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ABSTRACT

Introduction: Experiencing acute and chronic stress can contribute to adverse health outcomes. Responses to acute stress differ between individuals (i.e., stress reactivity) and the experience of chronic stress has been discussed to be associated with acute stress responses and stress recovery. This study thus aims to investigate whether hair cortisol concentration (HCC), being an indicator for hypothalamus–pituitary–adrenal (HPA) axis activity over a prolonged period of time, is associated with acute stress responses and recovery in a sample of medical students.
Material and methods: From July 2020 to July 2021, medical students (n = 54) underwent a virtual-reality Trier Social Stress Test in which their blood pressure and heart rate variability (HRV) were measured, and hair samples were taken to determine HCC. Piecewise linear growth analyses were used to investigate whether HCC (categorized into low, medium and high levels) is associated with acute stress responses and recovery regarding blood pressure and HRV.
Results: Significant interaction effects in piecewise linear growth analyses showed that participants with higher levels of HCC had lower systolic and diastolic blood pressure responses compared to participants with medium levels of HCC. No significant interaction effects were observed for HRV responses or for recovery measures.
Conclusions: The study suggests that higher levels of HCC are associated with a lower cardiovascular response in terms of blood pressure to an acute stressor in medical students. Therefore, long-term HPA-axis activity may contribute to different magnitudes of acute stress responses in the autonomic nervous system. As the shown lower blood pressure responses to acute stress in individuals with increased long-term HPA-axis activity may represent inadequate stress responses, these should be further studied in order to find out more about their interaction and potential subsequent disease risks.

1. Introduction

The exposure to a stressor is associated with a range of physiological and psychological responses and the magnitude of those responses differs between individuals (Chida and Hamer, 2008; Schlotz et al., 2011). Investigating this inter-individual variability (i.e., stress reactivity) has been an integral part in research for many years as there is much evidence that exaggerated cardiovascular responses to stress are associated with cardiovascular diseases, but also that blunted cardiovascular responses may have adverse health consequences such as obesity or depression (Phillips et al., 2013; Turner et al., 2020). In addition, recovery after stressful situations is suggested to have relevant health effects (Panaite et al., 2015). For example, poor heart rate (HR) and blood pressure recovery were associated with an increased cardiovascular risk status (Chida and Steptoe, 2010).

Acute stress responses, recovery, and potential moderators thereof are most often investigated in various laboratory settings in which individuals are for example exposed to situations that are usually

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perceived as stressful or threatening (Dickerson and Kemeny, 2004). The Trier Social Stress Test (TSST) has thereby been established as the gold standard of psychosocial stress tests by combining a public speech and mental arithmetic task with social evaluation in front of a selection committee (Kirschbaum et al., 1993). Previous research provided evidence that it reliably induces stress responses of the hypothalamic-pituitary-adrenal (HPA) axis by measuring salivary cortisol levels and stress responses of the autonomic nervous system (ANS) by measuring HR, blood pressure or heart rate variability (HRV) responses (Allen et al., 2014; Man et al., 2023). Over the years, several modifications and adaptations of the TSST were introduced, including virtual reality (VR) versions that increase standardization and enable resource-efficient research (Helminen et al., 2019; Liszio et al., 2018). A meta-analysis showed that VR versions of the TSST (VR-TSST) induce a cortisol response with medium effect size (Helminen et al., 2019). Compared to the original TSST, cortisol responses may be slightly reduced (Helminen et al., 2019).

Past research identified several moderators that may contribute to altered physiological values in psychosocial stress tests, such as lower baseline and recovery cortisol levels for higher body mass index (BMI), higher cortisol responses for higher age and for males (Herhaus and Petrowski, 2018; Zänkert et al., 2019). Another potential moderator may be chronic stress. Chronic stress can have adverse consequences on its own but may also contribute to the variation of acute stress responses through various pathways that can go along with increased or blunted acute stress responses (Chida and Hamer, 2008). For example, the allostatic load model suggests that long-term stress leads to a wear and tear of the body that can result in inadequate acute stress responses or prolonged responses resulting in health impairments (McEwen, 1998). In addition, Dienstbier (1989) developed a model of toughness in which he suggested that experiencing moderate stress levels could help to "toughen" the individual via an increased but adaptive response. These tough individuals would then show lower baseline values, increased sympathetic nervous system (SNS) responses and a faster recovery compared to less fit individuals (Chatkoff et al., 2010; Dienstbier, 1989; Wirtz et al., 2013).

In support of the mentioned pathways, experimental studies have found that higher self-reported general chronic stress and chronic stress at work were associated with lower systolic blood pressure and HR responses to laboratory stressors in healthy adults (Cavanagh and Obasi, 2021; Hamer et al., 2006; Matthews et al., 2001; Siegrist and Klein, 1990) and in patients with high risks for psychosis (Pruessner et al., 2013). In contrast, other findings showed no associations between chronic stressors at work and blood pressure responses (Wirtz et al., 2013). Furthermore, momentary stress during daily life and concurrent blood pressure were only positively associated with each other among individuals reporting chronic work stress (Lumley et al., 2014). Less research exists regarding the impact of chronic stress on cardiovascular recovery after acute stress. One study showed that blood pressure and HR recovery after a laboratory stress test was not associated with general chronic stress (Matthews et al., 2001). Conversely, when using general life stress as an overall measure including ongoing stressors and past life events, a meta-analysis found that general life stress was associated with poorer cardiovascular recovery (Chida and Hamer, 2008).

