



Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day

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Summary There is evidence showing that HPA axis responses to pharmacological provocation depend on time of day with larger cortisol responses in the afternoon and evening compared to the morning hours. However, it is still unknown whether HPA axis responses to psychological stress are affected by time of day and whether they can be assessed with equal reliability in the morning and afternoon, respectively.

The present reanalysis is based on five independent studies conducted in the same laboratory by Kirschbaum et al. (1999); Kudielka et al. (1998, 1999, 2000) and Schommer et al. (2003). All subjects were confronted with the Trier Social Stress Test (TSST) either in the morning or in the afternoon. The total sample consisted of 180 adults with 115 younger (49 females, 66 males) and 65 older adults (32 females, 33 males). All ANCOVA results controlled for possible age and gender effects. Stress-related free salivary cortisol, total plasma cortisol and ACTH net increases did not differ according to time of day (all $p = n.s.$). However, as expected pre-stress free salivary and total plasma cortisol levels differed significantly between the morning and afternoon group (both $p < 0.005$), leading to a significantly higher free cortisol area under the curve (AUC) in the morning ($p = 0.02$). Taken together, these observations suggest that the adrenal glands may be more sensitive to ACTH in the morning. Additionally, higher basal salivary cortisol levels were related to a lower stress-related net increase in salivary cortisol ($p = 0.02$), total plasma cortisol ($p < 0.0001$), and marginally ACTH ($p = 0.09$). Stress-related heart rate increases did not

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differ between groups ($p = n.s.$). The finding that the TSST-induced mood change was differentially affected by time of day requires further exploration.

We conclude that comparable HPA axis and heart rate stress responses to psychosocial stress can be measured in the morning and afternoon.

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1. Introduction

It is well-known that HPA axis activity follows a pronounced circadian rhythm with highest hormone levels in the early morning hours and continuously decreasing levels over the course of the day under unstressed conditions. The frequency and amplitude of ultradian secretory cortisol bursts change as a function of time of day (Weitzmann et al., 1971; Veldhuis et al., 1989; van Cauter et al., 1996; see also overviews by Kirschbaum and Hellhammer, 1989, 1994 and van Cauter, 1995). Therefore, if a stress stimulus is applied in the morning, pre-stress cortisol levels are usually higher compared to an afternoon or evening session. Based on the assumption that the subsequent endocrine response after stimulation depends on the preexisting baseline levels, it would be expected that the subsequent endocrine response is smaller in the morning when ACTH and cortisol levels are relatively high. One might expect that a ceiling value could be reached in case of high endocrine baseline levels, flattening the extent of the superimposed stress response, for example by providing less "space" for a stress effect.

Over the last decades numerous studies have investigated the effect of time of day on HPA axis responses applying different pharmacological provocation tests. Most studies triggered ACTH and cortisol responses by the injection of CRF (oCRF/hCRF), the exogenous administration of synthetic vasopressin (CRA-41) and ACTH (Synacthen₁₋₂₄), or insulin. Most CRF studies reported that the maximum cortisol increase after CRF administration was higher in the afternoon or evening than in the morning, although ACTH responses to CRF did not differ as a function of time of day (Copinschi et al., 1983; DeCherney et al., 1985; Schulte et al., 1985). However, two studies could not observe a difference in the responses of the HPA axis to CRF in the morning and in the eve-

ning although basal hormonal concentrations were higher in the morning (Tsukada et al., 1983; Watabe et al., 1985). Stimulation with ACTH or insulin led to greater maximum cortisol increases in the afternoon or evening compared to the morning (Takebe et al., 1969; Ichikawa et al., 1971; Nathan et al., 1979; Dickstein et al., 1991). Concerning vasopressin administration the picture is inconsistent (Clayton et al., 1963; Salata et al., 1988).

Beside the activation of the HPA axis by chemical agents, more recent studies investigated diurnal effects in physical exercise or meal intake. A standardized meal affected the HPA axis differently according to time of day, surprisingly showing an attenuated (Follenius et al., 1982) or even absent (Quigley and Yen, 1979) response in the evening. Relative to the observed baselines HPA axis responses after physical exercise appeared not to show a significant effect of time of day (Thuma et al., 1995; Galliven et al., 1997; Kanaley et al., 2001; Dimitriou et al., 2002). However, Scheen et al. (1998) could only evoke a significant cortisol response in the afternoon but not early in the morning (0500 h) using moderate-intensity exercise. Kanaley and coworkers found that a cortisol increase over control days were greatest at midnight, intermediate at 0700 h and smallest at 1900 h after high intensity treadmill exercise.

