Ulm University Medical Center
Clinic for Psychosomatics and Psychotherapeutic Medicine
Medical Director: Prof. Dr. med. Harald Gündel

Cardiovascular risk assessment and biopsychological effects of acute and repeated social stress in German soldiers

Cumulative Dissertation submitted to obtain the doctoral degree of Human Biology of the Medical Faculty of Ulm University

Tanja Maier
From Ehingen
2023

Acting dean: Prof. Dr. Thomas Wirth

First referee: Prof. Dr. Christiane Waller

Second referee: Prof. Dr. Harald Baumeister

Day of graduation: 15.12.2023

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Abbreviations

ASCVD = atherosclerotic cardiovascular disease

AUC = area under the curve

AUCg = AUC with respect to the ground

AUCi = AUC with respect to increase

BEST = German Armed Forces Deployment and Stress

CAD = coronary artery disease

CVD = cardiovascular disease

DNA = deoxyribonucleic acid

GSI = Global Stress Index

HPA = hypothalamic-pituitary-adrenal

HRV = heart rate variability

MI = myocardial infarction

PTSD = post-traumatic stress disorder

RMSEA = the root-mean-square errors of approximation

RMSSD = root mean square of successive differences between normal-to-normal inter-beat intervals

sAA = salivary α-amylase

SAM = sympathetic-adrenal-medullary

SEM = structural equation modeling

SDNN = standard deviation of all normal-to-normal inter-beat intervals

TSST-G = Trier Social Stress Test for groups

1 General Introduction

1.1 Social stress in soldiers

Soldiers face a work environment with challenging tasks, especially during foreign deployment. Work-related stressors like exhaustion due to workload can also be found at home base (Hsieh & Tsai, 2019). However, foreign deployment has been reported to provoke stressful experiences over a short and demanding time frame with changes in work hours/station as well as limited privacy or (witnessing) injury and coming under fire (Pflanz & Sonnek, 2002; Vogt et al., 2013). Research regarding military personnel mostly focuses on traumatic stress and the development of post-traumatic Stress Disorder (PTSD) (Hoge et al., 2004). In recent years, missions abroad and domestic deployment result in less frequent direct traumatic combat experiences (Kumar et al., 2013) and only 1% of German soldiers suffer from PTSD after deployment (Wittchen et al., 2012). Operating in low-intensity conflict zones, like peacekeeping missions, is more and more common and is characterized by unpredictability, diffuse threat, the feeling of powerlessness and micro-stressors such as boredom, sleep deprivation or long working hours (Sharma & Sharma, 2012). Away from their known social network and life these challenges result in a higher risk for perceived social stress during job performance (Pflanz & Sonnek, 2002). Social stress also shows an important influence on soldiers' well-being and overall health (Cohen, 2000; Kumar et al., 2013). Thus, shifting focus towards this factor could prove beneficial. Research on the impact on physical health showed that psychosocial stress can adversely affect cardiovascular health by increasing the risk for coronary artery disease (CAD) in healthy individuals (Ferrie et al., 2013). Cardiovascular diseases (CVD) still remain one of the leading causes of death worldwide (Mendis et al., 2011) but some of the potential influencing factors are not fully understood yet. Acute and chronic psychological stress are closely linked to CAD risk and atherosclerosis (Lagraauw et al., 2015) and psychosocial stress is significantly related to myocardial infarction (MI) (Yusuf et al., 2004). Military personnel faces high rates of CAD as well as health-threatening behavior like smoking or alcohol consumption (Tien et al., 2010) and soldiers with combat experiences in the past are more likely to suffer from hypertension (Granado et al., 2009) and stroke at an advanced age (Thomas et al., 2017).

1.2 Cardiovascular risk scores and social stress

In order to assess cardiovascular risk in individuals, a variety of risk factors must be taken into account. For the 10-year risk prediction of CAD events well-known classical risk factors include gender, age, sex, smoking, family history of MI, blood-pressure, fat and sugar metabolism which all are part of the internationally established cardiovascular risk scores like the Framingham-score (Wilson et al., 1998), the PROCAM-score (Assmann et al., 2002) and the ASCVD (atherosclerotic cardiovascular disease)-score (Goff et al., 2014). Not only biologically based factors but also stress at work also results in a 2.2-fold higher cardiovascular mortality risk (Kivimaki, 2002). Heavy drinking (Foerster et al., 2009) or a lack of physical fitness (Bijnen et al., 1998) show an increase in 10-year CAD/CVD risk. However, only a few approaches try to improve the precision of the scores through

psychosocial risk factors by including depression, stress or trauma scores, as well as consumption of fruits and vegetables, alcohol and regular physical activity which leads to a more accurate prediction (Yusuf et al., 2004). Due to the complexity of incorporating every single aspect of social stress into the risk scores, it could be advantageous to model a single index. The challenge within the development of a psychosocial stress index is to create a holistic statistical model of different aspects like stressful life events, job stress or anxiousness from early life to adulthood, which also considers potential relationships on an item level. Therefore, the first publication aimed to develop a global stress index in soldiers through structural equation modeling (SEM) using different psychological questionnaires displaying social stress (Maier et al., 2021).

1.3 Biopsychological response to social stress

Social stress causes specific bio-physio-psychological body reactions. Experimental approaches can provide a better understanding of the stress response mechanisms which can enable further research into whether these responses are potential moderating factors that link social stress with CVD. This is why social stress tests were used to induce the activation of biopsychological stress systems. This allowed us to measure the systems' ability to adapt to the stressor and to maintain homeostasis. The interindividual differences in how body functions and mental state change whilst being exposed to the same repeated stress over time provides insights into functioning coping mechanisms. Exposure to social stress leads to activation of the hypothalamic-pituitary-adrenal (HPA)- and sympathetic-adrenal-medullary (SAM)-axis and to changes in hormone levels, heart rate variability (HRV) and blood pressure, while chronic stress activation can even deregulate immune function (Henry, 1992; Padgett & Glaser, 2003). The influence of social stress on mental conditions includes negative emotions like anxiety, tension or anger (Cohen, 2000). It impacts cognition and behavior to properly deal with the challenging situation by the ability of focused attention, memory formation, higher alertness and quick decision making to actually fight or flight from the threat (Cohen, 2000). The dysregulation of stress systems and the resulting maladaptive neuroendocrine responses due to chronic or repeated stress (Cohen, 2000) highlight the importance of functioning adaptive mechanisms to repeated stress, the so-called habituation. Habituation describes the reduction of an expressed response to a repeated stimulus (Thompson & Spencer, 1966), in this case it describes the decrease of bio-physiological stress response patterns in response to social stress exposure. As mentioned above, soldiers are often exposed to social stress, which led to the analyses in the second publication, where soldiers' mental habituation patterns and differences on bio-physiological signals after a mission abroad were observed.

The biological impacts of social stress can even be found on the genetic level and therefore the third publication tries to establish the relation of social stress and radical formation (especially reactive oxygen species) with oxidative deoxyribonucleic acid (DNA) damage. It also led to considerations of possible links between DNA-damage and CVD risk in soldiers. The (para-) sympathetic response to acute social stress, mirrored for example by HRV, can cause autonomic imbalance, which is known to be directly related to CVD risk (Thayer et al., 2010). Exposure to social stress modulates immune, endocrine and metabolic pathways and is associated with an increase in CVD risk (Lagraauw et al., 2015). CVD risk is linked to increased short-term DNA damage, mainly visible as single or double strand breaks

(Kliemann et al., 2012). Yet, the role of social stress for oxidative DNA damage remains unclear so far. Patients with coronary artery disease show higher levels of oxidative DNA damage than healthy controls (Møller et al., 2020). Therefore, DNA damage could give an indication for risk stratification in patients with known CVD risk (Federici et al., 2008).

1.4 Experimental investigation

The interdisciplinary BEST (Bundeswehreinsatz und Stress/German Armed Forces Deployment and Stress)-study is a part of the joint project between the military hospital and the university in Ulm which prospectively investigated biopsychosocial stress effects of foreign deployment on cardiovascular health in the German Armed Forces. The study was approved by the Ethics Committee of the University of Ulm under the number 253/12 in 2012. It is registered in the German register of Clinical studies (DRKS00022345) and was conducted according to the guidelines of the Declaration of Helsinki. Soldiers were recruited from the German Armed Forces hospital in Ulm and from military barracks in Dornstadt, Laupheim and Ulm. All participants underwent a well-established social stress-test paradigm three-times, the Trier Social Stress Test for groups (TSST-G) (von Dawans et al., 2011), where data was collected prior to (t0), 4-6 weeks after (t1) as well as one year after (t2) a mission abroad. Therefore, soldiers with (experimental group) and without (control group) a mission abroad during the study time were compared and all participants underwent the same procedure.

During the TSST-G, 3-6 participants were interviewed successively for a mock job interview and a mental arithmetic task in front of a judging panel in lab coats and in the presence of the other participants. Prior to, during and after the stress-inducing procedure various psychological and biological parameters (figure 1 (Maier et al., 2022, p.4)) were collected to calculate cardiovascular risk and to show subjective and individual adaptation over time. Therefore, blood and saliva samples, blood pressure, HRV as well as questionnaires measuring perceived stress, mood and anxiousness were recorded. The activation of the SAM- and HPA- axes were determined together with oxidative DNA-damage at rest and during the experimental challenge. The data also enabled the development of a stress index to complement classical cardiovascular risk assessment.

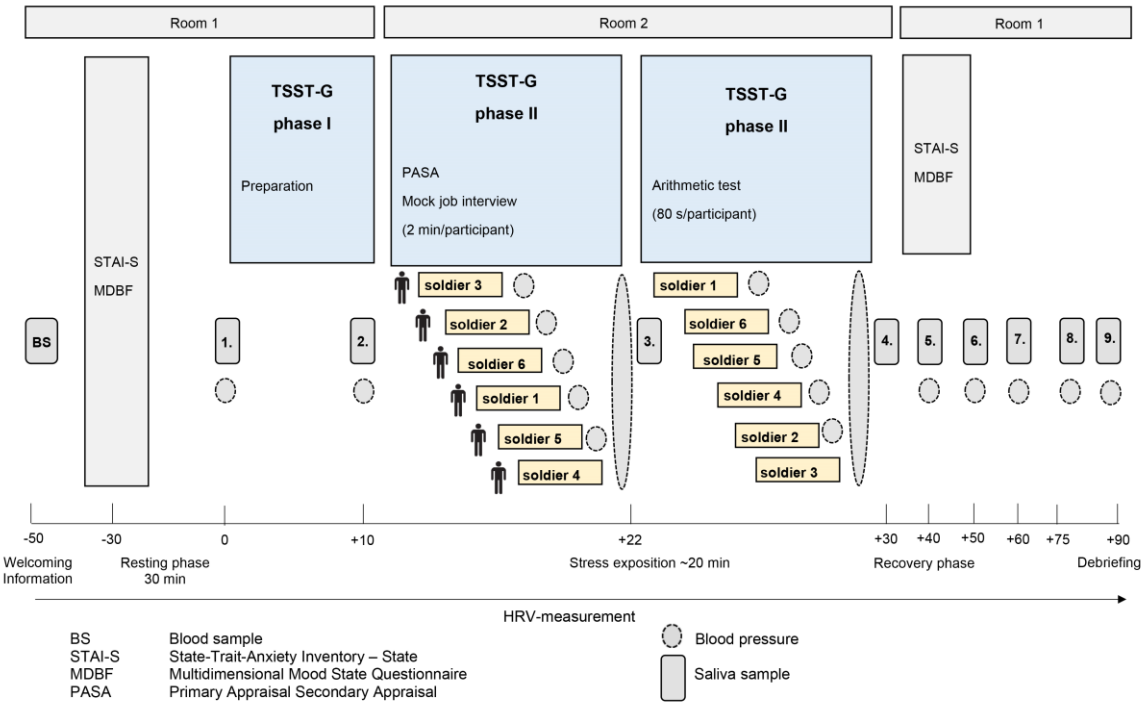

Figure 1. Flowchart of the Trier Social Stress Test for groups (TSST-G)
Detailed description of the trial design used in the BEST-study adapted according to the procedure from von Dawans, Kirschbaum and Heinrichs (von Dawans et al., 2011) displayed with used psychological questionnaires and bio-physiological measurement points. Source: (Maier et al., 2022, p.4); https://doi.org/10.3389/fpsyt.2022.1011181; CC-BY 4.0 - https://creativecommons.org/licenses/by/4.0/

In order to generate conclusive statements and hypotheses, the stress response has to be induced experimentally, as otherwise confounding variables and bias can lead to inaccurate results. This was realized in the BEST-study by using the TSST-G. Measuring resting situations alone is not suitable and valid. The effects of environmental acute stressors can be unpredictable. They consolidate, change or subside over longer time periods after first occurrence, which for example is well-known for acute stress disorders, memory consolidation or autonomic alterations (Shalev, 2002). Therefore, it becomes necessary to examine the biopsychological impacts of social stress repeatedly which prompted the repeated measure study design to evaluate soldiers subsequently after a mission abroad as well as one year later for long-term effects. The repeated use of the TSST-G to induce stress over a prolonged timespan is noteworthy because so far experimental stress research is mostly focused on short-term observations. For this dissertation these characteristics enabled the evaluation of biopsychological effects of acute and repeated social stress and the assessment of cardiovascular risk in German soldiers.

2 Research Objectives

The aim of this thesis was to provide an overview about risk assessment and biopsychological effects of social stress in healthy German soldiers. Questionnaires about social stress led to a stress index for cardiovascular risk investigation. Experimentally induced acute and repeated social stress enabled the measurement of physiological and psychological reactions. Furthermore, changes within and between the three points of measurement for soldiers with and without a mission abroad as well as associations with cardiovascular risk could be investigated in this cohort. All published papers refer to the data-basis of the BEST-study which provided a broad range of psychological, physiological and biological parameters collected in soldiers between 2016 and 2020.

Original manuscript 1 (Maier et al., 2021)

The statistically well-founded combination of various factors of social stress to a global stress index was the goal of the first mentioned paper. The GSI has the potential to enhance classical cardiovascular risk scores.

Maier, T., Kugelmann, M., Rhee, D.-S., Brill, S., Gündel, H., Friemert, B., Becker, H.-P., Waller, C., & Rappel, M. (2021). Structural Equation Modeling of a Global Stress Index in Healthy Soldiers. *Journal of Clinical Medicine, 10*(8), 1799. https://doi.org/10.3390/jcm10081799

Research hypothesis:

A global stress index can be generated by using different questionnaires for acute and chronic social stress.

Abbreviated conclusion:

The GSI was implemented with structural equation modeling.

Original manuscript 2 (Maier et al., 2022)

The concept of this paper was to test the hypothesis if soldiers with and without a recent mission abroad differently react to induced social stress. The focus was on the habituation patterns over the three repeated TSST-Gs. Moreover, differences between physiological and psychological habituation were investigated.

Maier, T., Rappel, M., Rhee, D.-S., Brill, S., Maderner, J., Pijahn, F., Gündel, H., Radermacher, P., Friemert, B., Becker, H.-P., & Waller, C. (2022). Mental but no bio-physiological long-term habituation to repeated social stress: A study on soldiers and the influence of mission abroad. *Frontiers in Psychiatry, 13*, 1011181. https://doi.org/10.3389/fpsyt.2022.1011181

Research hypothesis:

1) The bio-physio-psychological stress response in soldiers habituates over induced repeated social stress.
2) Foreign deployment results in attenuated stress habituation patterns compared with no mission abroad.

Abbreviated conclusion:

1) Psychological but not bio-physiological habituation could be found.
2) No differences in the stress response between the two groups was detectable over time.

Original manuscript 3 (Waller et al., 2020)

The aim of this paper was to evaluate oxidative DNA damage during experimental stress in relation to cardiovascular risk. For this purpose, single cell gel electrophoresis ("*tail moment*" of the so-called "*comet assay"*) was used, which allows quantification of oxygen radical-induced DNA single- and double strand breaks. Oxidative DNA damage during experimental stress was measured and related to cardiovascular risk, defined by the PROCAM-score in our study cohort. The paper dealt with the question whether the damage was related to stress-induced changes in sympathetic tone, measured by HRV.

Waller, C., Rhee, D.-S., Gröger, M., Rappel, M., Maier, T., Müller, M., Rottler, E., Nerz, K., Nerz, C., Brill, S., Becker, H.-P., & Radermacher, P. (2020). Social Stress-Induced Oxidative DNA Damage Is Related to Prospective Cardiovascular Risk. *Journal of Clinical Medicine, 9*(11), 3783. https://doi.org/10.3390/jcm9113783

Research hypothesis:

1) Induced social stress is associated with an increase in oxidative-DNA damage.
2) Cardiovascular risk is positively associated with increased DNA damage.
3) DNA damage is linked to sympathetic nervous system activation induced by social stress.

Abbreviated conclusion:

1) Social stress significantly increased DNA damage.
2) Soldiers with high prospective cardiovascular risk showed more DNA damage whereas no increase was found for individuals with no prospective cardiovascular risk.
3) The degree of DNA damage was related to an increase in sympathetic tone.

3 Overview of published manuscripts

3.1 Structural Equation Modeling of a Global Stress Index in Healthy Soldiers (Maier et al., 2021)

3.1.1 Introduction

The development of the Global Stress Index (GSI) aimed to model social stress in soldiers by analyzing different types of social stress as potential stressors. The motivation for generating the GSI was to gain an understanding of these potential stressors, how they are influencing each other and to properly combine them. The GSI is structured to be applicable as an extension of classical CAD risk factors due to the strong influence of social stress on CAD (Yusuf et al., 2004). The used questionnaires were selected according to their ability to describe certain social stressors with a proven influence on CVD. So far, external stressors were assessed by questions about stress at work or home, financial stress, locus of control, and occurrence of major life events like divorce, illness or family conflicts whereas depression was included as a potential reaction to stress (Rosengren et al., 2004). Child maltreatment as a strong form of social stress is also known to show a link to depression, obesity and CHD in adulthood (Felitti et al., 1998).

3.1.2 Material and Methods

As part of the BEST-study, 192 soldiers (27% female) were included in the statistical analyses. The data used for the development of the GSI were obtained from the first measurement time point (t0). All participants were asked to fill out baseline questionnaires prior to the TSST-G, which included anamnestic information, standardized psychological questionnaires and daily habits like physical activity or sleep quality. Some participants did not answer all questionnaire items which led to missing data. The multivariate normal distribution was not fully given for all items which can lead to underestimated X^2-values. The used questionnaires reflected different aspects of social stress. Acute stress in the last month was measured with the PSS-4- Perceived Stress Scale (Cohen et al., 1983) and chronic stress in the last three months with the TICS- Trier Inventory for Chronic Stress (Schulz et al., 2004). Anxiousness and depressive symptoms were quantified with the HADS- Hospital Anxiety and Depression Scale (Herrmann et al., 1995). Stressful life events over lifespan were covered with the sum score of the section live events from the PDS - Posttraumatic Stress Diagnostic Scale Checklist (Ehlers et al., 1996), the CTQ- Childhood Trauma Questionnaire (Bader et al., 2009) and the sum score of the section “prior stressors” from the DRRI-2- Deployment Risk and Resilience Inventory (Vogt et al., 2013).

A noteworthy part of this manuscript was the used statistical method. The SEM allowed us to find the statistically best possible structure reflected through one single index by taking into account all possible links between the used items and questionnaires. In a first step, theoretical considerations based on the validated structure of each questionnaire served as a template to create latent variables. This allowed us to simplify and to bundle different items into higher-level variables and led to the GSI. In a second step, the relationships between items and variables on the same and on different levels were tested to improve

the model fit exploratory. Different variations had to be calculated to reach a fair fitting model, where the root-mean-square errors of approximation (RMSEA) was below 0.08.

3.1.3 Results

For the first step described in the methods section, it took six theoretical based approaches to come up with a satisfactory model. In the beginning, a model with all items only loading on the GSI was tested. Thereafter, the structure and therefore the model fit was improved step-by-step through implementing latent factors of higher order.

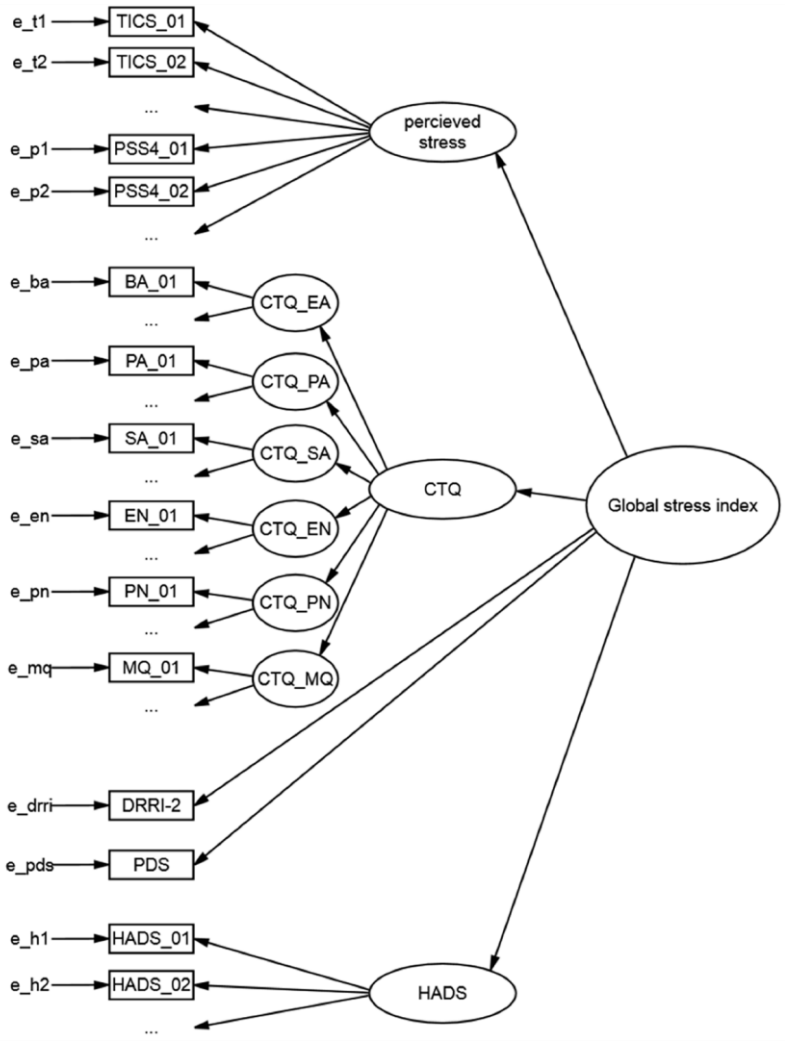

Figure 2. Multilevel structure of the Global Stress Index (GSI) model
Squares represent manifest variables; circles represent latent factors; e = error; PSS4 = Perceived Stress Scale; TICS = Trier Inventory for Chronic Stress; HADS = Hospital Anxiety and Depression Scale; PDS = Posttraumatic Stress Diagnostic Scale Checklist; DRRI-2 = Deployment Risk and Resilience Inventory; CTQ = Childhood Trauma Questionnaire; EA = Emotional Abuse; PA = Physical Abuse; SA = Sexual Abuse; EN = Emotional Neglect; PN = Physical Neglect; MQ = minimization questions. Source: (Maier et al., 2021, p.6); https://doi.org/10.3390/jcm10081799; CC-BY 4.0 - https://creativecommons.org/licenses/by/4.0/

The final structure of the GSI (figure 2 (Maier et al., 2021, p.6)) revealed that the latent factors HADS, CTQ and perceived stress as well as the manifest sum score variables PDS and DRRI-2 were directly loading on the GSI. Perceived stress consisted of all TICS and PSS4 items, which is in line with the content-related preliminary considerations. The HADS items directly loaded on the latent factor HADS whereas the factor CTQ consisted of the six CTQ subscales which then led to the manifest item level. In a further step, modifications were realized to reach a fair fitting model. Through excluding a critical CTQ-item with an extremely low standard deviation, implementing intercepts to the GSI factor, CTQ_MQ, PSS4_02, PSS4_03 and by allowing links between various items and factors, the calculations finally led to a satisfactory fair fitting model (χ^2 (5331) = 11.758; $p < 0.001$) that represented the underlying data well. This final model was superior to the previous ones ($\chi^2(1) = 158.47$, $p < 0.001$) with an RMSEA of 0.079.

Generating the GSI was an approach to combine different stress-based questionnaires in soldiers for easy application that may be used to complement classical cardiovascular risk scores. Social stress and anxiety are well-known to be associated with higher CAD risk and mortality (Ghadri et al., 2018; Stalnikowicz & Tsafrir, 2002; Vogelzangs et al., 2010), mostly explained through an increase in inflammatory mediators, reduced HRV and an increase in oxidative stress (Olafiranye et al., 2011). These findings underline the importance to include stress into CAD risk calculation. So far social stress was mostly determined by using one single questionnaire or a few questions (Arnold et al., 2012; Rosengren et al., 1991) which do not properly reflect the complex construct. Few approaches combined questionnaires through regression models and dichotomously measured constructs (Gallo et al., 2014; Yusuf et al., 2004), however, valid calculations considering various questionnaires on an item level which allowed inter-item and inter-factor relationships were lacking.

