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Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women

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Abstract

In 70 middle-class mothers of 2-year-old children, individual differences in mothers' morning cortisol levels, cortisol decreases across the day and average cortisol levels were predicted from demographic and medical control variables, maternal relationship functioning and home and work demands. For two days, salivary cortisol levels were measured in the morning immediately after wakeup, four times in the afternoon, and in the evening immediately prior to bedtime. Hierarchical linear modeling (HLM) growth curve analyses were used to estimate the intercept (early morning level), slope (steepness of decline in cortisol values across the day), and the average height of each mother's cortisol curve across the waking hours. HLM and multiple regression techniques were then used to predict individual differences in these parameters from the variables of interest. Time of day accounted for 72% of the variation in mothers' observed cortisol values across the day. After controlling for demographic and medical variables, positive relationship functioning was associated with higher morning cortisol levels and a steeper decline in cortisol across the day, while greater hours of maternal employment and a greater number of children in the household were associated with lower morning cortisol values and a less steep decline in cortisol levels across the day. Variables predicting higher morning values also predicted higher average cortisol levels, while variables predicting lower morning cortisol predicted lower average cortisol levels. The full model including selected control, relationship functioning and home and work demand variables accounted for 40% of the variance in mothers' morning cortisol values, 43% of the variance in cortisol slopes

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and 35% of the variability in mothers' average cortisol levels. This study presents the first evidence of associations between psychological variables and individual differences in the organization of cortisol levels across the waking day in normal adult women.

Keywords: Cortisol; Stress; Relationships; Employment; Women; Diurnal rhythms

1. Introduction

Recent evidence has shown relationships to be both powerful instigators (Flinn and England, 1995) and powerful buffers (Nachmias et al., 1996) of acute stress hormone reactivity in humans. There has been less research, however, on the role of relationships in understanding the basal organization of stress hormone activity. The current study examines the associations between relationship functioning and the organization of cortisol levels across the waking day in a healthy sample of adult women. In addition, it examines the associations between women's home and paid work demands and the organization of their diurnal cortisol patterns.

Cortisol is the main product of the hypothalamic–pituitary–adrenal (HPA) axis, one of the primary stress-responsive systems in humans. Cortisol may be non-invasively and reliably measured in small samples of human saliva (Kirschbaum and Hellhammer 1989, 1994), making it an attractive method for repeated measurement and for collection in naturalistic settings. In adults, cortisol levels typically have a strong diurnal pattern: they are highest in the morning soon after waking, drop rapidly in the first few hours after waking, then continue to drop more slowly across the day, reaching a nadir in the first few hours of sleep (Weitzman et al., 1971; Lacerda et al., 1973; Van Cauter, 1990; Schmidt-Reinwald et al., 1999). This diurnal pattern is established early in life, first emerging at about three months of age (Price et al., 1983).

While considerable research exists on factors predicting cortisol reactivity to stressors in adults, very little research is available on factors predicting individual differences in diurnal cortisol rhythms, particularly in normal adults in their naturalistic settings. When investigators have examined cortisol activity in nonclinical populations, they have tended to control for time of day by gathering data at a constant time of day rather than considering it a variable of interest. The way in which cortisol levels change across the day is however a potentially important indicator of the functioning of the HPA axis (Smyth et al., 1998). In general, because a strong diurnal rhythm in cortisol (with high morning levels, low evening levels, and a strong negative slope) is the normative or expected pattern, researchers have assumed an extremely weak, inconsistent or absent diurnal cortisol rhythm to be a sign of HPA dysregulation (Caplan et al., 1979). Whether or not more subtle individual differences in diurnal cortisol patterns have any psychological, physiological or clinical significance, however, remains an open question.

In clinical populations, evidence of a flattening of the diurnal cortisol rhythm (usually indicated by a smaller drop in cortisol from morning to afternoon or evening compared to a control group) has been reported for a variety of groups, including depressed adults (Carrol et al., 1976), children with a history of maltreatment and current symptoms of depression (Kauffman, 1991; Hart et al., 1996) and children reared in institutional settings (Carlson and Earls, 1997). A flattening of the diurnal cortisol rhythm has also been found in several physical disorders, including fibromyalgia (Crofford et al., 1994; McCain and Tilbe, 1989), chronic fatigue syndrome (MacHale et al., 1998) and severe rheumatoid arthritis (Neeck et al., 1990).

Very few studies have systematically examined factors predicting diurnal cortisol patterning in normal adults. An early study of this nature found lower morning cortisol levels and less of a drop to afternoon levels among individuals experiencing high levels of work stress (Caplan et al., 1979). A recent study on the relations between employment and diurnal cortisol activity reported unemployed subjects to have higher morning levels and lower evening levels than employed subjects (Ockenfels et al., 1995). In another analysis of these data, individuals who were judged to show flat diurnal cortisol cycles were no different from individuals with normal or inconsistent cycles on a wide variety of demographic and psychological variables (Smyth et al., 1997). Another recent study also found no significant associations between personality variables and change in cortisol levels across the day (Schommer et al., 1999).