To the best of our knowledge there is no published report about the impact of time of day on physiological stress responses after psychosocial stress, investigating the question whether these stress responses can be assessed with equal reliability in the morning and afternoon hours, respectively. This is surprising facing the broad literature on HPA axis, cardiovascular and subjective stress responses after laboratory psychological stress in humans which has significantly accumulated over the last years. Therefore, the present reanalysis aims to investigate the impact of time of day on acute HPA axis stress responses, heart rate, and mood ratings before and after the Trier Social Stress Test, a widely-used standardized laboratory stress protocol which can reliably induce psychosocial stress in humans.

Abbreviations: ACTH = adrenocorticotropin; BMI = body mass index; bpm = beat per minute; CI = confidence interval; HPA axis = hypothalamic pituitary adrenal axis; MCR = metabolic clearance rate; MDBF = Mehrdimensionaler Befindlichkeitsfragebogen (German mood questionnaire); Std.Dev. = standard deviation; TSST = Trier Social Stress Test; SEM = standard error of mean; VAS = visual analog scale; yrs = years

2. Methods

2.1. Subjects

The present reanalysis is based on data from five independent studies conducted in the same laboratory by Kirschbaum et al. (1999, young women and men tested in the afternoon); Kudielka et al. (1998, elderly men and women tested in the morning; 1999, 2000 elderly women, respectively men, tested in the afternoon), and Schommer et al. (2003, young women and men tested in the morning). The accepted age range for young adults was 18–32 years, for the older subjects age was ≥ 59 years. Only placebo-treated subjects were included in the present reanalysis. Since in premenopausal women, salivary cortisol responses are significantly larger in the luteal phase than in the early follicular phase of the menstrual cycle (Kirschbaum et al., 1999), only women in the luteal phase were included here. On a first appointment, eligibility was double checked in a medical examination (exclusion criteria: psychiatric, endocrine, cardiovascular, or other specific chronic diseases, intake of psychoactive drugs, beta-blockers, estrogens, or glucocorticoids, or enrollment in another TSST study). The total sample consisted of 115 younger adults (mean age: 23.6 yrs. \pm 0.3; age range 19–32 yrs.; 49 females, 66 males) and 65 elderly adults (mean age: 67.4 yrs. \pm 0.7; age range 59–81 yrs.; 32 females, 33 males). Table 1 summarizes the sample composition according to time of day, age, and gender.

Neither mean age nor body-mass-index (BMI) differed between the morning and afternoon groups (age: $t(177) = 1.4$; $p = 0.20$; BMI: $t(177) = -0.50$; $p = 0.64$). All study protocols were approved by the ethics committee of the University of Trier and all participants provided written informed consent.

2.2. Study protocol

At the second appointment, subjects were confronted with the Trier Social Stress Test (TSST). Morning sessions begun between 0900 and 1000 h. The afternoon sessions started between 1500 to

1600 h. To avoid any interference with the morning cortisol response to awakening (Pruessner et al., 1997; Wüst et al., 2000; Kudielka and Kirschbaum, 2003; Federenko et al., 2003), subjects participating during the morning hours were instructed to wake up at least at 0700 h the same day. Wake-up or reminder calls were partly provided. After arrival, an intravenous catheter was inserted in the subjects forearm to obtain blood samples before and after the stress task (see below). Additionally, subjects were equipped with the Sport-Tester-Profi (Polar Instruments, Gross-Gerau, Germany) for wireless transmission of heart rates with electrocardiogram precision. After a rest period of at least 45–60 mins, all subjects filled out a mood questionnaire. Directly afterward, pre-stress samples were gained and the stress session was started. Post-stress samples were gained immediately after cessation of the task. After the post-stress samples, subjects again filled out the mood form and provided ratings of the stressfulness of the TSST using visual analog scales (see below). Further blood and saliva samples were collected as described below.