Limitations for the here presented study were the non-multivariate normal distribution, the missing data and a relatively small sample size. These problems were met with the interpretation of RMSEA instead of X^2-value and the FIML method for dealing with missing data. To be able to generalize these findings, validation on an independent sample is inevitable. For this reason, first efforts to collect data from soldiers in the military hospital in Ulm already started within the BEST-study. The GSI combines information about negative life events, anxiety and depressive symptoms as well as acute and chronic stress perception in soldiers. This potentially enables it to complement the classical CAD risk assessment for higher accuracy and precision in CAD prediction.

3.2 Mental but no bio-physiological long-term habituation to repeated social stress: a study on soldiers and the influence of mission abroad (Maier et al., 2022)

3.2.1 Introduction

While the manuscript above deals with the underlying structure of social stress, this paper focuses on the soldiers' reaction to repeated acute social stress (habituation). To maintain homeostasis, coping mechanisms are needed and the human body is equipped with distinct biological and physiological reactions to stress (Padgett & Glaser, 2003) as well as mental adaptation programs (Cohen, 2000). Among others, social stress initially activates the SAM-

and HPA-axis response within seconds and minutes to appropriately deal with the stressor (Cohen, 2000). Ongoing overstimulation of the stress response increases the release of glucocorticoid hormones and catecholamines which may lead to changes in gene expression and consequently a poorer health status (Padgett & Glaser, 2003). The repeated presentation of a stimulus which is experienced as stressful can be followed by habituation, which is a reduction of the intensity of the stress response to this specific stimulus (Thompson & Spencer, 1966). The response of the HPA-axis has been found to habituate to repeated social stress (Gerra et al., 2001) whereas the SAM-axis response only partially habituates (Schommer et al., 2003). Behavioral changes and the decrease of subjective stress perception towards the stimulus are characteristics of mental habituation to social stress (Frankenhaeuser et al., 1967). Biological and mental habituation can occur independently of each other (Ruys et al., 2004). As already mentioned above, especially deployed soldiers are facing challenging situations and intense job stress (Pflanz & Sonnek, 2002). The sole selection of soldiers as study subjects enabled the research of the effects of foreign deployment. Differences in the stress response between soldiers with and without a mission abroad were tested.

3.2.2 Material and Methods

As part of the BEST-study 91 soldiers (23% female) with complete data sets could be included in the calculations. All participants underwent the TSST-G protocol (von Dawans et al., 2011) three times. Forty-four participants had a mission abroad during study time, more precisely between t0 and t1. Descriptive data showed an average stay of 3 months for foreign deployment. Before, during and after social stress induction with the TSST-G various stress-sensitive biological, physiological and psychological data were collected (figure 1 (Maier et al., 2022, p.4)). The following variables were used for the analyses in this manuscript: Salivary cortisol [nmol/l] concentrations and α-amylase (sAA) [U/ml] activity, heart rate, HRV, diastolic and systolic blood pressure [mm/Hg] as well as STAI-S- State-Trait-Anxiety Inventory – State (Laux et al., 1981) before and after mental stress, PASA-Primary Appraisal Secondary Appraisal (Gaab, 2009) and MDBF- Multidimensional Mood State (Steyer et al., 1994) questionnaires before and after mental stress with its subscales good/bad mood, calmness/restlessness and alertness/tiredness. To determine HRV, the standard deviation of all normal-to-normal inter-beat intervals (SDNN) and root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD) were calculated. Only men were taken into account into the group x time calculations because the percentage of women with a mission abroad was too small resulting in an unbalanced proportion. Hence, the control and experimental group comprises 37 men with a mission abroad and 33 men without.

ANOVAs were conducted to examine the stress reaction within one point of measurement as well as habituation effects over the three measurement time points (t0, t1, t2). To evaluate habituation the area under the curve (AUC) was used for bio-physiological variables. The AUC reflects the area under the concentration-time curve and can be generated over all measures within one TSST-G session. The AUC with respect to increase (AUCi) examines sensitivity, the AUC with respect to the ground (AUCg) examines overall intensity of the stress response (Pruessner et al., 2003). Mixed ANOVAs for interaction effects of repeated measures and group comparison were used to test the hypothesis of group x time differences in male soldiers.

3.2.3 Results

As expected, elevated stress reactions for the bio-physiological variables could be found within the TSST-Gs, time effects were mostly significant with $p < 0.001$. Habituation effects across the three TSST-Gs were tested on all soldiers (female and male). None of the bio-physiological variables showed any significant results, that means that no decrease or changes in the stress reaction occurred over time. However, the majority of psychological variables showed significant differences between the three time points. The STAI-S value for anxiousness significantly decreased after mental stress (F (2, 174) = 10.11, $p < 0.001$, partial $\eta^2 = 0.104$) between t0 and t1 (Bonferroni-adjusted post-hoc*:* $p = 0.03$, 2.10, 95%-CI [0.15, 4.06]) and between t0 and t2 ($p < 0.001$, 3.59, 95%-CI [1.58, 291 5.60]). Similarly, the PASA values also significantly decreased (F (1.77, 148.46) = 11.65, $p < 294$ 0.001, partial $\eta^2 = 0.12$) between t0 and t1 ($p < 0.01$, 0.40, 95%-CI [0.12, 0.67]) as well as between t0 and t2 ($p < 0.001$, 0.59, 95%-CI 296 [0.24, 0.95]). The good/bad mood values before mental stress significantly decreased over time (F (2, 178) = 3.41, $p = 0.04$, partial $\eta^2 = 0.04$) between t0 and t1 ($p = 0.02$, 0.69, 95%-CI [0.10, 1.28]). The good/bad mood values after mental stress significantly increased (F (2, 170) = 4.94, $p = 0.01$, partial $\eta^2 = 0.06$) between t0 and t1 ($p = 0.02$, -0.74, 95%-CI [-1.41, -0.08]) as well as between t0 and t2 ($p = 308$ 0.03, -0.79, 95%-CI [-1.52, -0.06]). Calmness/restless after mental stress also significantly increased (F (2, 172) =11.84, $p < 0.001$, partial $\eta^2 = 0.12$) between t0 and t1 ($p < 0.01$, -1.13, 95%-CI [-1.83, -0.43]) as well as between t0 and t2 ($p < 0.001$, -1.38, 95%-CI [-2.12, -0.63]).

Group effects for the variable with and without a mission abroad were tested to show psychobiological differences in the stress reaction in those two groups within and across the TSST-Gs. However, no significant group x time effects for mission abroad in male soldiers were detectable (figure 3 (Maier et al., 2022, p.6)) within or across the social stress paradigms, neither in the biological or physiological nor the psychological variables.

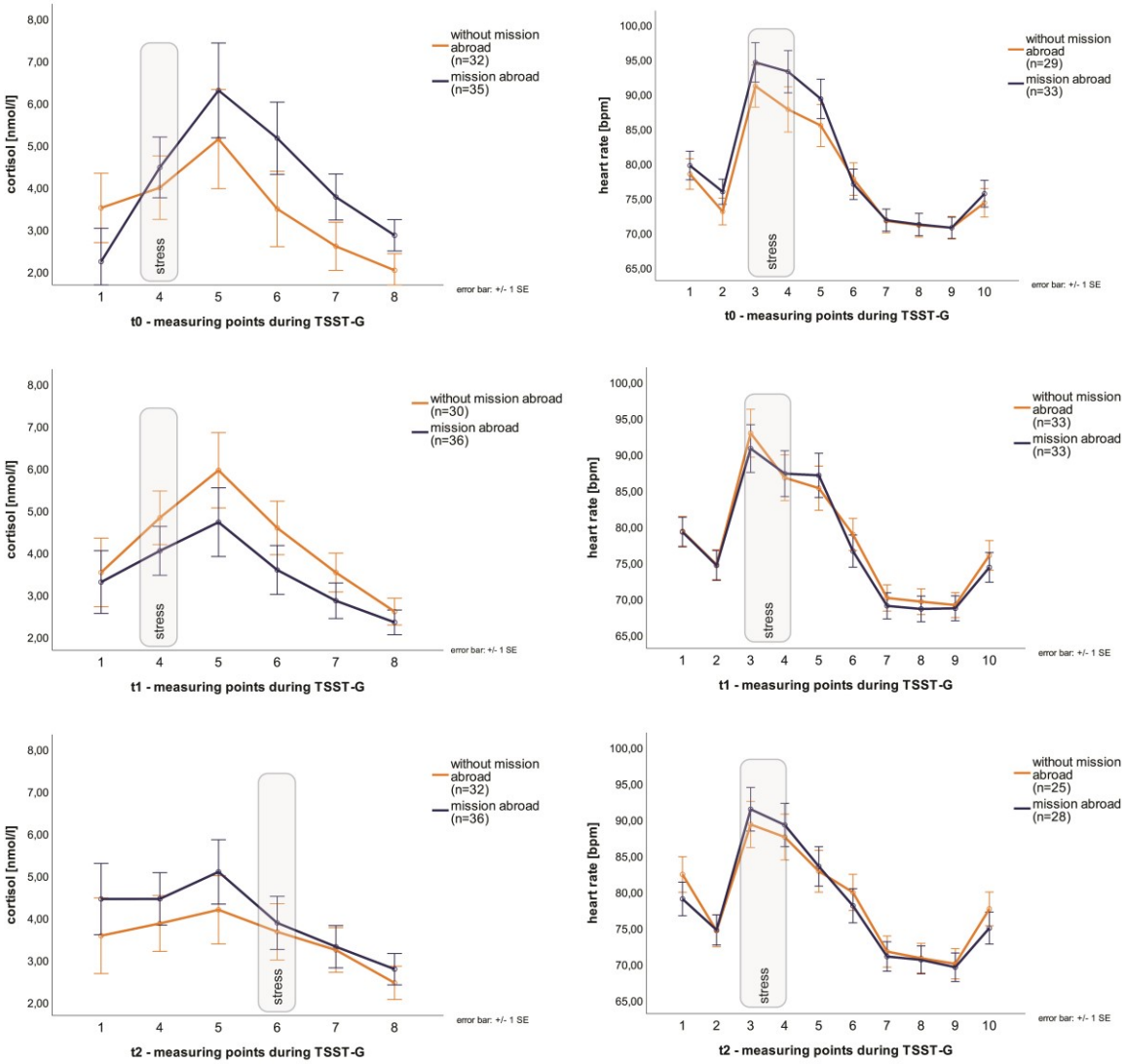

Figure 3. Bio-physiological stress response in male soldiers with and without a mission abroad
Stress induced with the Trier Social Stress Test for groups (TSST-G) for t0, t1, t2; width of stress bars reflects the number of measurement points within the variable. Y-axis values: absolute values for cortisol (nmol/l) and heart rate (bpm); values are means (+/- SE). Source: Adapted from (Maier et al., 2022, p.6); https://doi.org/10.3389/fpsyt.2022.1011181; CC-BY 4.0 - https://creativecommons.org/licenses/by/4.0/

Therefore, the evaluation of bio-physiological stress effects in soldiers showed a robust and unchanged stress response to repeated social stress whereas psychological parameters habituated over time. The soldiers mentally adapted to the procedure, however, the bio-physiological stress axes remained fully responsive to the social stress over a long period of time between the three stress tests. Furthermore, missions abroad did not significantly impact habituation in soldiers, both groups reacted in the same way to the stressor. This result could be an important finding to military research because it quantifies resilience in soldiers towards the mental stresses of foreign deployment.

3.3 Social Stress-Induced Oxidative DNA Damage Is Related to Prospective Cardiovascular Risk (Waller et al., 2020)

3.3.1 Introduction

In order to enhance the examinations on the biological reaction of a special application area this manuscript deals with the association of oxidative DNA damage and acute social stress. Oxidative DNA damage can be triggered by the natural cellular metabolism, the excess formation of reactive oxygen species as well as by oxidation of the nucleic acid bases and many more (Cooke et al., 2003). It is known that genomic instability, visible as DNA damage, is strongly associated with vascular aging and enhanced CVD risk (Bautista-Niño et al., 2016). Therefore, the link between prospective CV risk profile and DNA damage was tested in this study. The well-established relationship between cardiotoxic effects of catecholamines and increased superoxide radical formations (Neri et al., 2007; Rump & Klaus, 1994) led to the question if oxidative DNA damage triggered through social stress is also related to CAD. Higher perceived workload and stress are associated with oxidative DNA damage (Irie et al., 2001; Shimanoe et al., 2018). As already mentioned, soldiers are more likely to experience challenging workloads (Pflanz & Sonnek, 2002) than other professions. Most approaches used questionnaires in a retrospective manner for the evaluation of perceived social stress, which fails to identify direct changes in response to stress. In this study, social stress in soldiers was generated under experimental conditions. Therefore, oxidative DNA damage could be examined directly and related to the activity with the autonomic nervous system during the immediate response to stress (Shimanoe et al., 2018).

3.3.2 Material and Methods

As part of the BEST-study 95 soldiers (12 females) could be included in the calculations. The data from the first point of measurement (t0) was used. Blood samples for the oxidative DNA damage were collected right before the stress induction, as well as immediately and 60 min after stress and evaluated using single-cell gel electrophoresis. With this method the nucleic acid strands are separated by length which allows identification of single- and double DNA strand breaks as quantified by using the so-called "*tail moment*" (= DNA percentage tail length). Calculated tail moments below 0.1 were referred to undamaged (Witte, et al., 2014). To examine the 10-year risk of CAD the PROCAM-score (Assmann et al., 2002) was calculated with the risk factors age, smoking history, systolic blood pressure, family history of MI, diabetes mellitus, HDL cholesterol, LDL cholesterol, and triglyceride levels. Soldiers with an at least 1% prospective risk of MI were assigned to the "risk +" group, soldiers without this risk to the "risk - "group. SDNN and RMSSD were used to define parasympathetic and sympathetic stress reactivity. Furthermore, changes within the TSST-G were examined for sAA, systolic and diastolic blood pressure and heart rate using ANOVAs. Differences between the risk groups over time were calculated with mixed ANCOVAS. For the link between HRV measured with SDNN/RMSSD and DNA damage generalized linear modeling with repeated measures and partial correlations were used. The analyses were controlled for the variables age and gender.

3.3.3 Results

Prior to the TSST-G, soldiers in the risk + group showed significantly higher diastolic and systolic blood pressure ($p < 0.05$). Directly after stress exposure diastolic/systolic blood pressure, heart rate and sAA were significantly increased ($p < 0.05$) in comparison to the baseline measure. Blood pressure did not show intergroup differences anymore. After the 60 min resting period, all values returned to baseline levels and for systolic blood pressure only the group differences from the start could be shown again.

Social stress significantly increased tail moments and hence, oxidative DNA damage in the risk + group with F (1.85; 164.92) = 5.425, p = 0.006, whereas no significant association could be found in the risk - group. Inverse correlations could be found for the relation of tail moment and SDNN and RMSSD (r = -0.216, p = 0.046; r = -0.269, p = 0.012).

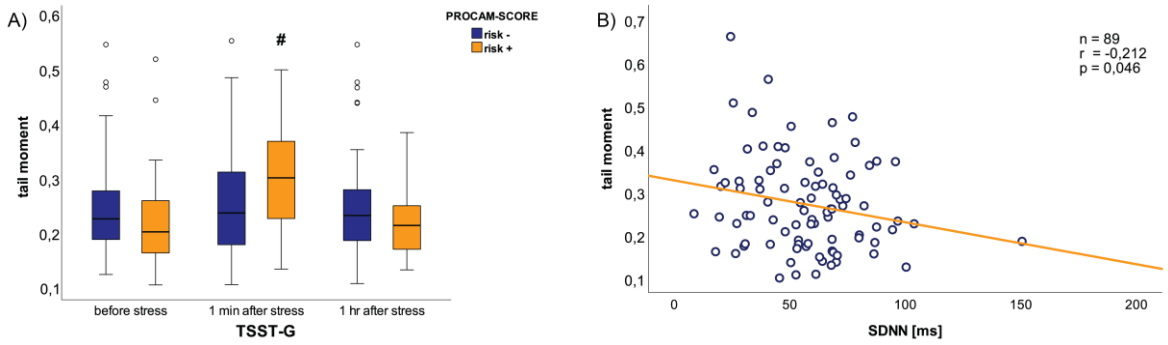

Figure 4. DNA-damage in soldiers during stress exposure
A) DNA-damage during stress induction was measured through tail moment in the comet assay of whole blood samples from 59 subjects without (risk-) and 33 with (risk+) 10-year cardiovascular risk according to PROCAM score. Data are presented as median, interquartile range, minimum, maximum; **#** represents significance ($p < 0.05$) in the group x stress ANCOVA. B) Tail moment 1 min after stress exposure (TSST-G) plotted as a function of the normal-to-normal inter-beat intervals (SDNN). Source: Adapted from (Waller et al., 2020, p.6); https://doi.org/10.3390/jcm9113783; CC-BY 4.0 - https://creativecommons.org/licenses/by/4.0/

As a limitation, it has to be mentioned that other explanations for the rise in DNA damage are possible, apart from the presumed link through superoxide radical formation in this study. SAA was used only as a marker for catecholamine concentrations, in a broader sense sympathetic tone, which is known to be directly related to superoxide radical formation. A direct measurement was not possible in the study design. The 1% threshold for 10-year cardiovascular risk was selected due to the relatively young and healthy participants in this study, whereas this threshold may not be suitable for other populations.

In conclusion, acute psychosocial stress did indeed cause DNA damage, especially in soldiers with enhanced cardiovascular risk, likely as a result of increased sympathetic tone.

4 General Discussion

4.1 Findings

The reported manuscripts aimed to promote the understanding of the impact of social stress in German soldiers. The bio-physiological and psychological stress responses and the development of a social stress index for cardiovascular risk assessment was the main focus of this synopsis. The research was performed on the data of the BEST-study and used the established TSST-G to experimentally induce social stress in a well-defined healthy population. The examinations showed that social stress influenced the soldiers' well-being and that bio-physiological and psychological reactions were neither linked nor showed similar adaptation patterns (Maier et al., 2022). Nevertheless, it is of high importance to emphasize the role of social stress in addition to biological factors regarding cardiovascular risk calculation. Therefore the development of the Global Stress Index underlines the importance and practicability as it is simply applicable and statistically well-founded (Maier et al., 2021).

In detail, the research question can be answered as follows: The GSI was implemented through SEM as a fair fitting model based on questionnaires about social stress. The final model consisted of the questionnaires CTQ, HADS, DRRI-2 and PDS and used the items of TICS and PSS4 combined to the "perceived stress" scale (Maier et al., 2021). The GSI offers the ability to enhance cardiovascular risk assessment. The evaluation of acute social stress showed a reliable bio-physiological stress response with significant time effects over the measurement points within the TSST-G. Significantly increased oxidative DNA damage in response to the mental stress task could be found only in soldiers with enhanced cardiovascular risk (Waller et al., 2020). The repeated presentation of social stress to the same cohort over a prolonged time period showed no bio-physiological habituation effects for HRV, cortisol, sAA or blood pressure, whereas the majority of psychological variables (PASA, MDBF, STAI-S) significantly habituated over time. Experiencing foreign deployment did not influence the answer patterns to social stress at all, neither for the stress reaction within one TSST-G nor for habituation across the three points of measurement. No differences between soldiers with and without a mission abroad could be found (Maier et al., 2022).

4.2 The role of missions abroad and habituation patterns

In contrast to the initial expectation and hypothesis that a mission abroad influences the acute stress response and habituation patterns, no differences were found (Maier et al., 2022). This could be an indicator for resilience in the study cohort. Postdeployment social support and resilience (e.g., positive acceptance of change) have been shown to be inversely related to depression or PTSD (Pietrzak et al., 2009) and these factors could also influence the bio-physiological response to mental stress. The percentage of 6% depression and 1% of PTSD in the BEST-cohort during the study time supports the assumptions of healthy and resilient participants: the 12-month prevalence in the overall general population is 8% for depression and 2% for PTSD (Jacobi et al., 2016). It has also been shown that

positive leader behavior (e.g., supportive, provides clear structures) is directly buffering stress effects (e.g., lack of sleep, environmental conditions, high workload) in the military (Britt et al., 2004). Other possible compensatory mechanisms are specific personality traits like hardiness and group cohesion, in addition the “healthy soldier effect” implicates reduced mortality in soldiers due to regular physical exercise and medical care (Thomassen et al., 2015; M. Waller & McGuire, 2011). Overall, it becomes clear that adequate coping mechanisms before, during and after a mission abroad play an important role in long-term stress development and can influence stress-related outcomes.

The fact that only mental habituation and adaptation to the stress tasks of the TSST-G was found shows an absence of any connection between the bio-physiological and psychological reactions (Maier et al., 2022) in this case. The subjective perception of social stress differed from the initially triggered stress sensitive systems in the body. Soldiers knew what to expect for the second and third TSST-G procedure which was displayed in the habituation response measured with the psychological questionnaires. In contrast, the bio-physiological activation to the potential threat (stress stimulus) remained unchanged. This clearly unaltered response to a social stress stimulus supports the well-founded mind-body connection (Littrell, 2008) but also shows that the affective and biological response do not necessarily show the same reaction patterns. Our approach is one of few that actually examined habituation effects over a longer time period with months to a year in between the stress inductions. For shorter intervals like days or weeks the mind and body can remember the stress stimulus more easily but long-term effects are only detectable if the effort is made of testing participants with considerably more time in between. By these considerations it became clear that it is not possible to extrapolate perceived stress relief to bio-physiological adaptation. Measuring three times over a prolonged time period showed that experimental stress induction with the TSST-G is able to provoke a robust bio-physiological stress response without habituation. The soldier’s body showed repeated and consistent bio-physiological reactions to occurring stress, regardless of assessments of mind or intellect. This makes it possible that a person’s bio-physiological stress systems are activated even though the person is already mentally adapted to the stressor and decided to categorize it as not harmful. These bio-physiological reactions are essential for survival by being fully prepared to respond to potential danger immediately, also known as the fight or flight response (Fink, 2016). The flight or fight response enables to react quickly through an increase in energy metabolism and muscle strength which is particularly important for deployed soldiers and their ability to operate in the field (Clemente-Suarez et al., 2018). Cumulative mental stress and chronic activation of the fight and flight response can become harmful by leading to CVD (Romero & Butler, 2007). Therefore, it can be hypothesized that in the long term, this lack of biological adaptation could lead to a direct negative impact of these bio-physiological responses on the cardiovascular system. The ability to habituate and reduce stress activation to repeated social stress mentally as well as bio-physiologically should support mental and physical health as well as resilience (Bennett et al., 2018). The challenge is the balance between a robust response to acute stress and adaptation when stressors become chronic (Romero & Butler, 2007). However, this is not specific to soldiers but to anyone who is exposed to social stress in their job. Regarding the biological basis it could be shown that soldiers with enhanced cardiovascular risk had more DNA damage in response to the acute mental stress than non-risk soldiers (Waller et al., 2020). This is in

line with current literature that shows an at least 2-folded higher level of strand breaks in CVD patients (Møller et al., 2020).

4.3 Limitations

In the BEST-study the cardiovascular risk was calculated using the PROCAM-score (Assmann et al., 2002) which gives a good overview for the potential risk profile of soldiers. Moreover, the duration of the study was not long enough to make general statements about the risk development of a manifest CVD which requires a minimum of ten years (like classical risk factor scores). The time periods of the two measurement points after a mission abroad differed between participants due to the allocation all over Germany and the different time slots and length for the foreign deployments. Between a mission abroad and the follow-up TSST-G an average of *M* = 4,6; *SD* = 4.0 months elapsed, between t1 and t2 it was *M* = 14,55; *SD* = 5.07. Another potential shortcoming is the reduced sample size of soldiers who completed all three points of measurement (t0:192 – t2:91). The non-randomized control and experimental group (missions abroad were appointed by the German military) may have led to an unintended selection bias. Moreover, more male than female soldiers participated in the study which led to unbalanced gender distribution; however, this reflects the actual circumstances in the German military. Generalization of the results towards the whole population has to be made cautiously because of our specific study-group. Nevertheless, the findings could also be highly relevant outside the military context. German soldiers generally represent a healthy group of subjects with regular medical examinations above average which makes it easier to extrapolate the results and serve as a comparison group for other healthy cohorts.

To consolidate the presented research, a subsequent step should be to validate the GSI on an independent (non-military) sample (Maier et al., 2021) to maximize the usability of this index for cardiovascular risk assessment. Furthermore, according to other stress literature not all participants respond to the repeated TSST similarly; individual differences for high and non-responders exist (Wüst et al., 2005). This could also partially explain the findings in the second manuscript, where none of the biological variables habituated over time (Maier et al., 2022). Therefore, for further assessments it could be interesting to search for different response patterns between individuals and to figure out what characterizes each type.