Thus while there is some evidence that current job strain may be associated with the organization of cortisol levels across the day, the available evidence does not point to associations between more stable aspects of psychological functioning and diurnal cortisol patterns. Yet prior studies have not focused on one aspect of psychological functioning that may be particularly important for stress system functioning: the quality of an individual's functioning in relationships. Interpersonal relationship style or functioning may be important because stress hormone activity is dependent not only on the nature of the stressors faced in the current environment, but also on one's assessment of available coping resources (Kirschbaum and Hellhammer, 1989). Relationships are a central resource for coping with life's challenges from infancy through to adulthood (Sroufe, 1996; Cohen and Wills, 1985). Importantly, it is not just the presence of relationships, but the quality of relationships that matters for understanding stress hormone activity (Spangler and Grossmann, 1993; Hertsgaard et al., 1995; Gunnar, 1999). For example, temperamentally fearful toddlers with secure attachment relationships with their mothers have been shown to have reduced cortisol reactivity to a novel stimulus compared to fearful infants with insecure attachments (Nachmias et al., 1996). In another study, men (but not women) who received social support from their romantic partners prior to a social stress paradigm had reduced cortisol reactivity to the stressor compared to those who received social support from a stranger or no support (Kirschbaum et al., 1995). In addition to serving as a coping resource or buffer against stress reactivity, relationships can be a significant source of emotional stress and a powerful initiator of physiological stress reactivity. Flinn and England (1995) found that traumatic family events (such as conflict, punishment, shaming, quarreling and fighting) predicted elevations in cortisol in naturalistic settings more strongly than any of the other variables measured.

What role might relationships play in the diurnal organization of cortisol activity?

Several of the studies mentioned previously suggested associations between early and severe relationship stress (maltreatment, institutional rearing) and the diurnal pattern of cortisol activity (Hart et al., 1996; Kauffman, 1991; Carlson and Earls, 1997). No prior studies have focused on the links between more subtle variations in relationship experience and the organization of cortisol activity across the day in normal adults. In a primarily middle-class sample of mothers of two-year old children, the current study examines the associations between measures of relationship functioning and the patterning of mothers' cortisol levels across their waking day. Because several prior studies have found effects of employment on diurnal cortisol patterning, aspects of mothers' employment and the demands placed on mothers in their home environments are assessed. Finally, a variety of medical and demographic control variables are examined.

In addition to examining associations between relationship functioning, work and home demands, and diurnal cortisol activity, this study adds to the literature by using Hierarchical Linear Modeling (HLM) growth curve analysis techniques (Bryk and Raudenbush, 1992) to better model and predict individual differences in the patterning of cortisol across the day in a normal sample of adult women.

2. Method

2.1. Participants

Participants were 70 mothers of toddlers who were recruited from a larger study of factors contributing to parenting stress in mothers of young children. They were predominantly Caucasian (98%), married (96%), middle-class (mean income $54,000) and college-educated (mean years of education 16.4). Most were employed either part-time (37%) or full-time (29%); the remainder (34%) were full-time homemakers. The mean age of mothers was 34 years (range 24 to 42), the mean age of their toddler was 25 months (range 21 to 36), and they had an average of 1.9 children (range 1 to 4).

Four (6%) mothers were in their first or second trimester of pregnancy at their time of participation. Twenty-percent used oral contraceptives. While most (86%) of participants regularly drank caffeinated beverages, none of the seventy participants smoked. Two mothers took medication for thyroid problems, five mothers used anti-depressant medications, and one mother regularly took medication for asthma. The effects of these medical factors, where present, were controlled for statistically rather than serving as exclusion factors for study participation.

The data of nine additional participants were however excluded for the following reasons: four were in the 3rd trimester of pregnancy, during which cortisol levels have been shown to be substantially elevated above normal (Kirschbaum and Hellhammer 1989, 1994), three did not provide any valid morning cortisol samples, which have an important influence on the shape of the diurnal curve, and two participants were missing substantial questionnaire or interview data. For the remaining

70 study participants, occasional missing data points (less than 3% of the total data) were replaced with mean values for each variable.

2.2. Procedures

Questionnaire and interview data were collected from each participant during a 3-hour afternoon appointment at the Institute of Child Development, University of Minnesota. Salivary cortisol samples were collected by mothers in their own homes or workplaces on two separate typical days selected by each participant.

2.2.1. Salivary cortisol data

Mothers gathered samples of saliva six times a day for two days to allow estimation of the typical daytime patterning of their cortisol levels. Participants were instructed to collect cortisol samples at home or work each day at the following times of day: immediately after wake-up, just before going to bed, and at 1400 h, 1500 h, 1600 h, and 1700 h. (The frequent afternoon sampling was designed to provide home comparisons for laboratory samples taken at the same times of day during a different component of this study). Less than 1/8 tsp of sweetened Kool–Aid crystals were used to stimulate saliva, which was then absorbed with sterile cotton and expressed through a needleless syringe into a sterile vial. Participants refrigerated their samples until sampling was complete, then mailed their samples back to us. In past research, no significant differences in cortisol levels were found between split samples when one half was frozen within an hour and the other half exposed to widely varying temperature and motion over a five day period, conditions mimicking a postal trip (Clements and Parker, 1998).

Samples were frozen until data collection was complete for all participants, and then assayed by technicians at the University of Minnesota Hospital Endocrine laboratory using the Ciba MAGIC radioimmunoassay. This assay has been shown to be minimally affected by changes in sample pH due to Kool–Aid stimulant use (Schwartz et al., 1998). Aliquots from each saliva sample were assayed in duplicate. Inter- and intra-assay coefficients of variation were 12% and 5% respectively. Where possible, all the samples for a single participant were assayed together in the same batch.

