# Self-Reported Depressive Symptoms and Stress Levels in Healthy Young Men: Associations With the Cortisol Response to Awakening

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**Objective:** There is evidence that clinical depression and negative mood are associated with elevated basal cortisol levels. Recently, measuring the cortisol response during the first hour in the morning with strict reference to the time of awakening was established as a reliable marker of individual adrenocortical activity. In studies using this marker, a relationship with self-reported stress levels and psychosomatic symptoms has been found. The goal of the present study was to investigate the association of self-reported depressive symptomatology with early morning free cortisol levels and their relationship to measures of stress. **Methods:** We assessed the severity of depressive symptoms using the Hamilton Depression Inventory and chronic and acute stress perception in 40 healthy young men. Once a week, for 4 consecutive weeks, subjects provided saliva samples collected at 0, 30, and 60 minutes after awakening. **Results:** Higher levels of depressive symptomatology were associated with a greater cortisol response after awakening. This association seemed to be stronger when only subjects in the nonclinical range of depression were included. Furthermore, cortisol levels and depressive symptomatology were significantly positively correlated with measures of chronic and acute stress perception. **Conclusions:** The present study extends earlier findings of hypothalamus-pituitary-adrenal axis hyperactivity in clinical depression to healthy young men with mild levels of depressive symptomatology. Measuring the cortisol response to awakening is proposed as an economical alternative to traditional approaches for determining basal hypothalamus-pituitary-adrenal axis activity. Associations between depressive symptomatology and chronic stress, as well as implications for future studies, are discussed. **Key words:** depression, mood, chronic stress, hypothalamus-pituitary-adrenal axis, salivary cortisol, awakening response.

ANOVA = analysis of variance; DSM-III-R = *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition revised; HDI = Hamilton Depression Inventory; HPA = hypothalamus-pituitary-adrenal axis; TICS = Trier Inventory for the Assessment of Chronic Stress; CRF = corticotropin-releasing factor.

## INTRODUCTION

Hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis in major depression has been frequently reported in recent decades (for reviews see Refs. 1 and 2). Alterations at different levels of the HPA system are believed to contribute to the observed elevation in cortisol levels. First, there seems to be an increased central drive with hypersecretion of CRF from the paraventricular nucleus of the hypothalamus (3), probably potentiated by the action of arginin vasopressin (4). Second, negative feedback control of the HPA axis is impaired in depression, probably as a result of altered capacity or function of glucocorticoid receptors (5). Using the dexamethasone suppression test as a tool to examine glucocorticoid-mediated feedback of the HPA axis, studies have shown that 50% to 60% of patients with depression (primarily severe endogenous depression) fail to display a subsequent suppression of cortisol (6, 7). Third, patients with major depression show an enlargement of the adrenal glands (8), which seems to be reversible on remission of the acute depressive episode after treatment (9). Apart from HPA dysregulations in association with clinical depression, numerous studies report negative affect in conditions characterized by hypercortisolism (10–15).

Traditionally cortisol secretion in depression or negative mood is determined as a diurnal profile in plasma (16–21) or urine (22–24). Only a few studies have used saliva samples (25–27). Some investigators have reported an association between depressive symptomatology and elevated cortisol levels especially in the morning hours (17, 21, 24, 26), whereas others have concluded that the evening cortisol levels show the highest association with depression (28).

In recent years the observation of a pronounced cortisol response to awakening (29–31) has triggered a series of studies testing the usefulness of that marker to determine basal HPA regulation. Cortisol levels in saliva with strict reference to the time of awakening increased by 50% to 70% during the first 30 minutes and showed test-retest correlations ($r$) of 0.45 to 0.70 (32). These results clearly demonstrate higher intraindividual stability than single morning cortisol assessment or measurement at predefined times (32, 33). Several studies investigated the association of the cortisol response to awakening with psychological vari-

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ables and found elevated cortisol levels 30 to 60 minutes after awakening in subjects with high levels of chronic stress during the past year (34, 35). In a similar way, perceived stress during the last month was correlated with increases of cortisol levels during the first hour after awakening after dexamethasone pretreatment (36). In contrast, a diagnosis of burnout (36) or chronic pain (37) was characterized by attenuated cortisol levels after awakening.

