CORTISOL SECRETION THROUGHOUT THE DAY, PERCEPTIONS OF THE WORK ENVIRONMENT, AND NEGATIVE AFFECT[1,2]

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ABSTRACT

The effects of explanatory variables derived from a work stress model (the effort–reward imbalance model) on salivary cortisol were assessed. A multilevel analysis was used to distinguish the effects of single occasion and multiple occasion measurements of work stress and effect on cortisol. The single (or cross-sectional) factors include Effort–Reward Imbalance (ERI), need for control, negative affect, and other enduring factors (type of occupation, gender, and smoking). The multiple occasion measurements include momentary negative mood, Momentary Demand–Satisfaction Ratio (MD–SR), sleep quality, work load (workday versus day off), at work (versus not being at the workplace), and lunch. The effect of time of day on cortisol was controlled for before the effects of these variables were determined.

Momentary negative mood but not trait negative affect was positively associated with ambulatory measured cortisol. The variables from the work stress model—effort, reward, need for control, and the multiple occasion measurements of demand and satisfaction—did not affect cortisol. As could be expected, time of day had an effect on cortisol, but a hypothesised interaction with momentary negative mood was not found. Additionally, the results show that the time course of cortisol differs between individuals and that the effect of sleep quality on cortisol can vary from person to person. This points to the necessity of continued efforts to single out sources of individual variability.

The finding that variables derived from the effort–reward imbalance model are not related with cortisol does not support the hypothesis that ERI leads to short-term changes in cortisol, indicating no relation with hypothalamic–pituitary–adrenal (HPA) axis activity. On the other hand, the present results invite further qualification of negative affect as a potential determinant of HPA activity, at least, as far as can be deduced from cortisol measurements.

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INTRODUCTION

It has been established that a chronic increase in hypothalamic–pituitary–adrenal (HPA) axis activity and the subsequent increase in cortisol is associated with negative health outcomes (1). HPA axis activity and increases in cortisol have been associated with life stress (2–5), work stress (6,7), negative affect (8–10), and loss of sleep (11–13). A number of authors argue that affect is the major cause of cortisol increases (8), but the evidence for this, particularly in daily life, is still lacking (8,10). The present study focuses on this issue, also providing information about the relative contribution of affect and work stress on cortisol throughout the day.

The Effort–Reward Imbalance (ERI) theory (14) provides a model for enduring work stress, resulting from low reward considering the demanded efforts. The work stress theory also states that conditions of high effort and low reward induce adverse emotional consequences and possibly affect the physiological functioning of an individual (15). Employees with a high ERI have been shown to have a higher risk of developing cardiovascular disease (16). To date, the short-term underlying mechanisms of this process still remain unclear. It has been demonstrated that high chronic work stress is associated with a slower decrease of cortisol towards the end of the day (17). This is of potentially great importance, since the shape of the cortisol curve (lower than normal values in the morning and higher than normal in the evening) has been related to lower well-being and health (18). The same is hypothesised for ERI. Furthermore, the ERI theory states that individuals with a high need for control are less likely to disengage from stressful experiences caused by high ERI (19). Therefore, the effect of ERI on cortisol is expected to be moderated by need for control (i.e. an interaction is expected between ERI and need for control).

As was mentioned above, repeated measurements of ongoing situations have also revealed that daily stressors are associated with increased cortisol (9,10). In the present study, the ratio of high demand and low satisfaction is considered a momentary assessment of stress throughout the day at work and at home. Thus, a high self-reported Momentary Demand–Satisfaction Ratio (MD–SR) at a given time of the day is expected to be associated with a higher cortisol at that moment. By controlling for time of day, it is determined whether this effect is superimposed on the well-known cortisol rhythm throughout the day. Evaluations of events throughout the day can be performed using an Ecological Momentary Assessment (EMA) technique (20). To our knowledge, this is the first study that measured demand and satisfaction using EMA.

Reich and coworkers (21,22) also measured demand and satisfaction, but the ratings were given at the end of the day. In their studies, subjects had to rate the demand frequency of 30 daily events and whether he/she had actually responded to the event. The outcome of the activity was then rated on a 5-point dissatisfaction/satisfaction scale. An average demand/satisfaction ratio was obtained by dividing the number of activities reported by the total of all satisfaction ratings. Another difference with the present study was that an estimate of occurrence frequency was used to reflect demands rather than an appraisal of the distress caused by the demands. The effect of daily work load on cortisol dynamics has yielded equivocal results. Lundberg et al. (23,24) found a higher cortisol on days with a higher work load in comparison to days with a low (or no) work load, but Pollard (7) did not find cortisol differences between a day off and working days. A possible explanation is the mediating effect of negative affect (8).

According to the literature, increases in cortisol are the result of negative affect involving fear, anxiety, helplessness, and loss of control (25–28). Negative affect is the experience of a wide range of traits and negative emotions like trait anxiety, depression, and negative mood (29,30). It is described as "... a general dimension of subjective distress and unpleasurable engagement ..." (31). Popular instruments used to indicate negative affect are the Positive Affectivity/Negative Affectivity Schedule (PANAS) (31) or the Spielberger State–Trait Anxiety Inventory (STAI) (30). In the present study, trait negative affect was measured by seven items reflecting anxiety and depression (32). In addition to this, momentary negative mood was also measured. This is a person's self-report of mood at different moments of the day (9,33). In the literature, negative affect is considered to cause inflated correlations between stressors and self-reported health symptoms (34,35). Buchanan et al. (8) argue that negative affect rather than stress is expected to be associated with increases in cortisol.

Cortisol has been found to increase if the amount of sleep in the preceding night was small (11). Subjects with lower durations of sleep who experienced less recovery after sleep showed altered cortisol curves during the day, reflected as a delay in the recovery of the hypothalamic–pituitary–adrenal axis from early morning circadian cortisol stimulation (12,13). This is interesting, as sleep is important in restoration of biological functioning (36), and a delay in recovery may involve an alteration in negative glucocorticoid feedback regulation (13). In this respect, sleep loss can be assigned a role in the stress–health relation.