2.2.2. Medical and demographic control variables

Because cortisol values may be affected by medical factors other than stress (Kirschbaum and Hellhammer 1989, 1994) the following information was obtained: caffeine use (number of cups or cans per day), nicotine use (number of cigarettes per day), whether birth control pills or other estrogens are taken (1=yes), number of days since last menstrual period, number of weeks of pregnancy (before the 3rd trimester; individuals in their third trimester were excluded), and whether or not mothers used any form of asthma medication, antidepressant medication, or other medications (1=yes for each medication). Basic demographic data were also collected, including maternal age, race-ethnicity, marital status, years of maternal education and family income.

2.2.3. Relationship functioning

Maternal relationship functioning was measured using several convergent measures, including both interview and questionnaire methodologies. The most in-depth measure was the Adult Attachment Interview (AAI; George et al., 1985). Participants were asked a series of questions regarding their early relationship experiences with their parents, and how they currently feel about those experiences. Interviews were transcribed word for word (including non-speech sounds) and each interview was scored by the first author, who is trained and certified as a reliable adult attachment coder by Dr. Mary Main. In addition to the traditional categorical coding method (Main and Goldwyn, 1994) each interview was scored on a 9-point scale measuring the individual's degree of "security" in their state of mind about past and present relationships (Adam, 1998). A secure individual values relationships, is insightful and balanced in their discussion of relationships, and is neither too distancing of nor too over-involved in past and present relationships (Main and Goldwyn, 1994). Twenty-five percent of the transcripts were independently scored by a second coder and adequate inter-rater reliability was obtained (an intraclass correlation coefficient of 0.70; Shrout and Fleiss, 1979). Mothers also completed several self-report questionnaire measures of relationship functioning. The Social Closeness scale of the Multidimensional Personality Questionnaire (MPQ; Tellegen, 1982) is a 22 item scale measuring the degree to which the person "is sociable, likes people; takes pleasure in, and values interpersonal ties; is warm and affectionate; turns to others for comfort and help". The Relationship with Spouse scale of the Parenting Stress Index (PSI; Abidin, 1995) measures the person's perception of the amount of emotional and active support received in the marital relationship since the birth of the child(ren). In addition, the Family Supports and Stresses Scale (FSS; designed for this study), has participants report on 5-point Likert scales their overall degree of satisfaction with their marital or partner relationship, the degree to which their husband/partner was a source of support for them, and the degree to their husband/partner was a source of stress in their life. While these measures capture slightly different aspects of maternal relationship functioning, they all in some way assess the extent to which the participant tends to have positive feelings about relationships and effectively uses them for support and comfort.

2.2.4. Home and work demands

While the focus of the study was on maternal relationship functioning, several variables provided some indication of the demands placed on mothers by their home and work environments. In terms of home demands, mothers reported the number of children in the household and estimated the percentage of the household childrearing duties they are responsible for (compared to their husbands and others). They also reported the number of hours they were employed, and their occupations. Maternal hours of work were coded for 1990 census codes and occupational status scores were assigned. Mothers not working outside the home were assigned the occupational status score associated with working as a childcare provider.

2.2.5. Data analysis

Hierarchical linear modeling growth curve analysis techniques were used (Bryk and Raudenbush, 1992) to describe mothers' diurnal cortisol rhythms and to predict individual differences in their diurnal rhythms from the control variables, maternal relationship functioning and work and home demand variables. The HLM/2L program was used (Bryk et al., 1996). A rough diurnal cortisol pattern for each indivdual was described by estimating lines of best fit through the available time-of-day by cortisol data of each person in a Level 1 HLM model. The obtained intercept and slope parameters of these lines (representing estimated early morning cortisol values and the rate of change in cortisol levels across the day) can then be related to the characteristics of the individual in a Level 2 HLM Model. The complexity of the modeling of individual diurnal cortisol rhythms is obviously limited by the relatively few data points per person; clearly ultradian variations in cortisol are not captured in the current study.

Use of HLM growth curve analysis has the following advantages. First, individuals are not required to sample at exactly the same times of day because they are not compared on individual cortisol samples, but rather on the line of best fit through all their data points. The effect of time of sampling is estimated and automatically controlled in the Level 1 model. Second, missing data points are not as critical, so long as sufficient points remain to accurately fit the line to the data. Third, estimates of the lack of fit in modeling each person's data are derived (which may be of interest in and of themselves) and less reliable data are weighted less heavily. In this manner, the influence of extreme data points is reduced. Fourth, it is parsimonious, allowing the description of each individual's diurnal curve with a few simple parameters, which can then be related to characteristics of the individual and his or her context. This seems preferable to comparing individual characteristics to multiple measures of cortisol across the day. Fifth, the technique is efficient in that all of these parameters for all individuals can be estimated simultaneously in a single model run. Finally, the degree to which the model fits the data can be determined by examining the percent of total variance in cortisol levels explained by time of day and characteristics of the individual.