4.4 Outlook: social stress and health implications

The mental and physical well-being of soldiers is of great importance to achieve good job performance regardless if they have a mission abroad or are currently working in domestic deployment. As already mentioned, social stress, especially when becoming chronic is known to negatively influence health with an increased risk of the development of mental illnesses (Siegrist, 2008), obesity (Scott et al., 2012), cardiovascular risk (Yusuf et al., 2004) and a lowered resistance against disease in general (Rahe et al., 1964). High demand and low control situations in jobs lead to a 1.8 elevated odds ratio of depression (Siegrist, 2008). Stress research including soldiers as a target group is mostly focused on the development of PTSD after a mission abroad or the role of social support after their return (Blais et al., 2021; Hines et al., 2014; Hoge et al., 2004; Wittchen et al., 2012). Approximately 1-13% of

soldiers, depending on their area of operation and work field, are facing the diagnosis of PTSD (Hines et al., 2014; Wittchen et al., 2012) which shows the importance of this phenomenon. However, a considerably high amount of soldiers are reporting (social) stress in their daily work-life (Hsieh & Tsai, 2019; Pflanz & Sonnek, 2002) and thus can potentially suffer from long-term negative outcomes. This aspect is underrepresented in the literature so far. Social stress can be a conglomerate of various causes, daily hassles as well as major live events and military-specific challenges (Adler et al., 2004). It gets clear that it is important to more frequently consider social stress as an important factor influencing health in the military. This can be achieved by complementing the classical risk factors for CAD or investigating the effects of social stress on a biopsychological level like in the presented manuscripts. To fully understand underlying mechanisms and the influence of other moderating variables, more research is essential in this field.

The findings in this study about social stress can support the physical and mental health of soldiers in the military in the following ways: it could have a positive impact to perform regular cardiovascular risk assessments including the psychosocial risk factors at an early stage, especially prior to foreign deployment. High-risk candidates could then be adequately supported beforehand, with health measures like stress reduction programs, physical exercise, nutrition counseling and with proper education on the relationship of stress and health. This could reduce the accumulating stress effects during missions abroad. Improving stress tolerance to develop resilient and hardy soldiers already is a key element in military pre-deployment training (Sharma & Sharma, 2012) and could be complemented by social stress reduction in general military training. In general, to raise more awareness in the military context about (social) stress in everyday life and the possible impacts could be an essential step to support and preserve soldiers' well-being.

5 Abstract

Soldiers face a work environment which can provide challenging tasks, especially during foreign deployment. Away from their known social network and life the challenges result in a higher risk for perceived social stress during their job performance but work-related stressors can also be found at domestic deployment like exhaustion due to workload or long hours. It has been found that acute and chronic psychosocial stress can adversely affect cardiovascular risk and is significantly related to myocardial infarction. Military personnel are facing high rates of coronary artery disease (CAD) and health-threatening behavior and soldiers with combat experiences are more likely to suffer from hypertension. Furthermore, CAD risk is directly linked to increased short-term DNA damage, mainly visible as single or double strand breaks. The adaptation to social stress over time also provides insights into functioning biopsychological coping mechanisms. Exposure to social stress can cause physiological changes like altered hormone rates or blood pressure and influence mental health including negative emotions like anxiety and tension.

This synopsis aimed to provide an overview about risk assessment and biopsychological effects of social stress in healthy German soldiers. Different questionnaires about social stress led to a stress index for cardiovascular risk investigation. Experimentally induced acute and repeated social stress enabled the measurement of physiological and psychological reactions and changes. Especially the comparisons to the baseline and between three points of measurement for soldiers with and without a mission abroad were of interest. Associations with cardiovascular risk were also assessed in this cohort. The investigations were conducted as part of the BEST (German Armed Forces Deployment and Stress)-study, which used the Trier Social Stress Test for groups (TSST-G) to experimentally induce stress response. Two hundred thirty-four soldiers were recruited from the German Armed Forces hospital in Ulm and from military barracks in Dornstadt, Laupheim and Ulm. Participants underwent the TSST-G three times, with data collected before (t0), 4-6 weeks after (t1), and one year after (t2) a mission abroad. Therefore, soldiers with (experimental group) and without (control group) a mission abroad during study time were compared and all participants underwent the same procedure. During the TSST-G, 3-6 participants simultaneously prepared and were interviewed for a mock job interview and a mental arithmetic task in front of study personnel in lab coats and in the presence of the other participants.

Data analyses led to a Global Stress Index as a fair fitting model which was generated with structural equation modeling using the questionnaires HADS, TICS, CTQ, PSS4, DRRI-2 (section prior stressors) and PDS for acute and chronic social stress. It could also be found that the psychological responses (MDBF, STAI-S, PASA) to social stress significantly habituated over the three points of measurement whereas the bio-physiological stress response (HRV, heart rate, blood pressure, sAA, cortisol) did not. No significant differences in soldier's habituation patterns between the groups with/without foreign deployment during study time could be found. Furthermore, results showed that induced social stress significantly increased oxidative DNA damage, especially for soldiers with a higher 10-year cardiovascular risk, with no effects for non-risk individuals. The degree of DNA damage was dependent on the increase in sympathetic tone. The examinations showed that bio-physiological and psychological reactions are not linked or show similar adaptation patterns

for repeated social stress. Nevertheless, emphasizing the role of social stress alongside biological factors in cardiovascular risk calculation is crucial, and the Global Stress Index highlights its importance and practicality as a simple and statistically well-founded tool. It becomes clear that it is important to frequently consider social stress as a key factor affecting mental and physical health in the military.

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7 Published manuscripts

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Declaration on own contributions:

- Psychobiological data collection
- Conduct of the TSST-G study protocol
- Data transmission, preparation and statistical analyses
- Literature research and conceptualization of the manuscript
- First draft and writing of the manuscript

Journal of
Clinical Medicine

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Structural Equation Modeling of a Global Stress Index in Healthy Soldiers

Tanja Maier [1,*], Melanie Kugelmann [1], Dae-Sup Rhee [1], Sebastian Brill [2], Harald Gündel [1], Benedikt Friemert [2], Horst-Peter Becker [3], Christiane Waller [1,4] and Manuela Rappel [1]

[1] Clinic for Psychosomatics and Psychotherapeutic Medicine, Ulm University Medical Center, 89081 Ulm, Germany; melanie.kugelmann@gmx.de (M.K.); dae-sup.rhee@uni-ulm.de (D.-S.R.); Harald.Guendel@uniklinik-ulm.de (H.G.); christiane.waller@klinikum-nuernberg.de (C.W.); manuela.rappel@uni-ulm.de (M.R.)
[2] Military Hospital, 89081 Ulm, Germany; sebastian.brill@web.de (S.B.); bendiktfriemert@bundeswehr.org (B.F.)
[3] Military Hospital, 10115 Berlin, Germany; HorstPeterBecker@bundeswehr.org
[4] Department of Psychosomatics and Psychotherapeutic Medicine, Paracelsus Medical University of Nuremberg, 90419 Nuremberg, Germany
* Correspondence: tanja.maier@uni-ulm.de

Abstract: Accumulation of stress is a prognostic trigger for cardiovascular disease. Classical scores for cardiovascular risk estimation typically do not consider psychosocial stress. The aim of this study was to develop a global stress index (GSI) from healthy participants by combining individual measures of acute and chronic stress from childhood to adult life. One-hundred and ninety-two female and male soldiers completed the Perceived Stress Scale (PSS4), Trier Inventory for Chronic Stress (TICS), Hospital Anxiety and Depression Scale (HADS), Childhood Trauma Questionnaire (CTQ), Posttraumatic Diagnostic Scale Checklist (PDS), and the Deployment Risk and Resilience Inventory (DRRI-2). The underlying structure for the GSI was examined through structural equation modeling. The final hierarchical multilevel model revealed fair fit by taking modification indices into account. The highest order had a g-factor called the GSI. On a second level the latent variables stress, HADS and CTQ were directly loading on the GSI. A third level with the six CTQ subscales was implemented. On the lowest hierarchical level all manifest variables and the DRRI-2/PDS sum scores were located. The presented GSI serves as a valuable and individual stress profile for soldiers and could potentially complement classical cardiovascular risk factors.

Keywords: psychosocial stress; structural equation model; cardiovascular disease; risk factors; global stress index

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Citation: Maier, T.; Kugelmann, M.; Rhee, D.-S.; Brill, S.; Gündel, H.; Friemert, B.; Becker, H.-P.; Waller, C.; Rappel, M. Structural Equation Modeling of a Global Stress Index in Healthy Soldiers. *J. Clin. Med.* **2021**, *10*, 1799. https://doi.org/10.3390/jcm10081799

Academic Editor: Giuseppe Santarpino

Received: 4 February 2021
Accepted: 14 April 2021
Published: 20 April 2021

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1. Introduction

Psychosocial stress is a common term in everyday life and was originally defined as a nonspecific reaction of the body to a noxious environmental stimulus [1]. Lazarus described a more psychological approach by analysing appraisal and coping mechanisms [2]. Over the years, the importance of stress for psychological and physical health and well-being has been widely accepted and stress-related research has increased considerably [3,4]. Psychosocial stress has been found to be a cardiovascular (CV) risk factor [5] for physically healthy individuals and patients with manifest diseases [6,7] and has been shown to be directly linked to the incidence of myocardial infarction (MI) [8]. Additionally, psychosocial stress is closely linked to depression and traumatic experiences, as well as specific life events and private/occupational burden [9–11].

Depressive episodes have been explained as a maladaptive phenomenon due to chronic stress or environmental changes [12]. Depressive and anxiety disorders were significantly associated with increased CV incidents in a 6-year follow up [13]. Higher odds ratios were also shown for depressive symptoms measured with the Hospital Anxiety

and Depression Scale (HADS) in predicting incident cardiovascular disease (CVD) [14]. Stress and stressful live events showed robust causal associations to depression [10] and anxiety [15]. Kendler and Gardner [16] combined recent stressful live events within the last month like job loss, marital problems, or death and found associations to depressive vulnerability and depression. Work stress acts as an independent risk factor for depression and anxiety, especially high job demands like time pressure [17,18] and is associated with CVD risk factors like triglycerides, cholesterol, smoking, and systolic blood pressure [19]. Furthermore, critical events over the lifespan have an impact on experienced psychosocial stress levels: major negative life events in adulthood like divorce or death of a loved one led to a significant increase in daily life stress [20] and the accumulation of major life events showed a dose-response relationship with the risk of stroke [21]. Among depression and negative life events, psychosocial stress also implies experiences of acute and chronic stress: daily hassles like work interruptions which causes an increase in experienced stress had a mediating impact on major life events and psychological symptomatology [22]. Sources of self-reported chronic stress can be interpersonal difficulties, (work) overload or goal-striving stress [23], and in a 21-year follow-up it was found that it significantly increases the risk of stroke, coronary heart disease (CHD), or CV events [24]. However, not only events during adulthood showed associations, in individuals with experiences of child maltreatment (CM) manifestations in the biological stress response was found—e.g., higher and prolonged levels of cortisol after social stress induced by the Trier Social Stress Test compared to individuals without CM-experiences [25]. Neuroendocrine changes in the stress response were linked to CM and current stress was a mediator of CM and depression [26]. Additionally, CM served as a predictor of increased psychological stress levels in adulthood [27] and showed a strong graded relationship with leading causes of death like smoking, obesity, depression, or CHD in adults [28] with a marked dose–response impact on CVD [29]. Especially military personnel have a higher risk for perceived job-stress than civilians [30]. They face high rates of cardiovascular disease and deployed soldiers are more likely to suffer from hypertension due to increased mental stress than non-deployed soldiers [31,32].

Classical risk factor scores consisting of gender, age, smoking, blood pressure, family history of MI, lipoproteins, and diabetes mellitus have been widely used for the calculation of the 10-year risk of a fatal CV disease event [33–35]. It is considered as a simple and accurate way for predicting MI in clinical practice [36]. New factors have emerged in recent years that can also trigger MI or predict CV risk precisely. Yusuf et al. [8] showed that regular consumption of fruits/vegetables, moderate alcohol consumption and moderate physical exercise are protective and associated with MI risk reduction, whereas psychosocial factors have an additional influence on the risk of MI in both sexes and at all ages. Multivariate analyses revealed that smoking and raised ApoB/ApoA1 lipid ratio—followed by history of diabetes, hypertension, and psychosocial stress—measured via positive exposure to depression, perceived stress at home or work, low locus of control and major life events were the strongest risk factors. High perceived mental stress was linked to a 1.5-fold increased CHD risk compared to low stress levels [37,38]. For instance, patients with an acute MI and high acute stress (PSS4), showed increased 2-year mortality [39].

The approach to define one global stress index for soldiers needs to consider a multidimensional conceptualization of stress. To potentially use this index in further research as a cumulated stress trigger for CVD events, the structure was developed following Yusuf et al. [8]. However, research that analyses the underlying structure and not only the construct level of psychosocial stress is lacking. It is not fully understood how the items between different stress-based questionnaires are linked to each other.

Therefore, the aim of the study was to develop a latent 'global stress index' (GSI) based on the aspects of acute and chronic perceived stress, depressive, and anxiety symptoms, stressful life events and CM as part of the BEST study (German armed forces deployment and stress) on healthy male and female German soldiers.

2. Materials and Methods

2.1. Study Design and Recruitment

The analysis was performed on data from the BEST study. The prospective design aimed to investigate bio-psycho-social stress effects of foreign deployment on CV health in German Armed Forces. Therefore, soldiers were asked to participate at three different time points prior to (t0), 4–6 weeks after (t1) and one year after (t2) mission abroad. The control group with similar age and gender distribution participated at the given time points without mission abroad. Both groups performed consecutive social stress tests using the Trier Social Stress Test for Groups (TSST-G [40]) and psychological as well as biological measures at baseline and during the stress task. This paper is based on the psychological baseline data prior to foreign deployment for both groups. Soldiers were recruited from German military barracks in Dornstadt, Laupheim, and Ulm as well as in the German Armed Forces hospital in Ulm, Germany.

2.2. Participants and Missing Values

In the study t0 was completed by 234 participating soldiers. Due to missing questionnaires 42 soldiers were excluded as a result of a study recess. In the SEM analysis missing data were estimated using the full-information maximum likelihood (FIML) method (Enders & Bandalos, 2001) considering all information of the observed data like mean and variance. In total, data of 192 male and female soldiers were used for further calculations. The mean age of the soldiers was M = 30.07 SD = 6.88 (range 19–56) years. 27.1% of the achieved participants were females.

2.3. Measures

To measure different aspects of psychosocial stress the following questionnaires were used. Chronic perception of stress in the last three months was covered by the Trier Inventory for Chronic Stress (TICS [41]) and acute stress in the last month by the Perceived Stress Scale (PSS4 [42]). The scales included questions to work-related stress, social stressors and daily hassles (e.g., high expectations, disputes and tension, lack of respect). In the PSS4 item 1 and 4 are phrased as negative questions. The Posttraumatic Stress Diagnostic Scale Checklist (PDS [43]) quantified potential traumatizing events (e.g., violence, sexual abuse, natural disasters) over lifespan. The Childhood Trauma Questionnaire (CTQ [44]) quantified childhood maltreatment and consisted of six subscales which were emotional abuse (EA), physical abuse (PA), sexual abuse (SA), emotional neglect (EN), physical neglect (PN), and a scale with minimization questions (MQ). The section "prior stressors" from the Deployment Risk and Resilience Inventory (DRRI-2 [45]) asked for highly stressful live events (e.g., divorce, financial problems, death of related persons). The two subscales of the Hospital Anxiety and Depression Scale (HADS [46]) measured symptoms of anxiety and depression (e.g., worrying about the future, loss of joy, feeling anxious). All instruments were validated. Due to the binary item structure (1 = occurrence vs. 0 = non-occurrence) a total sum score for all items of the PDS and DRRI-2 was used. For PSS4, TICS, HADS, and CTQ all single items were considered in the SEM. Reverse coded items were inverted prior to the analysis with higher values indicating more negative outcomes. All item statistics are described below (Table A1).

2.4. Data Analysis

Data were edited using open-source software R [47]. The software packages foreign [48], psych [49], lavaan [50], and nvnormtest [51] provided additional functionality. Graphics were generated with AMOS [52]. In advance, calculations of means, standard deviations and Shapiro–Wilk tests for item distribution were performed. The Pearson correlation matrix was used to show variances between items. To examine multivariate normality in the whole item set the Mardia and Henze-Zirkler test for multivariate normality were conducted. The structural equation model was used to analyze the relationships between all stress-related items. Within R all intercepts in the SEM were set to zero by

default. A two-step modeling approach was adopted. First, the structure of all variables was modified through creating latent variables based on the measured variables. The validated structure of questionnaires served as a basis for initial iterations of calculating the GSI. Comparing the fit of unnested models in SEM the Akaikes and Bayesian Information Criteria (AIC/BIC [53,54]) were taken into account. For this comparison lower values indicated better model fit. Calculating X^2 tests allowed verifying the significance of improvement within nested models. The root-mean-square errors of approximation (RMSEA) provided additional information about the fit within all nested models. Referring to several authors [55,56] values above 0.10 show poor model fit, values below 0.10 suggest mediocre fit, values below 0.08 indicate fair fit and values below 0.05 are a sign for close fit. The smaller the value the better the fit. We aimed to reach at least a fair fitting model. Second, relationships among the variables on different levels were tested exploratory to get an adequate fit. Modification indices were calculated. They indicate which links between variable can improve the model fit. Adequate RMSEA fit was reached by iteratively observing increased modification indices after every change. Step by step the highest values that could be theoretically justified were implemented in the model until adequate RMSEA, fair fit, was reached.

3. Results

3.1. Descriptive and Initial Data Analyses

Mean, minimum, maximum and standard deviation of all items are presented in Table A1. A very low standard deviation was found for item CTQ_21: "Someone threatened to hurt me or tell lies about me unless I did something sexual with them" (SD = 0.39), accompanied by a relatively low mean (M = 1.05). None of the items achieved normal distribution, the Shapiro–Wilk test was significant ($p < 0.001$) over all items (Table A1). Similarly, the multivariate normal distribution was not fully given for the whole item set, except for the skewness in Mardia test (HZ = 464, $p < 0.001$; skewness: $X^2 = 185785$, $p = 1.00$; kurtosis: z = −17.84, $p < 0.001$). The Pearson correlation coefficients measured for all items ranged between approximately zero (e.g., r = 0.006 PDS and tics18) and r = 0.75 (ctq28 and ctq19). There were also some highly negative correlations as r = −0.79 (ctq28 and ctq22). Within the items of the same questionnaire the correlations were expectedly higher than between items of different questionnaires (correlation tables are available on request from the corresponding author).

3.2. Structural Equation Model (SEM)

The next step in the calibration of a GSI model was to perform SEMs. The presumed model structure for the SEM was influenced by the given scales and subscales of the questionnaires that had already been validated. An overview of the statistics and fit values of all models is given in Table 1 At first a g-factor model was established with all items loading on the GSI factor. As it is shown in Table 1 the RMSEA of 0.108 indicated poor model fit. All items showed significant loadings on the general factor. It was consistent throughout all models 1–17 that all variable loadings were highly significant ($p < 0.001$). As a next step (model 2) all items belonging to one questionnaire were summarized to one latent factor. For example, all 12 HADS items were loading on one latent HADS factor. Furthermore, all latent questionnaire factors and the DRRI-2/PDS sum scores were directly loading on the GSI factor. For model 2, no valid results could be calculated, some estimated variances were negative. In model 2a, the latent variables TICS and PSS4 were loading on a stress factor of higher order but this approach was not identified. As this also did not lead to a proper solution model 3 was proposed similar to model 2 with the difference that all TICS and PSS4 items were directly combined to one factor labeled perceived stress. AIC and BIC proved model 3 to be superior to model 1. RMSEA was improved to 0.100. Consequently, the higher order factor perceived stress was retained in the following models. Differentiating HADS into its subscales deteriorated the AIC/BIC (model 4, Table 1). Combining the two HADS subscales to a higher order factor in model 4a led to

an unidentified model. Based on model 3 differentiating CTQ into its subscales improved model fit (AIC/BIC). RMSEA in model 5 indicated mediocre fit of 0.091. Therefore, the subdivision was also part of subsequent models. The last model based on theoretical considerations was model 6, all CTQ subscales were combined to one CTQ higher order factor (Figure 1). Model 6 was superior to model 5 comparing the AICs and BICs. Again, a mediocre fit was achieved (RMSEA = 0.091).

Table 1. Fit indices for all models

Model	Specification	*df*	X^2	RMSEA	AIC	BIC	X^2 Test *
Model 1	G-factor	5460	17,615.05 ***	0.108 *** (0.106; 0.109)	51,043.96	51,728.03	
Model 2	One factor for each questionnaire			No result			
Model2a	TICS and PSS4 factor with a factor of higher order			Model not identified			
Model 3	TICS and PSS4 items with perceived stress factor	5457	16,008.86 ***	0.100 *** (0.099; 0.102)	49,443.77	50,137.61	
Model 4	HADS divided in its subscales	5456	16,012.89 ***	0.100 *** (0.099; 0.102)	49,449.80	50,146.90	
Model 4a	HADSA and D with a factor of higher order			Model not identified			
Model 5	Model 3 and CTQ subscales	5452	14,203.58 ***	0.091 *** (0.090; 0.093)	47,648.49	48,358.63	
Model 6	Model 5 and CTQ factor	5451	14,164.89 ***	0.091 *** (0.089; 0.093)	47,611.80	48,325.19	
Model 7	Exclude CTQ_21	5347	13,776.91 ***	0.091 *** (0.089; 0.092)	47,459.57	48,166.44	
Model 8	Intercept: GSI	5346	13,294.53 ***	0.088 *** (0.086; 0.090)	46,979.18	47,689.31	$X^2(1)$ = 482.39 ***
Model 9	Intercept: CTQ_MQ, PSS4_02, PSS4_03	5343	12,560.59 ***	0.084 *** (0.082; 0.086)	46,251.24	46,971.15	$X^2(3)$ = 733.94 ***
Model 10	CTQ_EN~~CTQ_MQ	5342	12,460.37 ***	0.083 *** (0.081; 0.085)	46,153.02	46,876.18	$X^2(1)$ = 100.22 ***
Model 11	TICS_10 ~~ TICS_21 TICS_10 ~~ TICS_41 TICS_10 ~~ TICS_53	5339	12,311.28 ***	0.082 *** (0.081; 0.084)	46,009.93	46,742.87	$X^2(3)$ = 149.09 ***
Model 12	TICS_07 ~~ TICS_22 TICS_07 ~~ TICS_43 TICS_07 ~~ TICS_49	5336	12,182.09 ***	0.082 *** (0.080; 0.084)	45,886.74	46,629.45	$X^2(3)$ = 129.19 ***
Model 13	CTQ_23 ~~ CTQ_24	5335	12,107.42 ***	0.081 *** (0.079; 0.083)	45,814.08	46,560.04	$X^2(1)$ = 74.67 ***
Model 14	TICS_42 ~~ TICS_51	5334	12,003.64 ***	0.081 *** (0.079; 0.083)	45,712.29	46,461.52	$X^2(1)$ = 103.78 ***
Model 15	TICS_22 ~~ TICS_43	5333	11,916.08 ***	0.080 *** (0.078; 0.082)	45,626.73	46,379.21	$X^2(1)$ = 87.56 ***
Model 16	TICS_25 ~~ TICS_36	5332	11,829.89 ***	0.080 *** (0.078; 0.082)	45,542.55	46,298.29	$X^2(1)$ = 86.18 ***
Model 17	CTQ_SA =~ CTQ_25	5331	11,757.61 ***	0.079 *** (0.077; 0.081)	45,472.26	46,231.26	$X^2(1)$ = 158.47 ***

Note. *df* = degrees of freedom; RMSEA = Root Mean Square Error of Approximation; AIC = Akaike's Information Criteria; BIC = Bayesian Information Criteria; AIC and BIC should only be compared for same item set; ( ) = 90% Confidence Interval; *** = $p < 0.001$; Specification = modifications to the previous model; ~ = loading; ~~ = covariance. PSS4 = Perceived Stress Scale; TICS = Trier Inventory for Chronic Stress; HADS = Hospital Anxiety and Depression Scale; HADSA = HADS Anxiety; HADSD = HADS Depression; CTQ = Childhood Trauma Questionnaire; SA = Sexual Abuse; EN = Emotional Neglect; MQ = minimization questions. Until model 6 theoretical derivations were made. From model 6 onwards, modification indices were taken into account until adequate fit was reached. * X^2 test allows model comparisons to the previous model and shows the significance of changes between nested models.

