

Differential Effects of Body Posture on Biological and Psychological Responses in the Trier Social Stress Test

Miriam Kurz, MSc, Luca Abel, MSc, Felicitas Burkhardt, MSc, Robert Richer, PhD, Veronika Ringgold, MSc, Lena Schindler-Gmelch, PhD, Bjoern M. Eskofier, PhD, and Nicolas Rohleder, PhD

Objective: Acute stress protocols are important tools for laboratory research on acute stress mechanisms. Several reasons necessitate varying body posture. However, the effect of such variations has not been systematically studied. We therefore examined the impact of body posture (sitting vs. standing) on biological and psychological stress responses induced by the Trier Social Stress Test (TSST) and its control condition (friendly TSST; f-TSST).

Methods: One hundred four participants (57.69% female, mean age: 22.28, SD = 3.44) were randomly assigned to the sitting versus standing group and underwent the TSST and f-TSST on 2 separate days in randomized order. Stress responses were measured through salivary cortisol and alpha-amylase, HR, HRV, and self-reports.

Results: Biological stress responses were higher in the TSST condition and the standing group (eg, maximum cortisol increase: $p = .004$). Psychological stress response was higher in the stress condition (eg, negative affect: $p < .001$), but was not different between posture groups (eg, negative affect: $p = .819$). No differences were observed in any markers after adjusting the stress-response measures for the non-stress condition (smallest $p = .173$).

Conclusions: Although biological, but not psychological stress responses were affected by body posture overall, these effects did not persist when responses in the stress condition were adjusted by subtracting the non-stress values from the f-TSST. This implies that the effect of body posture can be neglected in studies with a non-stress control condition, but that its effects should be taken into account in studies with only the stress protocol.

Key Words: body posture, TSST, stress, cortisol, HPA axis, salivary alpha-amylase, heart rate

Abbreviations: ANS = autonomic nervous system, AUC = area under curve, BP = blood pressure, ECG = electrocardiogram, FAU = Friedrich-Alexander-Universität Erlangen-Nürnberg, f-TSST = friendly Trier Social Stress Test, HPA axis = hypothalamic-pituitary-adrenal axis, HR = heart rate, HRV = heart rate variability, PANAS = Positive Affect Negative Affect Scale, PASA = Primary Appraisal Secondary Appraisal, PNS = parasympathetic nervous system, RMSSD = root mean squared sum of successive RR interval differences, sAA = salivary alpha-amylase, SNS = sympathetic nervous system, SSSQ-G = Short Stress State Questionnaire German, TSST = Trier Social Stress Test.

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Address correspondence to Miriam Kurz, MSc, Department of Psychology, Chair of Health Psychology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Nögelsbachstr. 49a, 91052 Erlangen, Germany. E-mail: miri.kurz@fau.de

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INTRODUCTION

Stress has been recognized as the health epidemic of the 21st century.¹ Although responses to acute stress situations are evolutionary beneficial and crucial in successfully mastering threatening situations, experience of chronic stress has been linked to several adverse health conditions.^{2,3} To understand underlying determinants and consequences, laboratory stress protocols are useful to establish causality under controlled conditions.^{4–6} The Trier Social Stress Test (TSST)⁷ is the most widely used protocol and has reliably been shown to activate the stress response in humans.⁴ Despite the wide-spread use of the TSST in several thousand studies (a PsycNet search in February 2025 yielded 2473 results) and the availability of research on protocol variations,⁸ many aspects remain unclear, that, if un-researched, may confound study results. One of these is the question, if and how different body postures during the TSST affect the biological and psychological stress response.

The biological stress response is mediated by 2 distinct systems. When a stimulus is perceived as a threat or as harmful, a cascade of reactions is triggered to cope with the stressor.^{9,10} Firstly, the autonomic nervous system (ANS) is influenced, with an activation of the sympathetic nervous system (SNS) and a deactivation of the parasympathetic nervous system (PNS). The ANS response is crucial for the fight-or-flight response, and leads to increased heart rate (HR), elevated blood pressure (BP), or accelerated respiration, thus preparing the body to react quickly to perceived threats.¹¹ The SNS also includes the sympathetic-adrenal-medullary system, which induces the release of adrenaline and noradrenalin from the adrenal glands.^{12,13} Furthermore, the hypothalamic-pituitary-adrenal axis (HPA axis) is activated, which results in the release of the glucocorticoid cortisol. This hormone helps the body to mobilize and distribute resources, to effectively cope with the stressor.¹⁴ Although responses to acute stress are essential for survival, chronic and prolonged stressors can lead to altered basal activity of the HPA axis and ANS, potentially increasing the risk of hypertension, diabetes, asthma, or cancer.^{2,3,9} The altered basal activity is often accompanied by persistent low-grade inflammation, which not only contributes to cardiovascular and metabolic diseases, but also weakens the immune system.^{15,16}

Stress system reactivity is measured through various parameters. ANS reactivity is assessed through HR,¹¹ HR variability (HRV),^{17,18} BP, respiration rate,¹¹ or salivary alpha-amylase.¹⁹ HPA axis reactivity is typically quantified by cortisol levels in blood or saliva.²⁰ Psychological stress responses are evaluated using questionnaires like the Positive Affect Negative Affect Scale (PANAS),²¹ the Short Stress State Questionnaire (SSSQ),^{22,23} or visual analog scales (VAS).^{24,25}

Given the adverse effects of stress, research aims to understand its determinants and consequences. Laboratory experiments with acute stress induction are essential for identifying causal mechanisms, leading to the development of various stress protocols and measurement approaches. Among laboratory stress protocols, the TSST is widely recognized as the gold standard. Research has consistently demonstrated its effectiveness in activating stress response systems,¹² and, by combining elements of social evaluative threat and uncontrollability, it is the most effective laboratory protocol for HPA axis activation.^{4,26} The TSST has been used in and adapted for various populations and settings, including healthy individuals,⁸ patients affected by psychiatric or physiological diseases,^{27–30} children,³¹ and groups.³² Moreover, modified low-stress versions such as the friendly TSST^{33–35} have been developed to minimize or attenuate stress responses while maintaining the overall structure of the task (Burkhardt et al., *f*-TSST revised: evaluation of a modified version of the friendly TSST; manuscript in preparation).