To provide a more stable estimate of individual diurnal (daytime) cortisol curves, available data from both days of collection were combined, such that the estimates were based on a range of six to twelve samples per person (average was eleven), including at least one early morning and one late evening sample for each person. It was not considered feasible to ask participants to gather more than six data points on any single day without excessively interrupting their daily routine. Combining data across the two days also reduces the effect of the events of each individual day on the estimate of the diurnal curve. In order to justify combining the data across days, we first demonstrated that reasonable within-individual stability in cortisol levels existed across days. Correlations between Day 1 and Day 2 cortisol levels at various points in the day were examined, controlling for exact time of sampling.

Additional HLM analyses were conducted examining individual differences in evening cortisol levels rather than morning levels. Finally, based on the HLM intercept and slope parameters, daytime average cortisol levels were calculated for each participant and multiple regression procedures were utilized to predict these average levels from the control, relationship and home and work demand variables.

3. Results

3.1. Stability in cortisol levels from Day 1 to Day 2

Participants' average cortisol levels (mean of all available samples each day) from Day 1 to Day 2 were strongly correlated (r=0.65; $P<0.001$). Correlations between individual morning, afternoon and evening samples (controlling for exact time of sampling within each time period) were more variable, as would be expected given that individual values will reflect trait level cortisol as well as fluctuations due to immediate stressors and/or ultradian spikes (Kirschbaum et al., 1990). The correlation between the Day 1 and Day 2 morning samples was r=0.38, $P<0.001$ and the correlation between Day 1 and Day 2 evening samples was r=0.56, $P<0.001$. Correlations between individual Day 1 and Day 2 afternoon samples ranged from r=0.15 to 0.47 (average 0.35); the correlation between the average of the four afternoon samples from Day 1 to Day 2 was r=0.58, $P<0.000$. In addition, HLM Level 1 models (described in more detail below) were computed separately for Day 1 and Day 2; the correlation between the HLM estimated morning values and slope coefficients from the separate Day 1 and Day 2 models were r=0.36, $P<0.01$ and r=0.27, $P<0.05$ respectively. Stability across days seemed sufficiently high to justify combining the data from Day 1 and Day 2, for reasons outlined above. While further research should examine day to day variability in cortisol level and cortisol slopes in relation to the events of the day, the current study chose to examine trait level characteristics of the individual in relation to cortisol data aggregated across days.

3.2. Average form and degree of variability in mothers' diurnal cortisol cycles

As described, typical daytime basal HPA activity involves high levels of cortisol in the morning at shortly after wake-up, followed by a rapid drop in cortisol in the several hours after waking, then a more gradual decline throughout the day to near-zero values at bedtime. Fig. 1 illustrates that the cortisol data for the women in this study follows this basic pattern, but that considerable variability exists in the association between cortisol and time of day. Two of the notably high evening values were from a woman in the study whose spouse had recently died; exclusion of the data for this individual does not substantially alter the results.

As described above, hierarchical linear modeling procedures (HLM; Bryk and Raudenbush, 1992) were used to fit separate lines of best fit to the time-cortisol data of each participant, producing estimates of the morning value (B0) and slope (B1) of each mother's daily cortisol curve, and an estimate of the average morning value and slope of the diurnal curve across all participants. Because the relationship between time of day and cortisol was not linear, it was necessary to transform either the time data or the cortisol data prior to analysis of the data in HLM. An inverse

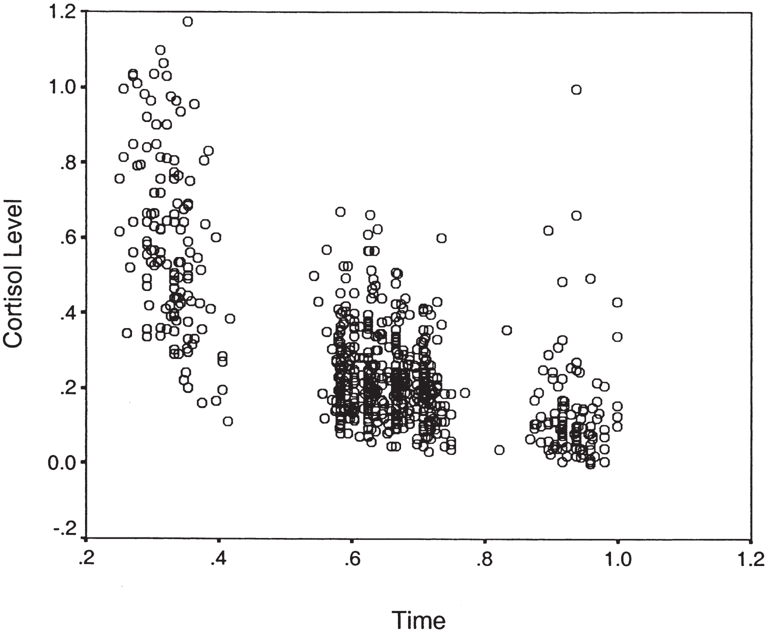

Fig. 1. Observed cortisol values for n=70 participants by time of day across two days of data collection. Time is represented on a scale from 0 (0000 h) to 1 (2400 h), and cortisol values are in μg/dl.

transformation of the time data provided the best fit to the data, because it had the effect of stretching out the time scale more at the low end of that scale, where the time-cortisol slope was steeper. The data were centered such that the intercept of the regression of time on cortisol represents an estimated 0600 h or approximate early morning peak cortisol level for each individual.