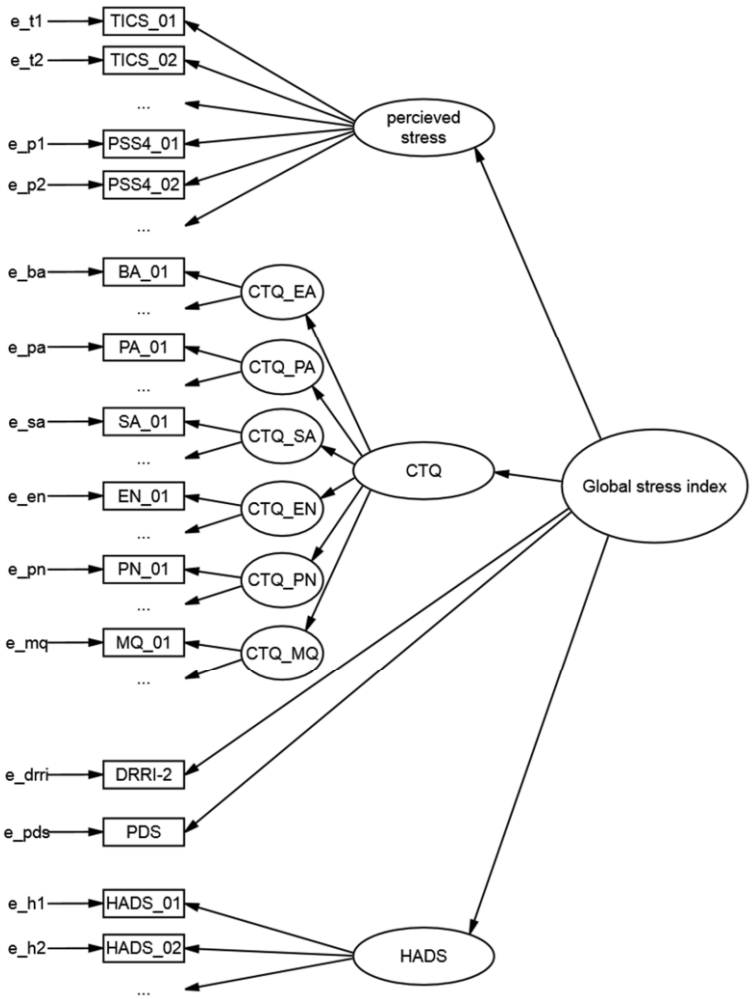

Figure 1. Illustration of the multilevel model results from structural equation modeling. All manifest variables are presented in squares. All latent factors are presented in circles. e = error; PSS4 = Perceived Stress Scale; TICS = Trier Inventory for Chronic Stress; HADS = Hospital Anxiety and Depression Scale; PDS = Posttraumatic Stress Diagnostic Scale Checklist; DRRI_2 = Deployment Risk and Resilience Inventory; CTQ = Childhood Trauma Questionnaire; EA = Emotional Abuse; PA = Physical Abuse; SA = Sexual Abuse; EN = Emotional Neglect; PN = Physical Neglect; MQ = minimization questions.

As the theoretically based, deductive method did not lead to a satisfactory model fit, the model was further improved by taking modification indices into account until a fair fit was reached (see Table 1). First, a critical item (CTQ_21) was identified and removed from the dataset. Modification indices suggested that the item was not represented adequately by the assumed factor. Many cross loadings would be required indicating that the item was not measuring the proposed construct. Prior analyses already revealed that item CTQ_21 had the lowest standard deviation. Considering all evidence, the item did not provide enough information and was therefore excluded from model 7. The following models were all nested. X^2 tests revealed that all improvements were significant (Table 1).

Modification indices suggested improving the fit through implementing an intercept to the GSI factor (model 8) followed by adding it to CTQ_MQ, PSS4_02 and PSS4_03 (model 9). Adding the link between two scales of higher order (CTQ_EN and CTQ_MQ) to model 10 also improved the RMSEA significantly. The expectable negative correlation between emotional neglect "I didn't feel loved" and minimization questions like "There was nothing I wanted to change about my family" highlighted the content related relation of the family background. Including correlations of mainly TICS items led to better fit in the following models. The highest values of modification indices suggested cross loadings of TICS_10 ("I'm missing interesting tasks which fill the day") with semantically closely linked items like "There are times where I don't do anything meaningful" (models 11,12). In model 13–16 other item correlations were added to the SEM on condition that high modification indices were detected and the combinations were reasonable in the way of a content-related fit (Table 1). In a last step the high effect between CTQ_SA and item CTQ_25 was considered as suggested in the statistic of modification indices. This led to the final model (model 17) with a fair fit of 0.079, so there was no need for further modifications. All parameter statistics and a figure of the overall model are shown in Table A2 and Figure 1. This version of the structural equation modeling analysis indicated that the final model 17 fit the underlying data well.

4. Discussion

The aim of the study was to develop the GSI for soldiers based on different stress-related questionnaires and to reveal the underlying item structure. This was reached by successfully establishing a structural equation model measuring the GSI. The final hierarchical multilevel model revealed fair fit. On the highest order was a g-factor called the "GSI", the newly developed global stress index. On a second level the following latent variables were directly loading on the GSI: perceived stress, HADS [46] and CTQ [44]. The variable perceived stress was directly measured by the combination of all TICS [41] and PSS4 [42] items, which means that the items of these questionnaires are closer linked than with the remaining questionnaires and consequently covered by a higher-order factor. Compared to that, the CTQ was divided into its six subscales. Therefore, a third level was implemented containing the underlying constructs EA, PA, SA, EN, PN, and MQ which seemed to have an additional informative influence on the GSI. All manifest variables were located on the lowest hierarchical level. This level also encompassed the DRRI-2 [45] and PDS [43] sum scores which were also directly loading on the GSI. Even though they both asked for major live events, the analysis showed that they independently loading on the GSI and both had an additional influence on the index. In a next step the described structure of the model was improved. By allowing relationships between all latent and manifest variables based on modification indices the statistical adjustments finally led to a fair fitting model. This step showed the importance to consider possible links between all items, factors, and questionnaires [57].

The sample in this study included healthy, middle-aged German soldiers that are regularly examined. This positively affected the extrapolation of the GSI to general population. Regarding to the stress-related questionnaires soldiers showed average values in mean and standard deviation comparable to normative samples [41,46]. Referring to recent findings the PSS4 in our sample was even lower than in the normative sample [58] and the mean of prior stressors was also lower than in a sample of veterans [45]. The similar answer patterns in the questionnaires compared to general population enables the SEM analysis to be extrapolated to findings in the overall population.

4.1. Structural Equation Modeling

A beneficial feature of the chosen procedure was the deductive implementation of theory-based questionnaires in the model leading to an inductive examination of the empirical underlying data structure. It is noteworthy that the study was based on a complex statistical method, the SEM analysis. Using SEM, we enabled to examine the links between

psychosocial stressors on an item basis by taking the underlying structure of different questionnaires into account. Gallo et al. [59] compiled a variety of separate questionnaires that showed associations between chronic stress burden and prevalence of CHD and stroke. They measured acute stress with the PSS10 and the total number of past and present traumatic events such as natural disaster, combat exposure, and sexual assault; as well as ongoing problems like financial burden, work, or relationship problems. The psychosocial index from Yusuf et al. [8] was developed through a multivariate regression model where all constructs were measured dichotomously. A combination of the psychosocial risk factors with the structural equation modeling method enables the compilation of an individual multidimensional value for stress. In the field of psychosocial stress various studies showed the applicability of using the SEM method for their research (e.g., [60,61]). However, the used content highly depends on the study-context: Ostovar et al. [62] combined stress, anxiety, depression and loneliness to verify the links to internet addiction; Woods-Giscombé and Lobel [63] combined generic stress, race-related stress and gender-related stress in an SEM and developed a multidimensional stress factor. Focussing on CV risk, the combination of specific stress factors is needed as mentioned above [6,64]. The SEM approach has been rarely used to create stress-related indexes in soldiers or in the context of CVD. Another common statistical approach to combine questionnaires is the use of multivariate regression models [8,65]. In comparison to that method, SEM analysis has to take potential causal links or effect chains between variables into consideration which positively affects the precision of a model [66]. Further advantages using the SEM are the following: it takes measurement errors in the observed variables into account, is able to show both direct and indirect effects, enables to develop, estimate, and test complex multivariable models, has a clear conceptualizing of the underlying theory, confirmatory approach and hypothesis testing and is so far the best method for modeling multivariate relations [57,66–68]. To our knowledge, the present study is the first attempt to quantify psychosocial risk factors for soldiers as a GSI through SEM.

4.2. GSI and Cardiovascular Risk

Apart from the sample and the used statistical method, the range of psychosocial factors and the questionnaire survey method has an important influence on the quality of a stress index which could potentially be used as a MI trigger marker in further research. Many approaches already dealt with the estimation of 10-year CV risk [34,35] as a basis for interventions and risk mitigation. A classical measuring tool for CV-risk is the PROCAM (prospective cardiovascular Münster)-score including already well-established risk factors like gender, age, smoking, systolic blood pressure, and diabetes mellitus [33]. It has recently been shown that also psychosocial stress can trigger MI or cardiac death [69]. It is an independent risk factor for CVD [8] and can increase the risk through moderating biological changes [70]. Consequently, subjective stress measurements should be considered for CV risk and supplementary complement the PROCAM-score as well as other classical risk scores like Framingham [34] or ASCVD [35]. In this context, the standard procedure is that most of the publications focussed on one specific stressor—i.e., earthquake or job stress [71]. However, psychosocial stress is a concept combining different kinds of stress. In the context of CHD specific psychosocial stress factors are closely linked: depressive and anxiety symptoms, negative life events/CM and acute and chronic perceived stress [6,64]. Especially anxiety disorders were found to increase the prevalence of CVD about three-fold [72] and high anxiety levels were associated with the risk of stroke, ventricular arrhythmias, cardiac death, and CVD [73]. It was shown that state-anxiety is associated with higher respiratory sinus arrhythmia magnitude [74]. The same pattern occurred in patients with Generalized Anxiety Disorders linked to lower cardiac vagal control [75] and furthermore higher phobic anxiety was significantly related to ventricular arrhythmias [76]. As underlying mechanism e.g., sympathetic activation, oxidative stress, increased inflammatory mediators and reduced heart rate variability are discussed [73]. Depression or depressive symptoms occur regularly after CV events and are associated with higher mortality rates and increased fol-

lowing CV events [77,78]. In addition, the Takotsubo syndrome should be mentioned here as a specific case of mental stress [79]. It appears from current events like sudden emotional stressors and is known to result in acute heart failure or MI [80]. Anxiety disorders were also found to be significantly more frequent in Takotsubo patients than in patients with an acute coronary syndrome, this association is linked through inflammation leading to sympathetic activity [81]. The long-term mortality consequences are comparable to patients with acute coronary syndrome [82] which underlines the importance to consider mental stress and anxiety in CVD. Some of the physiological and psychological mechanisms of anxiety disorders and the Takotsubo syndrome seem to be comparable therefore both can lead to cardiac arrhythmias or CV events. The choice of using stressful live events as well as the HADS combining anxiety and depressive symptoms among other questionnaires could possibly make the GSI applicable as a screening tool in a wider sense regarding arrhythmia, anxiety, and Takotsubo cardiomyopathy.

A wider range of stressors which then provided more information about the psychosocial aspect as a risk factor was, e.g., collected by Gallo et al. [59]. Amongst PSS10 [42] and the Traumatic Stress Screener [83], the "chronic stress burden" was used, a combination of eight items assessing the number of current ongoing problems. Yusuf et al. [8] and Rosengren et al. [84] expanded the range of aspects in the INTERHEART study and developed a psychosocial index using a multivariate regression model. They created a "general stress scale" that combined the answers for two single questions for stress at home or work and found associations to MI along with depression, locus of control, financial stress, and life events [84].

Following Rosengren et al. [84] and Yusuf et al. [8], our GSI analysis used a similar pattern: perceived acute and chronic stress at work or home was combined to an overall factor while depression, major live events and CM were loading independently on the GSI. In comparison to previous research, the SEM analysis for developing the GSI seems promising to be a future approach in the context of CHD and serves to combine stress questionnaires on an item basis. In our study, only well-established and validated questionnaires were used. To keep the GSI practicable, all single components of work and private stress were measured as a whole by using the questionnaires TICS and PSS4 [41,42] for the assessment of perceived acute and chronic stress.

4.3. Limitations of the Study

The exploratory concept of this study was indispensable but further investigations on a different dataset are needed to confirm the structure of the GSI and to increase the extern validity. The sample size was limited and consisted of considerably more male than female soldiers. This distribution reflected the unequal gender distribution in military context, even if 27.1% women in our sample were higher compared to the female proportion in the German army, which was 12.3% in 2019 [85]. These circumstances have to be taken into account when applying our findings to an evenly distributed population. Smaller sample sizes may lead to an underestimated X^2 value, lower RMSEA values and increased standard error of parameter estimation [86,87]. This may have an impact on lower model fit and underestimated significance levels. Furthermore, the multivariate normal distribution was not given in the sample. This is consistent with the Shapiro–Wilk tests showing non-normality for all items. However, studies showed that RMSEA and the parameter estimation are less influenced than the X^2 value by non-normality distribution. As the X^2-value was not interpreted in this study the results are expected to be just slightly biased [57,87–89]. The missing data in the item set may cause biasing problems when being ignored. The FIML method provided the most efficient unbiased solution of estimating missing variables and has been shown to be superior to listwise or pairwise deletion [90].

5. Conclusions

We implemented a GSI to cover mental stress in soldiers through one global factor. In a further step it may enable to focus on psychosocial risk as an additional trigger next

to the well-established CVD scores. Therefore, the validation of the GSI is mandatory to prove its suitability for clinical application. The GSI is not just a reflection of the current perception of a person but a holistic index combining information about current stress perception, negative life events, depression, anxiety as well as chronic stress and CM. The GSI globally combines this stress-related information for an easy implementation in clinical practice. All aspects influence each other and play a main role for stress-related mental health and disease.

Author Contributions: Conceptualization: T.M., C.W., and M.R.; Methodology: C.W. and M.R.; Formal analysis: M.K., and T.M.; Investigation: D.-S.R., M.R., and T.M.; Writing—original draft preparation: T.M.; Writing—review and editing: C.W., M.K., and M.R.; Funding acquisition: C.W., H.G., S.B., B.F., and H.-P.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the German MoD (E/U2CA/FA159/FF552). The investigation was authorized by the BMVg (Ministry of Défense—registration number 3/06/17).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the University of Ulm (number 253/12, date of approval: 18.12.2012). This investigation is registered under the number DRKS00022345 in the German register of Clinical studies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We gratefully thank Dawne Vogt of the National Center for PTSD (VA) for the accordance to use the Deployment Risk and Resilience Inventory (DRRI-2).

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Item statistics.

Item	*n*	MIN	MAX	*M*	*SD*	Shapiro–Wilk
TICS_01	192	0	4	2.16	1.12	$W = 0.91$ ***
TICS_02	191	0	4	1.78	0.97	$W = 0.87$ ***
TICS_03	192	0	3	0.85	0.67	$W = 0.77$ ***
TICS_04	191	0	4	1.53	0.98	$W = 0.90$ ***
TICS_05	192	0	4	2.11	0.99	$W = 0.91$ ***
TICS_06	192	0	4	1.56	0.77	$W = 0.85$ ***
TICS_07	192	0	4	2.54	1.14	$W = 0.89$ ***
TICS_08	192	0	4	1.87	0.94	$W = 0.90$ ***
TICS_09	192	0	4	1.57	0.98	$W = 0.88$ ***
TICS_10	192	0	4	1.51	1.09	$W = 0.89$ ***
TICS_11	192	0	4	1.15	1.03	$W = 0.84$ ***
TICS_12	192	0	4	1.68	1.01	$W = 0.90$ ***
TICS_13	192	0	4	1.90	0.87	$W = 0.87$ ***
TICS_14	192	0	4	2.09	0.97	$W = 0.90$ ***
TICS_15	192	0	4	1.11	0.91	$W = 0.85$ ***
TICS_16	192	0	4	1.35	1.05	$W = 0.88$ ***
TICS_17	192	0	4	1.56	1.08	$W = 0.91$ ***
TICS_18	163	0	4	1.28	1.09	$W = 0.88$ ***
TICS_19	192	0	4	1.61	1.03	$W = 0.90$ ***
TICS_20	192	0	4	0.81	0.80	$W = 0.77$ ***
TICS_21	192	0	4	1.47	1.04	$W = 0.90$ ***
TICS_22	192	0	4	2.37	1.03	$W = 0.90$ ***
TICS_23	192	0	4	2.36	1.02	$W = 0.88$ ***
TICS_24	190	0	3	0.63	0.68	$W = 0.76$ ***
TICS_25	192	0	4	1.11	1.01	$W = 0.86$ ***

Table A1. *Cont.*

Item	*n*	MIN	MAX	*M*	*SD*	Shapiro–Wilk
TICS_26	192	0	4	1.06	0.94	$W = 0.84$ ***
TICS_27	192	0	4	2.26	1.09	$W = 0.90$ ***
TICS_28	192	0	4	1.58	1.05	$W = 0.90$ ***
TICS_29	192	0	4	1.34	0.99	$W = 0.89$ ***
TICS_30	192	0	4	1.72	0.98	$W = 0.90$ ***
TICS_31	192	0	4	1.29	1.09	$W = 0.89$ ***
TICS_32	191	0	4	2.47	0.89	$W = 0.88$ ***
TICS_33	192	0	4	1.01	0.98	$W = 0.82$ ***
TICS_34	191	0	4	0.90	0.96	$W = 0.83$ ***
TICS_35	191	0	4	1.00	0.81	$W = 0.80$ ***
TICS_36	192	0	4	1.25	1.08	$W = 0.86$ ***
TICS_37	192	0	4	1.24	1.00	$W = 0.88$ ***
TICS_38	192	0	4	1.57	1.01	$W = 0.90$ ***
TICS_39	192	0	4	1.80	1.26	$W = 0.91$ ***
TICS_40	192	0	4	1.38	1.05	$W = 0.89$ ***
TICS_41	191	0	4	1.46	1.02	$W = 0.89$ ***
TICS_42	191	0	4	1.14	0.97	$W = 0.85$ ***
TICS_43	191	0	4	2.57	0.92	$W = 0.89$ ***
TICS_44	191	0	4	1.26	0.98	$W = 0.86$ ***
TICS_45	191	0	4	0.76	0.87	$W = 0.78$ ***
TICS_46	190	0	4	1.37	1.11	$W = 0.88$ ***
TICS_47	191	0	4	1.21	0.97	$W = 0.87$ ***
TICS_48	191	0	4	1.63	1.00	$W = 0.89$ ***
TICS_49	190	0	4	2.07	1.19	$W = 0.91$ ***
TICS_50	191	0	4	1.49	1.03	$W = 0.89$ ***
TICS_51	191	0	4	1.15	0.96	$W = 0.86$ ***
TICS_52	191	0	4	0.86	0.85	$W = 0.81$ ***
TICS_53	191	0	4	1.63	0.93	$W = 0.90$ ***
TICS_54	191	0	4	1.09	1.02	$W = 0.84$ ***
TICS_55	191	0	4	0.74	0.78	$W = 0.79$ ***
TICS_56	191	0	4	1.38	1.11	$W = 0.90$ ***
TICS_57	191	0	4	0.97	0.93	$W = 0.83$ ***
PSS4_01	191	0	4	1.63	1.03	$W = 0.91$ ***
PSS4_02	190	0	4	2.87	0.84	$W = 0.81$ ***
PSS4_03	191	0	4	2.56	0.91	$W = 0.87$ ***
PSS4_04	191	0	4	1.23	1.05	$W = 0.84$ ***
CTQ_01	192	1	5	1.18	0.69	$W = 0.33$ ***
CTQ_02	192	1	5	1.63	1.02	$W = 0.66$ ***
CTQ_03	191	1	5	1.53	0.92	$W = 0.57$ ***
CTQ_04	192	1	5	1.17	0.64	$W = 0.29$ ***
CTQ_05	191	1	5	1.79	1.19	$W = 0.68$ ***
CTQ_06	192	1	5	1.09	0.43	$W = 0.20$ ***
CTQ_07	192	1	5	1.76	1.10	$W = 0.71$ ***
CTQ_08	192	1	5	1.29	0.84	$W = 0.39$ ***
CTQ_09	192	1	5	1.22	0.74	$W = 0.38$ ***
CTQ_10	192	1	5	3.22	1.34	$W = 0.89$ ***
CTQ_11	192	1	5	1.32	0.82	$W = 0.45$ ***
CTQ_12	192	1	5	1.44	0.94	$W = 0.54$ ***
CTQ_13	191	1	5	2.04	1.10	$W = 0.83$ ***
CTQ_14	192	1	5	1.77	1.06	$W = 0.70$ ***
CTQ_15	192	1	5	1.19	0.73	$W = 0.26$ ***
CTQ_16	190	1	5	3.38	1.19	$W = 0.89$ ***
CTQ_17	191	1	5	1.13	0.61	$W = 0.23$ ***
CTQ_18	191	1	5	1.32	0.90	$W = 0.39$ ***
CTQ_19	189	1	5	2.20	1.13	$W = 0.86$ ***
CTQ_20	191	1	5	1.16	0.70	$W = 0.17$ ***
CTQ_21	191	1	5	1.05	0.39	$W = 0.11$ ***
CTQ_22	191	1	5	3.50	1.25	$W = 0.87$ ***

Table A1. *Cont.*

Item	*n*	MIN	MAX	*M*	*SD*	Shapiro–Wilk
CTQ_23	191	1	5	1.14	0.66	*W* = 0.18 ***
CTQ_24	191	1	5	1.13	0.64	*W* = 0.18 ***
CTQ_25	190	1	5	1.21	0.70	*W* = 0.34 ***
CTQ_26	191	1	5	1.40	0.93	*W* = 0.51 ***
CTQ_27	191	1	5	1.11	0.57	*W* = 0.15 ***
CTQ_28	190	1	5	2.22	1.17	*W* = 0.84 ***
HADS_01	192	0	3	0.86	0.71	*W* = 0.80 ***
HADS_02	192	0	3	0.81	0.88	*W* = 0.80 ***
HADS_03	192	0	3	0.74	0.88	*W* = 0.78 ***
HADS_04	191	0	3	0.41	0.63	*W* = 0.68 ***
HADS_05	192	0	3	0.70	0.76	*W* = 0.78 ***
HADS_06	192	0	3	0.45	0.71	*W* = 0.65 ***
HADS_07	192	0	3	0.85	0.79	*W* = 0.82 ***
HADS_08	192	0	3	0.88	0.68	*W* = 0.76 ***
HADS_09	192	0	3	0.52	0.58	*W* = 0.69 ***
HADS_10	191	0	3	0.43	0.71	*W* = 0.67 ***
HADS_11	192	0	3	0.98	0.79	*W* = 0.83 ***
HADS_12	192	0	3	0.54	0.72	*W* = 0.74 ***
HADS_13	192	0	2	0.31	0.56	*W* = 0.58 ***
HADS_14	192	0	3	0.40	0.72	*W* = 0.59 ***
DRRI_2	178	0	9	2.04	2.10	*W* = 0.83 ***
PDS	163	0	5	1.80	1.45	*W* = 0.92 ***

Note: *N* = sample size; *MIN* = minimum; *MAX* = maximum; *M* = arithmetic mean; *SD* = standard deviation; *W* = Shapiro–Wilk test statistic; PSS4 = Perceived Stress Scale; TICS = Trier Inventory for Chronic Stress; HADS = Hospital Anxiety and Depression Scale; PDS = Posttraumatic Stress Diagnostic Scale Checklist; DRRI_2 = Deployment Risk and Resilience Inventory; CTQ = Childhood Trauma Questionnaire; *** = $p < 0.001$; higher values indicate more negative outcomes.

Appendix B

Table A2. Additional statistics and results for Model 17: loadings, variances, and covariances.