In addition to protocol modifications aimed at reducing resource demands—such as varying the number and gender of panel members or incorporating video

recordings—there might be other reasons and additional ways to change the protocol. One such modification is conducting the TSST in a seated body posture, which may be necessary for individuals who are unable to stand or for reducing movement artifacts. Although some stress paradigms, like the Yale Interpersonal Stress Task³⁵ or the Montreal Imaging Stress Task,³⁶ are conducted with participants seated, it remains unclear how body posture may impact the stress response specifically in the context of the TSST.

Several studies have shown that sympathetic markers such as HR and BP vary depending on body posture. These changes reflect physiological adaptations of the body that counteract the effects of gravity to maintain appropriate blood flow and ensure the proper functioning of vital organs.³⁷ The impact of standing has been described as orthostasis. For instance, HR is generally higher in a standing posture compared with sitting, and higher in the prone compared with the supine posture.³⁸ Comparing prone and sitting postures, sitting has been associated with increased HR and decreased BP. BP has also been shown to vary across prone, sitting, and supine postures, whereas HRV remains unchanged between horizontal postures.³⁹ Postural changes alone can elicit physiological responses. For example, Hennig et al⁴⁰ reported a decrease in systolic BP and an increase in HR and cortisol levels after the subjects stood for 20 minutes as compared with when they were sitting or in a supine position. Similarly, Mlynarik et al⁴¹ found that a simulated stress task with all stressful elements emitted but involving postural changes (eg, sitting during the anticipation phase, standing up, walking to another room) led to changes in HR, BP, and plasma noradrenaline. In contrast, the evidence for posture-related effects on the HPA axis is less conclusive: although some studies found no significant influence of body posture on HPA axis markers,^{38,41,42} other findings suggest that salivary cortisol levels can be affected by posture.^{40,43} Taken together, these findings show that body posture can influence ANS activity and—to some extent—HPA axis activity, even in the absence of psychological stressors.

Given that protocol variations have been shown to potentially affect stress responses^{8,44} and that body posture can have an effect on stress response systems, it is likely that performing a stress experiment in a sitting versus standing condition can affect the magnitude of the stress response. Two main mechanisms should be considered: Biologically, differences could stem from orthostasis or related mechanisms.^{38,45,46} Psychologically, sitting at eye level with the panel may buffer perceived social evaluation (based on Craw et al¹³). Research suggests that body posture can influence ANS and HPA axis reactivity when no stress induction is present.⁴¹ Hence, it seems likely that such changes might become apparent in acute stress scenarios, such as the TSST, as well. Existing research involves both examining the influence of different body posture and facial expressions of the participant as well as the spatial position of the participant or the panel. For example, a standing compared with a supine

posture has been associated with higher baseline HR and muscle sympathetic nerve activity. Vascular responses to the stressor were attenuated in the standing condition as compared with the supine condition.⁴⁵ Similarly, a substantial part of the differences in salivary cortisol, adrenaline, and noradrenaline during a standing TSST and a control condition were attributed to orthostasis alone.³⁸ Transitions between supine, seated, and standing postures resulted in altered HR, BP, and plasma (nor)adrenaline.⁴⁷

Beyond biological responses, posture also influences the psychological stress response: an upright posture during the TSST was linked to higher self-esteem, more positive affect, and lower perceived threat.⁴⁸ However, cortisol levels remained unchanged when comparing dominant and submissive postures.⁴² Finally, spatial positioning of evaluators can modulate stress responses: participants facing evaluators during a social stress task exhibited greater anxiety, HR, and BP reactivity compared with an over-the-shoulder evaluation.¹³

The aim of this study, therefore, is to compare participants in the seated posture TSST and f-TSST against participants undergoing the standard TSST and f-TSST with regard to biological responses including salivary cortisol, sAA, and HR(V) as well as psychological stress. We hypothesize that participants undergoing the TSST and f-TSST in a standing posture will exhibit a stronger biological and psychological stress response compared with those in a seated posture. Specifically, we expect higher salivary cortisol and sAA levels, increased HR, and reduced HRV, as well as higher self-reported stress in the standing group.

METHODS

Transparency and Openness

This study was preregistered before data analysis and can be accessed under the following link: <https://doi.org/10.17605/OSF.IO/YC5DJ>. Analysis scripts and data are openly available here: <https://osf.io/ptuqf/>.

Participants and Study Design

Participants were recruited at Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and various public locations in Erlangen and Nuremberg. Interested individuals underwent a screening through an online questionnaire using Unipark (TIVIAN, Cologne, Germany). The following criteria resulted in exclusion: prior experience with laboratory stress tests and enrollment in a psychology master's program, where such experience was assumed, an age younger than 18 and older than 40, a BMI below 18 and above 30 kg/m², hormonal contraception and pregnancy, reporting to smoke > 5 cigarettes/day or using other drugs, certain physical (eg, cancer, or diseases of the nervous system) and mental illnesses, and intake of medication such as insulin, pain medication, or antidepressants. Furthermore, we only included participants that scored below 23 on the German version of the Centers for Epidemiological Studies Depression Scale,⁴⁹ the Allgemeine Depressionsskala,⁵⁰ to further assure to only include healthy individuals.