The Level 1 model predicting cortisol values from time of day was highly significant (see Table 1 Fixed effects). Comparison of the total variability in cortisol values to the variability remaining after time of day was entered in the Level 1 model revealed that 70% of the observed variability in cortisol values was accounted for by time of day. As would be expected, the estimated average 0600 h cortisol value was high and positive (0.78 μg/dl) and the estimated average slope value was negative (−0.23). Note that these values do not arise from a simple average of the individual intercepts and slopes, since HLM uses an algorithm that takes into account the reliability of the data for each individual, weighting more reliable (better fitting) data more heavily.

A significant amount of individual variation around these average slope and morning intercept values exists however (Table 1 Random effects), and could therefore potentially be explained by characteristics of the individual in a Level 2 model. Importantly for the interpretation of results, the slope and intercept residuals were very highly correlated (r=−0.96), indicating that higher morning cortisol values are almost perfectly associated with steeper slopes and therefore variables which signifi-

Table 1
Level 1 HLM model: Mothers' cortisol values predicted by time of day (n=70)

Fixed effect	Coefficient	SE	t-ratio	P-value
Average cortisol intercept[a]	0.783	0.032	24.8	0.000
Average cortisol slope[b]	−0.225	0.012	−18.7	0.000
Random effect	Variance component	Chi-squared	P-value	
Cortisol intercept	0.05599	353.93	0.000	
Cortisol slope	0.00761	280.47	0.000	

[a] An estimated average 0600 h cortisol value, based on a reliability-weighted average of all participants' data.

[b] Rate of decline in cortisol values from 0600 h to 2400 h, based on a transformed time scale ranging from 0 to 3.

cantly predict one parameter are likely to predict the other. The intercept and slope residuals were close to normally distributed, with no clear breaks in the distributions, suggesting that individual differences in diurnal cortisol rhythms are continuous, rather than falling into distinct categories or types. Can these individual differences be predicted from characteristics of the individual?

3.3. Predicting individual differences in diurnal cortisol cycles

Exploratory analyses were conducted in HLM to examine which of the control, relationship, and work and home demand variables might be important predictors of mothers' morning cortisol values and cortisol slopes as estimated by HLM (see Table 2).

Potentially important bivariate predictors were then entered together simultaneously in a multivariate HLM model. To ensure broad inclusion of control variables, each variable with a P-value less than 0.20 in the exploratory analyses was included in the final multivariate model. The relationship and work and home demand variables included in the final model were significant at $P<0.05$ or better in the exploratory analyses.

Of the medical control variables, only the use of medication for thyroid problems was potentially important in predicting the diurnal cortisol parameters. Maternal age was weakly related to both the intercept and slope parameters, and the dummy variable representing not being currently married (i.e. being single, divorced or widowed) was weakly related to cortisol curve slope. The age of the target child in the study was a significant predictor of both of the diurnal cortisol parameters. Of the work and home variables, the number of the children in the household and the number of hours of paid work were both significantly related to cortisol parameters in the exploratory analyses. All four of the relationship variables were related to the cortisol parameters in the same direction, with better relationship functioning predicting

Table 2
HLM exploratory analyses: cortisol morning values and slopes predicted from individual control, home and work demand and relationship functioning variables (n=70)[a]

	Cortisol Intercept Coefficient	*t*-value	Cortisol Slope Coefficient	*t*-value
Medical controls[b]				
Caffeine	0.014	0.825	−0.004	−0.668
Estrogens	−0.032	−0.339	0.015	0.645
Antidepressants	−0.053	0.048	0.034	0.973
Asthma medication	−0.189	−0.879	0.048	0.621
Thyroid medication	−0.298	−1.989*	0.088	1.634+
Days since period	0.002	0.773	−0.001	−0.819
Weeks pregnant	0.007	1.097	−0.002	−0.877
Demographic controls				
Age of mother	0.011	1.443+	−0.004	−1.351+
Age of child	0.308	2.448*	−0.134	−3.037*
Maternal education	0.002	0.196	−0.000	−0.086
Not married	−0.091	−0.725	0.059	1.316+
Family income	−0.000	−0.020	0.000	0.112
Home and work demands				
Number of children	−0.064	−2.021*	0.019	1.659+
% Childrearing	0.001	0.646	−0.000	−0.755
Hours of work	−0.003	−2.257*	0.001	2.201*
Occupational status	0.001	0.405	−0.000	−0.676
Relationship functioning[c]				
Adult attachment security	0.052	2.024*	−0.019	−2.048*
MPQ social closeness	0.056	2.137*	−0.022	−2.300*
PSI marital scale	0.037	1.502+	−0.013	−1.458+
FSS marital scale	0.040	1.451+	−0.014	−1.453+
Relationship composite[c]	0.109	2.831*	−0.022	−2.300*

[a] $*P<0.05$, $+P<0.20$.

[b] Nicotine use was dropped from the analyses due to insufficient variation; weeks pregnant represents weeks of pregnancy prior to the 3[rd] trimester, since mothers in their 3rd trimester were excluded from the study.