Items/Factors		Loading/Covariance/ Intercept	*SE* Loading	*p*	Variance	*SE* Variance	*p*
GSI ~					0.31	0.05	<0.001
	1	2.10	0.09	<0.001			
	perceived stress	1.00			0.24	0.05	<0.001
	HADS	0.40	0.03	<0.001	0.17	0.03	<0.001
	CTQ	0.71	0.04	<0.001	0.15	0.03	<0.001
	PDS	0.85	0.06	<0.001	1.98	0.23	<0.001
	DRRI_2	1.02	0.08	<0.001	3.73	0.41	<0.001
perceived stress~							
	TICS_01	1.00			0.94	0.10	<0.001
	TICS_02	0.84	0.04	<0.001	0.59	0.06	<0.001
	TICS_03	0.41	0.02	<0.001	0.33	0.03	<0.001
	TICS_04	0.74	0.03	<0.001	0.60	0.06	<0.001
	TICS_05	0.95	0.04	<0.001	0.92	0.10	<0.001
	TICS_06	0.72	0.03	<0.001	0.42	0.04	<0.001
	TICS_07	1.12	0.05	<0.001	1.26	0.12	<0.001
	TICS_08	0.85	0.04	<0.001	0.81	0.09	<0.001
	TICS_09	0.76	0.03	<0.001	0.59	0.06	<0.001
	TICS_10	0.67	0.04	<0.001	0.89	0.08	<0.001
	TICS_11	0.55	0.04	<0.001	0.89	0.09	<0.001
	TICS_12	0.79	0.04	<0.001	0.74	0.08	<0.001
	TICS_13	0.88	0.04	<0.001	0.55	0.06	<0.001

Table A2. *Cont.*

Items/Factors		Loading/Covariance/ Intercept	*SE* Loading	*p*	Variance	*SE* Variance	*p*
	TICS_14	0.95	0.04	<0.001	0.82	0.09	<0.001
	TICS_15	0.54	0.03	<0.001	0.61	0.06	<0.001
	TICS_16	0.54	0.03	<0.001	0.74	0.08	<0.001
	TICS_17	0.76	0.04	<0.001	0.75	0.08	<0.001
	TICS_18	0.63	0.04	<0.001	0.84	0.09	<0.001
	TICS_19	0.78	0.04	<0.001	0.60	0.06	<0.001
	TICS_20	0.41	0.03	<0.001	0.47	0.05	<0.001
	TICS_21	0.67	0.04	<0.001	1.04	0.11	<0.001
	TICS_22	1.06	0.05	<0.001	1.06	0.11	<0.001
	TICS_23	1.06	0.05	<0.001	1.01	0.11	<0.001
	TICS_24	0.33	0.02	<0.001	0.32	0.03	<0.001
	TICS_25	0.57	0.03	<0.001	0.61	0.06	<0.001
	TICS_26	0.52	0.03	<0.001	0.65	0.07	<0.001
	TICS_27	1.05	0.05	<0.001	0.87	0.09	<0.001
	TICS_28	0.77	0.04	<0.001	0.64	0.07	<0.001
	TICS_29	0.65	0.03	<0.001	0.71	0.07	<0.001
	TICS_30	0.78	0.04	<0.001	0.94	0.01	<0.001
	TICS_31	0.64	0.04	<0.001	0.81	0.08	<0.001
	TICS_32	1.10	0.05	<0.001	0.89	0.10	<0.001
	TICS_33	0.50	0.03	<0.001	0.71	0.07	<0.001
	TICS_34	0.46	0.03	<0.001	0.70	0.07	<0.001
	TICS_35	0.49	0.03	<0.001	0.48	0.05	<0.001
	TICS_36	0.63	0.04	<0.001	0.78	0.08	<0.001
	TICS_37	0.61	0.03	<0.001	0.68	0.07	<0.001
	TICS_38	0.78	0.03	<0.001	0.46	0.05	<0.001
	TICS_39	0.86	0.05	<0.001	1.20	0.12	<0.001
	TICS_40	0.67	0.04	<0.001	0.74	0.08	<0.001
	TICS_41	0.62	0.04	<0.001	1.25	0.13	<0.001
	TICS_42	0.55	0.03	<0.001	0.77	0.08	<0.001
	TICS_43	1.15	0.05	<0.001	0.84	0.09	<0.001
	TICS_44	0.64	0.03	<0.001	0.49	0.05	<0.001
	TICS_45	0.41	0.03	<0.001	0.53	0.05	<0.001
	TICS_46	0.68	0.04	<0.001	0.81	0.08	<0.001
	TICS_47	0.60	0.03	<0.001	0.62	0.07	<0.001
	TICS_48	0.74	0.04	<0.001	0.94	0.10	<0.001
	TICS_49	0.94	0.05	<0.001	1.26	0.13	<0.001
	TICS_50	0.73	0.03	<0.001	0.62	0.07	<0.001
	TICS_51	0.56	0.03	<0.001	0.67	0.07	<0.001
	TICS_52	0.43	0.03	<0.001	0.52	0.05	<0.001
	TICS_53	0.74	0.04	<0.001	0.78	0.08	<0.001
	TICS_54	0.57	0.03	<0.001	0.62	0.07	<0.001
	TICS_55	0.38	0.02	<0.001	0.41	0.04	<0.001
	TICS_56	0.66	0.04	<0.001	0.98	0.10	<0.001
	TICS_57	0.50	0.03	<0.001	0.56	0.06	<0.001
	PSS4_01	0.79	0.04	<0.001	0.65	0.07	<0.001
	PSS4_02	−0.42	0.08	<0.001	0.60	0.06	<0.001
	PSS4_03	−0.45	0.09	<0.001	0.71	0.07	<0.001
	PSS4_04	0.61	0.03	<0.001	0.74	0.08	<0.001
HADS~							
	HADS_01	1.00			0.30	0.04	<0.001
	HADS_02	1.01	0.07	<0.001	0.47	0.05	<0.001
	HADS_03	0.96	0.07	<0.001	0.46	0.05	<0.001
	HADS_04	0.59	0.05	<0.001	0.24	0.03	<0.001
	HADS_05	0.89	0.06	<0.001	0.31	0.04	<0.001
	HADS_06	0.63	0.05	<0.001	0.33	0.04	<0.001
	HADS_07	1.02	0.06	<0.001	0.39	0.04	<0.001
	HADS_08	0.95	0.06	<0.001	0.38	0.04	<0.001
	HADS_09	0.61	0.05	<0.001	0.25	0.03	<0.001

Table A2. *Cont.*

Items/Factors		Loading/Covariance/ Intercept	*SE* Loading	*p*	Variance	*SE* Variance	*p*
	HADS_10	0.56	0.05	<0.001	0.40	0.04	<0.001
	HADS_11	1.03	0.07	<0.001	0.59	0.06	<0.001
	HADS_12	0.73	0.05	<0.001	0.32	0.03	<0.001
	HADS_13	0.44	0.04	<0.001	0.23	0.02	<0.001
	HADS_14	0.56	0.05	<0.001	0.38	0.04	<0.001
CTQ~							
	CTQ_EA	1.00			0.14	0.03	<0.001
	CTQ_SA	0.77	0.04	<0.001	0.28	0.03	<0.001
	CTQ_PA	0.81	0.04	<0.001	0.12	0.02	<0.001
	CTQ_EN	1.19	0.07	<0.001	0.26	0.04	<0.001
	CTQ_PN	0.76	0.04	<0.001	0.04	0.01	<0.001
	CTQ_MQ	−1.20	0.11	<0.001	0.59	0.09	<0.001
CTQ_EA~							
	CTQ_03	1.00			0.56	0.06	<0.001
	CTQ_08	0.89	0.04	<0.001	0.29	0.04	<0.001
	CTQ_14	1.19	0.05	<0.001	0.52	0.07	<0.001
	CTQ_18	0.94	0.04	<0.001	0.24	0.03	<0.001
	CTQ_25	0.34	0.05	<0.001	0.22	0.02	<0.001
CTQ_SA~							
	CTQ_20	1.00			0.08	0.01	<0.001
	CTQ_23	0.97	0.02	<0.001	0.08	0.01	<0.001
	CTQ_24	0.96	0.02	<0.001	0.06	0.01	<0.001
	CTQ_27	0.94	0.02	<0.001	0.00	0.00	0.427
CTQ_PA~							
	CTQ_09	1.00			0.27	0.03	<0.001
	CTQ_11	1.11	0.04	<0.001	0.20	0.03	<0.001
	CTQ_12	1.18	0.05	<0.001	0.51	0.06	<0.001
	CTQ_15	0.99	0.04	<0.001	0.21	0.03	<0.001
	CTQ_17	0.93	0.03	<0.001	0.10	0.02	<0.001
CTQ_EN~							
	CTQ_05	1.00			0.81	0.09	<0.001
	CTQ_07	1.01	0.04	<0.001	0.43	0.05	<0.001
	CTQ_13	1.14	0.05	<0.001	0.45	0.05	<0.001
	CTQ_19	1.24	0.05	<0.001	0.36	0.05	<0.001
	CTQ_28	1.26	0.05	<0.001	0.26	0.04	<0.001
CTQ_PN~							
	CTQ_01	1.00			0.40	0.05	<0.001
	CTQ_02	1.46	0.07	<0.001	0.57	0.07	<0.001
	CTQ_04	1.02	0.05	<0.001	0.26	0.03	<0.001
	CTQ_06	0.93	0.04	<0.001	0.11	0.02	<0.001
	CTQ_26	1.25	0.07	<0.001	0.53	0.06	<0.001
CTQ_MQ~							
	1	4.98	0.18	<0.001			
	CTQ_10	1.00			0.95	0.11	<0.001
	CTQ_16	1.06	0.03	<0.001	0.35	0.05	<0.001
	CTQ_22	1.10	0.03	<0.001	0.26	0.04	<0.001
PSS4_02	1	3.50	0.19	<0.001			
PSS4_03	1	3.76	0.17	<0.001			
CTQ_SA~	CTQ_25	0.57	0.06	<0.001			
CTQ_EN~~	*CTQ_MQ*	−0.33	0.05	<0.001			
CTQ_23~~	CTQ_24	0.05	0.01	<0.001			
TICS_10~~							
	TICS_21	0.27	0.08	0.001			
	TICS_41	0.48	0.09	<0.001			
	TICS_53	0.24	0.06	<0.001			

Table A2. *Cont.*

Items/Factors		Loading/Covariance/ Intercept	*SE* Loading	*p*	Variance	*SE* Variance	*p*
TICS_07~~							
	TICS_22	0.53	0.09	<0.001			
	TICS_43	0.53	0.09	<0.001			
	TICS_49	0.43	0.10	<0.001			
TICS_42~~	TICS_51	0.47	0.06	<0.001			
TICS_22~~	TICS_43	0.58	0.08	<0.001			
TICS_25~~	TICS_36	0.42	0.06	<0.001			

Note: *SE* = Standard Error; *p* = *p*-value; ~ = loading; ~~ = covariance; Variables in normal type are items; words in Italic type are factors. Loadings for variables with a 1 in the third column were restricted to 1 or show intercepts. PSS4 = Perceived Stress Scale; TICS = Trier Inventory for Chronic Stress; HADS = Hospital Anxiety and Depression Scale; PDS = Posttraumatic Stress Diagnostic Scale Checklist; DRRI_2 = Deployment Risk and Resilience Inventory; CTQ = Childhood Trauma Questionnaire; EA = Emotional Abuse; PA = Physical Abuse; SA = Sexual Abuse; EN = Emotional Neglect; PN = Physical Neglect; MQ = minimization questions.

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Maier, T., Rappel, M., Rhee, D.-S., Brill, S., Maderner, J., Pijahn, F., Gündel, H., Radermacher, P., Friemert, B., Becker, H.-P., & Waller, C. (2022). Mental but no bio-physiological long-term habituation to repeated social stress: A study on soldiers and the influence of mission abroad. *Frontiers in Psychiatry, 13*, 1011181. https://doi.org/10.3389/fpsyt.2022.1011181

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Declaration on own contributions:

- Psychobiological data collection
- Conduct of the TSST-G study protocol
- Data transmission, preparation and statistical analyses
- Literature research and conceptualization of the manuscript
- First draft and writing of the manuscript

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 15 December 2022
DOI 10.3389/fpsyt.2022.1011181

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OPEN ACCESS

EDITED BY
Oswald David Kothgassner,
Medical University of Vienna, Austria

REVIEWED BY
Kimberly Urban,
Sartorius AG, Germany
Luca Carnevali,
University of Parma, Italy

*CORRESPONDENCE
Tanja Maier
tanja.maier@uni-ulm.de

SPECIALTY SECTION
This article was submitted to Psychological Therapy and Psychosomatics, a section of the journal Frontiers in Psychiatry

RECEIVED 03 August 2022
ACCEPTED 28 November 2022
PUBLISHED 15 December 2022

CITATION
Maier T, Rappel M, Rhee D-S, Brill S, Maderner J, Pijahn F, Gündel H, Radermacher P, Friemert B, Becker H-P and Waller C (2022) Mental but no bio-physiological long-term habituation to repeated social stress: A study on soldiers and the influence of mission abroad. *Front. Psychiatry* 13:1011181. doi: 10.3389/fpsyt.2022.1011181

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Mental but no bio-physiological long-term habituation to repeated social stress: A study on soldiers and the influence of mission abroad

Tanja Maier[1]*, Manuela Rappel[1], Dae-Sup Rhee[1], Sebastian Brill[2], Julia Maderner[1], Friederike Pijahn[1], Harald Gündel[1], Peter Radermacher[3], Benedikt Friemert[2], Horst-Peter Becker[4] and Christiane Waller[1,5]

[1]Clinic for Psychosomatics and Psychotherapeutic Medicine, Ulm University Medical Center, Ulm, Germany, [2]Military Hospital, Ulm, Germany, [3]Institute for Anesthesiological Pathophysiology and Process Engineering, Ulm University, Ulm, Germany, [4]Military Hospital, Berlin, Germany, [5]Department of Psychosomatics and Psychotherapeutic Medicine, Paracelsus Medical University, Nuremberg, Germany

Soldiers regularly participate in missions abroad and subjectively adapt to this situation. However, they have an increased lifetime cardiovascular risk compared to other occupational groups. To test the hypothesis that foreign deployment results in different stress habituation patterns, we investigated long-term psychological and bio-physiological stress responses to a repeated social stress task in healthy soldiers with and without foreign deployment. Ninety-one female and male soldiers from the BEST study (German armed forces deployment and stress) participated three times in the Trier Social Stress Test for groups (TSST-G) prior to, 6–8 weeks after and 1 year after the mission abroad and were compared to a control group without foreign deployment during the study period. They completed the State-Trait-Anxiety Inventory scale (STAI), the Primary Appraisal Secondary Appraisal questionnaire (PASA) and the Multidimensional Mood State Questionnaire (MDBF). Salivary cortisol and α-amylase, blood pressure, heart rate and heart rate variability were determined. Soldiers showed mental habituation over the three times with a significant decrease after the TSST-G in anxiousness (STAI) and cognitive stress appraisal (PASA), they were calmer and reported better mood (MDBF). Prior to the social stress part, the mood (MDBF) declined significantly. None of the biological and physiological markers showed any adaptation to the TSST-G. Mission abroad did not significantly influence any measured psychobiological marker when compared to soldiers without foreign deployment. Foreign deployment does not result in alterations in psychobiological social stress response patterns over 1 year after

 10.3389/fpsyt.2022.1011181

mission abroad which indicates that adaptation to acute social stress is highly maintained in healthy soldiers. The discrepancy between subjective perception and objective stress response has numerous clinical implications and should receive more attention.

KEYWORDS

social stress, mission abroad, TSST-G, habituation, biological stress axis response, salivary cortisol, salivary α-amylase, heart rate variability

1 Introduction

The importance of social stress in the general public awareness has strongly increased over the years and inspired research in different kind of fields (1). Chronic social stress is considered as a psychobiological risk factor (2) for cardiovascular diseases (CVD) (3), e.g., myocardial infarction or high blood pressure (4); moreover it shows robust causal associations with depression and anxiety (5, 6). Experiencing stress results from individual factors, e.g., lack of social support, time pressure, social conflicts, threats or hazardous situations (7–9).

Some professions are facing greater stress due to their job task, e.g., military personnel who are often exposed to various stressors like heavy workload, hierarchical structures or hazardous experiences during combat which may all lead to high stress perception (10). Not all missions abroad result in traumatic stress or experiencing direct mortal danger but soldiers are mostly facing social stress. Soldiers show higher risk for perceived job-stress than civilians and high rates of cardiovascular disease, particularly during foreign deployment (11). Soldiers with such missions abroad are also more likely to suffer from hypertension because of increased mental stress than non-deployed soldiers (12, 13). It is known that almost half of the deployed soldiers experience at least one traumatic event and hence have a two-to fourfold elevated risk of developing mental disorders like PTSD (14). This raises the question if soldiers that undergo missions abroad would react differently to social stress afterwards.

Various stress models demonstrated that the extent whether an external stressor is perceived as stressful or not depends on the individual coping mechanism, social support and resources (15). Especially psychosocial stress factors are closely linked to physical and mental wellbeing (16). Anxiety with the feeling of tension is the leading emotion linked to the stress response system which has an impact on cognition, emotions and behavior through the activation of the autonomic nervous response system (ANS) (16). The human body initiates elaborate reactions to stress and shows different coping mechanism to maintain homeostasis. Social stress activates the neurohumoral response of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system (17) which both modulate and show a bidirectional interaction with the immune system (18). The HPA axis releases cortisol which stimulates the release of fatty acids for providing energy and interacts through hormones with other systems (e.g., nervous, metabolic) (19). In contrast the SAM system provides epinephrine and norepinephrine to prepare for a fight or flight reaction (e.g., increase in heart rate and blood pressure) (20). Ongoing stress over an extended time period can lead to chronic modulation of the stress response system and modified gene expression which can cause dysregulated immune function (18).

However, recent studies have also focused on the adaptation process to a repetitive stressor (21) which is called "habituation." This term is generally referred to as a reduction of the measured variable due to a repeated stimulation (22) and is independent of the stimulus and the extent of habituation. Thompson and Spencer tried to define criteria for a more operational approach for habituation, which composes of e.g., the influence of frequency or strength of the stimulus as well as the phenomenon of spontaneous recovery or potentiation of habituation (22). It has been frequently shown that the HPA-axis response habituated to repeated psychosocial stress stimuli both in experimental animals (23, 24) as well as in human studies (21). However, the mechanism and stability of this phenomenon is not completely understood. Some research indicated that the attenuated HPA-axis response is a mixture between negative feedback mechanisms induced by cortisol and learning/memory and can only partially be explained by habituation (25). This resulted in the concept to differentiate between high and low responders (26). Results for the SAM axis are inconsistent with comparatively lower habituation compared to the HPA-axis, e.g., heart rate decreases whereas epinephrine and norepinephrine release showed no habituation (27). Mental habituation to repeated social stress can be measured by quantifying behavioral changes (28). Subjective perception, estimations of the situation like tenseness, irritation or the feeling of stress significantly decrease over time (28). Monkeys showed a decrease in behavioral stress reaction whereas no biological habituation (decrease in cortisol levels) was detected (29). These findings indicate that changes in mood or behavior may not necessarily reflect underlying

biological or physiological processes in response to repeated stress (30).

To properly examine changes in the biological, physiological and psychological stress response a valid and repeatable stress exposure protocol and considerations about the stress parameters are required. The Trier Social Stress Test for Groups [TSST-G (31)] is suitable for this purpose and is therefore used as a standard tool in psychobiological research. The applicability for inducing multidimensional stress in participants (HPA-axis response, SAM system activation and subjective emotional stress) has been frequently tested (32, 33). The TSST can be used for measuring repeated stress, whereas the protocol is often slightly adapted over time. To capture HPA-axis response and habituation the determination of salivary cortisol is standard practice whereas the SAM-system can be measured through salivary α-amylase (sAA) activity or adrenaline/noradrenaline concentrations (34, 35). Cardiovascular effects that indirectly mirror SAM activity can be revealed through heart rate variability (HRV) and blood pressure. Subjective psychological activation and differences over time include appraisal, anxiety, alertness, and mood (35). Recent studies indicate a habituation effect of cortisol to a repeated stressor whereas no decrease or even a slight increase over time has been described for sAA and HRV (21, 27, 36). Blunted cortisol concentrations have been described for deployed soldiers who experienced high stress (development of PTSD) in comparison to civilians or less stressed soldiers as measured with the Trier Social Stress Test (TSST) (37, 38).

To test the hypothesis that foreign deployment results in attenuated habituation patterns, we measured standardized psychological, physiological and biological markers in a TSST-group setting with a 1 year distance for the follow-up measure. Of further importance was the assessment of differences in the psychological and biological stress effects of the repeated mental stress tasks in healthy soldiers. The study is part of the BEST study (German Armed Forces Deployment and Stress).

2 Materials and methods

2.1 Recruitment and study design

The participants from the BEST-study were German soldiers that had been recruited from military barracks in Dornstadt, Laupheim, and Ulm as well as in the German Armed Forces hospital in Ulm. The aim of the prospective study design was to investigate biopsychosocial stress effects of foreign deployment on cardiovascular health in the German Armed Forces. Recruited soldiers with similar age and gender distribution without mission abroad served as the control group. All participants repeatedly passed through a social stress task, the TSST-G (31) at three different time points, prior to (t0), and 4–6 weeks (t1) as well as 1 year (t2) after mission abroad. The TSST is well-known and the protocol is widely used for various stress paradigms because it effectively leads to psychobiological stress response and has high reliability (31). All sessions took place and were scheduled at the same time in the early evenings and participants were asked not to eat or drink anything else than water at least 1 h beforehand. Each TSST-G lasted about 3 h in total, depending on the number of participants (3–6 per group). The standardized protocol (**Figure 1**) consisted of a 30 min resting period, followed by a stress exposition lasting for 30–40 min comprising of a 5 min mock job interview (describing their own personality traits) and a mental arithmetic task in front of two specialized evaluators in lab coats, a video camera and in the presence of the other participants. This procedure ensures stress induction not only during the tasks themselves but also through attending the other participants' tasks. After the stress exposure, a resting period of 60 min was allowed for downregulation of the neuroendocrine systems. Soldiers from the control and experimental group were tested under the same conditions. Various stress-sensitive psychological and biological values were measured at the three time-points for baseline and during stress exposure. The number and time frame of measurements within the TSST-G differed for each variable (**Figure 1**). Due to the time-sensitive characteristics of the examined variables the study protocol was thoroughly followed and group size did not influence measurement timing. Anamnestic, sociodemographic and psychological information was collected through questionnaires.

2.2 Participants

A total of 234 soldiers with regular medical examinations were included and completed t0. Due to administrative changes at the German Armed Forces, the survey of questionnaires had to be paused for 6 months so that 42 participants had to be excluded from final analyses due to missing questionnaires. A total of 91 soldiers completed all three measurements. The biological variables cortisol, amylase, HRV, systolic and diastolic pressure were available with varied quantities and case numbers because of one or more of the following reasons: errors within the technical devices, too little sample material for the analyses, transmission errors and conservative calculations for the changes over time where missing values were excluded and not extrapolated. Forty-four soldiers had a mission abroad between t0 and t1 during the study whereas 47 soldiers were in the control group. The experimental group was mostly deployed to Mali, Afghanistan and the Republic of Kosovo. The mission orders mainly included to build a "Safe and Secure Environment," securing peace and human security as well as humanitarian aid and training missions.

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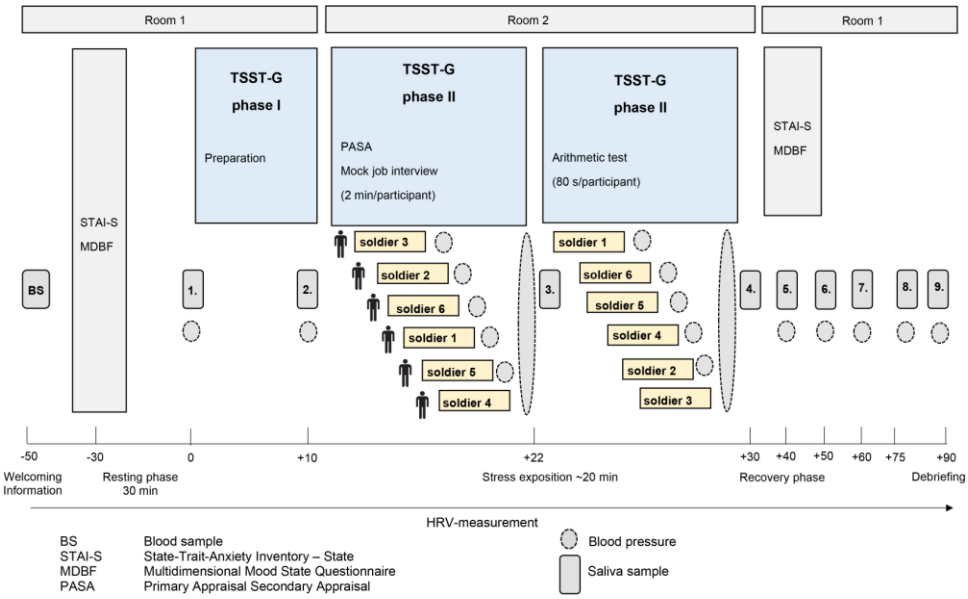

FIGURE 1
Illustration of the standardized process description of the trier social stress test for groups (TSST-G). The trial design was adapted according to von Dawans, Kirschbaum and Heinrichs (31).

2.3 Biological measures

During the TSST-G nine saliva samples were taken (Figure 1), immediately centrifuged at 3,000 rpm for 5 min, frozen and stored at −80°C until analysis. Cortisol concentrations (nmol/l) were measured using a chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). The intra- and interassay coefficients were below 8%. SAA concentrations (U/ml) were analyzed using an enzyme kinetic method and interference measurement was obtained at a wavelength of 405 nm using a standard ELISA reader. Increases in absorbance were calculated for unknowns and standards.

2.4 Physiological measures

Heart rate variability and heart rate was derived using the eMotion Faros 180° biosensor (BlindSight GmbH, Schlitz, Germany). All participants were monitored using a three-lead digital electrocardiogram with a sampling rate of 500 Hz during the whole procedure. At 10 time points of the TSST-G 40 s tracings of the HRV were used whereas the number of these 40 s segments varied between the time points. The shortest included interval was during the mental arithmetic task, the longest one during preparation phase (see Figure 1). Data were edited using the HRV Analysis Software (MindWareTechnologies; Version 3.1.7.). Artifacts were identified and manually removed and arrhythmic beats could be averaged with the midbeast function to avoid distorted values. Through time domain measures the standard deviation of all normal-to-normal inter-beat intervals (SDNN) and root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD) were calculated (39). Diastolic and systolic pressure (mm/Hg) were measured nine times during the TSST-G using the Boso Medicus Control Blood Pressure Monitor (Bosch & Sohn GmbH u. Co. KG, Jungingen, Germany). Soldiers had been instructed how to use the upper arm cuff and started the measuring by themselves after a signal. All values were stored automatically by the devices.