To limit confounding influences on biological markers, participants were required to refrain from physical activity in the 2 hours before testing, as well as from the consumption of food and caffeinated drinks one hour before testing. Female participants were tested during the luteal phase of their menstrual cycle.^{4,51}

Data collection took place between January and December 2023. A total of 105 participants were initially invited to the laboratory. During the course of the study, one participant ($n = 1$) decided to discontinue, leading to a final sample of $N = 104$ participants. Of these, 60 (57.69%) were female and 44 (42.31%) were male. The mean age was 22.28 years ($SD = 3.44$; range: 18 to 34) and the sample exhibited relative homogeneity in terms of educational level (97% with university entrance qualification or higher) and ethnicity (92% White). Participants' BMI ranged between 18 and 30 kg/m² ($M = 22.12$ kg/m²; $SD = 2.41$). The study was approved by the ethics committee of FAU on December 01, 2020 (protocol #493_20 B) and all participants gave written informed consent. All study procedures were in accordance with the declaration of Helsinki. After study completion, participants received a monetary compensation of 50 Euros or course credit. Participants underwent the TSST and its control condition, f-TSST, on 2 consecutive days in randomized order. Fifty-four participants (51.92%) performed the f-TSST on day 1, whereas 50 participants (48.08%) underwent the TSST on day 1. Participants were evenly distributed across the experimental conditions, with half of the participants ($n = 52$) completing the TSST and f-TSST in the sitting posture and the other half ($n = 52$) in the standing posture.

Acute Stress Induction and Control Condition

Acute psychosocial stress was induced using the TSST, which involves a free speech followed by a mental arithmetic task. To address the aim of our study, modifications were made to the original protocol described by Kirschbaum et al.⁷ Participants were either positioned standing, as per the original protocol, or seated in front of the panel. All participants were sitting at a desk during the anticipation phase. After 3 minutes, they were instructed by the panel to complete the Primary Appraisal Secondary Appraisal questionnaire.⁵² Participants were then instructed to move from the desk to the designated spot in front of the panel and to commence their speech.

An adapted version of the f-TSST³³ was used as a control condition. To enhance behavioral comparability with the standard TSST, we shortened the speech time from the originally proposed 8 minutes to 5, and added a slightly adapted mental arithmetic task from the Placebo TSST,³⁴ in which participants were instructed to add 10 and 20 alternately, starting at zero. Because of the within-subject design, participants were not informed that they were undergoing the control condition, and the procedure was video-recorded in the same manner as during the TSST.

During the entire course of the TSST and f-TSST, participants were filmed with a video camera (Sony SRG-

300H, Minato, Japan) to capture their face, and a mobile phone (Google Pixel 7, CA) that captured their body movements. Regardless of whether participants were seated or standing, the panel was seated behind a table across both experimental conditions.

Procedure

Participants were invited to the laboratory with appointments scheduled on 2 consecutive days at the same time between 12:30 and 19:00 to minimize biomarker fluctuations and to test participants in a diurnal window of relatively low cortisol concentrations.⁵³ The order of conditions was randomized. Upon arrival, participants were greeted and informed consent was obtained, followed by the collection of the first saliva (S0) sample using Salivettes (Sarstedt, Nümbrecht, Germany). Participants then consumed 200 ml of grape juice to minimize the probability of low blood sugar levels.⁵⁴ Biometric data, including height, body measurements, and body composition, were recorded, followed by the attachment of sensors for various biological measurements, such as an electrocardiogram (ECG). After a pre-questionnaire battery including the PANAS, and the second saliva sample (S1), participants were walked to a different room to undergo the TSST and f-TSST. After completion, they were brought back to the preparation room, provided 6 more saliva samples (S2 to S7), and completed a post-stress battery of questionnaires including the PANAS. Saliva samples were collected at 8 distinct time points relative to the TSST and f-TSST, specifically at -30, -1, +1, +10, +20, +30, +45, +60 minutes on both days. The procedure on day 2 was similar, with a final debriefing taking place after the last saliva sample, after which participants were compensated (50€ or course credit) and dismissed. In addition to the measurements described above, participants provided some other measurements for later exploration of additional research questions, including further questionnaires and 2 capillary blood samples taken from the finger.

MATERIALS AND PRE-PROCESSING

Cortisol and Salivary Alpha-Amylase

Saliva samples were stored at -18°C after collection and analyzed at the laboratory of the Chair of Health Psychology at FAU. After thawing, saliva samples were centrifuged at 2000g for 5 minutes at 20°C . Cortisol concentrations were then determined in duplicate using a chemiluminescence immunoassay (CLIA, IBL, Hamburg, Germany), as previously described.⁵⁵⁻⁵⁷ For sAA analysis, an in-house enzyme kinetic assay was performed using reagents from DiaSys Diagnostic Systems GmbH (Holzheim, Germany), following previously described procedures.^{19,55,57}

Raw data were then screened for missings and outliers. Missing values at S1 were imputed using the S0 value, whereas missing values at S7 were imputed using the S6 value. Participants were excluded from the respective condition if their cortisol concentration at S1

exceeded 15 nmol/l, because HPA axis activation at the beginning of a study protocol has been shown to prevent an HPA axis response to stress,⁵⁵ resulting in the exclusion of $n = 5$ participants. In addition, participants were excluded from the analysis if they had missing values in any sample required for computing the (maximum) increase (S1, S2, S3, and S4 for cortisol; S1 and S2 for sAA) or area under curve (AUC) measures. This criterion led to the exclusion of $n = 7$ participants in the TSST condition and $n = 9$ participants in the f-TSST condition for cortisol, and $n = 7$ participants in the TSST condition and $n = 6$ participants in the f-TSST condition for sAA. After this step, raw cortisol and sAA values, which exhibited right-skewed distributions, were transformed to approximate normality using a logarithmic transformation for cortisol and a square-root-transformation for sAA. These transformations were selected as they provided the best approximation to a normal distribution. On the basis of the transformed values, an increase measure was computed ($\Delta\text{cortisol} = \max(S_2, S_3, S_4) - S_1$; $\Delta\text{sAA} = S_2 - S_1$), and AUC with respect to ground (AUCg) and AUC with respect to increase (AUCi) were calculated following the approach outlined by Pruessner et al.⁵⁸ Outliers were detected using z -scores with values exceeding ± 3 SDs from the mean being considered outliers, resulting in the exclusion of $n = 3$ participants from the cortisol data and $n = 3$ participants from the sAA data.