The TSST protocol consists of a three to five minute preparation period, a five minute free speech and a five minute mental arithmetic task in front of an audience (Kirschbaum et al., 1993). In a recent meta-analysis, the TSST was found to provoke the most robust cortisol stress responses compared with several other laboratory stress tasks (Dickerson and Kemeny, 2002).

2.3. Blood and saliva sampling, biochemical analyses

In all studies, blood samples were drawn for ACTH (adrenocorticotropin) and total plasma cortisol analyses assayed by a two-site chemiluminescence assay (Nichols Institute, Bad Nauheim, Germany) or by radioimmunoassay (IBL, Hamburg, Germany), respectively. Saliva samples were obtained by the use of Salivettes (Sarstedt, Nümbrecht, Germany). Salivary free cortisol was measured using a time-resolved immunoassay with fluorometric detection as described in detail by Dresendörfer et al. (1992). For ACTH, samples were

Table 1 Number of subjects according to day time, age, and gender

Morning				Afternoon			
Young adults		Old adults		Young adults		Old adults	
Female	Male	Female	Male	Female	Male	Female	Male
28	46	18	17	21	20	15	15

available in all studies directly before onset of the stressor, directly after cessation of the stressor as well as 10 and 20 mins later (4 samples). Blood and saliva samples for total and free cortisol were available in all studies directly before and after the stressor as well as 10, 20, 30, 45 and 60 mins later (7 samples each). Inter- and intra-assay coefficients of variance were below 10–12% for all analytes.

2.4. Heart rate

Mean heart rate responses were available for a pre-stress period of five minutes duration, the task preparation period (3–5 min), as well as the speech task (5 min), the mental arithmetic (5 min) and a post-stress period of five minutes duration. Heart rate values within a period were averaged to generate means.

2.5. Psychological assessment

In all five studies, momentary mood was assessed before and after the stress task with a German mood questionnaire (MDBF; Steyer et al., 1994). This three-dimensional questionnaire measures elevated versus depressed mood, wakefulness versus sleepiness, and calmness versus restlessness.

Visual analog scales (VAS) were employed in all studies to measure the subjective perception of the stressor based on Mason's criteria (Mason, 1968; for review see Ursin, 1998). Subjects were asked to rate the novelty, difficulty, stressfulness, controllability, unpredictability of the task (speech and mental arithmetic) and the extent of ego involvement and challenge on a straight line ranging from 0 ('not at all') to 100 ('very much'). The applied items differed slightly in wording between studies as well as in the number of used items (6–14 items).

2.6. Statistical analyses

Students-t tests were applied to test for group differences in demographics (age, BMI) and endocrine pre-stressor baselines. For basal endocrine levels and pre-stress heart rates, the 95%-confidence intervals (95%-CI) and standard deviations (Std.Dev.) were determined for the total study sample and mean levels were presented for each group separately. ANCOVAs were used to analyze responses to the stressor with the independent factors 'time of day' (morning versus afternoon) and the repeated factor 'trial samples' (ACTH: 4 samples; total plasma and free salivary cortisol: 7 samples each; heart rate: 5 time periods); and the factors 'age' and 'gender' as covariates (cf. Kudielka et al., 2003). Where appropriate, results

were corrected by Greenhouse-Geisser procedure (Greenhouse and Geisser, 1959; Vasey and Thayer, 1987; indicated by uneven degrees of freedom). Post-hoc, planned comparisons were conducted in case of significant interaction effects following the procedure offered by the statistical software. The parameter 'increase' was computed as the difference between the highest individual endocrine value after stress and the individual pre-stressor baseline. The 'area under the curve' (AUC) was computed following the trapezoid formula with reference to zero. Both parameters were analyzed using one-way ANCOVAs. Mood ratings (before and after TSST) and VAS (only after TSST) were analyzed by ANCOVAs. Correlations were conducted following the Pearson product moment procedure. The significance level was set at $\alpha = 0.05$. All results shown are the mean \pm standard error of mean (SEM). For multiple comparisons the nominal alpha-level was adjusted by Bonferroni correction. Test power ($1-\beta$), which reflects the probability of finding an effect in the data when the effect actually exists in the 'real world', was analyzed using the GPower software (Faul and Erdfelder, 1992). All other statistical analyses were performed using the software package STATISTICA (version 5.1; StatSoft, Tulsa, USA).