Other factors like smoking (10), gender (37), and food consumption at lunch time (38–40) also influence cortisol dynamics. These effects are superimposed on the well-known time of day effect (10,41) and therefore will be taken into account in this study.

Summarising, cortisol patterning throughout the day is expected to vary as a function of negative affect, work stress, and sleep quality, superimposed on the effect of time of day. The following hypotheses are tested, after controlling for the well-known effects of lunch consumption, gender, occupation, and smoking: (a) Negative affect (i.e. trait negative affect and momentary negative mood measured throughout the day) is positively associated with cortisol; (b) Subjects with high work stress (i.e. high ERI, a high need for control [and their interaction], a high MD–SR) have higher cortisol; (c) Cortisol is elevated on workdays and when subjects are at the workplace; and (d) Cortisol is elevated in subjects that have some difficulty sleeping.

METHOD

Subjects

A total of 104 subjects in two companies were invited to participate in the study. After a meeting in which the objectives of the study were explained, 77 subjects agreed to participate in the present study. Thirty-six of the participants were health professionals (mean age = 39.8, *SD* = 4.7; 20 males, 16 females) and 41 were office clerks (mean age = 32.9, *SD* = 9.8; 23 males, 18 females). The age and proportion of male subjects did not differ significantly between the occupations. The work tasks of both professions were usually performed sitting down and had a low physical component. A large part of the day was spent answering telephone calls, communicating with clients, or typing data into a computer. The majority of the health professionals were nurses working at a "911" emergency line and a drug rehabilitation centre in Amsterdam (the Netherlands). The office clerks worked at the complaint department of a large telecommunication company in the Netherlands. All subjects reported the confrontation with clients (e.g. emergency telephone calls, drug addicts, and dissatisfied customers) as rather stressful. Based on the similarities between the groups, it may be concluded that the entire sample is homogeneous. However, to ensure this, the variable "occupation" was added to the analysis.

Materials

Effort, Reward, and Need for Control: Effort, reward, and need for control were measured using the revised Dutch Effort–Reward Imbalance Questionnaire (42). Effort was measured by 6 items that refer to demanding aspects of the work environment (e.g. "I have constant time pressure due to a heavy work load"). If the statement was affirmed, the subjects were then asked to rate its severity from "not at all distressed" (1 point) to "very distressed" (4 points). A negative answer to the statement scored 1 point. Reward was measured by 12 items that refer to the three following topics: esteem reward (6 items), monetary gratification (1 item), and status control (5 items) (e.g. "My promotion prospects are poor"). These items were scored in the same way as the effort items, so that a minimum score of 1 point and a maximum score of 4 points per item could be obtained. In this study (n = 77) an internal consistency (Cronbach's alpha) of .67 and .78 was found for respectively extrinsic effort and reward. A score for effort–reward imbalance was calculated by dividing the score on effort by the weighted score on reward: effort/(reward * (0.5)). The reward scale has twice as many items as the effort scale. Multiplying reward by 0.5 corrects for this. The need for control scale consists of 9 dichotomous items (e.g. "I don't let others do my work." Agree/Disagree). Affirmative answers to the question scored 1 and disagreement scored 0. The internal consistency of the need for control scale in this study (n = 77) was 0.92.

Trait Negative Affect: Negative affect was measured using a Dutch translation (33) of the Well-Being Questionnaire (32). The questionnaire consisted of four subscales: anxiety, depression, energy, and positive well-being. A factor analysis performed on the anxiety and depression subscales resulted in a new subscale (7 items) called negative affect. The items used to measure negative affect refer to feelings of depression (e.g. "I have crying spells or feel like it") and anxiety (e.g. "I feel nervous and anxious"). Both depression and anxiety are associated with negative affect (29,30). Each item was rated on a 4-point numeric scale (with the labels *never* and *always* on the extremes). The range of the scores was 21. A psychometric analysis performed on the scale revealed a satisfactory internal consistency (Cronbach's alpha = .86) (33). In the present study, an alpha of .82 (n = 77) was obtained.

Sleep Quality: The Groningen Sleep Quality Scale (14 items) was used to measure subjective sleep quality of the preceding night (43,44). The scale covers various complaints about sleep such as, sleep quality in the previous night, insufficient sleep, difficulty falling asleep, etc. Higher scores on the scale indicate a lower sleep quality. A score between 2–4 is considered normal in a healthy population. The internal consistency (Cronbach's alpha) was .85 on the first day and .87 on the second day.

Smoking, Lunch, and Occupation: Subjects were asked whether they smoked, at what time they went for lunch, and their occupation (i.e. office clerk or health professional). The time subjects went for lunch and whether they were smokers were coded as dummy variables and added to the analysis (0 = no lunch, 1 = lunch; 0 = nonsmoker, 1 = smoker).

Workday and Being at the Workplace: Cortisol samples were collected throughout the entire day, on a workday and a day off. On the workday, samples were collected at the workplace (at work) or elsewhere (not at the workplace, e.g. at home). The variables at work and workday were coded as dummy variables and added to the analysis (0 = at work, 1 = not at the workplace; 0 = workday, 1 = day off).

Momentary Demand, Satisfaction, and Negative Mood: An EMA diary was used to measure momentary demand, satisfaction, and negative mood. The diary contained three questions about the perceived demands: (a) "Since the last beep I was interrupted a lot." yes/no), (b) "Since the last beep I was under time pressure." yes/no), and (c) "Since the last beep I experienced physical demands." yes/no). Two questions referred to perceived satisfaction: (a) "Since the last beep my actions were worth the trouble." yes/no) and (b) "Since the last beep my input was acknowledged." yes/no). An appraisal was obtained reflecting the level of distress caused by each of the demand and satisfaction items. Distress was rated on a scale running from 1 (*Not at all distressed*) to 4 (*very distressed*). The scores for the total demand scale were obtained by summing the answers of the three items together with the scores on the yes/no items, leading to a minimum of 0 points and a maximum of 12 points. The scores for the total satisfaction scale were obtained by adding the answers of the two items together, leading to a minimum of 0 points and a maximum of 8 points. Finally, a score for the momentary demand–satisfaction ratio was obtained by dividing the scores on demand by the scores on satisfaction: ((⅔)*demand)/satisfaction. The demand scale has three items, and the satisfaction scale has two items. Multiplying demand by ⅔ corrects for this difference in number of items.