The above findings clearly encourage the measurement of salivary free cortisol after awakening to identify HPA hyperactivity in association with depression and negative mood. In a group of 40 healthy young men we assessed the relationship between self-reported depressive symptoms and the cortisol response to awakening and the association between depressive symptomatology and cortisol levels with measures of acute and chronic stress.

## METHODS

### Subjects

Forty healthy male university students aged 18 to 35 years (mean age = 24.3 ± 4.33) were recruited through postings on university bulletin boards. Woman were excluded from this study because of the possible confounding effects of menstrual cycle and use of oral contraceptives on HPA responsivity (38). Exclusion criteria were any history of psychiatric disorder, cardiovascular problems, and alcohol abuse. Subjects had to be medication free at the time of testing and were asked to indicate any history of chronic health problems, which were assessed to detect any factors that could affect cortisol reactivity. Six of the 40 subjects were light to moderate smokers. They reported smoking between 1 and 15 cigarettes per day. However, statistical analysis revealed that smoking was not associated with any of our dependent variables. The study was approved by the local hospital's ethics board, and written consent was obtained from each subject before participation.

### Cortisol Assessment

Cortisol was assessed from saliva collected with the Salivette sampling device (Sarstedt, Rommelsdorf, Germany). This noninvasive technique can be used at home and interferes only minimally with normal daily routines. Cortisol in saliva reliably reflects the free (unbound) fraction of cortisol in plasma (39).

Subjects collected saliva once a week (Wednesday or Thursday) for 4 consecutive weeks at 0, 30, and 60 minutes after awakening in the morning. Awakening was either spontaneous or by alarm clock. Previous studies have shown that the cortisol response is not affected by this variable (32). Subjects were asked to refrain from drinking caffeinated beverages and smoking before saliva sampling. Furthermore, they were instructed not to brush their teeth before the end of the sampling time in the morning, not to eat or drink in the 10 minutes before sampling, and to rinse their mouth with water before sampling. All psychological testing and an acute stress rating were performed within the month after cortisol assessment. The individual time delay between cortisol and psychological assessment did not create any effects.

### Psychological Assessment

*Depressive symptomatology.* Depressive symptomatology was assessed with the Hamilton Depression Inventory (HDI) (40), a revised and extended self-report version of the Hamilton Depression Rating Scale (41). The HDI consists of 23 items and serves as a measure of the severity of depressive symptoms. Subjects are asked to refer to their feelings and behavior during the past 2 weeks, which has become a standard on self-report measures of depression (40). According to the authors of the HDI, a cutoff score of 19 best distinguishes between nondepressed and clinically depressed persons rated with DSM-III-R criteria, whereas levels between 14 and 18.5 are regarded as subclinical and suggest a general level of psychological distress. The internal consistency reliability coefficient for the HDI is 0.93 (Cronbach's $\alpha$), and the test-retest reliability coefficient is 0.95 (Pearson product moment correlation). Criterion-related validity of the HDI was proven by high correlations ($r = 0.94$, $p < .001$) with the Hamilton Depression Rating Scale clinical interview; construct validity was demonstrated by strong correlations ($r = 0.93$, $p < .001$) with the Beck Depression Inventory.

*Chronic and acute stress.* Subjective stress levels were assessed with an English version of the Trier Inventory for the Assessment of Chronic Stress (TICS) (42). The TICS is a 39-item self-report scale for the assessment of chronic stress. It consists of six scales: work overload, worries, social stress, lack of recognition, work discontent, and intrusive memories. Internal consistency coefficients for the six scales vary between 0.76 and 0.88. Subjects are asked to assess on a five-point rating scale how frequently they experienced specific stressful situations during the past year. As a measure of acute stress, subjects were asked to indicate their actual stress level on a 10-point rating scale with 1 being very low and 10 being very high.