3. Results

3.1. Pre-stress baselines

As expected, basal salivary free and plasma total cortisol levels were significantly higher during the morning compared to the afternoon session (free cortisol: morning 10.1 ± 0.5 nmol/l versus afternoon 7.1 ± 0.4 nmol/l, $t(172) = -4.35$; $p < 0.0001$, total sample 95%-CI 8.26–9.65 nmol/l, Std.Dev. 4.67; total cortisol: morning 283.5 ± 10.8 nmol/l versus afternoon 236.1 ± 11.3 nmol/l, $t(165) = -2.84$; $p = 0.005$, total sample 95%-CI 250.3–282.6 nmol/l, Std.Dev. 105.7). Neither pre-stress ACTH levels (morning 22.5 ± 1.0 versus afternoon 25.2 ± 1.3 pg/ml, $t(169) = 1.66$; $p = 0.10$, total sample 95%-CI 22.0–25.1 pg/ml, Std.Dev. 10.3) nor basal heart rates differed by time of day (morning 79.4 ± 1.4 bpm versus afternoon 79.8 ± 2.0 bpm, $t(159) = 0.12$; $p = 0.87$, total sample 95%-CI 77.2–81.8 bpm, Std.Dev. 14.7).

3.2. Stress responses

Stress responses were analyzed following the outlined ANCOVA procedure (see above). The TSST

induced significant net increases in salivary free cortisol ($F(6,1026) = 75.2$; $p < 0.0001$), total plasma cortisol ($F(6,960) = 126.0$; $p < 0.0001$), ACTH ($F(3,501) = 92.6$; $p < 0.0001$) and heart rates ($F(4,636) = 74.6$; $p < 0.0001$; see Figs. 1(a)–(c), 2 and 4).

For salivary free cortisol a significant main effect of time of day (morning versus afternoon) could be observed ($F(1,169) = 6.3$; $p < 0.01$) while the interaction time of day by trial samples did not reach significance ($F(2.1,363.3) = 2.3$; $p = 0.10$; see Fig. 1(c)). The stress-related free cortisol net increase did not differ between morning and afternoon ($F(1,169) = 0.50$; $p = 0.48$; see Fig. 2), but the AUC was significantly higher in the morning ($F(1,169) = 6.0$; $p < 0.02$; see Fig. 3).

For total plasma cortisol there was neither a main effect of time of day ($F(1,158) = 1.02$; $p = 0.31$) nor a significant effect for AUC ($F(1,158) = 0.73$; $p = 0.40$); the interaction time of day by trial samples and the time of day effect for net increases only approached the significance level ($F(3.1,502.3) = 2.22$; $p = 0.08$ and $F(1,158) = 3.6$; $p < 0.06$; see Fig. 1(b)). For ACTH neither the main effect of time of day nor the interaction time of day by trial samples or the parameters net increase and AUC differed between the morning and afternoon sessions (all $F < 1.3$ all $p > 0.26$; see Fig. 1(a)).

However, correlation analyses in the total study sample showed that higher basal salivary cortisol levels were slightly but significantly related to a lower stress-related net increase in free salivary cortisol ($r = -0.19$; $p = 0.02$), total plasma cortisol ($r = -0.36$; $p < 0.0001$), and marginally in ACTH ($r = -0.13$; $p = 0.09$). Pre-stress total plasma cortisol levels were negatively related to total plasma cortisol net increases ($r = -0.27$; $p < 0.001$), while pre-stress ACTH levels showed small positive correlations with the subsequent ACTH net increase ($r = 0.19$; $p = 0.02$). Correlation analyses for each the morning and afternoon group separately revealed that basal salivary cortisol was significantly correlated with the stress-related salivary cortisol net increase in the morning ($r = -0.31$; $p = 0.001$), but not in the afternoon. Likewise, also stress-related heart rate net increases were similar in the morning and afternoon sessions ($F = 0.30$; $p = 0.58$; see Fig. 4).