Thus, research on the interaction between chronic stress and acute cardiovascular stress responses and recovery is mixed and indicates exaggerated, blunted, and unchanged responses to acute stressors under chronic stress (see for example Chida and Hamer, 2008). Some of these differences may occur partly due to the difficulty to conceptualize and assess chronic stress (Zänkert et al., 2019). A valuable addition can thus be a focus on a physiological measure which reflects cumulative activity of the body's stress system. In this regard, hair cortisol concentration (HCC) is a measure for cumulative cortisol secretion and therefore an indicator for HPA-axis activity over a prolonged period of time dependent on the analysed hair length (Russell et al., 2012; Stalder and Kirschbaum, 2012). Previous research has thereby found medium to large associations between chronic stress and increased HCC (Staufenbiel et al., 2013).

To the best of our knowledge, only a few studies investigated HCC and acute stress responses in the TSST in a sample of healthy adults (Sandner et al., 2020). In one study HCC was negatively correlated with salivary cortisol, indicating a lower stress response (Sandner et al., 2020). Similar findings were obtained by Wekenborg et al. (2019) who investigated men with various levels of burnout symptomatology and found blunted HR and HRV responses to acute stress for higher HCC levels. Despite the cross-sectional designs, valuable insights on the importance of HCC in research were already shown. However, more elaborate analyses that consider acute responses, recovery, potential confounders and relevant indicators of cardiovascular health, such as blood pressure, in a healthy adult sample performing a TSST are lacking so far. Blood pressure and HRV are chosen as relevant indicators for the investigation of cardiovascular responses to stress and potential subsequent resulting diseases.

2. Material and methods

Within this study, participants performed a laboratory VR-TSST, and got a hair sample taken in order to determine HCC. This study is part of a more elaborate study, which also included an ecological momentary assessment for which detailed information on design and procedure can be found at the pre-registered study protocol (Weber et al., 2022). Participants who completed the whole study received 280 EUR for compensation of their expenses. The ethics committee of the medical faculty of the Heinrich-Heine University Düsseldorf approved the study (study number: 2019–714).

2.1. Participants

Medical students who were enrolled in human medical studies at the Heinrich-Heine University Düsseldorf, Germany, participated in this study. Due to reports of high experienced stress levels among medical students, medical students were conceived as a suitable study population to investigate potential alterations of acute stress responses due to chronic stress (Dyrbye et al., 2005; Erschens et al., 2018). In addition, in a previous analysis of the study sample at hand, we analyzed whether self-reported adverse study conditions (i.e., demands, effort, and effort-reward imbalance) were associated with HCC (Heming et al., 2023). The positive associations found in this previous analysis thus support the notion that HCC reflects cumulative activity of the body's stress systems among medical students. Recruitment of a convenience sample took place between July 2020 and July 2021. The students were approached via distribution of digital flyers on social media channels (e.g. Facebook sites of the medical faculty and WhatsApp groups of different study years). During our study period about 2000 students were enrolled in human medical studies at the university. We received requests for further study information from 123 students. Of these, 33 students did not meet our eligibility criteria and 29 did not respond to the researchers after handing out further information regarding the study. This resulted in 61 students participating in the study. Inclusion criteria were enrollment within the second and ninth semester in human medical studies at the university and a minimum age of 18 years. The following exclusion criteria were applied: hair length less than two centimeters, any health condition that may increase health risks during participation of the study or may alter cardiovascular stress reactivity or HCC (such as cardiovascular diseases, hypertension, mental health disorders, endocrine disorders, epilepsy, lymphedema or other skin, bone or muscle disorders), self-reported substance abuse, heavy nicotine (more than 10 cigarettes per day) and alcohol consumption (more than one time per week with more than six drinks per occasion).

2.2. Procedure

Participants were invited to the study center between 8.30 am and 10.00 am to fill in a baseline questionnaire, to perform the VR-TSST and to get a hair sample taken. Participants received information one day before the study, that they should not consume caffeine or smoke one hour prior to the study. Once arrived, the participants gave written informed consent to participate in the study and were fitted with ambulatory blood pressure and ECG-monitors by a research assistant. They were given a period of 30 min to remain seated and fill in the baseline questionnaire. The questionnaire included confounders relevant to the study as well as questions about study conditions, health status and lifestyle. After completing this resting period, they were escorted to the soundproofed test room and performed the VR-TSST. Then, hair samples were taken from the participants after a five-minute recovery phase.

2.3. VR-TSST

This study applied the VR-TSST as described by Liszio et al. (2018). A pilot study showed similar stress responses of salivary cortisol, HRV, and psychological self-reported measures compared to the original TSST (Liszio et al., 2018). The VR-TSST was performed with an Oculus Rift S (Oculus VR). Upon arrival in the test room, participants were informed by a research assistant that they should prepare for a five minute interview to convince a selection committee about their personal qualification for a chief physician position in their preferred specialization. The research assistant also informed the participants that their performance would be compared with other medical student's performance and analysed later on by psychologists via audio recording. Participants were fitted with the VR headset while seated and were given five minutes to prepare for the interview. After the five minute preparation period, the research assistant started the interview on the VR headset. The research assistant chose responses for the virtual committee such as "the time is not up yet, please continue" or "speak loud and clearly" if the participants stopped their presentation for more than 20 s or if they asked questions. When the interview was finished, a math task automatically started on the VR headset. Within this test, the students had to start with the number 1022 and count backwards in steps of 13. When wrong answers were given, the research assistant again selected an answer for the virtual committee: "The answer was wrong. Please start from the beginning". If no wrong answers were given, the virtual committee could also say "Please speak with a louder voice". The math test was also performed for five minutes and afterwards the recovery phase of five minutes started in which students could take off their VR headsets and were asked to fill in further study questionnaires. These questionnaires asked participants if they had received information about the VR-TSST prior to the study. After this recovery phase, students were debriefed about the aims of the VR-TSST to dissolve the stress situation.