The subjects were asked to rate their momentary negative mood using four mood adjectives. The scores on negative mood (e.g. "I feel sad") were obtained using a numerical scale (ranging from 1 = *not at all* to 7 = *very much*). The items used to rate negative mood were a selection of 4 out of 5 variables used by Smyth et al. (9): sad, angry, unhappy, and worried. The minimum score for negative mood was 4 and the maximum score was 28 (range = 24). In this study, we found an internal consistency (Cronbach's alpha) of .80, .76, and .85 for negative mood, demand, and satisfaction, respectively.

Cortisol: Cortisol was determined from saliva samples. Salivary cortisol is considered a reliable index of free plasma cortisol (45). The secretion of cortisol is episodic and pulsatile (46), and the amount of cortisol in saliva increases within minutes after the occurrence of a stressful experience. The amount of cortisol found in saliva reaches its peak approximately 20 minutes after the stressor (47).

Cortisol samples were obtained as follows. Subjects were asked to chew on a cotton swab until it was saturated in saliva. This swab was then placed in a plastic tube (called "Salivette" manufactured by Sarstedt), capped, and placed in a refrigerator at the subject's home or workplace. After the experiment, the Salivettes were stored at −20°C. In the present study, cortisol collection rate was 85% (956 cortisol samples were collected). Five samples were discarded due to extreme values (>50 nM). Initially, salivary cortisol values were determined by employing a time-resolved immunoassay with fluorometric end point detection (see 48). To increase cost-efficiency, the remaining saliva samples were analysed using radioimmunoassay employing a polyclonal anticortisol–antibody (K7348). [1,2-3H(N)]-Hydrocortisone (NET 185,NEN-DUPONT, Dreiech, Germany) was used as a tracer following chromatografic verification of its purity. The lower detection limit of both assays was less than 0.43 nM. A separate test was performed to determine whether the two assay types produced the same results. To achieve this, cortisol was determined from the same saliva samples with both detection methods. The values did not differ significantly as was determined by a t-test, confirming that the assay types produced identical values. The intraclass correlation of the initial batch (0.11, n = 274) and the remaining batch (0.19, n = 576) showed some difference in the variance at the subject level. The difference between the batches was more likely the result of a larger variation in sampling moments than of assay types. Since the effects of sampling moments (time of day) were controlled for, it was concluded that the samples from the two batches could be combined in the analysis reported in the Results section.

Procedure

The data in this study were collected by means of questionnaires, Salivettes (test tubes for saliva collection), and diaries. Two days before the diary data collection started, the subjects were asked to complete questionnaires about effort, reward, need for control, and negative affect. They were also questioned about their medical history (hypertension, etc.), work environment (type of occupation), and personal characteristics such as sex and age. The diaries were used to obtain within-day measurements of demands, satisfaction, and mood. These measurements were carried out according to the principles of Ecological Momentary Assessments or the Experience Sampling Method (ESM) (6,10,49,50). Subjects were asked to fill out a diary several times a day. The diary questions were presented to the subjects via a palm-top computer (HP-100 LX) that beeped at semirandom intervals throughout the day. In total, 1,123 beeps were generated (for all subjects), 1,014 of which were answered (compliance rate = 90%). The first beep was after 8:00 a.m., and the last beep was not later than 10:30 p.m. In the subsample of health professionals, beeps were generated 6 times a day, at semirandom intervals of approximately 140 minutes. Beeps were clustered 20 minutes before and 20 minutes after these 140-minute intervals. In the subsample of office clerks, beeps were generated 10 times a day at semirandom intervals of approximately 90 minutes. In this subsample, beeps were clustered 20 minutes before and 20 minutes after the 90-minute intervals. Saliva was collected on 2 days, a workday and a day off (see measurement of cortisol). Subjects were instructed to collect saliva at the moment of the beeps and to label and preserve the salivettes.

Statistical Analysis

In the present study, cross-sectional data from questionnaires as well as within-day and daily diary data were collected (see Table 1). The questionnaires were used to measure effort, reward, need for control, and trait negative affect. The diaries were used to collect information about momentary negative mood, work stress, and sleep quality on 2 days (a workday and a day off). Erroneously, the relation between the independent variables and cortisol is sometimes determined by performing a series of regression analyses. Performing a large number of regression analyses to determine multilevel data may be problematic if not addressed adequately. Examples of problems that may arise are omission of entire subjects due to missing values or measurement points, aggregation bias, capitalisation of chance due to multiple testing, unequal timing of assessment, and correlated assessments (51,52). As can be understood from the previous sections, a subject was randomly prompted (by a beep) throughout the day. This leads to measurements that vary in time throughout the day (within-day level) as well as between subjects (subject level). To avoid the above-mentioned problems and to adequately analyse this multilevel data, a two-level linear model (or random coefficient model) (53–55) was performed.

In this paper, several models were explored to test the relationships between cortisol and explanatory variables at each level. Model 1 was used to decompose the total variance of cortisol$^{0.2}$ into between-person and within-person variance and serves as a baseline model. The effects of time of day were determined in Model 2, and the effects of the remaining variables were tested in Model 3. All nonsignificant effects were removed from the model (see Table 2).

All estimates were obtained using the program MLn (54). The significance of a fixed effect was determined by comparing it to its standard error. To achieve significance, the fixed effect should be at least twice the standard error. The significance of a random effect was determined by the likelihood ratio test (53). Only significant fixed effects are presented in the tables. Where necessary, explained variance and random effects are reported in the text.

RESULTS

Descriptive Statistics

To increase the comprehensibility of the data, some descriptive statistical analyses have been performed: mean, Standard Error of Mean (SE Mean), and their quartile scores (see Table 3). Mean and SE Mean for within-day variables were derived by aggregating the scores at each beep over subjects and days. The mean Effort–Reward Imbalance ratio (0.49) shows that the present sample was not highly stressed. According to a criterion provided by the theory, only subjects with an ERI ratio larger than 1 are at risk of developing cardiovascular disease. The average sleep quality is 3.73, which is normal for a healthy working population.