### Psychological, Endocrinological, and Statistical Analyses

The saliva samples were analyzed using a time-resolved immunoassay with fluorescence detection (43). The intra- and interassay variability was below 10% and 12%, respectively. One subject had to be excluded because of cortisol levels that were more than three standard deviations above the group mean. One subject obtained an HDI score of 36.5 (cutoff score was 19) and thus was referred to as potentially clinically depressed. Two subjects obtained a score of 19, and three subjects scored between 14 and 18.5, which corresponds to the subclinical range of depression. These subjects were excluded from analysis of the nonclinical range of depression.

To analyze the cortisol increase after awakening for the 4 sampling days, two-way within-subject ANOVAs were computed with repeated measures on the factors time (three levels) and day (four levels). To analyze the linear relationship between depressive symptoms or chronic stress and cortisol regulation, Pearson correlations were computed between the scores on the HDI and the TICS and the cortisol response after awakening. Pearson correlations were also calculated between HDI and TICS scores. Spearman rank correlations were used to determine associations between the 10-point rating scale for acute stress and HDI scores as well as cortisol levels. For all correlational analysis, cortisol levels were transformed into a single value by calculating the area under the curve for each day and then computing the median from the four individual cortisol (day) values.

Because previous studies had shown that cortisol levels 30 and 60 minutes after awakening best differentiate between groups (34, 35), the impact of cortisol levels at different times after awakening was assessed. Therefore, groups of subjects with high and low depressive symptomatology and high and low stress levels were deter-

mined by median split and entered into two-way ANOVAs (group by time) as independent variables. For the individual cortisol levels during the 4 weeks, the median was calculated for each sampling time (0, 30, and 60 minutes after awakening). These aggregated levels served as dependent variables in the ANOVA. Significant interaction effects were specified by Newman-Keuls post hoc tests.

## RESULTS

Cortisol levels after awakening showed a highly significant increase during the first 30 minutes on all 4 sampling days ($F(2,76) = 22.9$, $p < .001$). At the time of awakening, a mean cortisol level of 17.64 nmol/liter was observed; this level increased by approximately 40% to 24.55 nmol/liter 30 minutes after awakening. The response was not significantly different between the 4 sampling days (all $p$ values $> .10$). The effect size for the factor time was $f^2 = 0.26$, explaining 21% of the variability of the cortisol response to awakening ($\omega^2 = 0.21$). Figure 1 illustrates the cortisol response after awakening for the three sampling times and four sampling days.

To describe possible associations between depressive symptomatology and cortisol levels after awakening, first the HDI scores were determined. Analysis revealed a mean HDI score of 8.88, which is slightly lower than the mean score for male college students (9.44) reported by Reynolds and Kobak (40).

Pearson correlations between the total HDI score and the aggregated cortisol response after awakening showed a trend for significance ($r = 0.30$, $p = .06$) for the total group ($N = 39$). There seemed to be a stronger association ($r = 0.34$, $p = .05$) when subjects in the subclinical and clinical range of depression were excluded. Figure 2 shows the scatterplot between HDI score and the cortisol response after awakening for the nonclinical group.

Subjects were then assigned to groups with low and high depression scores by using a median split for the HDI scores (low: ≤7.5; high: >7.5). For the total group, an ANOVA (group by time) revealed a significantly higher cortisol response after awakening in subjects with high HDI scores ($F(1,37) = 7.56$, $p < .01$). The group-by-time interaction effect showed a trend to be significant ($F(2,74) = 2.91$, $p = .06$). Excluding all subjects in the subclinical and clinical range of depression still resulted in a significant group effect ($F(1,31 = 7.26$, $p = .01$). Moreover, a significant group-by-time interaction was observed ($F(2,62) = 5.4$, $p = .007$). Post hoc analysis revealed that the cortisol levels 30 and 60 minutes after awakening reflected the group differences best. Figure 3 shows the cortisol levels during the first hour after awakening for the total and nonclinical group in subjects with high and low scores on the HDI.