[c] An observational measure of parenting quality also related to the cortisol intercepts and slopes in the same way, however these data were only available for a small subset of the 70 mothers.

higher morning cortisol values and a steeper decline in cortisol values. In order to rule out the possibility that the observed effects were related to the timing of mothers' wakeup, partial correlations between each of the independent variables and the slope and intercept parameters were conducted, controlling for each participant's average time of morning sampling. None of the observed effects were substantially altered by controlling for time of morning sampling.

The potentially important predictors identified in the exploratory analyses were entered together simultaneously in the multivariate HLM model. Results are presented in Table 3. To simplify the model, rather than entering all four relationship functioning variables in the full HLM model, the four variables were standardized and aggregated to create a composite relationship functioning variable. Aggregation techniques have previously been demonstrated to improve the relations between personality variables and cortisol parameters (Pruessner et al., 1997a). Not surprisingly, in the current study the aggregate relationship functioning variable was a stronger predictor of diurnal cortisol curve parameters than any of the individual relationship functioning variables.

Many of the results obtained the exploratory analyses were significant in the multivariate HLM model; these coefficients now reflect the independent contribution of each variable beyond the effects of the other variables in the model. In terms of the control variables, the use of thyroid medication was significantly associated with lower morning cortisol values. Older mothers had marginally higher morning cortisol values, and mothers with older toddlers exhibited significantly higher morning cortisol values and steeper cortisol slopes. Mothers who worked a greater number of

Table 3
Level 2 HLM model: multivariate model predicting morning cortisol values and slopes from selected control, home and work demand and relationship functioning variables (n=70)[a]

Fixed effect	Coefficient	SE	*t*-ratio	*P*-value
Predicting cortisol intercept				
Intercept	−0.394324	0.401465	−0.982	0.326
Not married	−0.082530	0.137867	−0.599	0.549
Age of mother	0.015440	0.008073	1.913	0.055+
Age of child	0.422236	0.143783	2.937	0.004**
Thyroid medication	−0.314548	0.157669	−1.995	0.046*
Hours of work	−0.003283	0.001563	−2.100	0.035*
Number of children	−0.081940	0.032911	−2.490	0.013*
Relationship composite	0.126735	0.042430	2.987	0.003**
Predicting cortisol slope				
Intercept	0.259175	0.152564	1.699	0.089+
Not married	0.071234	0.053416	1.334	0.183
Age of mother	−0.004690	0.003069	−1.528	0.126
Age of child	−0.190072	0.054790	−3.469	0.001**
Thyroid medication	0.096458	0.059875	1.611	0.107
Hours of work	0.001016	0.000594	1.711	0.087+
Number of children	0.024288	0.012485	1.945	0.051+
Relationship composite	−0.049513	0.016088	−3.078	0.003**
Random effect	Variance	Chi-squared	*P*-value	
Component				
Cortisol intercept	0.03344	217.83	0.000	
Cortisol slope	0.00434	173.34	0.000	

[a] $**P<0.01$ $*P<0.05$, $+P<0.10$.

hours of work also had significantly lower morning cortisol values, and there was a trend suggesting their cortisol levels declined less steeply across the day. Having a greater number of children in the home also significantly predicts higher morning values and a steeper cortisol slope. Finally, mothers who were more secure in their relationship functioning had significantly higher morning values and significantly steeper cortisol curves (a stronger diurnal cortisol rhythm). When just the selected control variables were entered in the HLM Level 2 model (mother age, child age, marital status, use of thyroid medication), 22% of the variance in morning cortisol levels was accounted for, and 25% of the variance in cortisol slopes. With the addition of the relationship functioning composite and the selected home and work demand variables (number of children and hours of work), the amount of variance explained increased to 40% for morning cortisol values and 43% for cortisol slopes.

3.4. Predicting individual differences in average daytime cortisol levels

In the previous set of analyses, a variety of control, relationship and home and work demand variables predicted individual differences in participants' morning cortisol values and the slopes of their diurnal cortisol curves. What are the implications of this for understanding individual differences in mothers' average daytime cortisol levels?

Theoretically, the combination of lower morning cortisol values and shallower slopes could result in higher average values across the day, depending on whether the shallower slopes result in significantly higher evening cortisol values. As an initial test of this possibility, the HLM analyses were re-run with participants' midnight cortisol values serving as the intercept of the model, so that the model now examined individual differences in late evening cortisol values. There was far less variability in evening cortisol levels, and few significant associations between evening cortisol levels and the variables of interest. Mothers who were not married or in a stable relationship had significantly higher evening cortisol levels [($t(62)=2.531$, $P=0.01$]. Mothers whose children in the study were older had significantly lower evening cortisol levels [$t(62)=-2.834$, $P=0.001$]. There was a weak trend [$t(62)=-1.403$, $P=0.16$] for mothers with higher relationship quality to have lower evening cortisol levels. For the most part however, participants' cortisol levels converged to similar low levels in the evening hours (the HLM estimate of the average midnight value was 0.11 μg/dl), suggesting that morning cortisol levels contribute most strongly to individual differences in average cortisol levels.