Time of Day Effects on Cortisol

Before testing whether the explanatory variables had a significant effect on cortisol, the amount of variance at each level (the within-day level and subject level) was assessed. The amount of variance at each level was derived from an empty model (Model 1, Table 2). A simple calculation shows that 16% of the variance is at the subject level and 84% at the beep level (see Table 2). After controlling for time of day, the beep level or within-person variance decreases from 0.0348 (Model 1) to 0.0198 (Model 2). This means that time of day accounts for as much as 43% of the within-person variance.

TABLE 1
Measurement Levels and Variables

Variable	Within-Day Level	Subject Level
Time	*	
Cortisol	*	
Momentary negative mood	*	
Momentary Demand–Satisfaction Ratio (MD–SR)	*	
Sleep quality	*	
Lunch	*	
Work day	*	
Effort–Reward Imbalance (ERI)		*
Need for control		*
Negative affect		*
Occupation		*
Gender		*
Smoking		*
ERI * time	*	*
ERI * need for control		*
Momentary negative mood * time	*	

To adequately control for the effect of the time of day on cortisol, a cortisol curve was first estimated and plotted against time of day. Before this curve was estimated, a fifth root transformation was performed on cortisol (cortisol$^{0.2}$) data to correct for skewness. In accordance with Ockenfels et al. (6), this transformation resulted in normally distributed cortisol values (skewness = -0.19, minimum = 0.63, maximum = 1.91). As is shown in Table 2 (Model 2), the cortisol curve can be described by a third degree polynomial, including the time variables "time" and "time^3." Time^4 had no significant effect on cortisol. No other higher order time variable was calculated. Both the observed and the estimated curves are plotted in Figure 1. The estimated values closely follow the observed values, showing an adequate fit. A random term for time was introduced into the model, and its effect was tested. A random term allows for individual differences in cortisol at different times of the day. The effect of this term was significant. This means that the effect of time differs between subjects. The other time variables ("time^2" and "time^3") had no significant random effect.

Effects of Negative Affect on Cortisol

Negative affect was measured at two levels: trait negative affect (measured cross-sectionally) and momentary negative mood (measured throughout the day). The results show that momentary negative mood but not trait negative affect had a significant positive effect on cortisol, after controlling for time of day, food consumption, and smoker effects. To illustrate the size of the effects of momentary negative mood, observed cortisol levels of the highest and lowest quartile were calculated and presented in a figure (see Figure 2). The interaction between time and momentary negative mood was not significant. This means that although momentary negative mood has an effect on cortisol, this does not depend on the time of the day.

In the exploratory analysis performed during the preparation of statistical analyses, it was established that the variables trait negative affect and negative mood exhibited considerable skew (1.34 and 2.03, respectively). To determine whether this may affect the outcome of the analyses, skew was corrected for by performing

TABLE 2
The Effect of Explanatory Variables at the Beep Level on Cortisol

	Estimate + (s.e.)		
Fixed Effects	Model 1	Model 2	Model 3
Intercept	1.365 (0.0118)*	1.523 (0.0178)*	1.509 (0.0342)*
Time		−0.0144 (0.004)*	−0.0522 (0.0146)*
Time^2			0.0059 (0.0023)*
Time^3		−0.00008 (0.00002)*	−0.0003 (0.0001)*
Lunch			0.0504 (0.0168)*
Momentary negative mood			0.0050 (0.0017)*
Sleep quality			−0.0052 (0.0031)
Smoking			0.0324 (0.0105)*
Random effects	variance		
Subject level			
Var (intercept)	0.0068 (16%)	0.0068	0.0038
Var (time)		5.5^{-5}	
Var (sleep)			8.7^{-5}
Within-day level			
Var (intercept)	0.0348 (84%)	0.0198	0.0189
Δ deviance	—	392.66	435.71

For all models: *n* cases = 850, 77 subjects; * = $p < 0.05$. The deviance of each model with respect to the null model was calculated (Δ deviance).
Model 1: An intercept only model (empty model), for estimating variance at the subject and within-day levels. The percentage of the total variance is given within parenthesis ( ).
Model 2: The variables 'time,' 'time^2,' and 'time^3' (in hours after 8:00 a.m.) were introduced. The random effect of the 'time' and 'time^3' variable was significant (deviance change Var (time) = 14.70 $df = 1, p < 0.001$).
Model 3: Final model including all significant effects. The fixed effects of time^2, momentary negative mood, and the random effects of sleep quality (deviance change Var (sleep) = 8.39 $df = 1, p < 0.001$) are significant.

TABLE 3
Mean and Standard Error of Mean (SE Mean) for the Momentary Demand–Satisfaction Ratio (MD–SR), Effort–Reward Imbalance (ERI), Need for Control, Trait Negative Affect, Momentary Negative Mood, and Sleep Quality

	MD–SR	ERI	Need for Control	Negative Affect	Momentary Negative Mood	Sleep Quality
N	850	77	77	77	850	144
Mean	2.03	.49	3.70	2.95	6.45	3.73
Standard Error of Mean	.05	.01	.11	.10	.13	.10
Percentiles						
25	.83	.38	1.00	1.00	4.00	1.00
50	1.50	.46	3.00	2.00	4.00	3.00
75	2.50	.58	7.00	4.00	8.00	5.00

a square root transformation, and the analyses were repeated. These results support those reported in Table 2, confirming that momentary negative mood but not negative affect was related to cortisol.

Effects of Work Stress, Work Load, and Being at Work on Cortisol

The variables ERI, need for control, MD-SR, work load, and being at work had no significant effect on cortisol and neither did the interaction between ERI*time, ERI*need for control. To determine whether the effects of work stress, work load, and being at work could be masked by negative affect, their effects were determined after omitting negative affect. The results remained the same; no effect on cortisol was found.