The total HDI score was significantly correlated

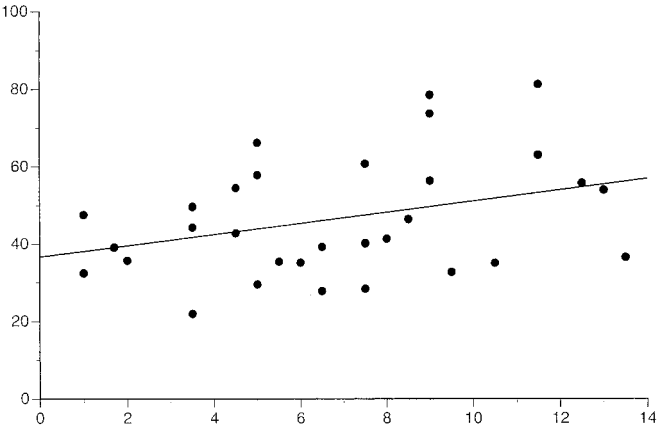


Fig. 2. Scattergram of cortisol response to awakening (area under the curve, median for 4 sampling days) plotted against the total score on the Hamilton Depression Inventory. Pearson correlation: $r = 0.34$, $p = .05$, $N = 33$.

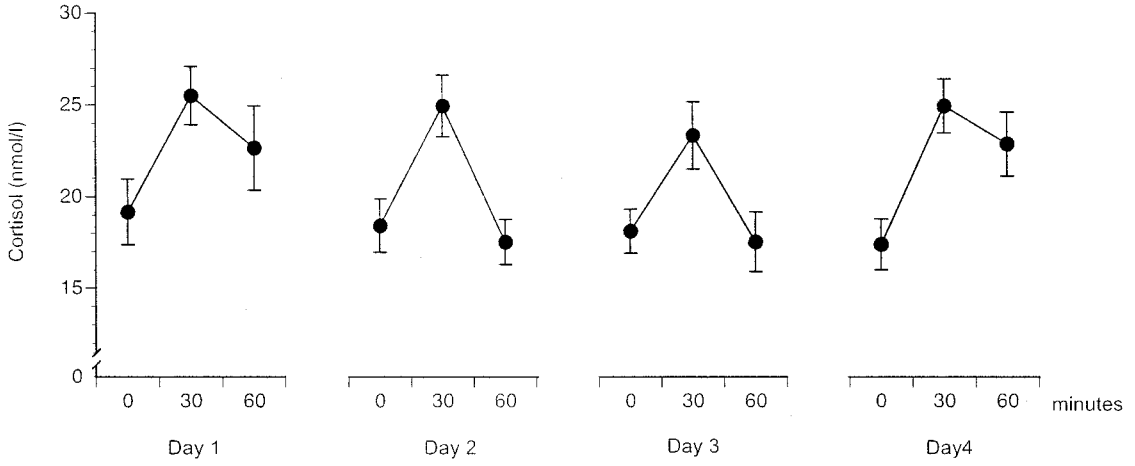


Fig. 1. Mean cortisol levels 0, 30, and 60 minutes after awakening on the 4 sampling days (1 day each for 4 consecutive weeks, $N = 39$).