Finally, post-hoc analyses of the test power ($1-\beta$) for the parameters net increase and AUC showed that the probability of revealing time of day effects in the present sample were 92 and 99% given big or medium sized effects of interest,

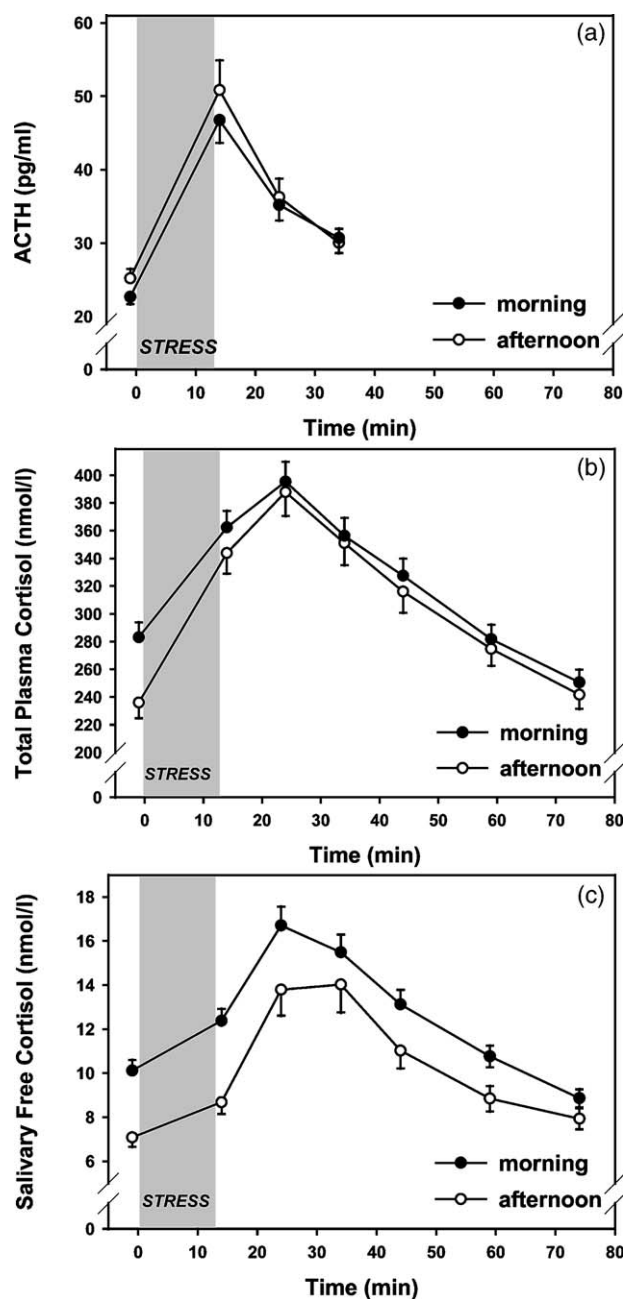


Fig. 1. Mean (\pm SEM) ACTH responses (pg/ml) (a), total plasma cortisol responses (nmol/l) (b) and free salivary cortisol (nmol/l) (c) in the morning and afternoon before and after stress (TSST). The shaded area indicates the period of stress exposure.

respectively. This shows that the given β -errors were relatively low for the present data set.

3.3. Psychological assessment

3.3.1. Mood

For elevated versus depressed mood (first scale of the mood questionnaire) ANCOVA results showed a significant main effect of trial (pre- versus post-

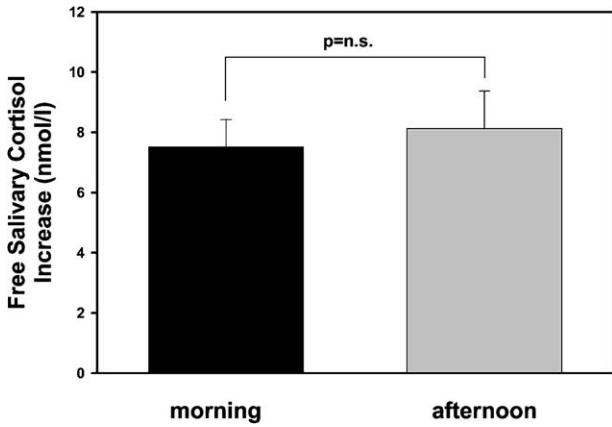


Fig. 2. Mean (\pm SEM) net increases above baseline in free salivary cortisol (nmol/l) after stress in the morning and afternoon.