The Effects of Other Explanatory Variables on Cortisol

After controlling for time of day, the effects of all other variables on cortisol were tested (Table 2, Model 3). The results show a significant fixed effect for momentary negative mood, lunch, and smoking. The variables sleep quality, trait negative

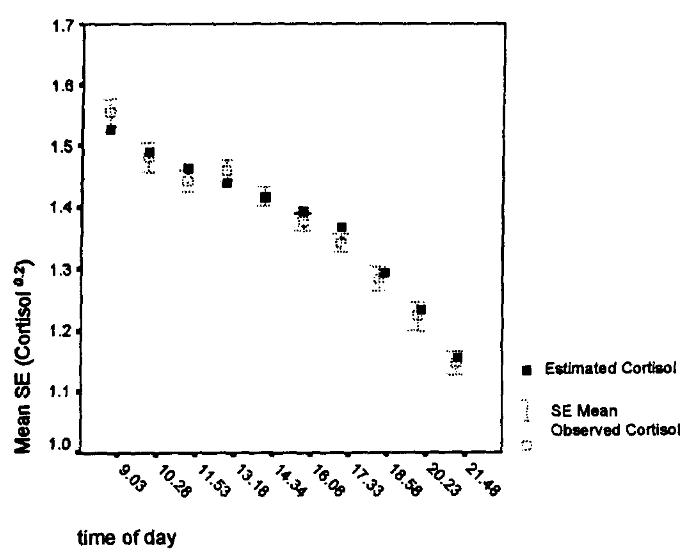

FIGURE 1: **Estimated and observed cortisol throughout the day.**

affect, occupation, and gender did not have an effect on cortisol; neither did the interaction between momentary negative mood and time. A random effect was found for sleep quality. This means that the effect of sleep quality differs between subjects. All other random effects were not significant.

DISCUSSION

Negative Affect

Momentary negative mood but not trait negative affect had a significant positive effect on cortisol, after controlling for time of day, lunch, and smoker effects. This means that a high momentary negative mood at a given time of the day is associated with a high cortisol at that moment. The interaction between negative mood and time was not significant. Thus, the relation between negative mood and cortisol does not vary systematically by time of day. Neither does it depend on a subject's trait negative affect (i.e. the tendency of subjects to perceive events as negative). These results are in contrast to the results of van Eck et al. (10). Possibly, cortisol is only affected if levels of trait negative affect are extremely high. As can be seen in Table 3, the mean trait negative affect is 2.95 which is rather low (minimum score = 0, maximum score = 18). Correcting this skew by performing a square root transformation does not change the observed relation between affect and cortisol. Another explanation may be the differences in questionnaires used to measure negative affect. Van Eck used the Spielberger Trait Anxiety Scale to indicate negative affect. The scale used in the present study has items referring to both anxiety and depression. Thus, it may be anxiety rather than depression that is related to cortisol.

An increase in momentary negative mood by 1 unit (e.g. from 4 to 5 on a scale ranging from 4–28) is associated with a $cortisol^{0.2}$ increase of 0.0050 (that is 0.13 nmol/L). The mean level of cortisol is 7.49 nmol/L. Thus, for every 1 unit increase in negative mood, a (7.49 + 0.13)/7.49 = 1.7% increase in cortisol is found above the mean. To enable comparison with other reports in the literature, the range of the mood scales have to be identical. For example, the mood scale in van Eck et al. (10) ranges from 1–7, because the mood score was divided by the number of items. After this transformation, a 1 unit increase in the revised mood scale (range 1–7) was associated with a $cortisol^{0.2}$ increase of (7.49 + 0.52)/7.49 = 6.9%. Van Eck et al. (10) found a 4.7 ng/dl increase of agitation above the mean (81.3 ng/dl), which is (81.3 + 4.7)/81.3 = 5.8%. According to van Eck et al. (10), the effects of the agitation scale (restless, irritated, hurried, and nervous) and negative mood on cortisol are of the same magnitude. Thus, it is concluded that the effects of mood on cortisol in both studies are comparable. Smyth et al. (9) estimated the magnitude of this effect by evaluating cortisol levels related to a shift in mood from the first to the fourth quartile (from <25% to >75%). Higher levels of negative mood were related to 0.81 nmol/l higher average levels (12% increase). In the present study, a shift from the first to fourth quartile represents a 4-point change in mood which is a 6.9% increase. However, it remains unclear exactly which items (if not all) are responsible for the negative mood effect on cortisol. Future studies should address this issue. In conclusion, the effect of momentary negative mood states—as measured in an ecological valid environment—on cortisol proves to be a robust one, found in at least three studies.

The relation between negative affect and cortisol is important, because of hypothesised relations with health (1). In the present study, we have seen that an increase in negative mood at any given moment of the day is related to a cortisol increase. Differences between subjects in trait negative affect, on the other hand, do not affect cortisol. Unfortunately, measures of negative health were not included in the present study. Without such measures, the true importance of within-day cortisol increases on health cannot be determined. We therefore suggest that future studies of cortisol throughout the day should also include indices of somatic symptoms of negative health outcomes.

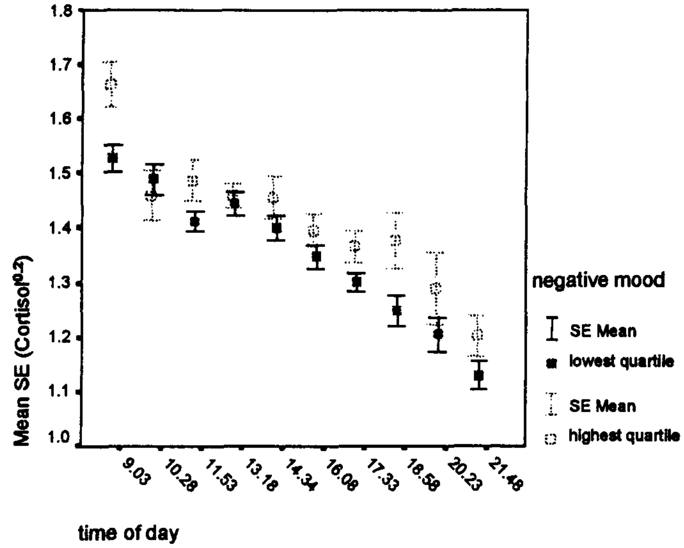

FIGURE 2: Cortisol throughout the day for the first and fourth quartiles of momentary negative mood.