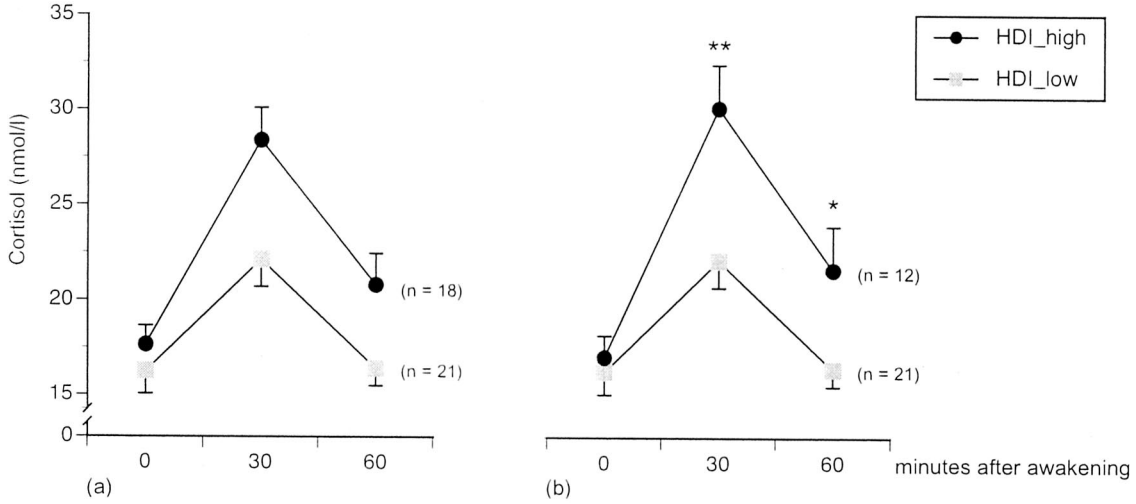


Fig. 3. Cortisol response during the first hour after awakening (median for 4 weeks) in subjects with high (>7.5) and low (≤7.5) scores on the Hamilton Depression Inventory (HDI). *a*, Total group. Main effect, $p < .01$; interaction effect, $p = .06$. *b*, Nonclinical group (all subjects in the clinical and subclinical range of depression excluded). Main effect, $p = .01$; interaction effect, $p < .007$. Difference according to Newman Keuls post hoc analysis: $*p < .01$; $**p < .001$.

with the total score on the TICS ($r = 0.67$, $p < .001$) and with scores on the work overload ($r = 0.44$, $p < .01$), worries ($r = 0.44$, $p < .01$), social stress ($r = 0.65$, $p < .001$), lack of recognition ($r = 0.59$, $p < .001$), and intrusive memories ($r = 0.67$, $p < .001$) subscales, suggesting similarities between the concepts.

Next, associations between chronic stress and the cortisol response after awakening were investigated. Correlational analyses revealed a trend for a significant association between the total score on the TICS and cortisol after awakening (area under the curve, median for 4 weeks: $r = 0.31$, $p < .06$). The only subscale that demonstrated a significant association with cortisol was work overload ($r = 0.37$, $p = .02$). However, after correcting for the number of comparisons, the result failed to remain significant.

Separating the group by median split according to the subjects' total score on the stress scale, a two-way ANOVA showed no main effect for the factor group but revealed a significant group-by-time interaction effect ($F(2,74) = 3.78$, $p = .027$). Newman-Keuls post hoc analysis revealed significant differences in the cortisol levels 30 minutes after awakening.

Assigning subjects to groups with high and low levels on each of the six subscales again revealed no main effect on cortisol for the factor group. However, for the scale work overload, a significant interaction effect was shown ($F(2,74) = 6.78$, $p = .002$); this effect was still significant after adjustment of the significance level according to the number of comparisons. Post hoc analysis revealed significant differences in the cortisol levels at the time of awakening and 30 minutes thereafter. Figure 4 shows the cortisol levels after awakening in the groups with high and low total scores on the TICS and in the groups with high and low scores for work overload.

Significant associations between the 10-point rating scale for momentary feelings of stress and the HDI were demonstrated by Spearman rank correlations ($r = 0.42$, $p = .008$). This result further demonstrates a partial overlap of self-perceived stress and feelings of depression. Also, this measure showed the highest correlation of all stress measures with cortisol levels after awakening ($r = 0.46$, $p = .004$; see Fig. 5).