2.5 Psychological measures

Acute anxiety was measured using the State-Trait-Anxiety Inventory—State [STAI-S (40)] scale right before and directly after the stress task. "I am tense," "I am worried," "I feel calm" are typical phrases for the current emotional state in the STAI-S. Reverse coded items were inverted prior to the analysis. The total sum score of all items was used, higher values indicated more severe anxiety. The Primary Appraisal Secondary

TABLE 1 Descriptive data for male soldiers with and without mission abroad.

Variable	*N* (cg/eg)	M (cg/eg)	SD (cg/eg)	Range (cg/eg)	*T*-test
Age	33/37	29/34	7.13/7.66	19–56/21–50	$t(68) = -3.08$*
BMI	32/36	24/26	2.81/3.12	19–29/21–35	–
Cortisol (nmol/l)	24/25	4/2	7.42/1.99	<1–30/<1–9	–
Alpha-amlyase (U/ml)	21/16	61/56	42.67/37.85	14–181/11–136	–
Heart rate (bpm)	21/21	78/80	11.72/13.27	54–99/57–101	–
HRV-RMSSD (ms)	21/21	26/27	11.10/15.73	9–53/7–65	–
HRV-SDNN (ms)	21/21	51/47	17.68/22.33	23–95/14–113	–
Diastolic pressure (mm/Hg)	20/32	78/79	11.22/10.16	55–100/46–102	–
Systolic pressure (mm/Hg)	20/32	125/127	13.04/13.80	89–142/83–153	–
STAI-S before	33/37	35.30/35.65	6.53/7.28	24–60/23–54	–
STAI-S after	33/37	39.97/38.76	6.98/8.96	23–55/25–67	–
PASA	32/36	−1.13/−1.19	1.25/1.30	−3.75 to 1.63/−3.63 to 1.88	–
MDBF good/bad mood before	33/36	17.39/17.31	2.02/2.56	13–20/8–20	–
MDBF good/bad mood after	33/37	15.15/15.51	2.80/3.02	9–20/5–20	–
MDBF alertness/tiredness before	33/37	13.18/13.00	2.81/3.76	8–20/5–19	–
MDBF alertness/tiredness after	33/36	12.76/14.03	2.39/3.19	8–18/5–19	–
MDBF calmness/restlessness before	32/37	16.28/16.73	2.13/2.76	10–20/9–20	–
MDBF calmness/restlessness after	33/37	14.70/14.97	3.29/3.40	8–20/6–20	–

N, number; *M*, mean; *SD*, standard deviation; cg, control group (no mission abroad); eg, experimental group (mission abroad); *significant at the <0.05 level.
STAI-S, state-trait-anxiety inventory: before and after mental stress task; PASA, primary appraisal secondary appraisal: overall stress index; MDBF, multidimensional mood state questionnaire: before and after mental stress task. Values for cortisol, amylase, systolic pressure, diastolic pressure, heart rate and heart rate variability (HRV) indicate resting values—first measurement after resting phase in the Trier Social Stress Test for groups (TSST-G) (t0).

Appraisal [PASA (41)] questionnaire covered situational anticipatory cognitive appraisal with statements like "The situation is important for me," "I do not feel threatened by the situation" before undergoing the stress task. The overall stress index derived from the PASA questionnaire is a difference calculation of a combination of the four primary scales challenge, threat, control expectancy and self-concept of own competencies. Reverse coded items were inverted prior to the analysis and higher outcomes indicated more pronounced stress-appraisal. Soldiers' mood before and after the stress task was quantified with the Multidimensional Mood State Questionnaire [MDBF (42)]. It is designed for follow-up measurement and consists of two parallel halves. The three bipolar dimensions are good/bad mood, alertness/tiredness and calmness/restlessness including questions like "Right now I feel rested," "Right now I feel alert." Negative items were inverted and item sum scores of the three bipolar dimensions were calculated and once again higher outcomes indicated better mood. All instruments are validated and widely-used in connection with the TSST-G and stress research (43–45).

2.6 Data analysis

For all biological and physiological data, the changes over the time period during the TSST-G were considered because the calculation of mean differences or using only one timepoint of measurement does not provide sufficient information. Therefore, the area under the curve (AUC) was used. It is an index reflecting the area under the concentration-time curve and generated over all measures of e.g., salivary cortisol during the TSST-G (46). Pruessner et al. (47) showed that this proceeding leads to compressed data for statistical analyses. To examine the sensitivity of the system and changes over time, the area under the curve with respect to increase (AUCi) was calculated. Negative outcome was possible due to mathematical characteristics of the calculations. To examine the overall intensity of the stress response the area under the curve with respect to the ground (AUCg) was calculated. Calculations, editing and graphics were realized using IBM SPSS Statistics 23 (48). Descriptive analyses, *t*-Test for baseline differences between female and male soldiers and Shapiro Wilk tests for item distribution were performed. Shapiro-Wilk test for normal distribution for mostly all stress parameters was significant ($p < .001$). Research showed that ANOVAs are robust against violation of the normal distribution (49). Furthermore, according to the central limit theorem (law of large numbers) the given sampling distribution was approximately normally distributed (50) because the sample size was $N \geq 30$ for each variable and group, thus leading to the conclusion that normal data distribution can be expected for our data. Mixed ANOVA and figures were provided to show the chronological sequence within the TSST-G for an overall view of stress reactivity. To examine the habituation effects across the three TSST-Gs

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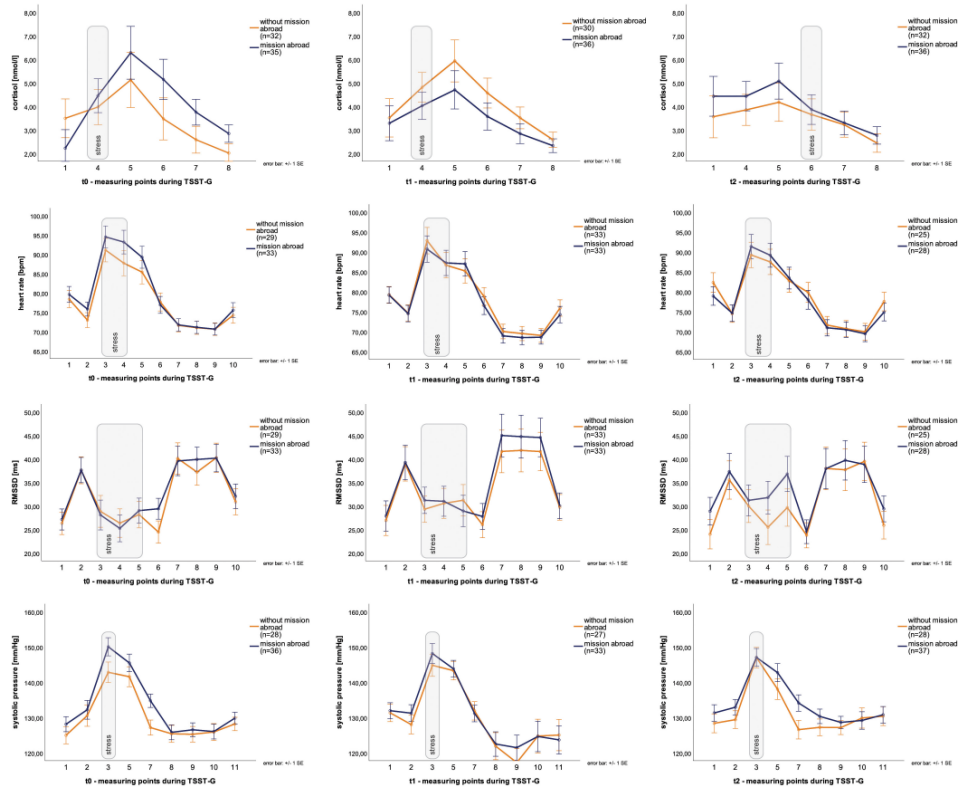

FIGURE 2
Stress response within Trier Social Stress Test for groups (TSST-G) (t0, t1, t2). Y-axis values: absolute values for cortisol (nmol/l), heart rate (bpm), RMSSD (ms), systolic pressure (mm/Hg). Stress: induced stress exposure following TSST-G protocol; width of stress bars reflects the number of measurement points within the variable; values are means (± SE).

analyses of variance (ANOVA) were conducted for cortisol, sAA, SDNN, RMSSD, systolic and diastolic pressure, STAI-S, PASA, and MDBF. All requirements were checked before calculation (e.g., normal distribution of residuals, sphericity/homoscedasticity, scaling). If requirements were not fulfilled alternative tests or correction methods (Greenhouse-Geisser for sphericity) were used. First, the ANOVA for repeated measures was used including all participants to show differences between the within-subjects factor time (three points of measurement: t0, t1, t2). All dependant variables were measured separately. Second, the mixed ANOVA for repeated measures was used to analyse the group x time effect of foreign deployment versus control group on the habituation effects to the repeated TSST-G. Therefore, the within-subject factor was time and the between-subject factor was group. Homogeneity of the error variances, as assessed by Levene's test ($p > 0.05$) was tested as well as homogeneity of covariances, as assessed by Box's test ($p > 0.05$). Due to the unbalanced proportions of the variable mission abroad in females (seven with and 14 without mission abroad) and the small sample size of women in the experimental group, only male soldiers were considered for the calculations of group x time effects. A χ^2-test for association was conducted between gender and mission abroad. The expected cell frequencies were less than the minimal required five for females for most biological variables in the experimental group. Therefore, we decided to exclude females from group analyses. For significant results bonferroni-adjusted *post-hoc* tests showed which variables (point of measurement/group) differed from each other. The covariates age and sex were taken into account in all calculations which involved biological variables to examine and control for any statistical influence on the outcome. The total number of missions abroad of a soldier prior to study inclusion was additionally taken into account as a covariate for the group x time effects in men. All reported analyses were tested

TABLE 2 Results for the group x time interaction effects (mixed ANOVA) in men within Trier Social Stress Test for groups (TSST-Gs).

Variable	Effect	*F*	*df*	*p*	Partial η^2
Cortisol—t0	Time x group	0.83	1.90, 121.53	0.43	–
	Time	1.90	1.90, 121.53	0.16	–
	Group	0.28	1, 64	0.60	–
Cortisol—t1	Time x group	0.63	1.77, 111.34	0.52	–
	Time	1.61	1.77, 111.34	0.21	–
	Group	0.11	1, 63	0.74	–
Cortisol—t2	Time x group	0.75	1.85, 120.18	0.46	–
	Time	1.91	1.85, 120.18	0.16	–
	Group	0.67	1, 65	0.42	–
Heart rate—t0	Time x group	0.93	2.82, 166.45	0.42	–
	Time	3.94	2.82, 166.45	0.01*	0.06
	Group	0.48	1, 59	0.49	–
Heart rate—t1	Time x group	0.34	2.85, 179.23	0.79	–
	Time	5.48	2.85, 179.23	<0.01*	0.08
	Group	<0.01	1,63	0.96	–
Heart rate—t2	Time x group	0.53	3.52, 175.85	0.69	–
	Time	5.03	3.52, 175.85	<0.01*	0.09
	Group	0.02	1, 50	0.89	–
RMSSD—t0	Time x group	0.47	5.85, 345.22	0.83	–
	Time	3.72	5.85, 345.22	<0.01*	0.06
	Group	0.38	1, 59	0.54	–
RMSSD—t1	Time x group	0.64	4.34, 273.63	0.65	–
	Time	4.64	4.34, 273.63	<0.01*	0.07
	Group	0.70	1, 63	0.41	–
RMSSD—t2	Time x group	0.84	4.77, 238.53	0.52	–
	Time	3.81	4.77, 238.53	<0.01*	0.07
	Group	0.90	1, 50	0.35	–
Systolic pressure—t0	Time x group	0.99	6.40, 390.08	0.44	–
	Time	1.29	6.40, 390.08	0.26	–
	Group	0.77	1, 61	0.38	–
Systolic pressure—t1	Time x group	0.70	2.78, 158.19	0.55	–
	Time	4.70	2.78, 158.19	<0.01*	0.08
	Group	0.13	1, 57	0.72	–
Systolic pressure—t2	Time x group	0.79	5.41, 335.45	0.56	–
	Time	2.03	5.41, 335.45	0.07	–
	Group	0.42	1, 62	0.52	–

Group, with/without mission abroad; time, measurement points within one TSST-G; effect sizes are shown if results are significant; cortisol (nmol/l), heart rate (bpm); root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD) (ms), systolic pressure (mm/Hg).
*Significant at the <0.05 level; df, degrees of freedom.

on the significance level of $p < 0.05$. As a measure for effect size for the ANOVAs the partial eta squared ($\eta 2$) was reported.

3 Results

3.1 Descriptive data

Descriptive data for male deployed and non-deployed soldiers at baseline measure is illustrated in Table 1. The average stay for a mission abroad was about 3 months long (m = 3.23, sd = 1.8). None of the participants had a posttraumatic stress disorder at any measurement time point (t0, t1, t2). Descriptive data representing all included male and female soldiers can be found in the Supplementary Table 1). The gender distribution was 21 (23%) female and 70 (77%) male soldiers. Among females, 14 were in the control group and seven in the experimental group, whereas 37 male soldiers had a mission abroad and 33 did not. The activation of biophysiological variables was tested (absolute values) to show

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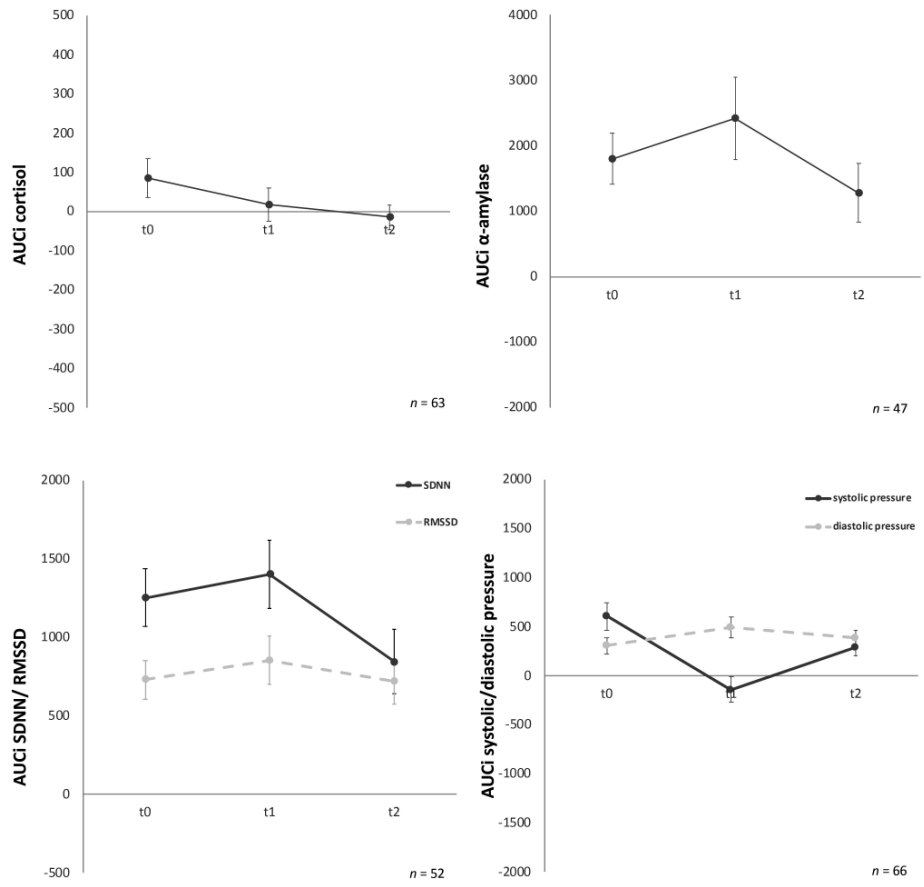

FIGURE 3
Stress response of cortisol, α-amylase, standard deviation of all normal-to-normal inter-beat intervals (SDNN) and root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD), systolic and diastolic pressure for the three time points (t0, t1, t2). Y-axis values: AUCi, area under the curve with respect to increase for cortisol (nmol/l), amylase (U/ml), SDNN/RMSSD (ms), systolic/diastolic pressure (mm/Hg). Values are means (± SEM).

changes in the groups with/without mission abroad within the TSST-G experiments for men. Exemplary results can be found in Figure 2. For cortisol, heart rate, RMSSD and systolic pressure with TSST-G t0, t1, and t2, the mixed ANOVA revealed non-significant results for all group and time x group effects. Most time effects were significant with $p < 0.001$ (Table 2).

3.2 Psychobiological habituation of stress response

Psychobiological habituation effects then were examined across the three TSST-Gs for all participants (female and male). For cortisol AUCg and AUCi no significant decrease between t0, t1, and t2 were given [F (1.36, 81.37) = 0.03, $p = 0.93$ and F (1.58, 95.03) = 0.36, $p = 0.65$], no habituation effects could be found (Figure 3).

For AUCg amylase no significant differences over time [F (2, 88) = 0.06, $p = 0.94$) were found either. Similarly, AUCi amylase (Figure 3) showed no significant effects over time [F (2, 88) = 1.43, $p = 0.25$].

For AUCg and AUCi SDNN, again no significant differences for the three time-points were given, F (2, 100) = 0.41, $p = 0.67$ and F (2, 100) = 0.19, $p = 0.82$, respectively (Figure 3). AUCg and AUCi RMSSD showed no significant effects either [F (1.78,

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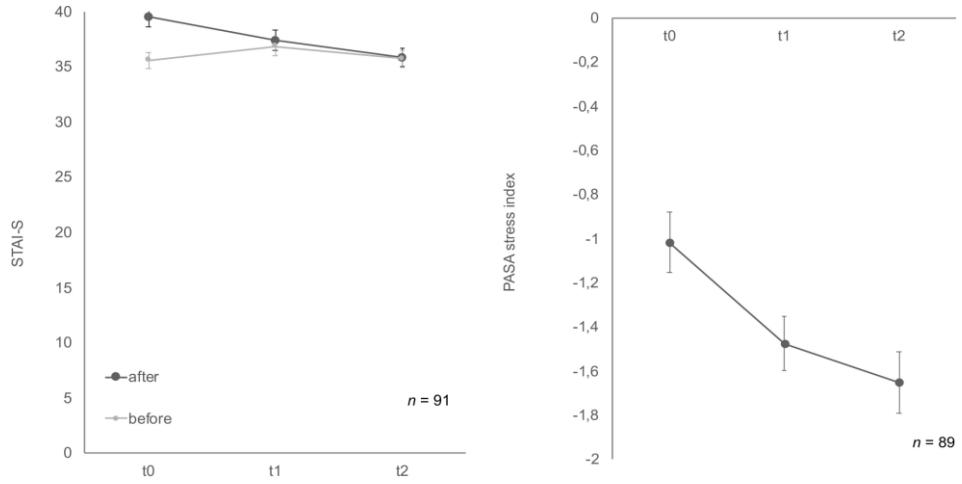

FIGURE 4
Stress response of state-trait-anxiety inventory: before and after mental stress task (STAI-S) before/after mental stress and primary appraisal secondary appraisal (PASA) during Trier Social Stress Test for groups (TSST-G) for the three measurement points (t0, t1, t2). Values are means (± SEM).

89.02) = 0.33, p = 0.70 and F (2, 100) = 0.56, p = 0.57, respectively).

Analyses of variance for systolic pressure determined that AUCg did not show any significant differences between the three time points, F (2, 126) = 2.83, p = 0.06. Similarly, AUCi systolic pressure (Figure 2) did not habituate over time either, F (2, 126) = 0.60, p = 0.55. No significant change over time for the three timepoints were given for AUCg diastolic pressure [F (2, 126) = 0.95, p = 0.39] and AUCi diastolic pressure [F (2, 128) = 1.57, p = 0.21] (Figure 3).

State-trait-anxiety inventory: before and after mental stress task for anxiousness revealed a significant habituation effect after the TSST-Gs for the three time points, F (2, 174) = 10.11, $p < 0.001$, partial $\eta^2 = 0.104$ (Figure 4), whereas the anticipatory anxiousness before stress did not show any differences, F (2, 180) = 1.75, p = 0.18. In the Bonferroni-adjusted post-hoc analysis STAI-S after mental stress showed a significant decrease ($p = 0.03$) in t0 and t1 [2.10, 95%-CI (0.15, 4.06)] and between t0 and t2 [$p < 0.001$, 3.59, 95%-CI (1.58, 5.60)].

Cognitive appraisal also habituated over time (Figure 4). The ANOVA determined that PASA showed a statistically significant difference between measurements, F (1.77, 148.46) = 11.65, $p < 0.001$, partial $\eta^2 = 0.12$. Bonferroni-adjusted post-hoc analysis revealed a significant decrease ($p < 0.01$) in t0 and t1 [0.40, 95%-CI (0.12, 0.67)] as well as between t0 and t2 [$p < 0.001$, 0.59, 95%-CI (0.24, 0.95)].

Soldiers' mood showed differences over time for MDBF scale (Figure 5) good/bad mood before and after mental stress as well as for the dimension calmness/restlessness after the TSST-G. Good/bad mood before indicated habituation effects over time, F (2, 178) = 3.41, $p = 0.04$, partial $\eta^2 = 0.04$. Bonferroni-adjusted *post-hoc* analysis revealed a significant decrease (p = 0.02) between t0 and t1 [0.69, 95%-CI (0.10, 1.28)]. In comparison, good/bad mood after mental stress increased F (2, 170) = 4.94, $p = 0.01$, partial $\eta^2 = 0.06$. Bonferroni-adjusted *post-hoc* analysis showed a significant difference (p = 0.02) in t0 and t1 [−0.74, 95%-CI (−1.41, −0.08)] as well as between t0 and t2 [p = 0.03, −0.79, 95%-CI (−1.52, −0.06)]. Calmness/restless after mental stress also significantly increased F (2, 172) = 11.84, $p < 0.001$, partial $\eta^2 = 0.12$. Bonferroni-adjusted *post-hoc* analysis showed a significant difference ($p < 0.01$) in t0 and t1 [−1.13, 95%-CI (−1.83, −0.43)] as well as between t0 and t2 [$p < 0.001$, −1.38, 95%-CI (−2.12, −0.63)]. For the bipolar dimension alertness/tiredness before no statistically significant differences for the three timepoints were given, F (2, 180) = 1.12, p = 0.33. Similarly, alertness/tiredness after showed no significant effects either, F (2, 168) = 2.24, p = 0.11. No habituation effects could be found for the dimension calmness/restless before the mental stress task, F (2, 178) = 2.41, p = 0.09.

3.3 Influence of mission abroad on TSST-G stress response

Group effects for male soldiers with and without mission abroad over t0, t1, and t2 were subsequently calculated. The covariates age for biological variables and number of missions

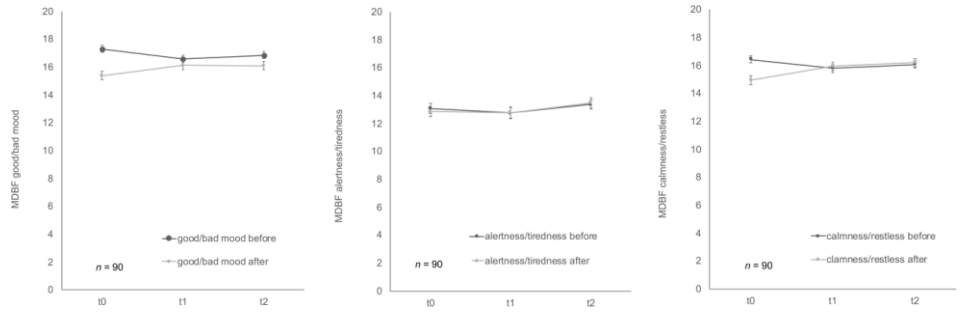

FIGURE 5
Stress response of multidimensional mood state questionnaire (MDBF) before and after stress exposure during Trier Social Stress Test for groups (TSST-G) for the three measurement points (t0, t1, t2). Values are means (± SEM).

abroad for all variables were taken into account. For AUCi cortisol a significant interaction between time and group, F (1.61, 72.5) = 4.13, p = 0.03, partial η^2 = 0.08 was found. On closer examination it got clear that the experimental and the control group showed differences as a trend from the start (t0), F (1, 47) = 3.88, p = 0.06 but adjust over time (Figure 6). Therefore, no significant difference in t1 or t2 could be found.

For all other biological, physiological and psychological parameters no significant time x group interaction effects could be detected (Table 3). Mission abroad did not show any significant influence on the stress response outcome when compared to non-deployed soldiers. Exemplary representations of these results can be found in Figure 6.