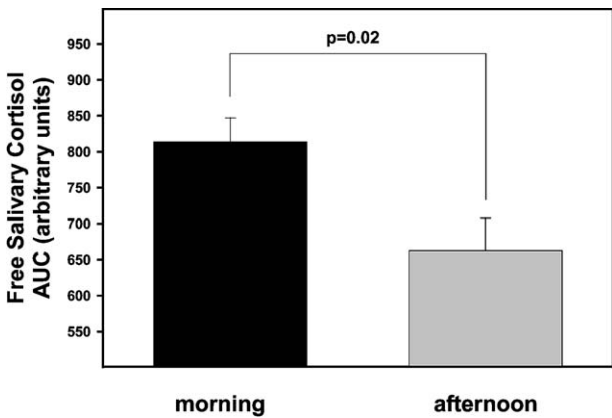


Fig. 3. Area under the free salivary cortisol response curve AUC (\pm SEM) after stress in the morning and afternoon.

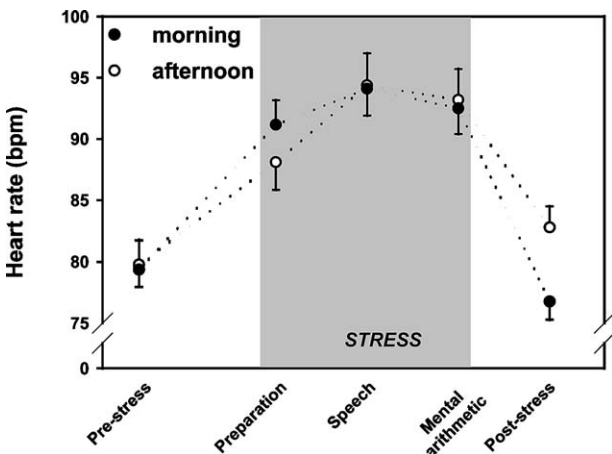


Fig. 4. Mean (\pm SEM) heart rate responses (beat per minute) in the morning and afternoon before and after stress (TSST). The shaded area indicates the period of stress exposure.

stress: $F(1,172) = 34.2$; $p < 0.0001$) as well a significant interaction time of day by trial ($F(1,170) = 5.2$; $p < 0.024$) while the main effect of time of day (morning versus afternoon) approached significance ($F(1,170) = 2.7$; $p < 0.10$). Planned comparisons revealed that mood before the stress task was significantly more elevated in the morning compared to the afternoon session ($p = 0.003$; see Fig. 5), whereas post-stress mood did not differ ($p = 0.9$). This result is reflected in the finding that the pre/post mood difference differed significantly between the morning and afternoon session ($F(1,170) = 5.8$; $p < 0.02$), indicating significantly more change in the morning.

For the second scale (wakefulness versus sleepiness) the main effect of trial (pre- versus post-stress) and the interaction time of day by trial reached significance (main effect trial: $F(1,172) = 8.7$; $p < 0.0001$; main effect time of day: $F(1,170) = 1.5$; $p = 0.22$; interaction: $F(1,172) = 4.4$; $p = 0.04$). Planned comparisons revealed that wakefulness did not differ before the stress task ($p = 0.7$), but was marginally higher after the stress situation in the morning compared to the afternoon session ($p = 0.07$; see Fig. 5). The pre/post difference in wakefulness did not reach the significance level ($p = 0.11$).

Analyses of the third scale (calmness versus restlessness) also resulted in a significant main effect trial (pre- versus post-stress) and interaction of time of day by trial (main effect trial: $F(1,172) = 51.7$; $p < 0.0001$; main effect time of day: $F(1,170) = 2.0$; $p = 0.16$; interaction: $F(1,173) = 3.9$; $p = 0.05$). Calmness before the stress task was significantly higher in the morning compared to the afternoon session ($p = 0.02$; see Fig. 5) whereas no post-stress differences emerged ($p =$