Heart Rate and Heart Rate Variability

To assess the electrophysiological response to the TSST and f-TSST, participants were fitted with a wearable ECG sensor node (Portables GmbH, Erlangen, Germany) to measure HR and HRV data. The sensor was integrated into a chest strap, which participants attached themselves. It featured an ECG unit that recorded a single-channel ECG at 256 Hz after lead I of the Einthoven triangle. The ECG signal was filtered as reported in previous work.^{59,60} First, a second-order FIR bandpass filter (3 to 45 Hz) was applied to reduce noise, such as powerline interference or baseline drifts. RR intervals were then computed based on the R peaks extracted from the ECG signal after applying the QRS detection algorithm proposed by Hamilton.⁶¹ Artifacts in the RR intervals were removed by excluding values corresponding to heart rates ≤ 45 bpm or ≥ 200 bpm, as well as statistical outliers ($\geq 2.576 \sigma$) and large differences between successive RR intervals ($\geq 1.96 \sigma$). Missing RR intervals were imputed through linear interpolation. Regarding HRV analysis, artefacts were corrected using an algorithm proposed by Lipponen & Tarvainen.⁶² The time-domain measure RMSSD (root mean squared sum of successive RR interval differences) was used to quantify HRV. HRV data were log-transformed to reach normal distribution.

Psychological Stress Response

To evaluate the psychological stress response, we utilized the PASA⁵² and the German version of the PANAS.^{21,63} Participants completed the PASA during

the anticipation phase of the TSST and f-TSST and the PANAS before and after the TSST and f-TSST. For the PASA questionnaire, threat and challenge mean scores were computed only if no more than one item per scale was missing. In this case, the mean was calculated based on the remaining items. On the basis of this criterion, we excluded $n = 7$ participants from the TSST condition and $n = 14$ participants from the f-TSST condition for both scales. In addition, one outlier was excluded from the Threat scale in the f-TSST condition.

Statistical Analysis

Data analysis was conducted using RStudio (version 2023.12.1+402). Analyses of variance (ANOVAs) were performed using the R package *afex*.⁶⁴ We performed a repeated measures analysis of variance with condition (TSST vs. f-TSST) as a within-subject variable, body posture (sitting vs. standing) as a between-subject variable, and sex and condition order as covariates. With regard to cortisol and sAA, we wanted to address the most commonly used reactivity indices and not only report results on individual samples, but also on (maximum) cortisol/sAA increase and AUCs (AUCg and AUCi). To account for potential orthostatic effects, that is, the contribution of the sitting/standing body postures on the stress response, we calculated difference indices between TSST and f-TSST for all reported measures and compared them between the 2 experimental groups, controlling for sex and condition order. The TSST data of each participant was adjusted for the respective f-TSST data: $\Delta\text{stress measure} = |\text{stress measure}_{\text{TSST}} - \text{stress measure}_{\text{f-TSST}}|$. These difference scores were analyzed using a univariate ANOVA with body posture as a between-subjects variable, and sex and condition order as covariates.

RESULTS

Cortisol

Regarding cortisol levels (Figure 1), our analysis revealed a significant interaction between sample and condition, $F(1.79, 146.74) = 29.42, p < .001$, indicating a significantly higher cortisol increase in the TSST condition compared with the f-TSST condition. We further found a significant interaction of sex and sample, $F(2.05, 167.96) = 4.49, p = .012$, with men showing a higher cortisol increase. In addition, there was a significant interaction between body posture and sample, $F(2.05, 167.96) = 3.30, p = .038$, with participants in the standing group showing a greater cortisol response to the TSST and f-TSST. Beyond these interactions, we also observed significant main effects for sample, $F(2.05, 167.96) = 123.64, p < .001$, condition, $F(1, 82) = 48.27, p < .001$, and body posture, $F(1, 82) = 6.21, p = .015$, whereas sex approached significance, $F(1, 82) = 3.31, p = .073$. When analyzing maximum cortisol increase, we found significant main effects for condition, $F(1, 82) = 43.00, p < .001$, sex, $F(1, 82) = 6.13, p = .015$, and body posture, $F(1, 82) = 4.20, p = .044$. Similarly, analysis of the AUCg revealed significant main effects for condition, $F(1, 82) = 47.50, p < .001$, and body posture, $F(1,$

$82) = 6.08, p = .016$, whereas sex approached significance, $F(1, 82) = 3.33, p = .072$. Further, analysis of AUCi indicated a significant main effect of condition, $F(1, 82) = 37.10, p < .001$, and sex, $F(1, 82) = 5.66, p = .020$, whereas body posture approached significance, $F(1, 82) = 3.92, p = .051$ (for full ANOVA results, see Table S1, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>). Overall, the cortisol response was more pronounced in the TSST condition than in the f-TSST condition, both over time and in terms of maximum cortisol increase, AUCg, and AUCi. Furthermore, men exhibited a more pronounced reactivity, as shown in maximum cortisol increase and AUCi, whereas overall cortisol output was not as strongly influenced by sex. The standing group exhibited a higher cortisol response than the sitting group in individual samples, maximum cortisol increase, and AUCg, whereas this effect was close to significant for AUCi.