Work Stress

The finding that neither trait ERI nor its within-day counterparts (demand and satisfaction) had a significant effect on cortisol is of particular importance for the ERI theory. In the introduction, it was argued that high trait ERI would be associated with higher cortisol and a slow cortisol decrease towards the end of the day. This relationship with cortisol was suggested because a high ERI is conceptually related to perceived chronic stress, which some authors have linked with higher cortisol (6). Furthermore, the effects of ERI were expected to be moderated by need for control. None of the hypothesised interaction effects were found. As mentioned in the introduction, the studies relating chronic stress with cortisol have yielded unequivocal results. Pollard et al. (7) found evidence for catecholaminergic effects of high demands in the work situation, but no effects of either demand or control on cortisol. In line with this, van Eck et al. (10) showed that chronically perceived stress did not affect cortisol. This is in contrast with Ockenfels et al. (6), who did find an effect of perceived stress on cortisol. Summarising, at closer look, the evidence for an association of chronic stress with cortisol is not uniform. As outlined by Hellhammer and Kirschbaum (56), in order to evoke or sustain HPA activation, it is necessary to have extremely stressful situations and/or situations characterised by novelty, taxing social interactions, or limited predictability. The present population is selected from a working population that was expected to suffer from high levels of work stress. The ERI scores that were obtained show that the population is probably not extremely stressed. By its very nature, the work situation—even for subjects high in Effort–Reward Imbalance—is not novel and cannot be considered unpredictable.

It was also hypothesised that the MD–SR measured throughout the day (an index derived from ERI [57]) would have a significant effect on cortisol. This was not the case and seems to be in contradiction with Smyth et al. (9) and van Eck et al. (10), both showing an effect of momentary stressors on cortisol. Again, it may be argued that the absence of an effect may be due to the characteristics of the perceived situation. Dissatisfying work situations are usually not novel or unpredictable and, as such, may be different from the kind of stressors assessed in the other studies. Additionally, there is increasing evidence (8,9,18,58) that negative mood mediates the effects of stressful events (i.e. only those events that evoke adverse affective changes result in cortisol enhancement). By their very nature, ongoing daily work-related stressors probably do not evoke strong enough emotional reactions to affect cortisol.

Alternatively, simultaneous psychological assessments and cortisol measurement is questionable, because peak cortisol secretion does not occur until 20 minutes after a stressful event. On the other hand, accounting for this by collecting saliva (for cortisol analysis) 20 minutes after being beeped (e.g. Smyth et al. [9]) is also debatable. Stressful events do not always occur at the moment of the diary beeps, but may have occurred several minutes before. Collecting saliva 20 minutes after the beep therefore does not guarantee that the time lag between event and saliva collection is 20 minutes, leaving some uncertainty. This time-lag uncertainty will remain as long as the exact times stressful events occur are unknown. Moreover, in the present study, appraisals of mood and saliva were collected simultaneously, indicating that the present set-up (i.e. not accounting for the time lag in peak cortisol response) clearly allowed detection of the effects of mood states on cortisol.

Workday and Being at the Workplace

The hypothesis that cortisol values on a workday are higher than on a day off could not be confirmed. Neither were there any differences between cortisol measured at work or elsewhere. While being at variance with Lundberg and Frankenhaeuser (24), this confirms the results of Sluiter et al. (59). A regular working day presumably—and fortunately—is not an extreme stressor, nor is it novel or unpredictable. Future studies should concentrate on assessing these situational characteristics in order to determine their effects on cortisol.

Sleep Quality

The relationship between sleep quality and cortisol shows some consistency with the results of studies (11–13,36) that showed that sleep deprivation resulted in elevated cortisol. Interestingly, our study shows the relationship between sleep quality and cortisol to vary from subject to subject (random effect). Differences between subjects regarding the circadian cortisol rhythm was also demonstrated by Hennig et al. (12). Atypical rhythms were associated with neuroticism. In future research, identification of these subjects will further improve our understanding of cortisol dynamics. From a similar point of view, it is worthwhile mentioning that the cortisol decrease throughout the day is probably not equally large for all subjects, because the effect of time of day on cortisol is also random. Slow unwinding is claimed to contribute to accumulating fatigue and to health problems (60). In the present study, we could not identify the individual differences accounting for these random effects, although we have determined that Effort–Reward Imbalance did not help explain them. We expect that clarifying the individual characteristics determining both the decrease during the day and the relationship between sleep quality and cortisol will contribute towards an understanding of the psychobiological concomitants of fatigue or burnout.

Future Studies

In conclusion, the present study has provided insight into cortisol dynamics throughout a working day and a day off. Data obtained from within-day measurements can be used in addition to other assessment methods, such as repeated exposure and aggregation (61). The results from the present study can be used to design future experiments in the field. More specifically, if most cortisol variance is to be explained at the subject level, the effects will be ascribed to differences between subjects on the explanatory variable. In this example, the effects vary with subjects and are therefore referred to as "subject-dependent." If most of the variance is to be explained at the within-day level, the effects are probably due to differences within a day. Thus, the effects vary from situation to situation, and are referred to as "situation-dependent." Table 2 shows that 84% of the variance in cortisol is at the within-day level, and 16% at the subjects. This means that even if all the variance is explained, only 16% is due to differences between subjects. The remaining 84% is situation-dependent. This variance can only be explained by other within-day variables including time of day, which may explain 40% of that variance. The finding that cortisol is substantially situation-dependent has implications for future research. The largest portion of cortisol variance is at the within-day level. To explain this variance, explanatory within-day variables, rather than subject-level variables, should be tested.

The present study shows ample leads to future studies that focus on ambulatory measurement of cortisol variations. The impact of a psychological state, momentary negative mood on cortisol could be confirmed and seems to be a robust one by now. This study confirms the hypothesis that affect rather than work-related stress (e.g. ERI or MD–SR) is related to cortisol in a healthy population. On the other hand, it is comforting to realise that experiencing minor stressors in normal daily life does not lead to increased cortisol that in the long run may be connected with negative health outcomes. Furthermore, the Effort–Reward Imbalance model explicitly states that although distress and negative emotions are important, a direct pathway (not via negative affect) to negative health also exists (16). The present study could not test this hypothesis, but could determine the relative importance between work stress and affect on cortisol throughout the day in a working population. The results show that, at least for the present healthy population, affect (momentary negative mood) is related to cortisol responses and not ERI or the demand/satisfaction ratio.