Average daytime cortisol levels were estimated by taking the area under each mothers' daytime cortisol curve as modeled by HLM, then dividing by the total time period. These average levels were predicted from the selected control, relationship and home and work variables, by entering these variables simultaneously in a multiple regression model. These results are presented in Table 4. As expected, most of the variables which were previously associated with lower morning cortisol values were also significantly associated with lower daytime average cortisol levels. Younger mothers had lower average cortisol levels than mothers who were older and there was a trend for mothers whose target child in the study was younger to

Table 4
Regression model predicting average cortisol values from selected control, home and work demand and relationship functioning variables (n=70)[a]

Variable	*B*	SE(*B*)	Beta	*t*-ratio	*P*-value
Intercept	0.086	0.167		0.517	0.607
Not married	0.034	0.055	0.065	0.620	0.538
Age of mother	0.072	0.003	0.235	2.155	0.035*
Age of child	0.103	0.059	0.191	1.750	0.085+
Thyroid medication	−0.143	0.066	−0.224	−2.176	0.033*
Hours of work	−0.0015	0.001	−0.261	−2.339	0.023*
Number of children	−0.039	0.014	−0.291	−2.822	0.006**
Relationship composite	0.043	0.018	0.257	2.440	0.018*

[a] $**P<0.01$ $*P<0.05$, $+P<0.10$.

have lower average cortisol levels than those with older children. Mothers taking medication for thyroid problems, mothers who worked a greater number of hours of paid work and those who had a greater number of children also had significantly lower average cortisol levels. There was no effect of being single on average cortisol levels, but mothers with a less positive approach to relationships (poorer relationship functioning) had significantly lower average daytime cortisol levels. In general, lower morning cortisol levels (and the variables predicting them) tend to be related to flatter diurnal rhythms and a lower daily average cortisol level. The full regression model including all of the selected demographic variables, home and work variables and the relationship functioning variable accounted for 35% of the variance in average cortisol levels.

4. Discussion

This study finds systematic associations between contextual and psychological variables and individual differences in the organization of cortisol levels across the normal waking day in a community sample of adult women. First it was shown that participants' cortisol levels were strongly predicted by time of day and their daytime cortisol curves could be modeled by parameters representing morning cortisol values, the rate of decline of cortisol across the day and average cortisol levels. It was then demonstrated that significant between-subjects variability exists in these parameters, and that these individual differences in diurnal cortisol rhythms could be predicted from medical, demographic, contextual (home and work demands), and psychological (relationship functioning) variables.

In this study, a flatter slope to cortisol levels over the day appeared to be the result of lowered morning cortisol levels (rather than increases in afternoon or evening levels) and these lower morning levels contributed to an overall reduction in average daytime cortisol levels. This result points to the importance of morning cortisol levels in determining the overall form and average daytime level of cortisol in adult women. Given the limitation that only two morning samples were gathered for each participant (one on each of two days), future research should attempt to replicate this finding with more extensive measurement during the morning hours. In addition, because cortisol levels were not measured over a 24-hour period, it cannot be determined when the lowered morning values began to emerge. They may reflect different patterns of cortisol excretion during the night time hours or a smaller elevation in cortisol concentration following wakeup. The latter explanation is less likely because participants were instructed to sample *immediately* upon waking and the peak cortisol response to waking is typically reflected in cortisol levels 30 minutes after waking (Pruessner et al., 1997b). The current study does not examine whether alterations in diurnal cortisol activity are associated with differences in cortisol reactivity to stressors, nor does it provide information on the health implications of the obtained individual differences in basal cortisol activity. Finally, the study includes only women; future research should examine whether these findings generalize to men as well. These limitations aside, this is one of the first studies to find consistent associations between individual differences in diurnal cortisol activity and medical, demographic, contextual and psychological variables in normal adults in their naturalistic environments.

Given that the data in the current study are cross-sectional and correlational, it is impossible to determine the origin of the observed individual differences in diurnal cortisol activity and the direction of effect in their association with the control, contextual and psychological variables. Some portion of the observed individual differences may be due to temporary differences in state such as differences in the immediate circumstances and psychological states faced by participants on their two days of testing. Future research should measure associations between diurnal cortisol parameters and daily events and psychological states. Some of the individual differences in basal cortisol activity may also be trait-level, reflecting either stable genetic differences or stable experiential effects on the organization of stress hormone activity. In support of a genetic contribution to basal cortisol activity, past research has shown identical twins to have a higher concordance in basal cortisol activity than non-identical twins (Meikle et al., 1988; Linkowski et al., 1993). Yet an accumulation of both animal and human evidence suggests that basal cortisol activity can also be altered by chronic exposure to stressors. The process by which prior stress experiences may have long-lasting effects on basal stress-system functioning and also on the functioning of other body systems has been called "allostatic load" (McEwen, 1998). While short-term stress system activation helps to preserve an organism's stability by preparing it for response to threat ("allostasis"), excessive activation of these allostatic mechanisms may in the long term be damaging ("allostatic load"). A history of stress exposure has been associated with stress system overactivity, including weakened HPA negative feedback and chronically heightened levels of glucocorticoids (Sapolsky et al., 1986; Sapolsky, 1996). A history of chronic stress has also been associated with low basal levels of cortisol (hypocortisolism) and increased risk for autoimmune and inflammatory type disorders due to a weakened ability to contain immunological responses (Heim et al., 2000). Whether less extreme differences in stress history are predictive of more subtle individual differences in basal stress system activity such as those found in the current study remains to be determined in future research. It is likely that individual differences in basal cortisol rhythms such as those found in the current study represent a combination of 1) immediate medical and psychological state effects and 2) trait-level differences determined partially by genetics and partially by cumulative stress history. The specific results of the study are discussed with these theoretical models in mind, emphasizing the possible contribution of the individual's history of stress and coping.