2.4. Measures of acute stress responses

2.4.1. Blood pressure

Systolic (SBP) and diastolic (DBP) blood pressure were measured with Spacelabs OnTrak Ambulatory Blood Pressure monitors (Spacelabs Healthcare) at the non-dominant arms. The research assistant started the measurements manually: five minutes (BL5) and 30 min (BL30) after the start of the resting period, directly after the interview (Int), directly after the math task (Math) and after the five-minute recovery period (Rec). Participants sat and did not talk during measurements.

2.4.2. Root mean square of successive differences (RMSSD)

HRV was measured with an ambulatory ECG monitor (eMotion Faros 180°) and gel electrodes (3 M Red Dot™ Electrodes) in a three-conductor chest configuration. A sampling rate of 250 Hz was used. RMSSD was chosen as the HRV measurement in this study and was calculated for five-minute intervals by the HRV-Scanner (BioSign GmbH). First, automatic artefact correction was performed using a plausibility control with HR_{max} = 208 - (0.7 * participant's age) and HR_{min} = 45 following previous recommendations (Tanaka et al., 2001). Furthermore, graphic filtering by the Poincare-Plot was used. ECG segments were manually edited if no sufficient data quality was obtained by those procedures. Baseline RMSSD measures were calculated for the intervals between five to 10 min (BL5) and 25 to 30 min (BL30) after the start of the resting period. Within the VR-TSST, the RMSSD measurements covered the five minute intervals during the interview (Int) and math task (Math). The first five minutes after the math test were used for the recovery period (Rec). As RMSSD was not normally distributed, log_{10}-transformation was applied.

2.5. Hair cortisol concentration

In order to determine HCC, participants' hair samples were cut by a research assistant (who was not involved in teaching or exams) as close to the scalp as possible. To reflect the last two months of cortisol secretion, the closest two centimeters were analyzed, as it is suggested that hair grows approximately one centimeter per month (Wennig, 2000). For analysis, 10 mg hair were used according to the laboratory protocol by Goreis et al. (2022). HCC was determined with a cortisol luminescence immunoassay (LIA; IBL International, a Tecan Group company, Hamburg, Germany) and inter- and intra-assay coefficients of variation were below 10%.

HCC was not normally distributed. To reduce skewedness, HCC was log_{10}-transformed. Further, the transformed HCC variable was divided according to percentiles and used as a categorical variable: the first category entailed values below the 25th percentile and the second category entailed values from the 25th percentile until the 75th percentile. A third category reflected values above the 75th percentile. The second category was used as the reference category in the analyses. This approach was chosen due to the possibility of a non-linear association between chronic stress and acute stress responses or recovery (i.e. potential of increased stress responses when HCC levels increase and attenuated responses for highest HCC levels) (Chatkoff et al., 2010; Dienstbier, 1989). In addition, very low HPA axis activity is thought to reflect hypocortisolism, i.e. a state that can occur when chronic stress is experienced for a long period (Miller et al., 2007). This issue was addressed by using three categories of HCC. While the categorization of variables can be helpful to simplify complex models and can enhance interpretability of results, it is inevitably accompanied by a loss of information (MacCallum et al., 2002). Therefore, HCC was used as a continuous variable in sensitivity analyses.

2.6. Statistical analyses

In order to determine to which extent HCC may moderate the between-subject differences in acute stress response and acute stress recovery, we applied piecewise linear growth analyses. With multilevel modelling, we can account for the five repeated measurements (Level 1) that are nested within individuals (Level 2). At first, an unconditional model (i.e., no inclusion of Level 1 or Level 2 predictors) was built in order to calculate the intraclass correlation (ICC). As part of preliminary analyses, growth plots were examined to observe the shape of the data in order to build appropriate models (Figure A.1 in supplements). According to the growth plots, two models were fitted: a linear mixed model with a quadratic function of measurement time and a piecewise linear growth model with one specified breakpoint at the measurement time point of the interview. With the latter model, the two segments acute stress response and recovery can be examined within one analysis. The piecewise growth model estimates change over time in different periods, in our case change of blood pressure levels or RMSSD during acute response and recovery in the VR-TSST. The acute stress response is reflected by two baseline measurements and the interview measurement and the recovery phase is reflected by the math and recovery measurement (see also Figure A.1 in supplements). In order to examine which model fits the data better, these two models were compared by Akaike information criterion (AIC; (Curran et al., 2010)), which suggested a better fit of the piecewise linear model (Table A.1 in supplements). Further analyses were thus performed with the piecewise linear model. In a next step, HCC (Level 2) was entered as a predictor variable as well as potential confounder variables (Level 2). Confounder variables with an ordinal or continuous scale were grand mean centered. Within a first model, a random intercept and fixed slopes for the effect of acute response and recovery were specified. In a next step, random slopes for response and recovery were added to the model to account for individual differences and in a final model cross level interactions between HCC and response and recovery were added to the model. These models and their fit indices were then compared using chi square test, due to the hierarchically structured data (Table A.1 in supplements; Curran et al., 2010). Analyses were adjusted for sex (female/male), BMI, and physical activity (< one hour/week; one to two hours/week; three to four hours/week; five to six hours/week; > six hours/week) as these variables may confound acute stress reactivity (Allen et al., 2014; Forcier et al., 2006; Herhaus and Petrowski, 2018). Age was not used as a confounder due to a small age range within participants. In preliminary regression analyses, contraceptive use (yes/no) in women was not associated with the outcomes and was therefore not added as a potential confounding variable.