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results did not reveal an effect of body posture for any of the cortisol parameters (smallest $p = .693$; Table S2, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Salivary Alpha-Amylase

Regarding sAA levels (Figure 1), our analysis revealed a significant 3-way interaction between sample, condition, and condition order, $F(4.98, 438.23) = 3.51, p = .004$. Among participants who underwent the f-TSST first, the sAA response to the f-TSST was slightly more pronounced than to the TSST. In contrast, among those who completed the TSST first, the sAA response to the TSST was stronger than to the f-TSST. We observed a further significant interaction of condition and condition order, $F(1, 88) = 7.28, p = .008$ as well as a significant main effect of sample, $F(4.47, 393.71) = 42.55, p < .001$. There was no significant main effect of body posture ($p = .142$). When analyzing the sAA increase, the main effect of condition approached significance, $F(1, 88) = 2.98, p = .088$, that is, the increase was slightly more pronounced in the TSST condition compared with the f-TSST condition. For both AUCg, $F(1, 88) = 7.56, p = .007$ and AUCi, $F(1, 88) = 5.71, p = .019$, we observed a significant interaction of condition order and condition. AUCi further showed a significant main effect of sex, $F(1, 88) = 4.10, p = .046$, and an effect of condition that approached significance, $F(1, 88) = 3.05, p = .084$. That is, sAA secretion was higher in men and in the TSST condition, respectively (for full ANOVA results, see Table S1, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results did not reveal an effect of body posture for any of the sAA parameters (smallest $p = .298$; Table S2, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

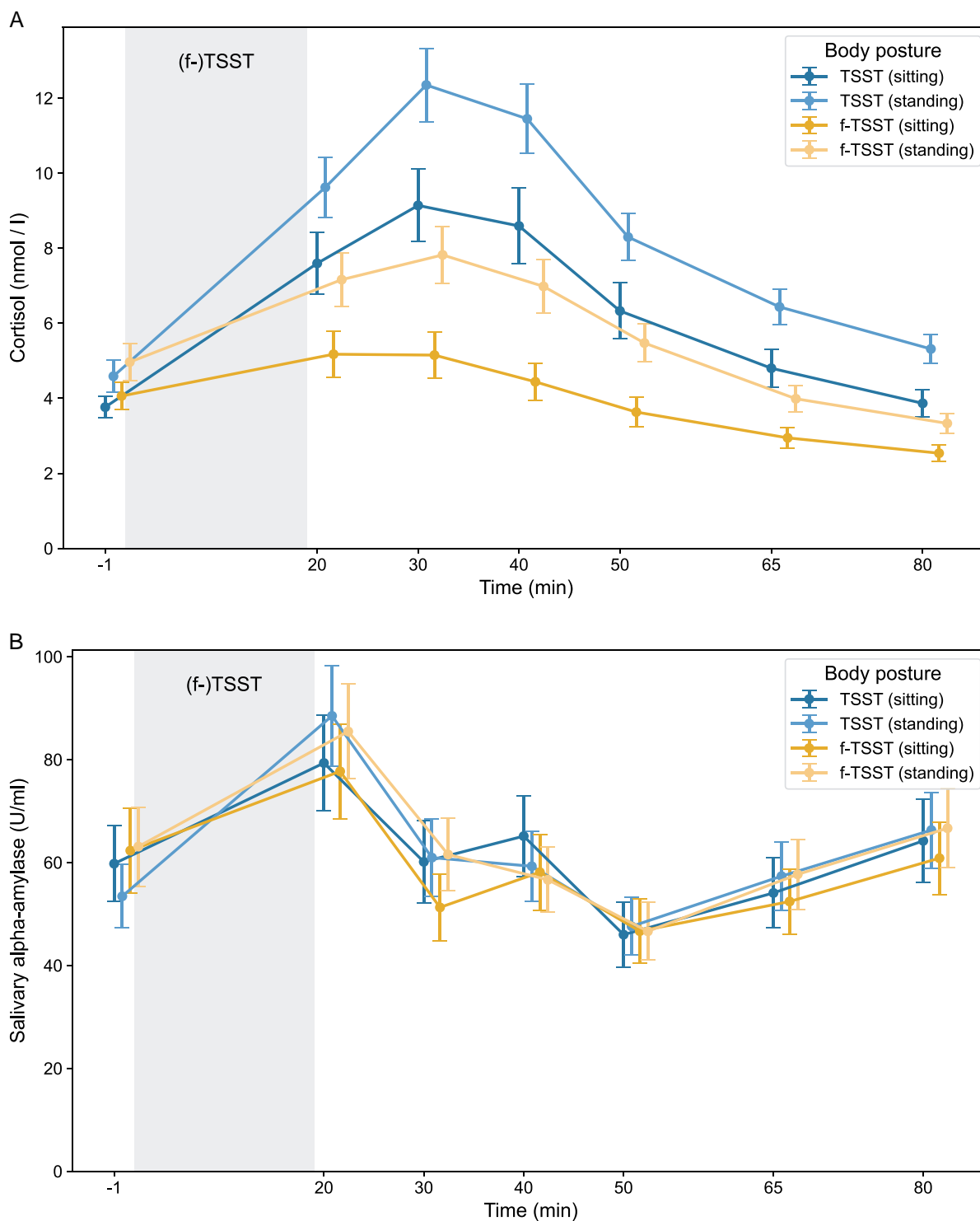


FIGURE 1. HPA axis responses (top plot) and salivary alpha-amylase responses (bottom plot) to the Trier Social Stress Test (TSST). Graph shows means and standard errors of the mean (SEM) of salivary concentrations (untransformed values) of both female and male participants in both the sitting and the standing group at baseline as well as 1, 10, 20, 30, 45, and 60 minutes after TSST. Color image is available online only at the journal's website.