Among the medical control variables, only use of thyroid medication (synthroid, taken by two of the seventy participants) significantly predicted lower morning cortisol levels and lower average cortisol levels. These findings could be the result of the condition necessitating medication use (typically hypothyroidism), or the effects of the medication itself. Previous findings of links between hypothyroidism and CRH hyposecretion in animals and humans (Chrousos and Gold, 1992) suggest the first possibility. Given that the result is based on the data of only two individuals, however, this finding should be replicated before further interpretation is warranted. In terms of the demographic control variables, older mothers (within a range of 24 to 42 years) had higher morning cortisol values and mothers with older toddlers (within a range of 21 to 36 months) had higher morning levels and steeper slopes. The relations between maternal and child age and diurnal cortisol activity are difficult to interpret — it is possible that slightly older mothers have better developed coping resources and older toddlers pose a lesser degree of challenge for mothers, although it is important to note that mothers may also have other older and/or younger children in the household.

Indeed, having a greater number of children in the household significantly predicted weaker diurnal rhythms and lower average cortisol levels in mothers. In a prior study (Luecken et al., 1997), working women with at least one child in the home were found to report greater strain at home than working mothers without children but in contrast were found to have higher urinary cortisol levels over a 24-hour period. In the current study, engaging in greater hours of employment outside the home was another variable predicting weaker diurnal rhythms and lower average daily cortisol levels in women. It is possible these differences are related to increased stresses associated with working a greater number of hours, including the demands of the work itself and less time to accomplish family and personal tasks (Frankenhaeuser, 1991). A competing possibility that the results were accounted for by differences in time of awakening and morning sampling was ruled out in our analyses. High levels of work stress have previously been associated with a flattening of the diurnal cortisol rhythm (Caplan et al., 1979). Also in line with our results, a recent study found that high levels of work-related burnout was significantly associated with lower morning cortisol levels in a group of teachers (Pruessner et al., 1999). Another recent study, however, found that chronic work-related burnout was associated with increased cortisol levels both at 0800 h and 1600 h, and found no interaction with time of day (Melamed et al., 1999). While intriguing, further investigation is needed into the relations between work-related demands and diurnal cortisol activity.

Such studies should also take into account the demands of childrearing parents encounter in the home setting.

As a result of the multiple methodologies used to assess relationship quality, and because of the consistency of the findings across the measures, the most robust finding of this study is the association between maternal relationship functioning and diurnal cortisol rhythms. All four of the relationship variables were related to the diurnal cortisol parameters in the same way, and a composite of the four relationship variables yielded the strongest associations. In all cases more positive or desirable relationship functioning was associated with higher morning values and steeper diurnal cortisol slopes (a stronger diurnal rhythm). Positive relationship functioning also predicted higher average cortisol. As suggested more generally above, there are three possible explanations for this finding: 1) poorer relationship functioning may have led to different immediate circumstances on the days of testing and temporary differences in cortisol patterning; 2) genetic differences may account for individual differences in relationship functioning and the associated differences in diurnal cortisol patterning; and 3) individuals with poorer relationship functioning may have had different histories of stress system activation, resulting in subtle but stable alterations in their basal stress system functioning.

In terms of the latter hypothesis, differential stress histories among individuals with poorer relationship functioning could reasonably have involved increased relationship conflict (higher stress) as well as reduced social support (poorer coping). With respect to the adult attachment measure in particular, it is thought that an insecure adult attachment style emerges from a history of difficult early relationship experiences (Main and Goldwyn, 1994). Insecure adult attachment has previously been linked to more negative attributions and expectations regarding others, as well as to more difficult observed interactions with others. Given that cortisol may be activated by anticipated, as well as present threat, individuals who have negative expectations regarding the behavior of others may experience stress system activity even in the absence of real relationship threat. In addition they may have experienced more frequent actual relationship difficulties, and be less effective at using others as a coping resource. Insecure attachment is also considered to reflect a repressive or defensive stance towards relationships; a coping style that Heim et al. (2000) suggested may represent a risk for hypocortisolism. These factors may help explain why relationship functioning appears to be a consistent predictor of diurnal cortisol organization in adult women.

Clearly, the investigation of factors predicting diurnal cortisol rhythms in normal adults is only just beginning (Smyth et al., 1997; Schommer et al., 1999). Our understanding of the mechanisms by which such associations might emerge and of the health significance of these individual differences in diurnal cortisol cycles is even younger. It is possible that they represent relatively benign individual differences with little significance for emotional or physiological health. It is also possible that subtle variations in diurnal cortisol rhythms are markers of initial HPA dysregulation that represent increased risk of or stepping stones along the path to more serious HPA dysregulation and associated mental and physical health problems. Future studies should examine links between diurnal rhythms and mental and physical health indicators in more detail. It will also be of interest to examine individual differences in diurnal cortisol rhythms in infants, children and adolescents to trace the developmental emergence of individual differences in diurnal cortisol activity. The current study provides initial evidence that their quality of functioning in relationships and the demands of their home and work environments may be important predictors of individual differences in the organization of cortisol levels across the waking day in adult women.

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