The final model was specified as followed:

Level 1:

$$SBP_{ij} = \beta_{0j} + \beta_{1j}a_{ij} + \beta_{2j}b_{ij} + r_{ij}$$

Here, SBP_{ij} is the SBP for individual *j* at time *i*; β_{0j} is the intercept, predicted mean SBP at baseline for individual *j*; β_{1j} is the mean SBP change in the acute stress response phase (a_{ij}); β_{2j} refers to the mean SBP change in the stress recovery period (b_{ij}) and r_{ij} is the residual of individual *j*'s value at time *i*.

Level 2:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}W_{1j} + \gamma_{02}W_{2j} + \gamma_{03}W_{3j} + \gamma_{04}W_{4j} + \gamma_{05}W_{5j} + u_{0j}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11}W_{1j} + \gamma_{12}W_{2j} + u_{1j}$$

$$\beta_{2j} = \gamma_{20} + \gamma_{21}W_{1j} + \gamma_{22}W_{2j} + u_{2j}$$

γ_{00} represents the intercept of SBP for individual *j*. γ_{10} and γ_{20} represent the intercept of random slope for acute stress response and recovery for individual *j*. $\gamma_{01}W_{1j}$ and $\gamma_{02}W_{2j}$ represent the direct between-subject effects of HCC on blood pressure. $\gamma_{11}W_{1j}$ and $\gamma_{12}W_{2j}$ represent the cross-level interaction effects between HCC and the acute stress response and $\gamma_{21}W_{1j}$ and $\gamma_{22}W_{2j}$ represent the cross-level interaction effects between HCC and recovery. $\gamma_{03}W_{3j}$, $\gamma_{04}W_{4j}$ and $\gamma_{05}W_{5j}$ are the intercept coefficients for effects of sex, BMI, and physical activity. u_{0j}, u_{1j} and u_{2j} represent the residuals for random intercept, response and recovery. In sensitivity analyses the same procedure was followed, but HCC was included as a continuous variable.

Descriptive statistics were performed in SPSS version 28. Multilevel analyses were performed with R Version 4.2.2 and by using the lme4 package (Bates et al., 2014) and lmer function. The analytical models were estimated with restricted maximum likelihood (REML), while maximum likelihood (ML) was used within chi square test for model comparison. Unstandardized regression coefficients are shown and statistical significance was assumed at a level of $p < 0.05$.

3. Results

In total, n = 61 medical students participated in our study. One person had to be excluded from all analyses, as the participant indicated to have received information about the procedure of the VR-TSST. Five participants were excluded from all analyses, as their hair was too short to determine HCC in the laboratory. Another participant was excluded because the hair cortisol value was considered as an outlier (exceeding three standard deviations from the mean). This resulted in n = 54 participants for the blood pressure analyses. Furthermore, six participants were excluded from the HRV analyses due to insufficient data quality. In addition, one person was excluded due to a great amount of ventricular ectopic beats. This resulted in n = 47 participants for the HRV analyses. Two values in the interview and recovery measurement were considered as outliers and two values for BL30 and the recovery measurement were missing. There was no other missing data.

3.1. Descriptive results of the study sample

Descriptive results of study variables are shown in Table 1. About

Table 1
Descriptive statistics of the study sample (n = 54).

	n (%)	Mean	SD[a]	Range
Sex				
female	41 (75.9)			
male	13 (24.1)			
Age		22.19	2.13	19-31
HCC[b]		5.65	2.93	1.80-15.43
pg/mg				
<25th percentile	13 (24.1)	2.55	0.44	1.80-3.30
25th-75th percentile	28 (51.9)	5.11	1.12	3.38-7.48
75th percentile	13 (24.1)	9.91	2.07	7.74-15.43
HCC[b]		0.70	0.22	0.26-1.19
log-transformed				
<25th percentile	13 (24.1)	0.40	0.08	0.26-0.34
25th-75th percentile	28 (51.9)	0.70	0.09	0.53-0.87
75th percentile	13 (24.1)	0.99	0.08	0.89-1.19
SBP[c]				
mmHg				
Baseline 5 min		117.31	12.68	
Baseline 30 min		115.31	12.27	
Interview		128.63	15.73	
Math		125.41	15.61	
Recovery		119.24	11.33	
DBP[d]				
mmHg				
Baseline 5 min		73.28	9.81	
Baseline 30 min		73.94	9.42	
Interview		80.35	10.73	
Math		79.37	10.21	
Recovery		76.59	9.45	
RMSSD[e]				
ms				
Baseline 5 min		43.38	26.02	
n = 7 missing[f]				
Baseline 30 min		45.00	25.43	
n = 8 missing[f]				
Interview		32.08	16.17	
n = 8 missing[f]				
Math		42.56	24.40	
n = 7 missing[f]				
Recovery		49.75	25.93	
n = 9 missing[f]				
RMSSD[e]				
ms; log-transformed				
Baseline 5 min		1.57	0.26	
n = 7 missing[f]				
Baseline 30 min		1.59	0.24	
n = 8 missing[f]				
Interview		1.45	0.23	
n = 8 missing[f]				
Math		1.56	0.25	
n = 7 missing[f]				
Recovery		1.64	0.23	
n = 9 missing[f]				

[a] Standard deviation. [b] Hair cortisol concentration. [c] Systolic blood pressure. [d] Diastolic blood pressure. [e] Root mean square of successive differences. [f] please see results section for details on missing data.

three quarters of the participants were female and the reported mean age was 22 years (SD= 2.13, Median= 22).