Heart Rate

Regarding HR (Figure 2), our analysis revealed a significant 3-way interaction of sex, condition, and task, $F(2.65, 169.75) = 3.61, p = .019$. A further significant interaction emerged for condition and task, $F(2.65, 169.75) = 35.45, p < .001$, whereas the interaction of condition order and condition approached significance, $F(1, 64) = 3.72, p = .058$. We further found a significant interaction of body posture and task, $F(2.24, 143.39) = 22.42, p < .001$. That is, although participants in both the sitting and the standing group showed a similar HR during the baseline, preparation, and recovery phase, the standing group exhibited a higher HR in the talk and math part of the TSST and f-TSST. Further main effects became apparent for phase (baseline, preparation, talk, math, and recovery), $F(2.24, 143.39) = 186.16, p < .001$, condition, $F(1, 64) = 52.29, p < .001$, and body posture, $F(1, 64) = 4.60, p = .036$ (for full ANOVA results, see Table S3, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results did not reveal an effect of body posture HR ($p = .381$; Table S4, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Heart Rate Variability

Regarding HRV (Figure 2), our analysis revealed a significant 3-way interaction of condition order, condition, and task, $F(2.66, 165.11) = 2.88, p = .044$. That is, participants who underwent the f-TSST on the first day showed a stronger HRV decrease in response to the f-TSST than did those who underwent the TSST on the first day. A 3-way interaction between sex, condition, and task approached significance, $F(2.66, 165.11) = 2.50, p = .069$, indicating that men show a stronger decrease in HRV in response to the TSST and f-TSST than women. Further interactions emerged for condition and task, $F(2.66, 165.11) = 10.95, p < .001$, and body posture and task, $F(3.26, 202.10) = 5.19, p = .001$, with a lower HRV in the preparation, talk, and math phases during the TSST as compared with the f-TSST and in the sitting as compared with the standing group. We further found significant main effects of phase, $F(3.26, 202.10) = 30.74, p < .001$, and condition, $F(1, 62) = 20.55, p < .001$, whereas the main effect of body posture approached significance, $F(1, 62) = 3.23, p = .077$ (for full ANOVA results, see Table S3, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results did not reveal an effect of body posture for HRV ($p = .179$; Table S4, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

PANAS

Regarding PANAS' negative affect scale (Figure S1, Supplemental Digital Content, <http://links.lww.com/>

PSYMED/B147), our analysis revealed a significant 3-way interaction of condition order, condition, and time point (pre vs. post), $F(1, 94) = 11.13, p = .001$. That is, participants who underwent the TSST on the first day showed a larger increase in negative affect after the TSST and a decreased in negative affect after the f-TSST. In contrast, participants who underwent the f-TSST on the first day showed a less pronounced increase after the TSST and a slight increase after the f-TSST in their negative affect ratings. Significant interactions became apparent for sex and time point, $F(1, 94) = 7.72, p = .007$, sex and condition, $F(1, 94) = 11.77, p < .001$, condition and time point, $F(1, 94) = 71.44, p < .001$, and condition and condition order, $F(1, 94) = 19.37, p < .001$, with negative affect being more pronounced in women and after the TSST, and in participants who completed the TSST on the first day. In accordance, we found significant main effects for condition, $F(1, 94) = 63.48, p < .001$ and sex, $F(1, 94) = 6.33, p = .014$, with negative affect being higher in the TSST condition and in women, respectively (for full ANOVA results, see Table S5, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results revealed a significant main effect of condition order, $F(1, 95) = 10.39, p = .002$, indicating a smaller difference between the TSST and f-TSST in participants who first completed the f-TSST. Body posture did not differ significantly ($p = .479$, Table S6, Supplemental Digital Content 1, <http://links.lww.com/PSYMED/B147>).

Regarding PANAS' positive affect scale (Figure S1, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>), our analysis revealed a significant 3-way interaction of condition order, condition, and time point, $F(1, 94) = 8.44, p = .006$. Positive affect decreased in all participants in response to the TSST. Participants who underwent the TSST on the first day showed a more strongly pronounced increase in positive affect after the f-TSST than did their counterparts who underwent the f-TSST on the first day. Our data showed a further 3-way interaction between condition, sex, and time point, $F(1, 94) = 4.70, p = .033$. Although both men and women showed an increase in positive affect in response to the f-TSST, women showed a more strongly pronounced decrease in positive affect after the TSST. Significant interactions emerged for condition and time point, $F(1, 94) = 31.11, p < .001$, condition order and time point, $F(1, 94) = 4.93, p = .029$, sex and time point, $F(1, 94) = 4.96, p = .028$, and condition order and condition, $F(1, 94) = 12.97, p < .001$. Our data further showed significant main effects of sex, $F(1, 94) = 5.07, p = .027$, and condition, $F(1, 94) = 9.00, p = 0.003$, with women and participants undergoing the TSST showing less positive affect, respectively. No effects emerged for body posture ($p = .202$). For full ANOVA results, see Table S5, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>.