4 Discussion

4.1 Habituation effects in soldiers and the influence of mission abroad

No habituation effects over the three time-points were detectable for any of the biological and physiological variables cortisol, sAA, RMSSD, SDNN, systolic and diastolic pressure in female and male soldiers. However, psychological stress data indicated habituation effects for PASA and STAI-S after mental stress as well as for MDBF dimension good/bad mood before mental stress. According to the psychological scales, soldiers tend to show less anxiety and stress-appraisal but showed impaired mood. After the mental stress task from the TSST-G the dimensions calmness/restlessness and good/bad mood increased significantly, therefore soldiers tended to be more relaxed and were in a better mood over the three time points.

Foreign deployment in male soldiers did not seem to have any significant effects neither on the physiological or biological nor on the psychological stress response, both groups reacted in the same way to mental stress over time. Only cortisol showed marginally significant differences between the groups before the intervention (t0) which may result from the lack of randomization of the groups due to our study design.

Our findings show a different pattern than findings of other research groups that found habituation mostly for cortisol/HRV and an increase in sAA as an anticipation reaction (21, 27, 51, 52). These contrary results could be explained by using different study designs. Most research on repeated mental stress focused on relatively short time periods between the repetition, mostly a few days or at most weeks (51, 52), whereas our study focused on long-time effects with 3 months between t0 and t1 and even a 1 year period for the t2 follow-up. Therefore, these results reflect the stress adaptation mechanism to a repeated stressor over a relatively longer time period. The biological fight or flight reaction stays stable over time and a reaction is provoked every single time a potential threatening stressor is presented, whereas an adaptation to the stressor can be expected if the stressor is presented more regularly within a shorter period of time (53). This possible explanation is supported by one of the habituation criteria from Thompson and Spencer (22) that implies that rapid frequency (time) of a stressor accelerates and enhances habituation. It could be possible that the HPA and SAM stress activation have a sensitive time frame for "remembering" repeated stress, which leads to already shown habituation or activation changes, whereas when the same stressor repeatedly occurs outside of this time frame no memorizing and changes in the biological stress answer patterns can be found anymore.

The group setting could be another possible impact on the different stress activation when compared to previous studies which had mostly been performed using the single TSST (52, 54, 55). The soldiers were confronted with other participants right from the start and they did not know who the other participants were going to be for each TSST-G. This generated a situation where the participants experienced social evaluation which increased social-self threat, shame and a decrease in

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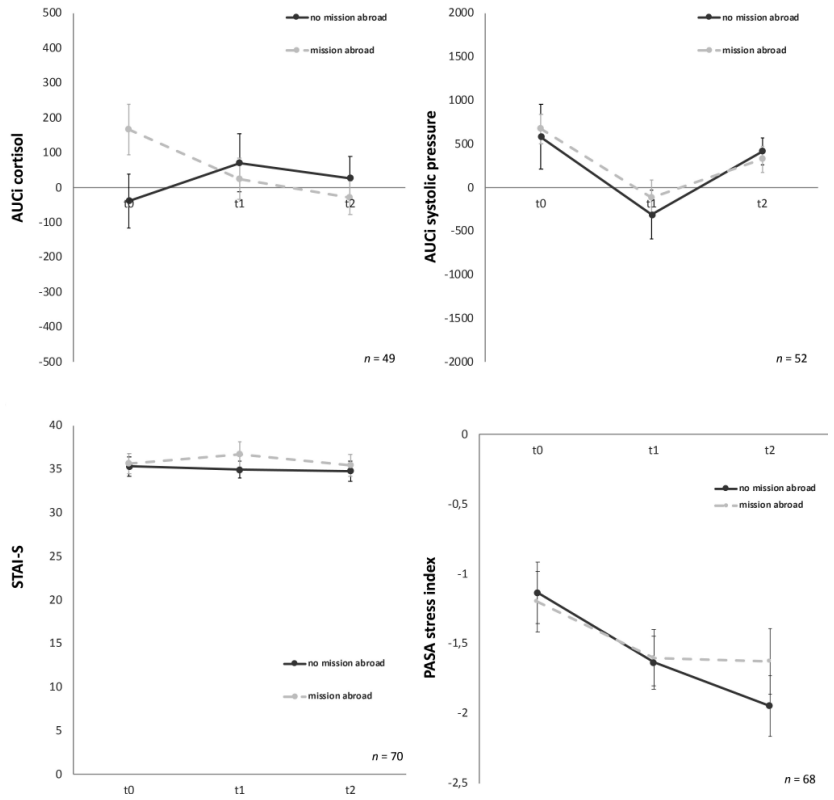

FIGURE 6
Stress response of cortisol, systolic pressure, state-trait-anxiety inventory: before and after mental stress task (STAI-S) and primary appraisal secondary appraisal (PASA) for the three time points (t0, t1, t2). Y-axis values: AUCi, area under the curve with respect to increase for cortisol (nmol/l) and systolic pressure (mm/Hg). STAI-S before mental stress and PASA during Trier Social Stress Test for groups (TSST-G); values are means (± SEM).

self-esteem through the possibility of their performance being judged by others (56). Psychosocial stress situations activate the HPA-axis response (57) and the effect of the group setting is well-established to provoke a robust psychobiological stress response (33). Especially the TSST-G evoked an even higher response to the stressor then in the single setting (31). This could lead to a suppressed biological habituation because of the instability of a changing group for each time-point. These conditions supported the usability of the repeated TSST-G in our case because in this study a stable biological stress response without significant changes occurred throughout the three measurements. The TSST-G seems to be a reliable instrument with the ability to provoke a stress response without biophysiological habituation effects even if used repeatedly on the same population after at least 3 months. Finally, the sample size from our BEST-study was larger than in other studies (36, 52, 58). Smaller sample sizes mostly result from the complex and time-intensive procedure of the single TSST.

The results of the psychological changes of anxiety and stress perception to a mental task measured with PASA and STAI-S are in line with the literature (51, 58, 59). The results in this study showed habituation of anxiousness after the mental stress task and cognitive appraisal for t1 and t2 in comparison to the baseline t0. These findings were expected and result from the already known task and procedure for the second and third stress test. Subjective indices of activation have been shown to decrease over time whereas the performance level even increases, due to the psychological habituation to the stimulus it is possible to concentrate more on the performance level (28). Consequently, this may lead to the conclusion that in this study the biological adaptation program is not as sensitive for delayed stress stimuli as the psychological answer. Briefly,

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TABLE 3 Results for the group x time interaction effects (mixed ANOVA) in men across trier social stress test for groups (TSST-Gs) (t0, t1, t2).

Variable	Effect	*F*	*df*	*p*	Partial η^2
Cortisol (AUCg)	Time x group	0.94	1.44, 64.79	0.37	–
	Time	0.42	1.44, 64.79	0.60	–
	Group	0.09	1, 45	0.76	–
Amylase (AUCg)	Time x group	0.12	2, 66	0.89	–
	Time	0.75	2, 66	0.48	–
	Group	0.22	1, 33	0.64	–
Amylase (AUCi)	Time x group	0.61	1.57, 51.65	0.51	–
	Time	0.45	1.57, 51.65	0.60	–
	Group	0.32	1, 33	0.58	–
SDNN (AUCg)	Time x group	0.46	2, 74	0.63	–
	Time	0.01	2, 74	0.99	–
	Group	<0.01	1,37	0.97	–
SDNN (AUCi)	Time x group	0.51	2, 74	0.60	–
	Time	0.33	2, 74	0.72	–
	Group	1.13	1, 37	0.29	–
RMSSD (AUCg)	Time x group	0.44	2, 74	0.65	–
	Time	0.08	2, 74	0.92	–
	Group	0.11	1, 37	0.75	–
RMSSD (AUCi)	Time x group	0.03	1.59, 58.88	0.95	–
	Time	0.16	1.59, 58.88	0.80	–
	Group	0.62	1, 37	0.44	–
Systolic pressure (AUCg)	Time x group	2.38	2, 94	0.10	–
	Time	4.96	2, 94	<0.01*	0.10
	Group	0.02	1, 47	0.89	–
Systolic pressure (AUCi)	Time x group	0.07	2, 94	0.94	–
	Time	1.75	2, 94	0.18	–
	Group	0.02	1, 47	0.89	–
Diastolic pressure (AUCg)	Time x group	0.98	2, 94	0.38	–
	Time	2.45	2, 94	0.09	–
	Group	<0.01	1,47	0.96	–
Diastolic pressure (AUCi)	Time x group	0.27	2, 94	0.77	–
	Time	0.88	2, 94	0.42	–
	Group	0.11	1, 47	0.75	–
STAI-S before stress	Time x group	0.77	2, 132	0.47	–
	Time	1.06	2, 132	0.35	–
	Group	0.27	1, 66	0.61	–
STAI-S after stress	Time x group	0.89	2, 126	0.41	–
	Time	5.13	2, 126	<0.01*	0.08
	Group	0.05	1, 63	0.83	–
PASA	Time x group	0.70	1.72, 104.85	0.50	–
	Time	5.88	1.72, 104.85	<0.01*	0.09
	Group	0.15	1, 61	0.70	–
MDBF good/bad mood before stress	Time x group	1.09	2, 130	0.34	–
	Time	2.85	2, 130	0.06	–
	Group	0.45	1, 65	0.51	–
MDBF good/bad mood after stress	Time x group	1.49	2, 124	0.23	–
	Time	3.49	2, 124	0.03*	0.05
	Group	0.14	1, 62	0.71	–

(Continued)

TABLE 3 (Continued)

Variable	Effect	*F*	*df*	*p*	Partial η^2
MDBF alertness/tiredness before stress	Time x group	0.05	2, 132	0.95	–
	Time	0.67	2, 132	0.52	–
	Group	<0.01	1,66	0.99	–
MDBF alertness/tiredness after stress	Time x group	0.25	2, 120	0.78	–
	Time	1.70	2, 120	0.19	–
	Group	2.81	1, 60	0.10	–
MDBF calmness/restlessness before stress	Time x group	0.90	2, 130	0.41	–
	Time	1.14	2, 130	0.32	–
	Group	0.05	0, 65	0.83	–
MDBF calmness/restlessness after stress	Time x group	0.04	2, 126	0.96	–
	Time	13.08	2, 126	<0.01*	0.17
	Group	0.98	1, 63	0.33	–

Group, with/without mission abroad; time, t0, t1, t2; AUC, area under the curve; effect sizes are shown if results are significant. STAI-S, state-trait-anxiety inventory; PASA, primary appraisal secondary appraisal: overall stress index; MDBF, multidimensional mood state questionnaire. *Significant at the <0.05 level; df, degrees of freedom.

participants remember the past stress situation in terms of mood, anticipation and the attitude towards it and react to the known stimulus differently (51, 58, 59), whereas the body does not change the adaptation program when the repetition of the stress stimuli is delayed (53). This leads to the conclusion that it could be important to consider the differentiation between the biological and psychological stress answer system to mental stress, especially when it comes to coping and buffering mechanisms.

There is a lack of research about changes of the psychobiological stress reaction after foreign deployment in soldiers, especially regarding to a stable and consistent stress response before and after mission abroad. To the best of our knowledge this is the first study showing psycho-physio-biological stress responses to acute mental stress in relation to a mission abroad. Our results show that the reaction and adaptation to repeated stress does not seem to be influenced by a mission abroad and the experiences coming along with it. Different reasons may play a role in this phenomenon. Resilience of the soldiers like family support can act as compensatory mechanisms during deployment experiences (60). It is known that the recovery phase and a supportive environment after stress has a major impact on processing negative experiences and consequently psychological health (61). For a wider perspective, psychosocial aspects after mission abroad which are also collected in the context of the BEST-study could be associated with stress response in a next step. Personality characteristics like hardiness or group cohesion may also function as helpful traits for coping (62). Furthermore, the term "healthy soldier effect" describes a lower mortality rate in military personnel than in the general population due to physical exercise, regular medical examinations and direct access to medical care (63). Nevertheless these findings depend on the type and area of deployment as well as the follow-up time (64). Clearly, more research considering potential moderating variables is mandatory to clarify the underlying mechanisms for the shown resilience.

4.2 Limitations

The sample size was limited and unbalanced with respect to the distribution of male and female participants. However, this distribution reflects the unequal gender distribution in the military in general, even if 23 % women in our sample is a higher proportion when compared to the overall distribution in the German army, which was 12.3 % in 2019 (65). Therefore, any generalization to the whole population has to be confirmed in a non-soldier sample to increase extern validity. The sample in this study included healthy, middle-aged German soldiers that undergo regular medical examination. This may also positively affect the extrapolation of the study results to the general population. The overall healthiness of the soldiers could allow the conclusion that the results could hold also for different samples. No randomisation for deployed and non-deployed soldiers was possible as the soldiers were appointed by the German military for a mission abroad which could lead to selection bias from the start. In this context the group x time interaction effect for cortisol could be explained like mentioned above. It must be taken into consideration that the participating soldiers were deployed to rebuilding zones, which cannot be compared with the stress response and habituation changes in soldiers experiencing acute mortal danger and continual front-line status with little to no opportunity for decompression and relaxation. The focus in this study group did not include major traumatic stress, it solely dealt with social stress factors e.g., sleep deprivation or being separated from family. Less than half of the participants went through all three stress tasks which could lead to selection bias towards more conscientious soldiers. Also, the time periods between measurements differed between

the individuals because the soldiers did not all go to a mission abroad at the same time. The admission to the second and third stress test could not precisely be planned because the soldiers were allocated all over the country, therefore the 3 months and 1 year time lag differed slightly. Further investigation and a different population are needed to reduce these biases and to confirm the presented results.

To our knowledge this study is one of the first to examine psychobiological habituation effects in a group setting on repeated mental stress over prolonged time periods in soldiers. The method of calculating AUCi and AUCg allowed us to display changes of the biological stress answer during the whole TSST-G session and therefore we were not limited to only one specific time point, which always leads to informational loss. This approach covered for individual differences like a prolonged or delayed stress response. It has to be mentioned that the AUC is not suitable for investigations within one TSST-G trial because it may mask differences at specific time points. The used TSST-G procedures provided a standardized protocol for great comparability and additionally we were able to address the question whether a mission abroad can lead to changed stress adaptation.

5 Conclusion

In summary our results suggest that the stress sensitive systems of our body do not habituate or adapt to repeated mental stress over longer time periods while the affective response shows changed experience and behavior patterns. In particular, no effects of foreign deployment on the habituation to mental stress in soldiers could be found which indicates the possible conclusion that the partially stressful experiences during a mission abroad have no direct effect on the psychobiological adaption program. These findings are important for the research to stress-related mental health and disease in military personnel and their resilience. To gain an insight in the potential underlying mechanism would be of great interest, especially for expanding and complementing suitable supportive interventions before and after missions abroad.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Commitee of Ulm University, Ulm, Germany. The patients/participants provided their written informed consent to participate in this study.

Author contributions

TM, CW, and MR: conceptualization. CW and MR: methodology. TM: formal analysis and writing—original draft preparation. D-SR, MR, and TM: investigation. CW, PR, and MR: writing—review and editing. TM, MR, D-SR, JM, and FP: formal analysis. CW, HG, SB, BF, and H-PB: funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding

This research was supported by the German MoD (E/U2CA/FA159/FF552). The investigation was authorized by the BMVg (Ministery of Defense—registration number 3/06/17).

Acknowledgments

We thank Clemens Kirschbaum, Institut für Allgemeine Psychologie, Biopsychologie und Methoden der Psychologie, Technische Universität, Dresden, Germany, for measuring the salivary cortisol and α-amylase activity.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.1011181/full#supplementary-material

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Waller, C., Rhee, D.-S., Gröger, M., Rappel, M., Maier, T., Müller, M., Rottler, E., Nerz, K., Nerz, C., Brill, S., Becker, H.-P., & Radermacher, P. (2020). Social Stress-Induced Oxidative DNA Damage Is Related to Prospective Cardiovascular Risk. *Journal of Clinical Medicine, 9*(11), 3783. https://doi.org/10.3390/jcm9113783

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Declaration on own contributions:

- Psychobiological data collection
- Conduct of the TSST-G study protocol
- Data transmission and data preparation
- Support with first draft and proofreading of the manuscript

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Social Stress-Induced Oxidative DNA Damage Is Related to Prospective Cardiovascular Risk

Christiane Waller [1,*], Dae-Sup Rhee [2,3], Michael Gröger [3], Manuela Rappel [2], Tanja Maier [2], Markus Müller [1], Edit Rottler [2], Katharina Nerz [3], Christopher Nerz [3], Sebastian Brill [4], Horst-Peter Becker [5] and Peter Radermacher [3]

1 Klinik für Psychosomatische Medizin und Psychotherapie, Universitätsklinik der Paracelsus Medizinischen Privatuniversität, 90419 Nürnberg, Germany; markus.mueller@klinikum-nuernberg.de
2 Klinik für Psychosomatische Medizin und Psychotherapie, Universitätsklinikum, 89081 Ulm, Germany; dae-sup.rhee@uni-ulm.de (D.-S.R.); manuela.rappel@uni-ulm.de (M.R.); tanja.maier@uni-ulm.de (T.M.); edit.arbeit@budape.st (E.R.)
3 Institut für Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinikum, 89081 Ulm, Germany; michael.groeger@uni-ulm.de (M.G.); katharina.nerz@tukaneph.de (K.N.); christopher@nerz.me (C.N.); peter.radermacher@uni-ulm.de (P.R.)
4 Klinik für Allgemein-, Viszeral- und Thoraxchirurgie, Bundeswehrkrankenhaus, 89081 Ulm, Germany; sebastian.brill@web.de
5 Bundeswehrkrankenhaus, 10115 Berlin, Germany; horstpeter.becker@t-online.de
* Correspondence: christiane.waller@klinikum-nuernberg.de; Tel.: +49-911-398-2839; Fax: +49-911-398-3675

Received: 6 October 2020; Accepted: 18 November 2020; Published: 23 November 2020



Abstract: Psychosocial stress increases cardiovascular risk, which coincides with enhanced oxidative DNA damage. Increased sympathetic tone-related catecholamine release causes oxidative stress, which contributes to catecholamine-related cardiotoxicity. Therefore, we tested the hypothesis whether acute psychosocial stress induces oxidative DNA damage, its degree being related to the cardiovascular risk profile and depending on the sympathetic stress response. After assessment of the prospective cardiovascular Münster score (PROCAM) to determine the risk of acute myocardial infarction, 83 male and 12 female healthy volunteers underwent the Trier social stress test for groups (TSST-G). Heart rate variability was quantified by measuring the standard deviation (SDNN) and root mean square of successive differences (RMSSD) between normal-to-normal inter-beat intervals. Salivary α-amylase (sAA) activity was assessed as a surrogate for noradrenaline plasma concentrations. Oxidative DNA damage was determined using whole-blood single-cell gel electrophoresis ("tail moment" in the "comet assay"). A total of 33 subjects presented with a prospective risk of myocardial infarction (risk+) vs. 59 subjects without risk (risk-). The TSST-G stress significantly increased blood pressure, heart rate, and sAA in both groups, while oxidative DNA damage was only increased in the risk+ group. Immediately after the TSST-G, the "tail moment" showed significant inverse linear relations with both SDNN and RMSSD. Acute psychosocial stress may cause oxidative DNA damage, the degree of which is directly related to the individual cardiovascular risk profile and depends on the stress-induced increase in the sympathetic tone.

Keywords: Trier stress test for groups; PROCAM score; single-cell gel electrophoresis; heart rate variability; noradrenaline

1. Introduction

Psychosocial stress is associated with cardiovascular risk [1,2], in part due to autonomic dysbalance resulting from a rise in sympathetic tone [3]. Increased superoxide radical formation contributes to catecholamine-related cardiotoxicity [4,5], and enhanced oxidative DNA damage coincides with

cardiovascular risk [6]. However, studies investigating the potential impact of social stress on oxidative stress-induced DNA damage are rare [7], and evidence of a putative association with sympathetic stress response is lacking.

Therefore, our study tested the hypothesis that (i) acute psychosocial stress may cause oxidative DNA damage, (ii) the degree of which is directly related to the individual cardiovascular risk profile and (iii) depends on the stress-induced increase in the sympathetic tone.

2. Methods

All subjects gave their written informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Ulm University (Antrag Nr. 253/12 - "Auslandseinsatz und Auswirkungen der daraus resultierenden Stresseffekte auf die kardiovaskuläre Gesundheit", approval date 18 December 2012; trial registration DRKS00022345). We investigated 83 male and 12 female healthy volunteers (mean (range) age: 31 (20–48) years) as a part of the "German Armed Forces Deployment and Stress" study. Three to six soldiers were scheduled to the lab (always at 5:00 p.m.), and a venous catheter was inserted prior to a 30-min resting period. Thereafter, subjects underwent the Trier social stress test for groups (TSST-G), which combines a social stress comprising a 10-min anticipatory stress following a short introduction, a 2-min mock job interview, and a 80-s mental and cognitive arithmetic task [8]. The prospective cardiovascular Münster (PROCAM) score was calculated from eight independent risk variables (age, smoking history, systolic blood pressure, family history of premature myocardial infarction, diabetes mellitus, HDL cholesterol, LDL cholesterol, and triglyceride blood level) to determine the risk of acute myocardial infarction within ten years [9]. Subjects with an at least 1% prospective risk of myocardial infarction were defined as "risk+" vs. subjects without prospective risk ("risk-").

Heart rate variability was derived from 3-lead digital electrocardiogram recordings using the eMotion Faros 180° biosensor (BlindSight GmbH, Schlitz, Germany) with a sampling rate of 500 hertz. Two time domain measures were computed for each subject using 40-s tracings at the stages of TSST-G: standard deviation of all normal-to-normal inter-beat intervals (SDNN), and root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD) [10,11]. PROCAM scores were lacking in three subjects due to missing smoking information and blood sampling, and in another six due to missing heart rate variability data due to technical problems. Oxidative DNA damage was assessed using single-cell gel electrophoresis of whole blood samples obtained before as well as immediately (within 1 min) and 60 min after the TSST-G. After overnight lysis at 4° C, alkaline (pH = 13, in order to transform alkali-sensitive parts of the DNA into DNA strand breaks) denaturation, electrophoresis performed at 300 mA and 25 V for 25 min, and ethidium bromide staining for the mean "tail moment" (=DNA percentage tail length) were calculated in 100 randomly selected nuclei using image analysis software (COMET Assay IV, vers. 4.3, Perceptive Instruments, Haverhill, UK) [12,13]. Nuclei with a calculated "tail moment" of <0.1 were qualified as "undamaged" [13]. Salivary α-amylase (sAA) activity was measured as a surrogate of plasma noradrenaline concentrations using an enzyme kinetic method as described previously [14]. Samples were obtained before as well as immediately (within 1 min) and 40 min after the TSST-G. Briefly, after dilution, saliva aliquots and standard solutions were incubated with the reagent (a-amylase EPS Sys; Roche Diagnostics, Mannheim, Germany) on a microplate. Thereafter, absorbance was measured at a wavelength of 405 nm using a standard ELISA reader (Anthos Labtech HT2, Anthos, Krefeld, Germany). Due to an insufficient amount of saliva available, data could be obtained in 77 of the participants only. A flow chart of the investigational protocol is shown in Figure 1.

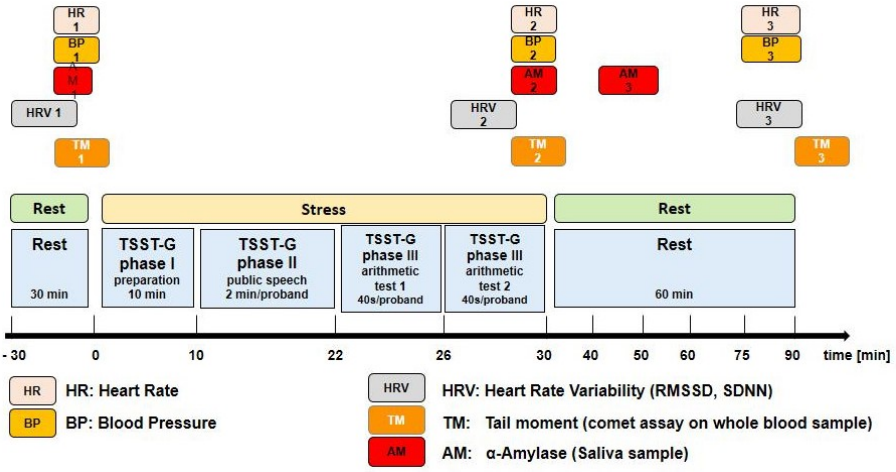


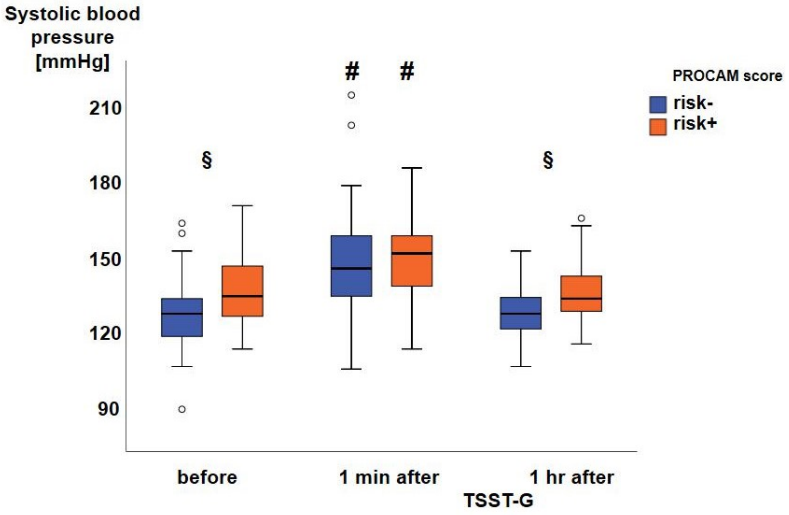
Figure 1. Flow chart of the investigational protocol.