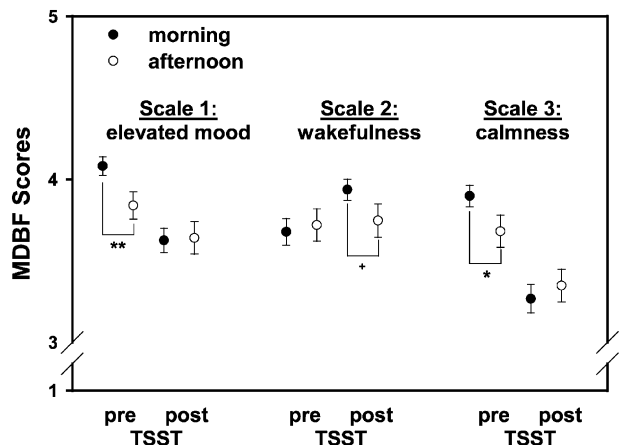


Fig. 5. Mean (\pm SEM) ratings of elevated mood (Scale 1), wakefulness (Scale 2) and calmness (Scale 3) in the morning and afternoon before and after stress (TSST). Planned comparisons: ** $p = 0.003$, * $p = 0.02$, † $p = 0.07$.

0.9). The pre/post difference in calmness approached the significance level ($p = 0.09$).

3.3.2. Subjective stress ratings

Analyses of the VAS can only be regarded as a crude approach because different versions of the visual analog scales were used in the different studies. Although, four items covering the stressfulness and novelty of the task, the uncontrollability of the situation, and the personal involvement, were applied in all five studies. Only the item uncontrollability approached the nominal significance level ($F(1,171) = 3.9$; $p = 0.065$; all other $F > 0.6$; $p = \text{n.s.}$). Taken into account a Bonferroni-correction of the nominal α -level for four comparisons to avoid α -error inflation ($\alpha' = 0.013$), this result cannot be considered as significant. Furthermore, nine identical VAS items were applied in the samples of older adults, while only one identical VAS item (unpredictability) was used in the younger adults samples in the morning and afternoon. None of the comparisons revealed significant differences. Test power for revealing day time effects in the VAS was $>92\%$ given a medium or big size effect of interest.

4. Discussion

The present reanalysis is the first report on the impact of time of day on HPA axis responses, heart rate, and mood changes after acute psychosocial stress in humans. However, it has to be acknowledged that the present data set is based on five independent studies. Although each of the five cohorts was exposed to the highly standardized TSST stress procedure in the same laboratory, there are some (in most parts inevitable) differences between the studies (e.g., personnel involved in the studies; possible seasonal effects or cohort differences in, e.g., socioeconomic status; younger adults had several additional appointments afterwards; elderly subjects received placebo treatment; etc.). Another limitation is that there is only data available comparing morning versus afternoon but not late evening or even night time (e.g., 1900 h to midnight). Therefore cautious interpretations of the present findings are warranted.

The results show that the ACTH, total plasma cortisol, and salivary cortisol stress responses to the TSST are comparable (pattern as well as net increase) when the stress test is performed between 0945 and 1900 h. This may be somewhat surprising given the higher pre-stress baseline levels for cortisol in the morning. Analyses of the test power for the parameter net increase con-

firmed that the probability of revealing an effect of time of day was satisfactorily high ($>90\%$) and underlines that medium to large effects of time of day could have been discovered with the present sample. It is still possible that small differences between morning and afternoon sessions may exist, since the test power of the present analysis was below 30% for small effects of interest.

On the other hand, we observed that higher basal salivary and total plasma cortisol levels were slightly (but significantly) related to a lower respective endocrine response after stress. This points to the possibility that for cortisol responses to psychosocial stimulation, too, higher baseline levels might reduce the net stress response to some degree. Negative correlations between basal cortisol and cortisol increases were also reported by Schürmeyer et al. (1987); DeCherney et al. (1985) and Hermus et al. (1984) applying oCRF, and more recently by Dimitriou et al. (2002) after swimming exercise. However, the associations found in the present data set were relatively small given the large sample size. These findings might point to ceiling effects in case of high baseline values but this effect seems to be especially relevant for pharmacological provocation tests which uses agents beyond the range of a normal psychophysiological experiment.