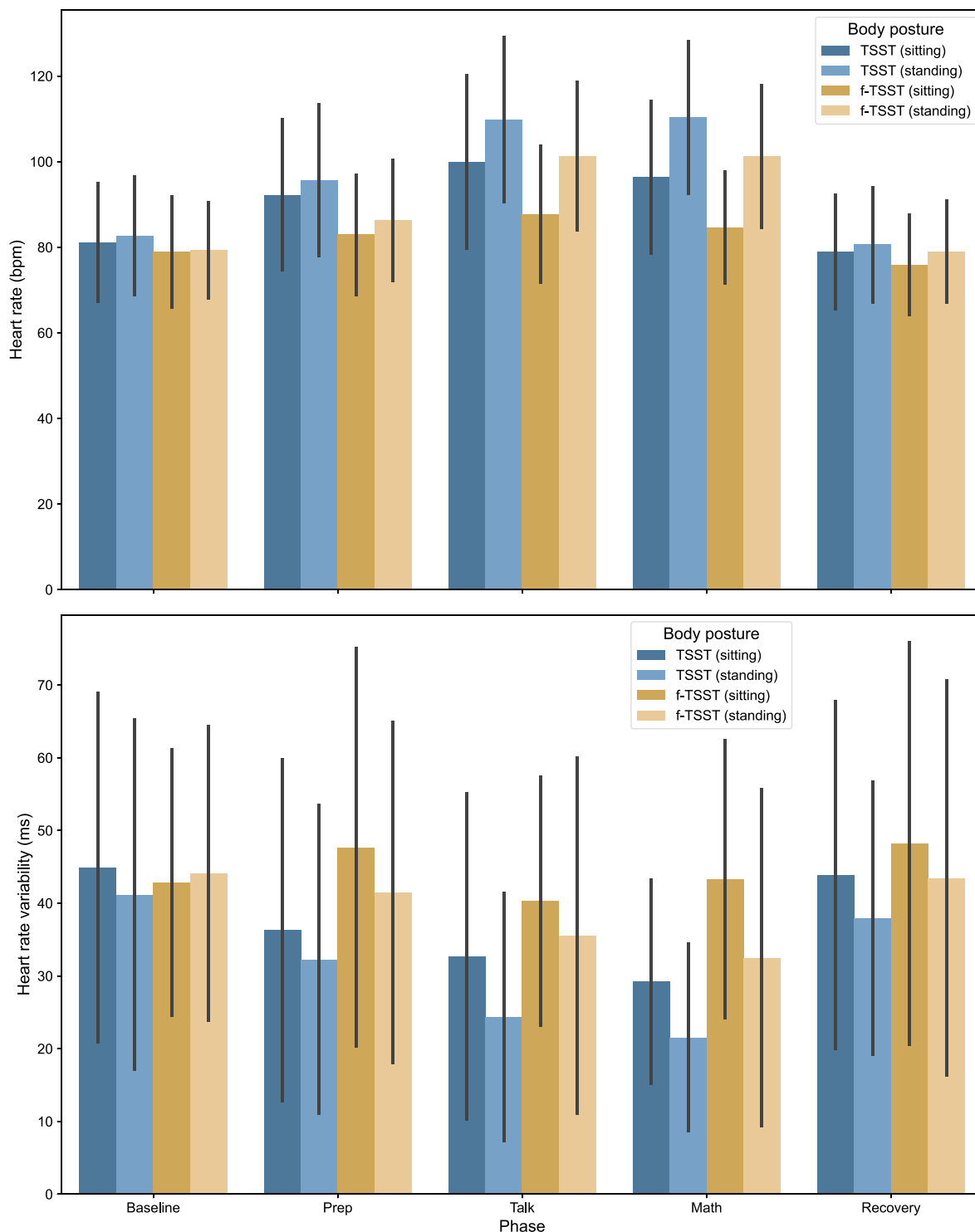


FIGURE 2. Heart rate (HR) and HR variability (HRV) response to the trier Social Stress Test (TSST). Graph shows an averaged HR(V) and SDs of the mean of both female and male participants in both the sitting and the standing group for the baseline phase, the preparation phase, the talk phase, the math phase, and the recovery phase. Color image is available online only at the journal's website.

Finally, we tested the effect of body posture on difference indices, that is, TSST data adjusted for f-TSST data, calculated for each individual participant. Results revealed significant main effects of condition order, $F(1, 95) = 7.48, p = .007$, and sex, $F(1, 95) = 5.19, p = .025$, indicating a smaller difference between the TSST and f-TSST in participants who first completed the f-TSST and in men, respectively. Body posture did not differ significantly ($p = .173$, Table S6, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

PASA

With regard to PASA's threat scale (Figure S2, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>), our analysis indicated a marginal significant interaction of sex and condition, $F(1, 81) = 3.22, p = .077$. Although in the f-TSST, men and women showed relatively similar levels of threat, the difference became more pronounced in the TSST with women indicating a higher threat perception. Threat perception was significantly different between the 2 conditions, $F(1, 81) = 90.80, p < .001$ with participants experiencing a higher amount during the TSST. Both sex, $F(1, 81) = 3.48, p = .066$, and body posture, $F(1, 81) = 3.85, p = .053$, approached significance with threat scores being higher in women and the standing group. With regard to the challenge scale (Figure S2, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>), there was a significant main effect of condition, $F(1, 82) = 59.59, p < .001$, with higher scores in the TSST condition. Body posture did not exhibit a significant effect ($p = .249$). Examining the difference indices, our data showed no significant effects of body posture in both the threat ($p = .763$) and the challenge ($p = .941$) scales (for full ANOVA results, see Table S5, Supplemental Digital Content, <http://links.lww.com/PSYMED/B147>).

DISCUSSION

The aim of this study was to identify potential effects of body posture during the TSST and the f-TSST. To confirm that the TSST effectively induced stress regardless of condition order, we analyzed changes in endocrine (salivary cortisol), autonomic (sAA, HR, HRV), and self-report (PANAS, PASA) stress markers. In line with previous work,^{11,12,57} participants showed a significant rise in salivary cortisol levels, HR(V), and the psychological stress response after the TSST compared with the f-TSST. However, it is important to note that the f-TSST is not a neutral or passive control condition but involves—to a certain extent—elements of socially evaluative threat, such as speaking in front of others, which may elicit mild stress responses. This might also be the reason why the reactivity of sAA, which has previously been found to be a reliable marker of the SNS,^{19,65} only approached significance between the conditions when considering the sAA increase, but was sensitive to the interaction of condition and condition order when taking all samples into account. Although previous literature does not report a carryover effect,³⁴ we did find this in our data. This

could be further evidence that the f-TSST as we used it does indeed contain social evaluative elements. We found previously described gender differences in both the psychological stress response,⁶⁶ operationalized through positive and negative affect, and cortisol.^{51,67} Although gender effects on HR(V)⁶⁸ and sAA^{34,69} have been described, these studies investigated women across all phases of their menstrual cycle. We tested women in their luteal phase and did not find any gender differences in any SNS markers, which is in line with another finding reported by Het et al³⁴ who did not observe gender differences in the luteal phase. Interestingly, the AUC with respect to increase of sAA differed significantly between the genders. That is, the temporal dynamic of the sAA response differs between men and women.

