some individuals, which may be operating in the women in our study. This phenomenon has received renewed interest as observations of adrenal gland hypoactivity have increasingly been observed in some stress-related states, even among healthy individuals (Gunnar & Vazquez, 2001; Heim, Ehlert, & Hellhammer, 2000). Although our study’s result may be due to chance, it is also consistent with prior literature showing differences between men and women in their cortisol responses to stress (Frankenhaeuser et al., 1978; Hellhammer & Wade, 1993; Kirschbaum et al., 1992b; Schaeffer
& Baum, 1984). For example, Ennis Kelly and Lambert (2001) showed that while cortisol levels increased in men anticipating an exam, cortisol levels actually decreased in the women; cortisol was not related to either cognitive appraisal (interpretation of exam as a threat or challenge) or self-reported anxiety. They interpret their results as support for the ‘tend-and-befriend’ hypothesis (Taylor et al., 2000), which suggests that in response to stress women may be more likely to engage in nurturing and network-building activities to promote safety and reduce distress. The sex differences in physiological responses to stress observed are intriguing and warrant further study.

**Conclusion**

Substantial variation in cortisol patterns was observed both within and between the healthy adults in our study. Our data show little evidence of a strong association between stress or psychological symptoms and the cortisol patterns of working parents. Instead, these data highlight the complexity and variability of the cortisol secretory system, including hinting at a sex difference in the relationship between stress and cortisol in day-to-day life.

**Acknowledgements**

This research was supported by the Alfred P. Sloan Foundation.

We thank Emma Adam, who was instrumental in study design and data collection, and who provided helpful comments. We also thank Holly Rice for her assistance with data preparation and statistical analyses and Jonathan Pritchard for helpful discussions.

**References**


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