SBP was on average 11.32 mmHg higher after the interview than at the first baseline measurement. Thereafter SBP decreased with on average 3.22 mmHg from interview until the end of the math test and 6.17 mmHg from the end of the math test until the end of the recovery period. The course of DBP was similar. Log-transformed RMSSD was on average 0.11 ms lower during the interview than during the first baseline measurement. RMSSD increased with on average 0.1 ms from interview to the math test and 0.09 ms from math test until the recovery period.

Mean values of SBP, DBP and RMSSD during the different VR-TSST periods for the three categories of HCC are shown in Fig. 1. The plots show that participants with medium levels of HCC had the highest blood pressure mean values after the interview and math task and lowest RMSSD mean values after the interview and math task. Participants with the highest levels of HCC had the lowest blood pressure mean values after the interview and math task. They had higher RMSSD mean values during the interview and after the math task than participants with medium HCC levels and comparable RMSSD mean values during the interview with participants with the lowest levels of HCC. Participants with the highest HCC levels showed a sharper decline in their RMSSD values from baseline to interview compared to the two other HCC categories. Blood pressure and RMSSD mean values after the interview and math task of participants with low levels of HCC were between those of the other two HCC categories.

3.2. Multilevel analyses

In all three unconditional models, the ICCs were suitable for considering multilevel analyses (Table A.1 in supplements). For example, 68.8% of the variance in SBP lies between the individuals, thus the hierarchy of data should be acknowledged.

Table 2 shows results of the final piecewise growth models estimating the effect of HCC on acute stress response and recovery for SBP, DBP, and RMSSD. SBP and DBP values increased during the acute stress response phase and decreased during the stress recovery period which was descriptively shown in Fig. 1. There was a significant interaction effect found for HCC and acute stress response on SBP (Table 2, model 1). Participants who had higher HCC levels (>75th percentile) showed lower changes in SBP in the acute stress response phase compared to participants who had medium levels of HCC levels (25th-75th percentile). There was another significant interaction effect found for HCC and acute stress response on DBP (Table 2, model 1). Participants who had higher HCC levels showed lower changes in DBP in the acute stress response phase compared to participants with medium HCC levels. The descriptive results for RMSSD were only partly confirmed by the piecewise growth analyses. No change in RMSSD during the acute stress response phase was observed, but results indicated an increase of RMSSD levels during the stress recovery phase. No significant interactions effects were found between HCC and the acute stress response or recovery phase on RMSSD levels. There were also no significant interaction effects found for lower levels of HCC (<25th percentile) and acute responses or recovery compared to medium levels of HCC.

Sensitivity analyses with inclusion of HCC as a continuous variable resulted in similar estimates as when HCC was treated as a categorical variable, but significant interaction effects were no longer significant (Table 2, model 2). There was an almost significant interaction effect found for HCC and acute stress response on DBP ($p = 0.07$).

4. Discussion

This study investigated whether HCC as an indicator for cumulative cortisol secretion is associated with the magnitude of acute