## DISCUSSION

The present study examined the relationship between depressive symptomatology, measures of stress, and the cortisol response to awakening in healthy male college students. Most importantly, a positive association between elevated cortisol levels after awakening and the self-reported severity of depressive symptoms could be demonstrated. The present finding differs from earlier studies in several aspects. First, whereas numerous studies in the past reported hypercortisolism in association with clinical depression (1, 2, 44), the present study investigated changes in HPA activation due to differences in the severity of depressive symptoms in a normal population. In contrast to the diagnosis of clinical depression, which regularly is confirmed with a structured clinical interview, the present study assessed the severity of depressive symptomatology with a self-report measure. However, because a clinical interview was not performed, we cannot exclude the possibility that clinically de-

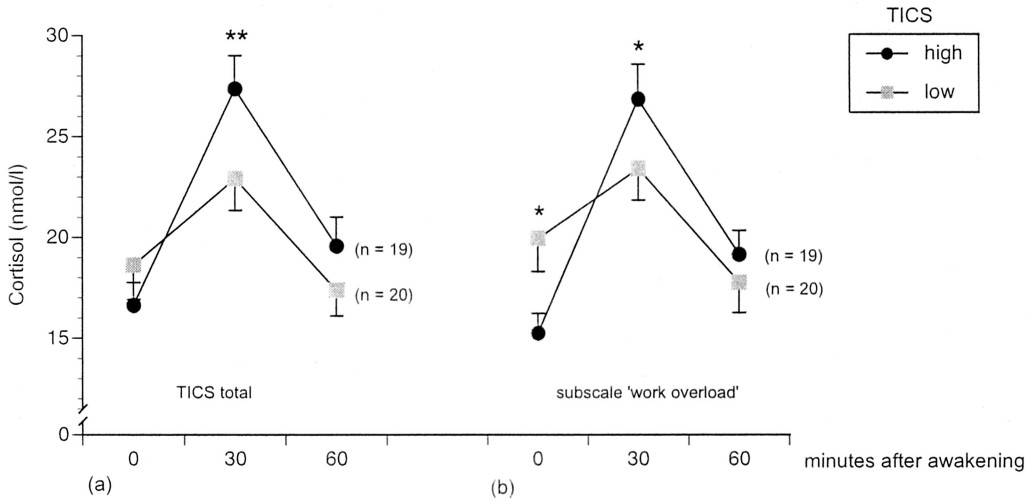


Fig. 4. Cortisol response during the first hour after awakening (median for 4 weeks). *a*, Subjects with high and low total scores on the Trier Inventory for the Assessment of Chronic Stress (TICS). Interaction effect of group by time, $p < .03$. *b*, Subjects with high and low scores on the subscale work overload. Interaction effect, $p = .002$. Difference according to Newman Keuls post hoc analysis: $*p < .05$; $**p < .01$.

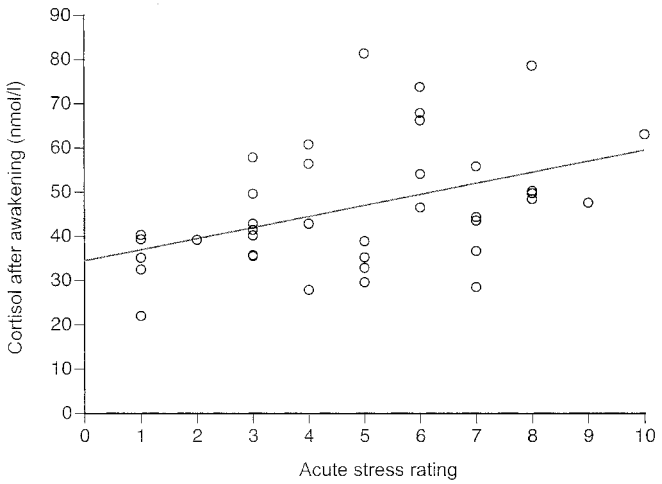


Fig. 5. Scattergram of the cortisol response after awakening (area under the curve, median for 4 sampling days) plotted against the acute stress rating. Spearman correlation: $R = 0.46$, $p < .004$.

pressed subjects were part of our population. Second, compared with previous studies measuring changes in HPA activation and mood due to predefined situational factors (14, 26, 45, 46), the HDI is believed to measure the severity of depressive symptomatology on a more general level. However, it could be argued that the 2-week period the HDI is referring to is too short to measure depressive symptomatology beyond a state. On the other hand, it must be noted that this reference time is consistent with other self-report measures of depression and the time period of symptom duration in the DSM-IV (40).