Previous research has shown habituation effects of the HPA axis.⁷⁰ We also expected that the f-TSST on the first day might trigger significant acute stress, which could weaken the response to the TSST on the second day. However, our data do not support this expectation. Cortisol, sAA, and HR(V) were unaffected by condition order. This also applied to the PASA, which, as a self-report, was administered during the anticipation phase of the TSST and f-TSST. However, positive and negative affect were affected by condition order: Participants who underwent the TSST on the first day reported a higher increase in positive affect after the f-TSST compared with those who completed the TSST on the second day.

The primary aim of this study was to investigate whether the body posture during acute psychosocial stress induction influences the stress response. On the basis of previous findings, we hypothesized that body posture would affect both endocrine and sympathetic pathways^{38–43} as well as the psychological stress response.¹³ To the best of our knowledge, this specific research question has not been answered before. Regarding cortisol as an indicator of HPA axis reactivity, we found significant differences between the sitting and standing body posture across individual samples, maximum cortisol increase, and AUC with respect to ground. Although AUC with respect to increase approached significance, the overall pattern suggests an amplified HPA axis reactivity and hence a greater cortisol output in participants who completed the TSST in a standing body posture. After isolating the relative contribution of body posture by subtracting f-TSST levels from TSST levels, posture-related effects were no longer statistically significant. Thus, although HPA axis reactivity was higher in participants who completed the f-TSST and the TSST while standing, our findings suggest that a substantial part of this effect may be attributed to body posture itself rather than an increased acute stress response induced by the standing protocol.

When examining sAA as a marker of ANS reactivity, no significant differences were detected between sitting and standing body postures across any measures (individual samples, sAA increase, AUC_i, AUC_g). We attribute this finding to the faster response of sAA compared with cortisol, with its peak occurring shortly after

the TSST.^{65,71} In contrast, individual samples, AUC_i, and AUC_g encompass all measurements taken throughout the experiment, including the pre-TSST and post-TSST phase, which were identical for all participants, regardless of body posture. After isolating the relative contribution of body posture by subtracting f-TSST levels from TSST levels, no significant differences were found in any sAA markers.

Heart rate was significantly higher when performing both the TSST and f-TSST in a standing posture compared with a seated one. This is in line with previous research that reported differences in HR depending on the body posture.^{38,39,41} After isolating the relative contribution of body posture by subtracting f-TSST levels from TSST levels, no significant group effects were found for HR. Similarly, when using HRV as an ANS marker, we observed a trend towards a lower HRV in the standing body posture group. After isolating the relative contribution of body posture by subtracting f-TSST levels from TSST levels, body posture did not significantly influence HRV. These findings suggest that the sympathetic contribution, which mainly determines HR, might be more strongly affected by body posture than the parasympathetic contribution, which mainly determines HRV, in acute stress scenarios.⁷² Furthermore, in line with the cortisol results, the observed differences in HR(V) seem to reflect the influence of body posture itself rather than an increased stress response. Although a within-subject design would be particularly beneficial for HR to account for body posture effects, practical constraints often make this difficult. Therefore, we conclude that HRV may be a more suitable measure for assessing acute stress, as it is less influenced by body posture.

Regarding the psychological stress response, body posture did not have a significant effect on either the PANAS (pre-post estimate) or the PASA, which was administered during the anticipation phase of the TSST. On the basis of findings by Craw et al,¹³ it could have been possible that being at eye level with the panel might reduce perceived stress. However, our data did not support this assumption. One possible explanation is that this effect was not yet noticeable during the anticipation phase (as measured by PASA) or was not strong enough to persist until the administration of PANAS after the TSST.

The findings we presented here should be interpreted in light of some strengths and limitations. Our findings contribute to the existing research on body posture and postural changes and their effects on biological processes. It is important to note as a strength that the differences between sitting and standing were limited to the talk and math phase of the TSST and f-TSST, but with otherwise identical experimental procedures. Baseline measures, particularly relevant for HR(V), were collected with all participants in a seated posture. This approach ensures comparability of baseline values across conditions and allows for within-subject comparisons between stress (TSST) and control (f-TSST) conditions. However, it also has a limitation: For participants in the

standing TSST group, changes in HR(V) reflect both stress-induced effects and the influence of orthostatic posture, making it more difficult to isolate the pure stress response.

In addition, although performing the TSST in a seated posture may be particularly relevant for subpopulations with mobility restrictions, such as older adults or individuals with health conditions, we tested our hypothesis using a healthy, young sample, which could lead to different results. In this study, we deliberately chose not to correct for multiple testing to reduce the risk of Type II errors, which could result in overlooking potentially meaningful effects due to an overly conservative significance threshold. However, we recognize that this approach may increase the likelihood of type I errors, meaning that some observed significant differences could be due to chance rather than representing true effects.

In conclusion, although we observed effects of body posture on cortisol, HR, and HRV, these effects did not persist after isolating the relative contribution of body posture by subtracting the markers of the non-stress condition from the stress condition. This implies that the effect of body posture, in particular a seated posture, can be neglected in studies with an adequate non-stress control condition, but that its effects should be taken into account in studies with only the stress protocol. We encourage researchers to explore in future studies the effect of body posture using a baseline measurement conducted in the corresponding body posture. In addition, future research could manipulate posture of both participant and panel members to disentangle effects of shared eye level from those of posture itself. Further, the influence of sex hormones on HPA axis reactivity⁷³ could be investigated in more detail. To ensure transparency and to uphold open science principles, we further recommend providing a detailed description of the experimental procedure, explicitly stating the body posture in which the stress induction occurred. By testing a larger sample in a within-subject design, we contributed to a deeper understanding of differential effects of body posture on biological and psychological responses in acute stress protocols. This knowledge enables researchers to make informed decisions about whether to conduct the TSST (and f-TSST) in a seated or standing body posture and which stress measures best align with their research goals. A seated TSST may be particularly beneficial when testing subpopulations that might otherwise face barriers to participation, such as older adults or individuals with limited mobility. Hence, this work contributes to inclusivity and respect for diversity.

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Availability of Data and Open Science Principles: The study was preregistered and data as well as analysis scripts were made available on OSF.

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