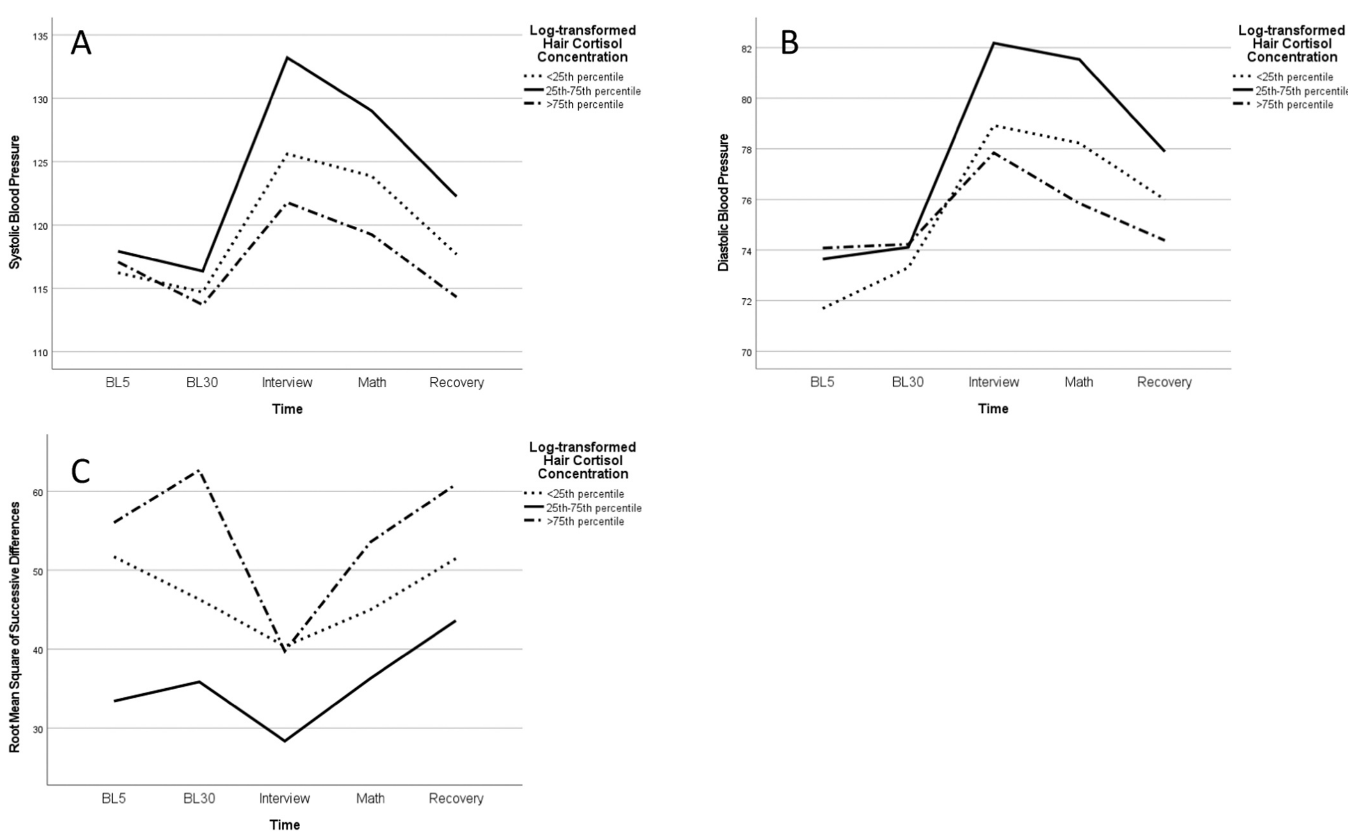

Fig. 1. Mean cardiovascular responses to the VR-TSST for different levels of HCC. BL5 refers to baseline measurement after 5 min and BL30 refers to baseline measurement after 30 min.

Table 2
Results from piecewise growth models estimating the association between stress response, stress recovery and HCC.

	Cross-level interaction model 1[a]			Cross-level interaction model 2[b]		
	B[c]	SE[d]	p-value	B[c]	SE[d]	p-value
	SBP[e] (n = 54)			**SBP[e] (n = 54)**		
Stress response	**8.12 * ****	6.43	< 0.001	**9.46 * ****	2.35	< 0.001
Stress recovery	**-3.87 * ****	0.89	< 0.001	-3.45	2.15	0.16
HCC[f]				4.16	6.96	0.55
HCC[g]						
< 25th percentile	0.31	3.80	0.94			
25-75th percentile	Ref.					
> 75th percentile	2.92	3.86	0.45			
HCC < 25th × Stress response	2.88[+]	1.62	0.08			
HCC > 75th × Stress response	**-5.41 * ****	1.62	< 0.001			
HCC[f] × Stress response				-4.77	3.21	0.14
HCC < 25th × Stress recovery	1.04	1.58	0.51			
HCC > 75th × Stress recovery	1.15	1.58	0.47			
HCC[f] × Stress recovery				0.15	2.94	0.96
	DBP[h] (n = 54)			**DBP[h] (n = 54)**		
Stress response	**4.63 * ****	0.63	< 0.001	**6.41 * ****	1.53	< 0.001
Stress recovery	**-1.45 ***	0.59	0.015	-1.27	1.41	0.37
HCC[f]				4.81	5.87	0.41
HCC[g]						
< 25th percentile	-1.14	3.20	0.72			
25-75th percentile	Ref.					
> 75th percentile	2.70	3.25	0.41			
HCC < 25th × Stress response	-0.83	1.12	0.46			
HCC > 75th × Stress response	**-2.75 ***	1.12	0.01			
HCC[f] × Stress response				-3.78[+]	2.09	0.07
HCC < 25th × Stress recovery	0.35	1.05	0.74			
HCC > 75th × Stress recovery	0.01	1.05	1.00			
HCC[f] × Stress recovery				-0.13	1.93	0.95
	RMSSD[i] (n = 47)			**RMSSD[i] (n = 47)**		
Stress response	-0.04	0.02	0.14	-0.05	0.06	0.36
Stress recovery	**0.08 * ****	0.02	< 0.001	**0.09 ***	0.04	0.02
HCC[f]				0.20	0.18	0.28
HCC[g]						
< 25th percentile	0.10	0.09	0.26			
25-75th percentile	Ref.					
> 75th percentile	**0.25 * ***	0.10	0.01			
HCC < 25th × Stress response	-0.02	0.04	0.55			
HCC > 75th × Stress response	-0.04	0.04	0.31			
HCC[f] × Stress response				-0.00	0.08	0.99
HCC < 25th × Stress recovery	0.00	0.03	0.91			
HCC > 75th × Stress recovery	-0.00	0.03	0.94			
HCC[f] × Stress recovery				-0.02	0.05	0.76

[a] Random intercept and random slopes plus cross-level interaction. Adjusted for sex, physical activity and body mass index. [b] Random intercept and random slopes plus cross-level interaction. Adjusted for sex, physical activity and body mass index. HCC is included as a continuous variable as a sensitivity analysis.[c] Unstandardized regression coefficients. [d] Standard error. [e] Systolic blood pressure. [f] Hair cortisol concentration, log-transformed, continuous variable. [g] Hair cortisol concentration, log-transformed, categorical variable. [h]Diastolic blood pressure. [i] Root means square of successive differences, log-transformed. Bold values indicate a p-value below 0.05. [+] <0.1, * < 0.05, ** <0.01, *** <0.001.