Third, cortisol regulation was assessed during 1 hour in the morning with strict reference to the time of awakening. We propose that this method has several advantages compared with traditional approaches determining cortisol regulation. Diurnal assessments of cortisol secretion in depressed patients and control subjects (16–21) led to inconsistent results concerning the time of day that best differentiates between groups. Whereas measurement of cortisol levels at predefined times underlies large inter- and intraindividual variation (33), cortisol sampling during the first hour in the morning with strict reference to the time of awakening seems to produce more reliable results (32). Moreover, determining early morning free cortisol in saliva is noninvasive, less time consuming than diurnal sampling, and can be done at home without disturbing individual morning habits.

It was intriguing to find group differences in the cortisol response to awakening already in the nonclinical range of depression. When we included subjects with more severe depressive symptoms according to the HDI, the association with cortisol was less clear than for the nonclinical group alone. The common finding that only a small proportion of clinically depressed subjects reacts with cortisol nonsuppression to the dexamethasone suppression test (6, 7) suggests that clinical depression is characterized by an increased variability of cortisol levels and that additional mechanisms might have an impact on HPA regulation.

Previous reports describing a significant relation between measures of stress and early morning free cortisol (34–36) could be confirmed and extended in the current study. Consistent with the results by

Schulz et al. (34), the present study reports significantly higher cortisol levels after awakening in subjects with high work overload. Moreover, we observed a significant cortisol difference between subjects with high and low total TICS scores 30 minutes after awakening, a finding that has not been reported before. Differences between our results and those of a previous study in which associations between other TICS subscales and cortisol were found (35) might be explained by the smaller sample size and the restriction to male subjects in the present study. Furthermore, the median cortisol level during a period of 4 weeks (assessed in this study) is expected to better represent baseline HPA activity than measurement on a single day.

Interestingly, the highest correlations of the cortisol response after awakening were found with a 10-point rating scale assessing momentary feelings of stress. Although a simple statement about the level of acute stress is rather unspecific and should not be overinterpreted, this finding points to the relevance of actual mood variations in determining cortisol differences. The observed correlation between measures of chronic stress and depressive symptomatology confirms similar evidence from other studies (47–50). The association between the acute stress measure and the HDI score was even stronger. It can be argued that the time frame both scales are referring to is overlapping. Thus, the acute stress measure might have better reflected the subject's actual situation than the TICS, which is referring to the past year.

We can only speculate about the causal relationship between stress, depression, and elevated cortisol levels, but a mediating role of life stressors for the development of depressive illness has been suggested (51, 52). The underlying dysregulations of the HPA axis in depression and chronic stress states seem to follow a similar pattern. Both conditions are characterized by increases in cortisol secretion, and in both conditions, increased central drive, impaired glucocorticoid feedback, and hypertrophy of the adrenal gland have been observed (3, 4). The consistent coappearance of hypercortisolism and depression led several authors to suggest that elevated cortisol levels cause depressive symptoms (1, 53, 54). Additional evidence for such a relationship comes from studies reporting that depressive symptomatology seems to be reversible by antiglucocorticoid therapy and antidepressant actions on HPA regulation (1, 4, 55–57).

Future studies still need to compare the usefulness of measuring the cortisol response to awakening with traditional approaches to assess HPA dysregulations. It will be particularly interesting to investigate if the proposed method is applicable for patients with clinical depression. Furthermore, subsequent studies should include more detailed questionnaires assessing the association between acute stress perception and the cortisol response to awakening. In light of the higher prevalence of depressive symptomatology in women (58), it would also be interesting to include both genders in similar investigations in the future.

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