



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

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Received 26 May 2003; received in revised form 30 June 2003; accepted 30 August 2003

## KEYWORDS

HPA axis; Time of day; Psychosocial stress; Salivary cortisol; Trier Social Stress Test (TSST); ACTH

**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 (Synacthen<sub>1-24</sub>), 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

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