Furthermore, there seems to be a difference in the adrenal cortex sensitivity to ACTH signals in relation to time of day. In the present data, comparable ACTH stress responses (overall pattern, net increase and AUC) in the morning and afternoon led to a larger salivary cortisol AUC response in the morning compared to the afternoon (see Fig. 1(a) and (c)). In contrast, DeCherney et al. (1985); Schulte et al. (1985) and Dickstein et al. (1991) observed higher adrenal cortex sensitivity in the afternoon. Contrary to our data, the latter studies applied pharmacological provocation tests (oCRF and ACTH administration) after dexamethasone pretreatment. The contrasting conclusions might be attributable to these important differences between the studies. One possible explanation for the results obtained with CRF might be that endogenous CRF levels at the level of the pituitary are high in the morning, so that additional exogenously administered CRF causes little further HPA axis responses (see Salata et al., 1988). Furthermore, the TSST is a central stimulus, whereas CRF acts at the pituitary level. It is possible that glucocorticoid negative feedback may operate differently for a central stimulus than for a pituitary stimulus (cf. Schürmeyer et al., 1987; Galliven et al., 1997), thereby making it possible to elicit a stress response despite

heightened cortisol levels. Also, vasopressin might play an important role in centrally activated HPA axis responses, like a confrontation with the TSST. Finally, it is unlikely that a difference in metabolic clearance rate (MCR) of total plasma cortisol is responsible for the present finding of higher pre-stress total cortisol levels, since MCR is, if at all, higher in the morning, and only pre-stress total cortisol levels differed according to the time of day (de Lacerda et al., 1973; DeCherney et al., 1985; see also Clayton et al., 1963).

Normative data reported on circadian variations in cardiac control (Hayano et al., 1990) with an acrophase for heart rate between 1300 and 1600 h and minimum values at night (Clarke et al., 1976; Adan and Sanchez-Turet, 1995; Scheer et al., 1999). Heart rate responses to exercise also appear to follow a 24 h pattern as reviewed by Trine and Morgan (1995). We did not observe any circadian variations in stress-related heart rate responses probably because we did not measure during the critical periods reported by Adan and Sanchez-Turet (1996). More importantly, circadian variations in cardiac control are very complex and much more fine-grained methods are necessary.

Furthermore, mood appeared to be differentially affected by time of day. Ratings of elevated mood and calmness were higher in the morning compared to the afternoon before stress but comparable after cessation of the TSST. Wakefulness was equal before onset of the stress task but marginally higher in the morning group after stress. These findings point to the idea that mood changes might be more pronounced in the morning hours and are therefore easier to assess in the morning. These results have to be interpreted with caution because we could not control for possibly confounding factors like e.g. individual differences in "morningness" and "eveningness". In respect to the visual analog scales, no group differences between the morning and evening groups in the subjective perception of the stressfulness of the TSST could be revealed. We suggest considering this observation as preliminary result that needs further exploration because this topic is until now seldom addressed in the literature. Furthermore, more elaborated measures are warranted. However, there appear to be diurnal variations in subjective activation and mood (Monk, 1989; Monk et al., 1992; Adan and Guardia, 1993; Trine and Morgan, 1995; Kerkhof, 1998). Recently, Adan and Sanchez-Turet (2000, 2001) found significant chronopsychological effects for activation (alertness, vigor, weariness, sleepiness) as well as mood and affect (happiness, calm, tension, but not sadness). Their observation that positive mood was higher in the morning than

afternoon and evening fits nicely to our finding of higher ratings of elevated mood during the morning session compared to the afternoon session before onset of the psychological stress task and is also in line with the earlier reviewed findings by Trine and Morgan (1995).

In summary, stress responses to the TSST can be assessed with comparable reliability in the morning or afternoon. However, stress-related mood changes might be easier detectable in the morning but this topic needs much further exploration. In terms of practical recommendations, one might prefer to conduct a stress study in the late afternoon to minimize minor (but in correlation analyses detectable) effects of baseline values on stress responses during the morning, unless there are other restrictions concerning study implementation. Researchers should be aware of possible meal-induced cortisol increases (Brandenberger and Follenius, 1975; Brandenberger et al., 1982; meal and stress responses are not additive) and heart rate or mood changes (Christie and McBrearty, 1979; Hayano et al., 1990; Adan and Sanchez-Turet, 2000, 2001). In the morning, any interference with the subjects cortisol response to awakening should be avoided (Pruessner et al., 1997; Wüst et al., 2000; Kudielka and Kirschbaum, 2003; Federenko et al., 2003). This could be guaranteed by fixed awakening times and/or extended resting periods before onset of the stress procedure.

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