It is interesting to see whether increases in negative affect may be responsible for other behavioural changes, such as sleep deprivation, and if relations with health can be established through such alternative routes. Individual differences in both daily variations and the effect of sleep quality on cortisol offer promising links to understand psychobiological mechanisms for fatigue, burnout, or even other health problems.

REFERENCES

(1) McEwen BS, Stellar E: Stress and the individual: Mechanisms leading to disease. *Archives of Internal Medicine.* 1993, *153:*2093–2101.

(2) Selye H: *Selye's Guide to Stress Research.* New York: Van Nostrand Reinhold, 1980.

(3) Mason JW: A review of psychoendocrine research on the pituitary cortical system. *Psychosomatic Medicine.* 1968, *30:*576–607.

(4) Anisman H, LaPierre Y: Neurochemical aspects of stress and depression: Formulations and caveats. In Neufeld RW (ed), *Psychological Stress and Psychopathology.* New York: McGraw-Hill, 1982.

(5) Endresen IM, Relling GB, Tönder O, et al: Brief uncontrollable stress and psychological parameters influence human plasma concentrations of IgM and complement component C3. *Behavioral Medicine.* 1991–1992, *17*(4):167–176.

(6) Ockenfels MC, Porter L, Smyth J, et al: Effect of chronic stress associated with unemployment on salivary cortisol: Overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosomatic Medicine.* 1995, *57:*460–467.

(7) Pollard TM, Ungpakorn G, Harrison GA, Parkes K: Epinephrine and cortisol responses to work: A tèst of the models of Frankenhaeuser and Karasek. *Annals of Behavioral Medicine.* 1996, *18:*229–237.

(8) Buchanan TW, Al'Absi M, Lovallo WR: Cortisol fluctuates with increases and decreases in negative affect. *Psychoneuroendocrinology.* 1999, *24:*227–241.

(9) Smyth J, Ockenfels MC, Porter L, et al: Stressors and mood measurement on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology.* 1998, *23:*353–370.

(10) Eck MMv, Berkhof H, Nicolson N, Sulon J: The effects of perceived stress, traits, mood states, and stressful events on salivary cortisol. *Psychosomatic Medicine.* 1996, *58:*447–458.

(11) Murawski BJ, Crabbe J: Effect of sleep deprivation on plasma 17-hydroxycorticosteroids. *Journal of Applied Physiology.* 1960, *15:*282.

(12) Henning J, Kieferdorf P, Moritz C, Huwe S, Netter P: Changes in cortisol secretion during shiftwork: Implications for tolerance to shiftwork? *Ergonomics.* 1998, *41:*610–621.

(13) Leproult R, Copinschi G, Buxton O, Van Cauter E: Sleep loss results in an elevation of cortisol levels the next evening. *Sleep.* 1997, *20:*865–870.

(14) Siegrist J: Adverse health effects of high effort–low reward conditions. *Journal of Occupational Health Psychology.* 1996, *1:*27–41.

(15) Siegrist J, Peter R, Junge A, Cremer P, Seidel D: Low status control, high effort at work, and ischemic heart disease: Prospective evidence from blue-collar men. *Social Science and Medicine.* 1990, *31:* 1127–1134.

(16) Siegrist J, Klein D, Voigt KH: Linking sociological with physiological data: The model of effort–reward imbalance at work. *Acta Physiological Scandinavia Supplement.* 1997, *640:*112–116.

(17) Caplan RD, Cobb S, French JRP: White collar work load and cortisol: Disruption of a cicadian rhythm by job stress? *Journal of Psychosomatic Research.* 1979, *23:*181–192.

(18) Smyth J, Ockenfels MC, Gorin AA, et al: Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology.* 1997, *22:*89–105.

(19) Siegrist J, Peter R: Job stressors and coping characteristics in work-related disease: Issues of validity. *Work & Stress.* 1994, *8:*130–140.

(20) Stone AA, Shiffman S: Ecological momentary assessment (EMA) in behavioral medicine. *Annals of Behavioral Medicine.* 1994, *16:* 199–202.

(21) Reich JW, McCall MA, Grossman RM, Zautra AJ, Guarnaccia CA: Demands, desires, and well-being: An assessment of events, responses, and outcomes. *Journal of Community Psychology.* 1988, *16:*392–402.

(22) Reich JW, Zautra AJ: Demands and desires in daily life: Some influences on well-being. *American Journal of Community Psychology.* 1983, *11:*42–58.

(23) Lundberg U, Granqvist M, Hansson T, Magnusson M, Wallin L: Psychological and physiological stress responses during repetitive work at an assembly line. *Work & Stress.* 1989, *3:*143–153.

(24) Lundberg U, Frankenhaeuser M: Pituitary–adrenal and sympathetic–adrenal correlates of distress and effort. *Journal of Psychosomatic Research.* 1980, *24:*125–130.

(25) Levine S, Weiner SG: Coping with uncertainty: A paradox. In Palermo DS (ed), *Coping with Uncertainty.* Hillsdale, NJ: Lawrence Erlbaum, 1989.

(26) Al'Absi M, Lovallo WR: Cortisol concentrations in serum of borderline hypertensive men exposed to a novel experimental setting. *Psychoneuroendocrinology.* 1993, *18:*355–363.

(27) Frankenhaeuser M: A biopsychosocial approach to work life issues. *International Journal of Health Services.* 1989, *19:*747–758.

(28) Arnetz BB, Fjellner B: Psychological predictors of neuroendocrine responses to mental stress. *Journal of Psychosomatic Research.* 1986, *30:*297–305.

(29) McCrae RR: Controlling neuroticism in the measurement of stress. *Stress Medicine.* 1990, *6:*237–241.