cardiovascular stress responses and recovery during a VR-TSST in a sample of medical students. In piecewise linear growth analyses, a significant effect of HCC on blood pressure responses was observed. Participants with higher levels of HCC had lower SBP and DBP responses compared to participants with medium levels of HCC. There were no significant effects observed for HCC on stress recovery or for HCC on any RMSSD responses during the VR-TSST. Sensitivity analyses for HCC as a continuous variable showed a similar direction of association to the observed interaction effects of higher HCC levels and acute blood pressure responses.

Our results of a blunted blood pressure response to the VR-TSST among individuals with higher levels of HCC could indicate that there may be an inadequate cardiovascular response to acute stress due to increased HPA axis activity. One study, which also investigated HCC, in a sample of employees with various degrees of burnout symptoms, found supporting results indicating that higher levels of HCC were associated with lower HR and increased HRV responses (Wekenborg et al., 2019). Sandner et al. (2020) showed that HCC was negatively correlated with salivary cortisol responses. Our findings are also supported by other studies that showed associations between higher self-reported general chronic stress or chronic work stress and lower cardiovascular responses to acute stressors in employees (Hamer et al., 2006; Matthews et al., 2001; Siegrist and Klein, 1990) and African American females (Cavanagh and Obasi, 2021). With our study we can add to these findings that potential inadequate responses may also occur for blood pressure in medical students that show higher levels of HCC. Our findings may represent one type of allostatic load where inadequate acute responses of one stress system (i.e., ANS) occur to compensate over activity of the other stress systems (i.e., HPA-axis) (McEwen, 1998). For example, experimental studies have found that suppressing one of the two main physiological response systems during TSST can result in increased activity of the other (Ali et al., 2020; Andrews et al., 2012). Our findings also partly support the proposed model of “toughness” by Dienstbier (1989). Due to the evidence that HCC is associated with adverse study conditions in our study sample (Heming et al., 2023), individuals with medium levels of HCC might be those students that were toughened by moderate stress levels and thus reacted with an increased blood pressure response to the VR-TSST compared to students with lower or higher levels of stress and thus HCC. However, the model of “toughness” by Dienstbier (1989) also suggests that individuals with medium stress levels show a faster or steeper recovery, which our data does not support. In addition, a recent review about different laboratory stress tests showed that there are indications of subsequent negative health consequences such as depression or obesity for healthy individuals predicted by lower cardiovascular responses (O' Riordan et al., 2023). The authors also presented thresholds of blunted cardiovascular responses (HR and blood pressure), that are suggested to predict these subsequent adverse health outcomes (O' Riordan et al., 2023). Applying their DBP thresholds to our results, the group of students with higher levels of HCC would be considered at risk for adverse health outcomes. However, it should be noted that stress responses induced by a VR-TSST can be lower compared to the original TSST (Helminen et al., 2019) and that there are no reference values for appropriate blood pressure responses in a VR-TSST. This limits statements on whether blood pressure responses of the group with medium HCC are actually adequate or exaggerated or if a robust stress induction took place in our sample.

Furthermore, we did not investigate coping strategies in our study. However, coping may moderate the association between HCC and acute stress responses. For example, it was suggested that emotion-oriented coping may only be beneficial for individuals that not yet suffer from high levels of chronic stress and thus a potential dysregulation of the SNS (Cavanagh and Obasi, 2021). A study among children showed that higher levels of HCC and avoidant coping strategies applied during a stressor were associated with a more efficient recovery compared to children with higher levels of HCC and distraction coping strategies applied during a stressor (Bendezú and Wadsworth, 2017). If certain types of coping strategies can moderate the association between HCC and acute stress responses, these should be further investigated to find appropriate coping strategies that help reduce potential negative health outcomes due to high levels of cumulative cortisol secretion.

We did not find any associations between HCC and recovery measures in our study. Prolonged responses to acute stressors have been shown to be particularly harmful for health (Panaite et al., 2015), and our results may thus indicate that cumulative cortisol secretion does not contribute to an increased risk of cardiovascular disease due to a prolonged recovery period after acute stressors in our study population. Another study could also not find associations between chronic stress and cardiovascular recovery from laboratory stressors (Matthews et al., 2001). These findings are in contrast to the allostatic load model (McEwen, 1998). The allostatic load model proposes sustained arousal after the end of a stressor due to long-term stress in individuals (McEwen, 1998). Supporting this model, a meta-analysis reported that general life stress was associated with slower cardiovascular recovery (assessed by a greater difference between recovery and baseline scores; Chida and Hamer, 2008). However, general life stress includes several different types of stressors (Chida and Hamer, 2008) and therefore different conceptualizations of stress may contribute to the mixed findings. In contrast to this, HCC is an indicator for cumulative cortisol secretion and is thus dependent on a large array of other variables that trigger cortisol secretion in addition to psychosocial stress (Stalder and Kirschbaum, 2012). As there are only few studies existing that examined potential associations of HCC and acute stress recovery, there is need for more research to gain more knowledge on potential associations, their directions, and potential pathways. Another possible explanation for our contrasting finding might include the sample size, which might have not been sufficient to find smaller effects. In addition, our study population was relatively healthy and young, which might indicate that consequences of long-term stress cannot yet be observed.