Statistical Analysis

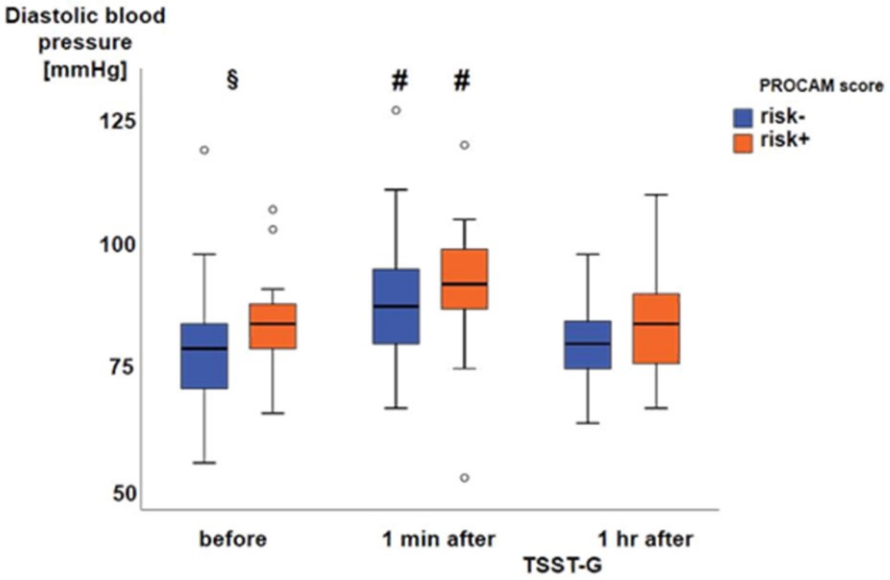
Data were analyzed with the statistic package SPSS (Ver.25, IBM, USA). Normal data distribution was tested using the Kolmogorov–Smirnov test. Data are presented as median (quartiles, range). Time-dependent within-group effects of the stress exposure on hemodynamic parameters and sAA were analyzed using a Friedmann ANOVA on ranks and a post hoc Mann–Whitney rank sum test with Bonferroni correction for multiple comparisons. Analysis of covariance (ANCOVA) for repeated measures (controlled for gender) was used to assess the group-by-stress effect on the "tail moment" in the "comet assay". In order to characterize the relationship between the "tail moment" plotted as a function of SDNN and RMSSD, generalized linear modeling with repeated measures and partial correlations was used. Non-linear (i.e., logarithmic, exponential, and/or hyperbolic) models were discarded since the respective calculated regression coefficients did not yield improved mathematical modeling when compared to linear relationships. Significance was stated at $p < 0.05$.

3. Results

Thirty-three subjects had an at least one percent prospective risk of myocardial infarction (risk+), whereas 59 subjects presented without prospective risk (risk-). Prior to the TSST-G, both systolic (Figure 2A) and diastolic (Figure 2B) blood pressures were significantly higher in the risk+ than in the risk- group, while heart rate (Figure 2C) did not significantly differ between the two groups. One minute after the TSST-G, both systolic and diastolic pressures as well as heart rate were significantly higher than before the TSST-G without any intergroup differences (Figure 2A–C). All hemodynamic values had returned to baseline levels at one hour after the TSST-G, however, the difference between the risk- and risk+ groups present prior to the TSST-G only persisted for systolic blood pressure (Figure 2A). Salivary α-amylase activity was also significantly increased at one minute after the TSST-G, again without difference between the risk- and risk+ groups (Figure 3). TSST-G stress exposure significantly increased oxidative DNA damage in the risk+ group as evidenced by the increased "tail moment" in the "comet assay" demonstrated by the significant group-by-stress effect in the ANCOVA for repeated measures ($F(1.85; 164.92) = 5.425$, $p = 0.006$). In contrast, no significant effect of the TSST-G could be detected in the risk- subjects (Figure 4). Linear correlations for all participants taken together showed significant inverse relationships of the "tail moment" plotted as a function of SDNN ($r = -0.216$, $p = 0.046$) (Figure 5A) and RMSSD ($r = -0.269$, $p = 0.012$) (Figure 5B) immediately after the TSST-G stress.

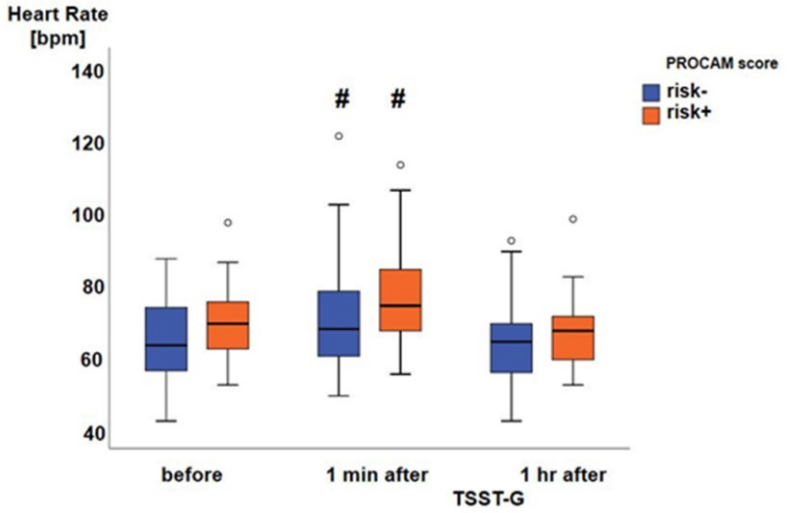


(A)



(B)

Figure 2. *Cont.*



(**C**)

Figure 2. Systolic (**A**) and diastolic (**B**) blood pressure as well as heart rate (**C**) from the 59 subjects without (risk-; blue boxplots) and 33 with (risk+; orange boxplots) cardiovascular risk according to the PROCAM score. Data are presented as median, interquartile range, minimum, and maximum, # denotes $p < 0.05$ vs. before and 1 h after the TSST-G, and § denotes $p < 0.05$ between the risk- and risk+ groups.

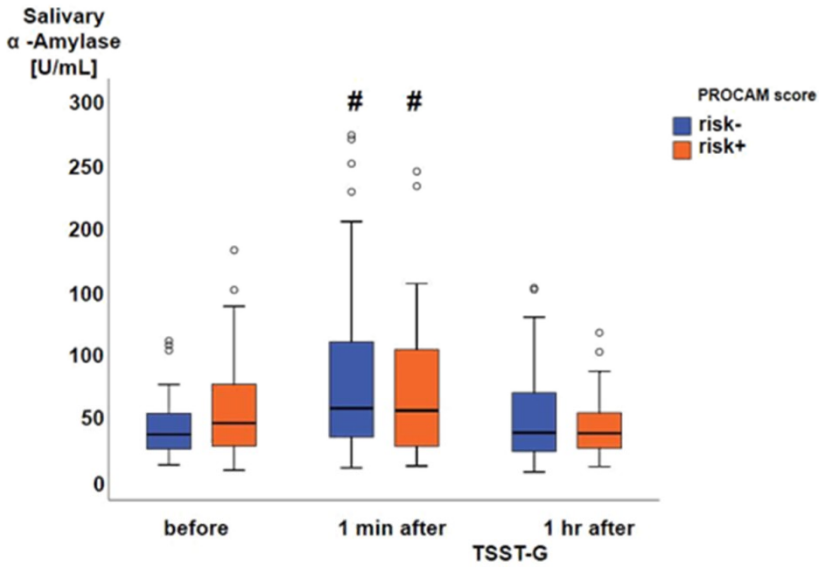


Figure 3. Salivary α-amylase activity from 52 subjects without (risk-; blue boxplots) and 25 with (risk+; orange boxplots) cardiovascular risk according to the PROCAM score. Data are presented as median, interquartile range, minimum, and maximum, and # denotes $p < 0.05$ vs. before and 40 min after the TSST-G.

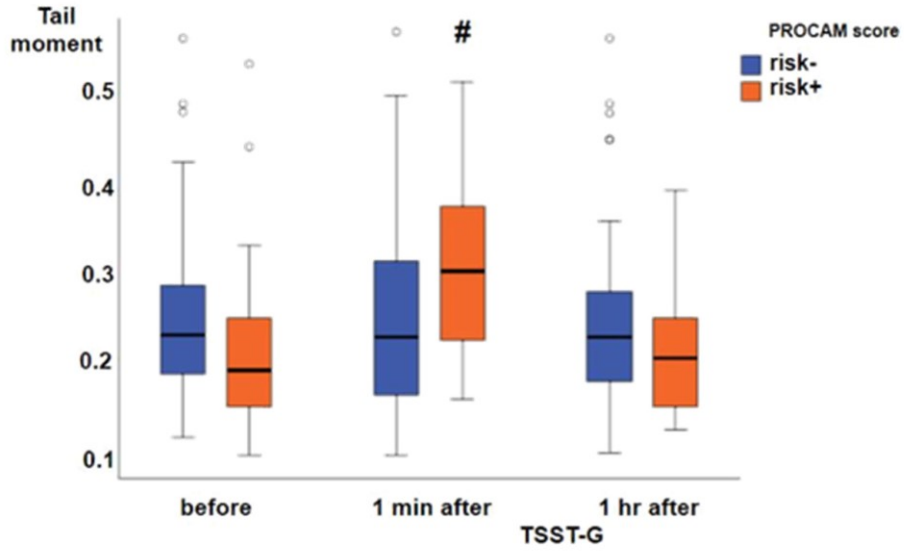
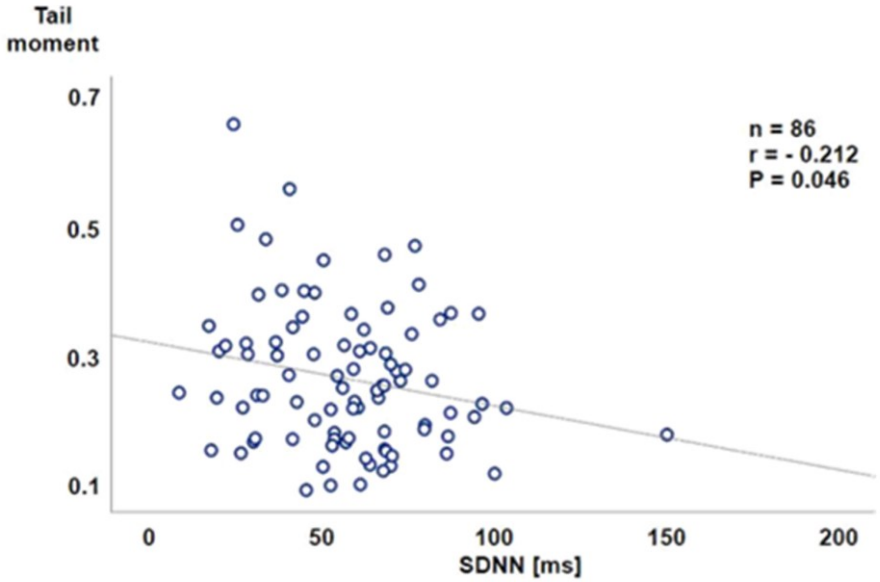
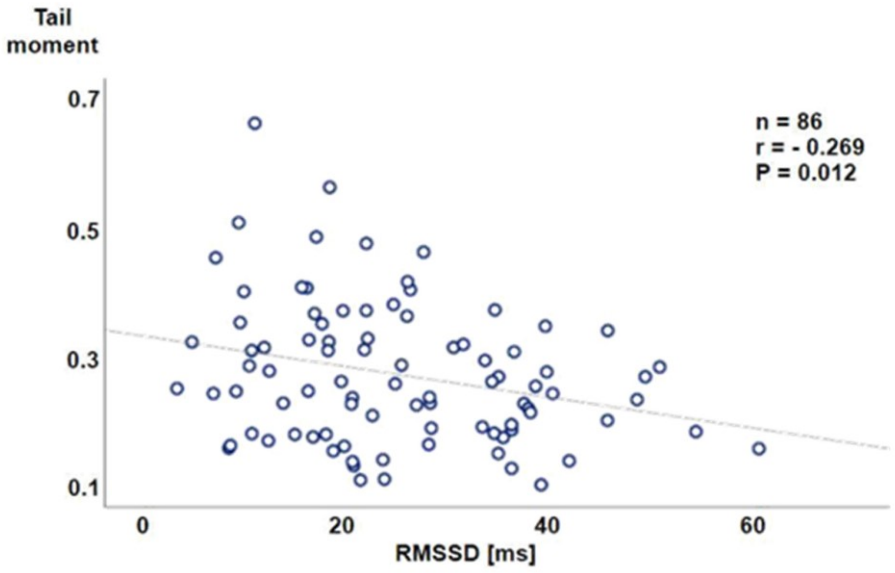


Figure 4. Tail moment in the "comet assay" of whole blood samples from 59 subjects without (risk-; blue boxplots) and 33 with (risk+; orange boxplots) cardiovascular risk according to the PROCAM score. Data are presented as median, interquartile range, minimum, and maximum, and # denotes $p < 0.05$ vs. before and 1 h after the TSST-G in the group-by-stress ANCOVA.



(**A**)

Figure 5. *Cont.*



(**B**)

Figure 5. Tail moment in the "comet assay" 1 min after the TSST-G stress plotted as a function of the normal-to-normal inter-beat intervals (SDNN; millisecond (ms)) (**A**, upper panel) and the root mean square of successive differences between normal-to-normal inter-beat intervals (RMSSD; milliseconds (ms)) (**B**, lower panel). Note that the highest immediate post-TSST-G tail moment values were observed with SDNN and RMSSD below the median of the normal values recently reported for healthy volunteers aged 30–39 years, i.e., the mean age of the study population [11,15].

4. Discussion

In the present study, we found that (i) acute psychosocial stress as induced by a TSST-G per se was able to cause oxidative DNA damage, (ii) which was predominant in subjects with increased prospective cardiovascular risk. Furthermore, (iii) there was an inverse relation between the amount of DNA damage and the post-TSST-G parasympathetic activity.

Prolonged psychological stress is associated with increased cardiovascular risk [1,2]. Prolonged psychological stress is also associated with increased oxidative DNA damage as assessed using the "comet assay" [16,17]. Transitory, short-term exposure to psychological stress, e.g., exam periods, may also lead to enhanced oxidative DNA damage [18]. To the best of our knowledge, the present study is the first to demonstrate that even acute psychological stress may exert comparable effects to acute physiological stress, e.g., exercise, in particular beyond the anaerobic threshold [19]. Finally, this finding was only present in study participants with increased prospective cardiovascular risk according to the PROCAM score, which is in good agreement with the more than doubled incidence of oxidative DNA damage in patients with coronary artery disease [6].

Albeit the comet assay was shown to yield robust and comparable results when following thoroughly defined protocols and standardized routine procedures [20], it may still be prone to potential inter- and intra-laboratory variation [21]. In the present study, the baseline tail moment values were comparable to those found in whole blood samples from different groups of slightly younger healthy volunteers using an identical experimental protocol and equipment [12]. Moreover, the median increase in the tail moment was nearly exactly as high as previously observed in healthy

volunteers after exposure to hyperbaric oxygen [22], which is well established to induce oxidative DNA damage that can be detected using the comet assay [23].

It could be argued that the Framingham risk score may have been more appropriate for risk estimation than the PROCAM score since it has been referred to as being better at estimating the general cardiovascular risk [24]. However, being based on a localized North American cohort, it was also reported to overestimate the cardiovascular risk in European populations when compared to the PROCAM score [25,26], at least in part due to health inequalities between populations of different ethnicities, socio-economic backgrounds, and family histories [27].

Assessment of heart rate variability, in particular RMSSD, allows determining cardiac vagal tone [28] and, hence, evaluating the relation between sympathetic and parasympathetic activity, i.e., autonomic nervous system balance. It is well established that autonomic dysbalance, which is ultimately associated with reduced heart variability, is directly related to increased cardiovascular risk [29]. In our study, the degree of oxidative DNA damage was inversely related to SDNN and RMSSD, and the highest immediate post-TTST-G tail moment values were observed with an SDNN and RMSSD below the median of the normal values of 37.5/43.4 and 37.7/47.7 (males/females) ms, respectively, as recently reported for healthy volunteers aged 30–39 years, i.e., the mean age of the study population [15]. We did not directly measure catecholamine concentrations, but salivary α-amylase (sAA), which is referred to as a surrogate of plasma noradrenaline concentrations [30], significantly increased at one minute after the TSST-G, however without difference between the risk- and risk+ groups. It should be noted in this context that we may have missed any group effect, because 20% of the sAA data were not available due to the insufficient saliva volume. Furthermore, baseline sAAs were about five to six times lower than in previous investigations [14,30], and the TSST-G exposure increased sAA by about 25 mU/mL only. However, investigating the effect of a TSST-G (rather than a single TSST) in healthy volunteers of a comparable age range as in the present study, other authors reported not only similar baseline sAA values, but also that the TSST-G only moderately increased sAA by about 10 mU/mL [31]. In addition, the direct association between the plasma noradrenaline and the sAA response has been questioned due to variations between types and models of stress as well as the responder patterns [32]. Finally, no matter whether the risk- or risk+ group, the TSST-G was associated with a significant increase in both systolic and diastolic blood pressure one minute after stress exposure, and, moreover, Jarczok et al. reported a significant inverse correlation between overnight urinary norepinephrine and nighttime heart rate variability [33]. Hence, despite the lacking effect on sAA, it is likely that the TSST-G led to increased catecholamine in a particular noradrenaline release: consequently, it is tempting to speculate that the more pronounced oxidative DNA damage indicated by the higher tail moment values in the risk+ group was due to higher TSST-G-induced catecholamine release. This conclusion agrees well with the enhanced superoxide radical formation that contributes to noradrenaline-related toxic effects on cardiomyocytes both in vitro and in vivo [4,5], and the observation that oral antioxidant treatment with vitamin C helps the sympathetic response to exercise by attenuating the increase in noradrenaline blood concentrations in patients after myocardial infarction [34]. In line with this finding, other authors showed that antioxidant pre-treatment also reduced DNA damage after strenuous exercise [18].

4.1. Limitations of The Study

Clearly, we did not assess additional parameters of oxidative stress other than the DNA strand breaks using the "comet assay". However, a recent consensus paper stated that although it "*does not provide insight into the nature of the oxidative modification*", the comet assay "*is simple and easy to use*" and provides "*general information about strand breaks*" and, thus, is suitable as a general measure of DNA damage [35].

Our study population was part of the "German Armed Forces Deployment and Stress" study. Consequently, the recruitment was unbalanced with respect to distribution between male and female participants. Moreover, the latter were only in the "risk-" group. Nevertheless, we decided to pool the

complete dataset: statistical analysis based on the data of the male participants alone yielded the same results (data not shown). Hence, any gender-related effect resulting from a skewed study population can be ruled out.

Our choice of using a 1% threshold for stratifying the study population may represent a study caveat. In fact, usually the PROCAM score differentiates between a low (i.e., <10%), intermediate (i.e., 10–20%), and high (>20%) 10-year risk of coronary events [36]. We chose to use this approach because, due to the participants' age and their aptitude for foreign deployment, a low overall cardiovascular risk was anticipated in the study cohort. Hence, validation of the findings in an additional study, preferentially in a cohort with at least "intermediate" risk, is warranted.

Clearly, our data cannot exclude that other effects than the catecholamine-related increase in superoxide radical formation caused the observed aggravation of oxidative DNA damage: using sAA as a surrogate for direct measurement of catecholamine concentrations has inherent weaknesses, and measurement of superoxide radical formation would have required electron spin resonance, which was not available for this study.

4.2. Clinical Implications

It is well established that (i) prolonged psychological stress is associated with increased cardiovascular risk [1,2], (ii) oxidative stress plays a critical role in the initiation and progression of atherosclerosis and coronary artery disease (CAD) [37], and (iii) CAD coincides with a two to three times higher incidence of oxidative DNA damage [6]. We now demonstrate that (i) acute psychosocial stress per se may cause oxidative DNA damage, and (ii) the degree of this effect is directly related to the individual cardiovascular risk profile and (iii) depends on the stress-induced increase in the sympathetic tone.

Author Contributions: Conceptualization, C.W. and P.R.; methodology, P.R. and C.W.; software, E.R.; formal analysis, M.M., E.R., K.N. and C.N.; investigation, D.-S.R., M.G., M.R., and T.M.; writing—original draft preparation, P.R. and C.W.; writing—review and editing, C.W., P.R., and H.-P.B.; funding acquisition, C.W., S.B., M.R. and H.-P.B. All authors have read and agreed to the published version of the manuscript.

Funding: Supported by the German MoD (E/U2CA/FA159/FF552).

Acknowledgments: We thank Clemens Kirschbaum, Institut für Allgemeine Psychologie, Biopsychologie und Methoden der Psychologie, Technische Universität, Dresden, Germany, for measuring the salivary α-amylase activity.

Conflicts of Interest: The authors declare no conflict of interest.

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8 Acknowledgments

This dissertation would not have been achievable without the support and direction of numerous people who had a vital role in my academic journey. I would like to express my heartfelt gratitude to those who made this experience enriching and purposeful. Special thanks to my doctoral supervisor, Christiane, who offered steadfast encouragement, inspiration, and invaluable perspectives throughout the whole process. Her mentorship and expertise were crucial in defining the path of my research and in successfully completing my PhD. Peter's direction, counsel and his knowledge were immeasurable as I faced the challenges of my publications. Furthermore, I want to acknowledge the whole BEST-study crew for their emotional encouragement and outstanding teamwork. Their inspiration and assistance made this experience truly fulfilling. I am especially grateful for my parents, who paved my way with unconditional love and encouragement. They were a significant source of strength, motivation and inspiration during my academic pursuits. Finally, I would like to extend my deepest gratitude to my partner, who was my rock and reliable supporter throughout this journey. I could not have achieved this without your love, comfort and understanding.

Thank you all for your assistance and guidance. This dissertation became a reality through your efforts and confidence in me.

9 Curriculum Vitae

Personal Information

Name	Tanja Maier
Date of birth	1994
Place of birth	Ehingen

Education and Professional Experience

Since 04/2022	Independent practitioner with health insurance accreditation, Psychotherapiepraxis Maier in Friolzheim: Guideline treatment of patients in individual & group therapies.
05/2021 – 02/2022	Psychotherapist (Coverage Assistance) at Psychotherapiepraxis Breitruck, Weil der Stadt
Since 01/2018	Research Fellow (until 03/2021) & Ph.D. candidate (Dr. biol. hum.) in the BEST (Bundeswehreinsatz & Stress) Project: Psychosomatic Medicine and Psychotherapy, University Hospital Ulm & University of Ulm.
10/2017 - 04/2021	Training as Psychological Psychotherapist at AWIP PT1 & PT2: University Hospital Ulm, Psychiatry III. Additional qualifications: Group Psychotherapy & Relaxation Techniques. Graduation: Cognitive Behavioral Therapy for Adults with a grade of 1.0.
2015 - 2017	Master's in Psychology (M.Sc.), University of Ulm. Specializations: Clinical Psychology, Personnel & Organizational Psychology. Graduation: Psychology (M.Sc.) with a grade of 1.3. Master's thesis: "The Influence of Inflammation on Lipid Metabolism Moderated by Psychosocial Stress in Bundeswehr Soldiers."
2012 - 2015	Bachelor's in Psychology (B.Sc.), University of Ulm. Graduation: Psychology (B.Sc.) with a grade of 1.5. Bachelor's thesis: "The Relationship between Hostility and DNA Damage in Leukocytes: A Comparison between Patients with Borderline Personality Disorder and a Healthy Control Group."

Publications

Maier, T., Kugelmann, M., Rhee, D.-S., Brill, S., Gündel, H., Friemert, B., Becker, H.-P., Waller, C., & Rappel, M. (2021). Structural Equation Modeling of a Global Stress Index in Healthy Soldiers. *Journal of Clinical Medicine, 10*(8), 1799. https://doi.org/10.3390/jcm10081799

Maier, T., Rappel, M., Rhee, D.-S., Brill, S., Maderner, J., Pijahn, F., Gündel, H., Radermacher, P., Friemert, B., Becker, H.-P., & Waller, C. (2022). Mental but no bio-physiological long-term habituation to repeated social stress: A study on soldiers and the influence of mission abroad. *Frontiers in Psychiatry, 13*, 1011181. https://doi.org/10.3389/fpsyt.2022.1011181

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