(30) Watson D, Clark LA: Negative affectivity: The disposition to experience aversive emotional states. *Psychological Bulletin.* 1984, *96:*465–490.

(31) Watson D, Clark LA, Tellegen B: Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Personality and Social Psychology.* 1988, *55:*1063–1070.

(32) Bradley C, Lewis KS: Measures of psychological well-being and treatment satisfaction developed from the responses of people with tablet-treated diabetes. *Diabetic Medicine.* 1990, *7:*445–451.

(33) Doosje S, Godaert GLR: De ontwikkeling van de welbevinden vragenlijst. In Godaert GLR, Doosje S, Pennings-Elderen van der L, et al (eds), *Psychologische en Biologische Stress in Diabetes. Regulatie, Complicaties an Kwaliteit van Level.* Utrecht, Netherlands: Universiteit Utrecht, 1994.

(34) Costa PT, McCrae RR: Neuroticism, somatic complaints, and disease: Is the bark worse than the bite? *Journal of Personality.* 1987, *55:*299–316.

(35) Watson D, Pennebaker JW: Health complaints, stress, and distress: Exploring the central role of negative affectivity. *Psychological Review.* 1989, *96:*234–254.

(36) Friess E, Wiedemann K, Steiger A, Holsboer F: The hypothalamic–pituitary–adrenocortical system and sleep in man. *Advances in Neuroimmunology.* 1995, *5:*111–125.

(37) Kirschbaum C, Wust S, Hellhammer DH: Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine.* 1992, *54:*648–657.

(38) Follenius M, Brandenberger G, Heitter B, Simeoni M, Reinhardt B: Diurnal cortisol peaks and their relationship to meals. *Journal of Clinical Endocrinology and Metabolism.* 1982, *55:*757–761.

(39) Malozowski S, Muzzo S, Burrows R, et al: The hypothalamic–pituitary–adrenal axis in infantile malnutrition. *Clinical Endocrinology.* 1990, *32:*461–465.

(40) Quigley ME, Yen SSC: A midday surge in cortisol levels. *Journal of Clinical Endocrinology and Metabolism.* 1979, *49:*945–947.

(41) Krieger DT, Allen W, Rizzo F, Krieger HP: Characterization of the normal temporal pattern of plasma corticosteroid levels. *Clinical Endocrinology and Metabolism.* 1971, *32:*266–284.

(42) Hanson EKS, Schaufeli WB, Vrijkotte T, Plomp N, Godaert GLR: The validity and reliability of the Dutch Effort–Reward Imbalance questionnaire. *Journal of Occupational Health Psychology.* 2000, *5:*142–155.

(43) Mulder-Hajonides van der Meulen REH, Hoofdakker van den RH: The Groningen Sleep Quality Scale. *Scandinavian Journal of Psychiatry.* 1990.

(44) Meijman TF, Thunnissen MJ, Vries-Griever AGHd: The after-effects of a prolonged period of day-sleep on subjective sleep quality. *Work & Stress.* 1990, *4:*65–70.

(45) Vining RF, McGinley RA, Symons RG: Hormones in saliva: Mode of entry and consequent implications for clinical interpretation. *Clinical Chemistry.* 1983, *29:*1752–1756.

(46) Gallagher TF: ACTH and cortisol secretory pattern in man. *Journal of Clinical Endocrinology and Metabolism.* 1973, *36:*1058.

(47) Kirschbaum C, Hellhammer DH: Methodological aspects of salivary cortisol measurement. In Kirschbaum C, Read GF, Hellhammer D

(eds), *Assessment of Hormones and Drugs in Saliva in Biobehavioral Research.* Seattle, WA: Hogrefe and Huber, 1992.

(48) Egloff B, Tausch A, Kohlmann C-W, Krohne HW: Relationships between time of day, day of the week, and positive mood: Exploring the role of the mood measure. *Motivation and Emotion.* 1995, *19:*99–110.

(49) Csikszentmihalyi M, Larson R: Validity and reliability of the Experience-Sampling Method. *Journal of Nervous and Mental Disease.* 1987, *175:*526–536.

(50) Delespaul PAEG: *Assessing Schizophrenia in Daily Life: The Experience Sampling Method.* Maastricht, Netherlands: Unicersity Pers IPSER, 1995.

(51) Jaccard J, Wan CK: Statistical analysis of temporal data with many observations: Issues for behavioral medicine data. *Annals of Behavioral Medicine.* 1993, *15:*41–50.

(52) Schwartz JE, Stone AA: Strategies for analyzing ecological momentary assessment data. *Health Psychology.* 1998, *17:*6–16.

(53) Bryk AS, Raudenbush SW: Applying the hierarchical linear model to measurement change problems. *Psychological Bulletin.* 1987, *101:* 147–158.

(54) Goldstein H: *Multilevel Statistical Models.* New York: John Wiley & Sons, 1995.

(55) Berry CC: Multilevel statistical models for psychosomatic research. *Psychosomatic Medicine.* 1997, *59:*350–351.

(56) Hellhammer DH, Kirschbaum C, Belkien L: Measurement of salivary cortisol under psychological stimulation. In Hingtgen J, Hellhammer D, Huppmann G (eds), *Advanced Methods in Psychobiology.* Toronto, Canada: Hogrefe Inc., 1987.

(57) Siegrist J: *Soziale Krisen und Gesundheit (Social Crises and Health).* Gottingen, Germany: Hogrefe, 1996.

(58) Al'Absi M, Bongard S, Buchanan TW, et al: Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology.* 1997, *34:*266–275.

(59) Sluiter JK, Van der Beek AJ, Frings-Dresen MH: Work stress and recovery measured by urinary catecholamines and cortisol excretion in long distance coach runners. *Occupational and Environmental Medicine.* 1998, *55:*407–413.

(60) Kuiper JI, Van der Beek AJ, Meijman TF: Psychosomatic complaints and unwinding of sympathoadrenal activation after work. *Stress Medicine.* 1998, *14:*7–12.

(61) Pruessner JC, Gaab J, Hellhammer DH, et al: Increasing correlations between personality traits and cortisol stress responses obtained by data aggregation. *Psychoneuroendocrinology.* 1997, *22:*615–625.