In contrast to Wekenborg et al. (2019) who showed a significant moderation of HCC on RMSSD responses in males with varying burnout symptoms, we did not find any significant interactions between HCC and RMSSD responses or recovery. Reasons for those contrasting findings could lie in the characteristics of the study samples, as our study sample was predominantly female. Furthermore, the lack of association between HCC and RMSSD response contrasts our findings of a blunted blood pressure response in the group of participants with high HCC levels. As RMSSD reflects parasympathetic activity (Hill et al., 2009), it thus seems that long-term HPA axis activity is associated with sympathetic but not parasympathetic activity in the study sample at hand.

As our study shows indications of a blunted blood pressure response during a laboratory stress test among medical students with higher levels of HCC, there are some theoretical implications that can be drawn from our findings. Today most research on TSST is done on measures that reflect acute HPA axis activity (i.e., salivary cortisol) (Allen et al., 2014; Man et al., 2023). This is partly due to the fact that the HPA axis response is suggested to be stronger to social-evaluative stressors than the SNS response and due to the large number of studies that have validated the TSST for HPA axis measures (Dickerson and Kemeny, 2004; Man et al., 2023). We have now shown a lower blood pressure response in individuals with higher HCC, which may indicate an inadequate response of the SNS as a compensatory response to high HPA-axis activity and highlights the relevance of investigating this cardiovascular measure. As there are indications for adverse health outcomes due to blunted blood pressure responses (O' Riordan et al., 2023) it would be of interest to further investigate the associations at hand by longitudinal studies. By assessing HCC over time it could be investigated how changes in HCC are associated with changes in stress responses and also with potential negative health outcomes. Therefore, larger samples of healthy adults with greater age ranges and an equal distribution of sex would be desirable. This could help to tailor prevention approaches more precisely to reduce future health risks.

4.1. Limitations

This study has several limitations that need to be addressed. First, generalizability of our results is limited due to recruitment at only one medical school. At the respective medical school, more women than men are enrolled which made it more difficult to recruit men. If an association between HCC and acute stress responses or recovery is stronger for men, our results may be underestimated. Second, due to the study design, statements on causality cannot be made. Although the assessment of HCC reflects the time before the VR-TSST and the VR-TSST is known to induce stress responses, we cannot know whether the associations shown are dependent on third variables that we have not accounted for. However, we did adjust for confounders such as sex, BMI and physical activity. We have also tried to minimize the risk that underlying conditions contributed to the shown effects by excluding participants with mental health disorders. Depressive symptoms, however, are more often reported in medical students than in a general population of similar age (Rotenstein et al., 2016) and could have thus altered our effects. However, findings on depressive symptoms and HCC are inconsistent so far. For example, one study has shown elevated mean levels of HCC in employed individuals with depressive symptoms (Janssens et al., 2017) while another study showed that young exercise and health science students with increased levels of HCC tended to report lower depressive symptoms (Gerber et al., 2013). Depressive symptoms showed no main or interaction effects on acute salivary cortisol, HR or HRV responses in a recent study (Wekenborg et al., 2019). Furthermore, it is important to mention that the sample size was rather small, which could made it more difficult to detect smaller effects. However, VR-TSST studies use similar sample sizes and researchers have shown valuable insights with less comprehensive analyses and similar sample sizes (Helminen et al., 2019; Sandner et al., 2020; Wekenborg et al., 2019). In addition, due to categorization of HCC information loss occurs. However, this approach was suitable to acknowledge a non-linear association between different levels of HCC and acute stress responses. To minimize the risk of drawing false conclusions due to the simplified model, we included sensitivity analyses with HCC as a continuous variable. Results could not show significant effects, but similar directions of associations and estimates. Third, research suggested that speaking during the TSST is associated with an increase of blood pressure and decrease of high frequency HRV, another

HRV-parameter of parasympathetic activity (Grimley et al., 2019). It is thus possible that the need to speak during the TSST might disguise effects of third variables and lead to an underestimation of the association between HCC and blood pressure or HRV responses. A neutral speaking period has therefore been recommended (Grimley et al., 2019), but has not yet been widely established for the TSST.

5. Conclusion

In summary, our findings show lower blood pressure responses to an acute stressor in medical students with higher levels of HCC compared to students with medium levels of HCC. Increased HPA axis activity over a prolonged period of time thus seems to be associated with lower acute stress responses of the sympathetic branch of the ANS. Inadequate stress responses may entail future health risks, such as cardiovascular diseases, due to the presence of long-term stress.

Ethical approval and consent to participate

Ethical approval for this study was obtained by the ethics committee of the medical faculty of the Heinrich-Heine University of Düsseldorf (study number: 2019-714). All participants gave written informed consent to participate in the study. All methods were carried out in accordance with relevant guidelines and regulations.

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CRediT authorship contribution statement

Heming Meike: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Angerer Peter:** Conceptualization, Writing – review & editing. **Apolinário-Hagen Jennifer:** Conceptualization, Writing – review & editing. **Liszio Stefan:** Software, Writing – review & editing. **Nater Urs Markus:** Methodology, Writing – review & editing. **Skoluda Nadine:** Methodology, Writing – review & editing. **Weber Jeannette:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Consent for publication

Not applicable.

Declaration of Competing Interest

On behalf of all authors, there are no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.106986.

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